Resident Handbook

Division of Transplantation and Hepatic Pathology
University of Pittsburgh Medical Center
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Anatomic Transplantation Pathology Rotation

Clinical Responsibilities of the Division

The Division of Transplantation Pathology is responsible for pathology support for the Thomas E. Starzl Transplantation Institute. This includes evaluation of primary recipient disease, resected donor organs, and resected allografts. Evaluation of post-transplant biopsies for rejection and other causes of graft dysfunction comprise the main daily workload. This Division also evaluates biopsies of native organs from transplant patients and handles all native liver biopsy specimens. Some native kidney biopsies are also performed in this Division; these are not incorporated into resident rotations.

The Division conducts six separate weekly clinicopathologic conferences to ensure quality control of biopsy results and to keep an open channel of communication between the clinical physicians and transplantation pathologists. In addition, there are two intradivisional quality assurance slide review conferences per week, to ensure agreement among the pathologists in grading rejection and to discuss interesting and/or difficult cases.

Categorization of Specimens and Structure of "Signout"

Specimens that come to the Division for review fall into five categories. They include "Bigs," of which the majority are diseased native organs removed at the time of transplantation; "Quicks," mainly biopsies such as surveillance gastrointestinal biopsies; native liver biopsies; skin biopsies for GVHD; lymph node biopsies to evaluate for PTLD, etc; "Stats," mainly organ allografts biopsies used to monitor rejection; and "Consults" which consist of outside slides submitted for review. The Division also handles a portion of medical kidney biopsies.
The priority ranking the specimens receive, the structure of signout and reporting of the results are designed to best serve the transplant patients and clinical physicians involved in their care. "Stat" specimens receive the highest priority. These biopsies are submitted to Pathology before 11 AM and permanent H&E slides are ready for review by 2:30-3:30 PM the same day. "Quicks" and "Consults" are next in priority, and have a one day or less turnaround whenever possible. "Bigs" receive the next highest priority, and are signed out as expeditiously as possible. Native kidney biopsy results are transmitted to physicians in a provisional manner and signed out as special studies become available.

The staff service responsibilities are divided as follows: One staff pathologist takes weekly responsibility for the Quicks and Stats, and also handles any frozens that occur during the workday. This rotation runs from 7:30 AM until 5 PM, Monday through Friday. A second pathologist takes responsibility for all Bigs and Consults for this time period. This second pathologist covers nightly call during the week, and additionally covers the entire service for the weekend. The services are staggered in the following way: Saturday and Sunday, pathologist A covers everything. Monday through Friday, Pathologist A covers Bigs/Consults/Night call and Pathologist B covers Quicks and Stats. Saturday and Sunday, Pathologist C covers everything. Monday through Friday Pathologist C covers Bigs/Consults/Night call and Pathologist D covers

Quicks and Stats…and so forth. Holidays are treated like any other day of the week or weekend. The turnover times between shifts are 7:30 AM and 5:00 PM.

Resident Responsibilities

The level of resident responsibility depends upon three factors: the level of training, competence, and the desire to assume responsibility. PGY-1 level residents are generally responsible for all "big" cases, including gross evaluation, organization, and review of the slides and finally, signout with the pathologists. The gross processing of cases can usually be accomplished by mid-morning or early afternoon. The resident is expected to sit in on signouts and to participate according to the level of his/her experience. When more than one resident is on rotation, it is the residents' responsibility to divide the workload between them. Residents >PGY-1 may want to assume more responsibility by reviewing "quicks" and "consults" to enhance learning opportunities. A satisfactory division of labor in the past has been for the PGY-1 to assume responsibilities for "bigs"and >PGY-1 to take "quicks" and "consults." The cases are then shared at signout time. Unfortunately, because of the urgency of Stat specimens, it is often not possible for the residents to review the cases before the official signout. The pathologist and resident review the cases together on a daily basis, and the preliminary results are recorded daily in the "Stat Book," immediately outside the signout room. A recent change has been to deliver the "quicks" at 10:30 AM. Depending upon the signout time, this may give the resident an opportunity to review these cases upon delivery. The "big" specimens offer excellent learning opportunities in inflammatory and neoplastic liver disease and cardiovascular pathology. Most renal disease tends to be endstage and native kidneys are often not resected at the time of transplant, in contrast to other organs. "Consult" cases offer excellent review of late posttransplant liver, kidney, and heart pathology, and review of native liver disease.

The resident will be provided with desk space in Transplantation Pathology, and should remain "on-site" during the rotation. If the resident will be away from the Division, it is his/her responsibility to notify the pathologist or secretary of this. This minimizes misplaced slides, reports, requisitions, etc. All slides, typed gross reports with requisitions, special stains, etc. will be delivered to Transplantation Pathology. These should go into the common signout basket and are not delivered to individual mailboxes. It will be the resident's responsibility to organize the cases for which she/he has "taken charge."

Learning Resources

The Division keeps glass slide study sets of liver, kidney, heart, intestine and pancreas transplantation, as well as special topic slide boxes for resident review. These cases may be photographed, but otherwise they are not to leave the Division. Older study slides are in individual binders and are maintained by Ms. Joyce Marcoz. These can be signed out for study from her. In addition we maintain an ongoing collection of slides placed in containers in the signout room and available for resident review. These slides may be photographed but should not be removed from the Division. We are currently transferring these cases to a whole slide digital imaging format for permanent reference via the internet.

Transplantation Pathology on the World-Wide Web

We have put great effort into producing an informative and up-to-date transplant pathology site on the World-Wide Web. This is designed to be a working resource for the practicing pathologist who must deal with transplant-related material. We urge you to take advantage of this site while you are with us. This will benefit you long after you leave the residency program, since you will be able to access it at any time and from any site. The address is http://tpis.upmc.edu. and is currently being transferred to a separate server under the address http://tpis.upmc.com. The grading schemas that appear throughout this handout have been copied from our web pages. You should check the website directly for the most recent versions of these schemas. There is much additional information on line that has not been reproduced for this handout. Remember that this material is copyrighted and cannot be copied for commercial use. You are welcome to use it in lectures and presentations, and we hope that you would give credit to us for this material. Your comments and suggestions for improvements to this site are welcome.

This handout itself contains copies of a number of published papers. We have not obtained copyrights for these, and this handout is strictly for your private use as a member of our Department. We wish you the best of luck in your training with us and in your career as a pathologist. You should always feel free to consult with us or to just stop by to say "Hello."

Weekly Schedule

The following is an "idealized" weekly work schedule. Signout times are variable and should be determined between staff and resident. Also, some signouts may occur in E733 and others may occur in the pathologist's office. Note that residents are not required to attend Sunday signout. Check with the signout pathologist for specific times of signout during a given week, as this schedule may also be modified by other conferences and commitments.
"Conf" refers to the Conference Room in the Division.

Day	Time	Room	Activity
Monday	2:30 – 5:00 p.m.	E733	Quicks, Consults, Bigs signout
Tuesday			
	10:30 – 11:30 a.m.	E733	Quicks signout
	1:00 – 1:30 p.m.	E733	Slide Review Conference
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	Conf	Kidney Transplant Conference
Wednesday			
	10:30 – 11:30 a.m.	E733	Quicks signout
	1:00- 2:00	Conf	Research/Admin Conf.
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	Conf	Liver Tumor Conference
Thursday	10:30 – 11:30 a.m.	E733	Quicks Signout
	1:00-1:30	E733	Slide Review Conference
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	E733	Liver Transplant Conference
Friday	10:00-12:00	Conf	Small Bowel Transplant Conference
	10:30 – 11:30 a.m.	E733	Quicks signout
	2:30 – 5:00 p.m.	E733	Stats, Consults, Bigs signout
Saturday	9:00 – 11:00 a.m.	E733	Bigs, Quicks signout
	2:30 – 3:30 p.m.	E733	Stats signout
Sunday	10:30 – 11:00 a.m.	E733	Quicks signout (Staff only)
	2:30 – 3:30 p.m.	E733	Stats signout (Staff only)

TRANSPLANT PATHOLOGY PHONE LIST					
	Room #	Telephone	Fax	Pager	Lab
Askren, Linda	E-742 MUH	412-647-2067	412-647-2084		
Batal, Ibrahim	E-732 MUH	412-647-2034	412-647-5237	412-958-0430	
Cappella, Nickie	N-753 MUH	412-647-5143	412-647-1434	4122158954@vtext.com	
Conference Room	E-724 MUH	412-647-2027			
CORE		412-963-3550	412-963-3596		
Demetris, Anthony	E-741 MUH	412-647-2072	412-647-2084	cell: 412-860-8351	
Duquesnoy, Rene		412-624-1075		Tissue Typing	7-6148
Esch, Lorrie	E-736 MUH	412-647-3169	412-647-5237	412-958-7562	
Farasati, Nousha	E-732 MUH	412-648-8403		412-958-4469	648-8403
Gross Room	A-625 PUH	412-647-6597	412-647-6251	412-393-9819 (Frank)	
Kitchen		412-647-9507			
Main (Sign out room)	E-733 MUH	412-647-7645	412-647-5237		
March, Jill	E-736 MUH	412-647-9509	412-802-8799		
Marcoz, Joyce	E-733 MUH	412-647-7645	412-647-5237		
McMichael, John	E-735 MUH	412-647-8375	412-647-5237	cell: 412-721-0621	
Nalesnik, Michael	E-738 MUH	412-647-2094	412-647-5237	412-565-8201	
Ochoa, Erin	E-739 MUH	412-647-9568	412-647-5237	412-958-5619	624-1304
Ramaswami, Bala	E-732 MUH	412-647-3378	412-647-5237	412 958 7670	648-8403
Randhawa, Parmjeet	E-737 MUH	412-647-7646	412-647-5237	412-565-1422	648-8403
Resident Room Wall	E-732 MUH	412-647-7641			
Sasatomi, Eizaburo	E-732 MUH	412-682-1981	412-647-5237	412-958-3242(3293)	
Student	E-733 MUH	412-647-2054			
Zeevi, Adriana (Monica Green)		412-624-1073 (412-647-3786) or, 412-624-6183	(412) 624-6666	(412) 392-7475	
Physician Services Help Desk		7-7748	Service for Copier	888-831-7400	Model #
RHS Lab		412-624-6603		SN - 27LE00083	7222

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Historic Landmarks in Clinical Transplantation: Conclusions from the Consensus Conference at the University of California, Los Angeles

Carl G. Groth, M.D., Ph.D.,[1] Leslie B. Brent, B.Sc., Ph.D.,[2] Roy Y. Calne, M.D.,[3] Jean B. Dausset, M.D., Ph.D.,[4] Robert A. Good, M.D., Ph.D.,[5] Joseph E. Murray, M.D.,[6] Norman E. Shumway, M.D., Ph.D.,[7] Robert S. Schwartz, M.D.,[8] Thomas E. Starzl, M.D., Ph.D.,[9] Paul I. Terasaki, Ph.D.,[10] E. Donnall Thomas, M.D.,[11] Jon J. van Rood, M.D., Ph.D.[12]

[1]Department of Transplantation Surgery, Karolinska Institute, Huddinge Hospital, SE-141 86 Huddinge, Sweden
[2]30 Hugo Road, Tufnell Park, London N19 5EU, UK
[3]Department of Surgery, University of Cambridge, Douglas House Annexe, 18 Trumpington Road, Cambridge CB2 2AH, UK
[4]Foundation Jean Dausset—C.E.P.H., 27 rue Juliette Dodu, 75010 Paris Cedex, France
[5]Department of Pediatrics, Division of Allergy and Immunology, All Children's Hospital, 801 Sixth Street South, St. Petersburg, Florida 33701, USA
[6]Department of Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115, USA
[7]Department of Cardiothoracic Surgery, Falk Cardiovascular Research Center, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, California 94305-5247, USA
[8]*The New England Journal of Medicine,* 10 Shattuck Street, Boston, Massachusetts 02115-6094, USA
[9]Department of Surgery, University of Pittsburgh, School of Medicine, Thomas E. Starzl Transplantation Institute, 3601 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA
[10]12835 Parkyns Street, Los Angeles, California 90049, USA
[11]Department of Medicine, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, PO Box 19024, Seattle, Washington 98109-1024, USA
[12]Department of Immunohematology and Blood Bank, University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands

Abstract. The transplantation of organs, cells, and tissues has burgeoned during the last quarter century, with the development of multiple new specialty fields. However, the basic principles that made this possible were established over a three-decade period, beginning during World War II and ending in 1974. At the historical consensus conference held at UCLA in March 1999, 11 early workers in the basic science or clinical practice of transplantation (or both) reached agreement on the most significant contributions of this era that ultimately made transplantation the robust clinical discipline it is today. These discoveries and achievements are summarized here in six tables and annotated with references.

The symposium making up this issue of the *Journal* was held at the University of California, Los Angeles (UCLA) and announced by the Department of Surgery hosts as "a unique and historic meeting at which pioneers of transplantation from around the world will present and discuss landmarks in the advancement of transplantation biology." The participants (in alphabetical order) were: Leslie B. Brent (London), Roy Y. Calne (Cambridge, UK), Jean Dausset (Paris), Robert A. Good (St. Petersburg, FL), Joseph E. Murray (Boston), Norman E. Shumway (Palo Alto), Robert S. Schwartz (Boston), Thomas E. Starzl (Pittsburgh), Paul I. Terasaki (Los Angeles), E. Donnall Thomas (Seattle), Jon J. van Rood (Leiden).

Each of these 11 pioneers provided for publication their reflections about their own unique contributions. The ultimate objective, however, was to reach a consensus by the group on what were the most critical historical discoveries that made transplantation a form of clinical therapy. Carl G. Groth (Stockholm) was invited to be the Chairman for these consensus deliberations and to prepare the executive summary.

Historical landmark status was restricted to contributions made at least a quarter of a century ago. By this time it had been established that rejection of organ allografts could be prevented or reversed with immunosuppressive drugs and that variable donor-specific immunologic tolerance of the graft subsequently developed in many patients. Long-term survival of human recipients of organ and bone marrow allografts had been repeatedly obtained, ensuring continuation of such clinical efforts. A large number of HLA antigens had been discovered, allowing efforts at tissue matching to proceed. The scientific articles annotating this progress are listed in six tables under the following headings: transplantation immunology, bone marrow transplantation, renal transplantation, liver transplantation, heart transplantation, and tissue matching. The material presented in these tables, including the citations, originated from the participants of the symposium.

It should be noted that transplantation could not have proceeded without contemporaneous advances in general and thoracic surgery, medicine, and anesthesia, such as open-heart surgery, renal dialysis, antibiotics, and intensive care technology. The

Correspondence to: C.G. Groth, M.D., Ph.D.

Table 1. Transplantation immunology.

Author	Discovery or application	Year published	Reference
Gibson	Defined the immunologic nature of skin allograft rejection in humans, confirmed subsequently with controlled rabbit experiments.	1943	1
Owen	Discovered that bovine dizygotic twins with placental vascular anastomoses (freemartin cattle) were red blood cell chimeras.	1945	2
Burnet	Based on Owen's observations and on studies of lymphocytic choriomeningitis virus by Traub, Burnet, and Fenner postulated "the development of tolerance . . . during embryonic life."	1949	3
Anderson	Demonstrated mutual tolerance to skin grafts by freemartin cattle twins and speculated that "actively acquired tolerance" was responsible.	1951	4
Billingham	Produced actively acquired donor specific tolerance to skin allografts in mice injected during late fetal life with donor hematolymphopoietic cells.	1953	5
Simonsen	Independently demonstrated GVHD in chick embryos (manifested as	1957	6
Billingham	pancytopenia) and mice (runt disease) after intravenous injection of adult spleen cells.	1957	7
Starzl	Reported evidence that human kidney allografts under azathioprine-prednisone induced variable donor specific nonreactivity.	1963	8

GVHD: graft-versus-host disease.

cardiopulmonary resuscitation procedures introduced during the 1950s were particularly influential because they mandated redefinition of death in terms of irreversible brain damage rather than the cessation of heartbeat and respiration. While salvaging countless victims of cardiac or pulmonary arrest, the new methods also resulted in brain-dead corpses on physiologic life support.

In 1966, at a symposium on medical ethics in London, G.P.J. Alexandre described the criteria of brain death that had been used in Belgium and France for discontinuing mechanical ventilation of "heart-beating cadavers." It became possible thereby to remove kidneys and other organs from cadaver donors with an intact circulation. The concept was further elaborated in a Harvard-based ad hoc committee report in 1968 in the *Journal of the American Medical Association.* The impact on transplantation of cadaver organs was immediate and lasting.

Transplantation Immunology

The modern age of transplantation immunology (Table 1) [1–8] began with three seminal observations. First, rejection is a host-versus-graft (HVG) immune reaction. Second, a similar immune reaction [graft-versus-host (GVH)] may occur in reverse and lead to lethal graft-versus-host disease (GVHD). Third, it is possible under well defined experimental conditions to avert rejection as well as GVHD and to induce tolerance of alloantigens, which is strongly associated with the persistence in the recipient of donor leukocyte chimerism.

The next step was the recognition that organ allografts are inherently tolerogenic, a property without which their transplantation with long survival in the recipient would not be possible (Table 1). The tolerance induced by organs usually is manifested only under an umbrella of immunosuppression, but it is not a prerequisite in some animal models, particularly if the allograft is the leukocyte-rich liver (see also Table 4).

The discoveries listed in Table 1 were made piecemeal over a period of 25 years, obscuring the fact that all three of the fundamental phenomena studied by early workers (i.e., HVG, GVH, and acquired tolerance) were involved, but to different degrees, in the "acceptance" of organ allografts and the tolerance induced by allogeneic bone marrow following recipient cytoablation. In 1992 the mechanistic linkage of engraftment after these two kinds of transplantation was established with the discovery of donor leukocyte microchimerism in long-surviving human organ recipients.

The clonal selection theory proposed in 1949 by Burnet and Fenner marked the beginning of a new wave in immunology, from which transplantation is often viewed as a mere stream. Instead, transplantation is a mighty tributary. It fostered research into the mechanisms of the destructive antigraft immune response and the control of this response. From these efforts, directly or indirectly, came the discovery of the function of the lymphocyte (1959–1961) and the role of the thymus in the ontogeny of the immune system (1961); delineation (1958–1963) of the human major histocompatibility complex (MHC); distinction of the T and B lymphocyte subsets (1967–1968); and mainly by study of antiviral immune responses, demonstration of the MHC-restricted nature of the adaptive immune response (1968–1974).

Bone Marrow Transplantation

Bone marrow transplantation (Table 2) [9–22] had its roots in radiobiology and hematology, and it was influenced by clinical studies of certain inherited immune deficiency diseases. Early in these efforts it was learned that engraftment of histoincompatible bone marrow can cause lethal GVHD in a recipient rendered immunologically defenseless by cytoablation, a complication also predicted in recipients with immune deficiency disease. Consequently, the preclinical and clinical development of bone marrow transplantation was delayed until reliable methods of HLA typing and matching became available.

The first completely successful bone marrow transplantations were in children with immune deficiency diseases whose family donors were selected with relatively primitive first-generation tissue-matching techniques. Because of their T cell deficiency, these recipients did not require the cytoablation and postgrafting immunosuppression needed with other indications for bone marrow transplantation. With the use of methotrexate as an immunosuppressant in cytoablated recipients, bone marrow transplantation subsequently was applied with steadily improving results in those with an array of benign and malignant hematolymphopoietic dis-

Table 2. Bone marrow transplantation.

Author	Discovery or application	Year published	Reference
Jacobson	Protection against lethal irradiation by spleen shielding, mistakenly ascribed to humoral factors.	1951	9
Lorenz	Protection against lethal irradiation by injection of bone marrow, mistakenly ascribed to humoral factors.	1951	10
Main	Protection against lethal irradiation in mouse by infusion of bone marrow cells and subsequent acceptance of skin allograft from the marrow donor (tolerance). Recognized analogy to neonatal tolerance.	1955	11
Ford	Proved with cytogenetic techniques that marrow cells of mouse reconstituted with bone marrow after lethal total body irradiation (TBI) were donor origin.	1956	12
Barnes	First attempt to treat leukemia in mice by bone marrow transplantation after lethal TBI.	1957	13
Thomas	First attempts to treat malignancy in human patients by high dose chemotherapy or TBI and an infusion of marrow, showing safety of the infusion and one example of transient engraftment.	1957	14
Thomas	Two children with leukemia given twice the lethal dose of TBI and bone marrow from an identical twin had benign hematologic recovery. Recurrence of leukemia led to the subsequent addition of chemotherapy to TBI.	1959	15
Thomas	First outbred animals (dogs) to be successfully engrafted with allogeneic marrow; conditioning with TBI and treatment after grafting with a short course of methotrexate. Graft rejection, other causes of graft failure, and GVHD described.	1962	16
Mathé	World's first prolonged engraftment of human allogeneic bone marrow; adult recipient with leukemia conditioned with TBI. Died without disease recurrence after 20 months, probably from complications of GVHD.	1963	17
Storb	After developing dog typing sera, achieved survival of most histocompatibility matched, but not of unmatched, recipients of bone marrow from littermate donors. Recipients cytoablated and treated with a short course of postgraft methotrexate.	1968	18
Gatti[a]	After initial illuminating analyses of the inborn errors of lymphocyte development [X-linked agammaglobulinemia, thymic alymphoplasia, and severe combined immunodeficiency disease (SCID)] as experiments of nature, Good suggested a new two-component concept of immunity and performed the world's first completely successful bone marrow transplant in a child with otherwise uniformly lethal X-SCID. A second marrow transplant from the same donor cured a complicating aplastic anemia in this patient, also for the first time.	1968	19
Bach[a]	This was followed by a partially successful allogeneic bone marrow engraftment in a child with Wiskott-Aldrich syndrome.	1968	20
deKoning[a]	Successful allogeneic bone marrow plus thymus engraftment was done subsequently in a child with lymphopenic immune deficiency.	1969	21
Thomas	Review of bone marrow transplantation, including description of first large series of patients with aplastic anemia or leukemia given allogeneic marrow grafts from matched siblings. Problems with GVHD and opportunistic infections defined, with emphasis on the importance of histocompatibility, and discussion of possible use of matched unrelated donors.	1975	22

[a]These three patients did not need myeloablation or postgraft immunosuppression.

eases, other kinds of malignancies, and numerous inborn errors of metabolism.

Kidney Transplantation

Three factors made the kidney a pathfinder organ in transplantation (Table 3) [8, 23–47]. One was the development of dialysis for the treatment of acute, and ultimately chronic, renal failure. The second was the fact that the kidney is a paired organ, ensuring a supply of surgically removed "free kidneys" and, increasingly after 1953, physiologically ideal live donor kidneys. Third, its technical simplicity and the ease with which allograft function could be monitored made kidney transplantation ideal for laboratory and clinical investigation.

By 1974 kidney transplantation had already gone through the four eras shown in Table 3 defined by: no immunosuppression, immunosuppression with total body irradiation (TBI), the first use of drugs to prevent rejection (azathioprine) or reverse it (prednisone), and the introduction of adjunct anti-lymphocyte antibody therapy. Each major improvement in immunosuppression up to 1974 and subsequently permitted goals in kidney transplantation to be reached that were not attainable before.

Thus the transition from no therapy to TBI corresponded with the step from identical to fraternal twin transplantation. The change to azathioprine-based treatment established kidney transplantation as a clinical service from 1963 onward, especially using kidneys from living related donors. Cadaver kidney transplantation burgeoned with the acceptance of brain death during the late

Table 3. Kidney transplantation during four eras.

Author	Discovery or application	Year published	Reference
Preimmunosuppression			
Carrel	Developed vascular anastomotic techniques used for organ transplantation today.	1902	23
Lawler	Surgically excised ("free") kidney allograft transplanted to recipient nephrectomy site. Function controversial.	1950	24
Küss	Free kidneys or kidneys from guillotined donors transplanted with surgical techniques still used today.	1951	25
Michon	First use of living related donor kidney (mother to son): good function before rejection at 3 weeks.	1953	26
Hume	Nine cadaveric or free kidneys transplanted, eight to thigh and one to an orthotopic location. One thigh kidney functioned for 5 months.	1955	27
Murray	First transplantation of identical twin kidney on 12/23/54,	1955	28
Merrill	reported first in abstract [28] and more completely the following year [29]. Later report of first nine cases included description of first posttransplant pregnancy.	1956	29
Total body irradiation			
Murray	Renal allograft from fraternal twin transplanted (1/24/59) to a	1960	30
Merrill	recipient preconditioned with sublethal TBI [30] more fully reported elsewhere [31]. This was the first long survival of an organ allograft, an objective not previously achieved in an animal model.		31
Hamburger	Second successful fraternal twin kidney transplantation using TBI, performed June 1959.	1959	32
Hamburger	Successful transplantations of two living related but nontwin kidney allografts using TBI; secondary steroid administration mentioned.	1962	33
Küss	Eighteen-month survival of two nonrelated kidney allografts using TBI; secondary steroid and 6-mercaptopurine (6-MP) administration noted, without details.	1962	34
Chemical immunosuppression			
Schwartz	Showed in rabbits given bovine serum albumin (BSA) while also being treated with 6-MP that the 6-MP suppressed the antibody response to BSA and rendered the animals tolerant of the foreign protein. The experiments were driven by the hypothesis that the proliferating immunocytes of an expanding antigen-specific clone would be selectively vulnerable to antimetabolite drug therapy.	1959	35
Schwartz	Independently demonstrated a 6-MP dose-related prolongation	1960	36
Meeker	of rabbit skin allograft survival.	1959	37
Calne	Moved from the skin to an organ allograft model and	1960	38
Zukoski	demonstrated (independent from each other) prolongation by 6-MP of canine kidney allograft survival.	1960	39
Calne	Further extensive preclinical studies (in Murray's Boston laboratory) of a report on efficacy in dogs of 6-MP and its analogue azathioprine.	1961	40
Murray	Clinical trials begun with 6-MP and azathioprine.	1962	41
Murray	Report of first 13 patients treated with 6-MP or azathioprine, one of whom reached 1 year with a still functioning but failing kidney allograft on 4/5/63.	1963	42
Starzl	First systematic use of azathioprine and prednisone with long survival of most of kidney allografts.	1963	8
Starzl	Clinical experience summarized with azathioprine/prednisone therapy in recipients of 67 kidney allografts and 6 baboon xenografts.	1964	43
Antibody immunosuppression			
Waksman	Demonstration of anti-lymphocyte serum (ALS) efficacy with skin allograft test model in rats.	1961	44
Woodruff	Showed additive protection of skin allografts in rats using ALS combined with thoracic duct drainage.	1963	45
Monaco	Convincing demonstration of the therapeutic value of ALS in the canine kidney transplant model.	1966	46
Starzl	First clinical trial of anti-lymphocyte globulin (ALG) as an adjunct to azathioprine and prednisone for human kidney transplantation. With the hybridoma technology of Kohler and Milstein (1975) monoclonal antibodies could be raised against discrete immunologic targets. In 1981 anti-CD3 antibody (OKT3) was introduced clinically.	1967	47

Table 4. Liver transplantation.

Author	Discovery or application	Year published	Reference
Preimmunosuppression			
Welch	First mention of hepatic transplantation in the literature, with insertion of an auxiliary liver in unmodified dogs.	1955	48
Moore	Independent studies in Boston and Chicago of liver replacement	1960	49
Starzl	(orthotopic transplantation) in unmodified dogs.	1960	50
Starzl	Transplantation in dogs of multiple abdominal viscera, including liver and intestine, nearly identical to human procedures done three decades later.	1960	51
Immunosuppression era			
Starzl	World's first three attempts at orthotopic liver transplantation in humans (March 1, May 5, and June 24, 1963) with maximum survival of 21 days.	1963	52
Starzl	Discovery that splanchnic venous blood of dogs contained hepatotrophic factor(s), the most important of which was later proved to be insulin; the finding dictated methods of liver allograft revascularization.	1964	53
Starzl	First >1-year survival after liver replacement in any species (here mongrel dogs) with recognition of the liver's unusual ability to induce tolerance under a 3- to 4-month course of azathioprine, or in this canine model after only a few perioperative injections of ALS or ALG [47].	1965	54
Cordier	Observed that liver allografts in untreated pigs frequently were not rejected. This finding of spontaneous tolerance to livers was promptly confirmed by Peacock and Terblanche in Bristol and by Calne in Cambridge.	1966	55
Starzl	First report of prolonged survival of four (of seven) children after orthotopic liver transplantation between July 1967 and March 1968.	1968	56
Calne	Report of first four patients in the Cambridge (England) liver replacement series, including an adult with >4 months survival.	1968	57
Calne	Showed that spontaneous tolerant pig liver recipients also were tolerant to skin and kidney allografts from the same donor.	1969	58
Starzl	Text summarizing experience at the University of Colorado with 25 liver replacements to March 1969 and 8 cases elsewhere.	1969	59
Starzl	Metabolic abnormality of Wilson's disease corrected, first of more than two dozen liver-based inborn errors cured or ameliorated with liver replacement. These liver recipients and patients cured of mesoderm-based inborn errors by bone marrow transplantation were the first examples of effective genetic engineering.	1971	60

1960s and the subsequent establishment of organ procurement agencies, usually associated with clinical immunology laboratories for tissue (HLA) matching. By 1974 renal transplantation had become a government-financed component of health care in most Western countries.

Liver Transplantation

After a failed trial in 1963, liver transplantation was successfully performed in humans in July 1967 (Table 4) [48–60]. Hepatic replacement was initially viewed as too difficult to be technically feasible, particularly in terminally ill patients for whom artificial organ support comparable to renal dialysis was not available. Instead, challenges generated by its surgical difficulty and physiologic complexity made liver transplantation the co-leader after 1963 (with the kidney) or the leader in the development of broadly applicable advances of surgical technique, immunosuppression, and means of multiple organ procurement and preservation.

Despite a high mortality rate during the first year after liver transplantation, nearly two dozen recipients from this early era have been stable for 20 to more than 29 years using immunosuppression with azathioprine, prednisone, and antilymphocyte globulin (ALG). The proof of the liver's unusual tolerogenicity (Tables 1, 4) is that most of these patients have been able to discontinue immunosuppressive therapy without rejecting their grafts.

The ripple effects of liver transplantation included discovery of the first hepatotrophic factors (beginning with insulin) that are involved in hepatic growth control and regeneration. More than two dozen liver-based inborn errors of metabolism have been corrected by liver transplantation, with clarification of disease mechanisms in some.

Heart Transplantation

The landmarks of heart transplantation are summarized in Table 5 [61–69]. Studies of heart transplantation were carried out at Stanford University in dogs and subhuman primates from the late 1950s to 1967. The results justified the decision by this group to proceed clinically, as announced by interview in the November 20, 1967, issue of the *Journal of the American Medical Association.* On December 3, heart replacement was carried out in Cape Town following an extended visit by the South African team leader to Stanford and other American transplant centers. The first South African recipient died from infection after 18 days, but the second patient (January 2, 1968) lived several years. On January 5, 1968,

Table 5. Heart transplantation.

Author	Discovery or application	Year published	Reference
Cass	Described standard current practice of combining the multiple pulmonary venous and venacaval anastomoses into two large atrial anastomoses. No dogs survived the operation.	1959	61
Lower	Independently developed same procedure as Cass/Brock, preserving allografts with immersion hypothermia. Dogs recovered.	1960	62
Lower	Technically successful canine heart-lung transplantation in nonimmunosuppressed dogs with 5-day survival. With long survival the same operation was done under cyclosporine two decades later, first in monkeys and then in humans.	1961	63
Lower	Immersion hypothermia of canine allografts at 2°–4°C adequately preserved dog hearts for 7 hours.	1962	64
Dong	Demonstrated normal heart function and reinervation of cardiac autografts 2 years after transplantation in dogs.	1964	65
Hardy	Transplantation of chimpanzee heart to human recipient. The heart was too small to support the circulation and failed after 2 hours.	1964	66
Lower	First long survival (up to 9 months) of heart allografts in any species (here dogs). Azathioprine-based immunosuppression was guided by electrocardiogram (ECG) voltage changes, especially R-wave diminution.	1965	67
Barnard	Description of the world's first transplantation of a human heart in Cape Town on 12/3/67, with 18 days survival. A second attempt in New York on 12/6/67 failed after 6 hours. A third recipient, operated in Cape Town on 1/2/68, survived for several years.	1967	68
Stinson	The world's fourth human heart transplantation at Stanford on 1/5/68 was successful and inaugurated the long-standing thoracic organ transplant program at that institution.	1970	69

the Stanford program recorded its inaugural human case, which was successful.

Graft survival after heart transplantation using triple-drug immunosuppression (azathioprine, prednisone, ALG) was essentially equivalent to that of cadaver kidney transplantation. As with kidney and liver transplantation, many of the pioneer cardiac recipients enjoyed an excellent quality of life, ensuring prompt acceptance and widespread application of all these operations when better immunosuppression became available.

Tissue Matching

The ABO blood groups, the compatibility of which was later found to be a requirement for transfusion and for bone marrow and organ transplantation, were discovered in 1901 [70]. Similarly, it was necessary to develop methods to type human tissue antigens and then to determine which were compatible or incompatible with those of the donor (Table 6) [70–94]. This was made possible with the discovery in transfused patients, and in women who had been pregnant, of leukoagglutinating and lymphocytotoxic antibodies that recognized alloantigens.

The introduction of computer-assisted search systems allowed delineation of families of antibodies that reacted with individual alloantigens and also made feasible the grouping of alloantigens into the two closely associated series that are now called HLA-A and HLA-B. The demonstration of crossover of the A and B antigens established HLA as a closely linked supergene. After 1964 use of the microcytotoxicity test greatly facilitated the standardization of HLA typing and the search for HLA antigens. The method was adapted for donor-recipient crossmatching and subsequently for the detection of pretransplant sensitization to HLA alloantigens.

HLA matching has been a stringent requirement for bone marrow transplantation (Table 2). For organ transplantation, the lymphocytotoxic crossmatch has been of crucial importance. Although there is clear evidence that the HLA system contains the dominant histocompatibility antigens, it has not been possible to identify which mismatches would result in failure. Nonetheless, HLA-identical sibling kidney allografts provide the highest graft survival rates. These are approached by survival rates of zero HLA-mismatched cadaver kidneys, justifying kidney sharing.

Quarter Century after 1974

The advent of cyclosporine two decades ago was a watershed for both bone marrow [95] and organ [96] transplantation. When the new drug was substituted for azathioprine, allograft survival and the quality of recipient life improved dramatically. In particular, the transplantation of cadaver organs was upgraded from a frequently feasible but unpredictable service to a reliable one. The results of organ transplantation were further enhanced after another decade with the introduction of tacrolimus [97]. Other promising drugs and monoclonal antibody preparations have been introduced more recently or are in various stages of preclinical or clinical evaluation. However, the therapeutic principles have remained essentially the same as were originally developed with azathioprine, prednisone, and ALG.

With more potent immunosuppressive agents, the field of transplantation has expanded continuously over the last 25 years. Heart–lung and lung transplantations were extensions of the heart procedure. Although survival of a lung recipient for 10 months had been accomplished as early as 1969 [98], the first examples of survival exceeding 1 year were not reported for heart–lung transplantation until 1982 [99] and for lung transplantation until 1987 [100]. Efforts at transplantation of abdominal organs expanded from the liver-only to the liver combined with small bowel [101]

Table 6. Tissue matching.

Author	Discovery or application	Year	Reference
Landsteiner	Discovery of ABO blood groups.	1901	70
Gorer	Described single dominant histocompatibility locus (later H-2) in mouse, analogous to the human leukocyte antigen (HLA) system.	1948	71
Dausset	Discovered first HLA antigen (MAC) using antiserum from transfused patients.	1958	72
Van Rood	Independently demonstrated HLA antibodies in pregnant	1958	73
Payne	women.	1958	74
Van Rood	First use of computers to make sense of the complex reactions produced by human antibodies, allowing identification of antigens currently known as HLA-B 4 and 6, as well as leukocyte antigen grouping.	1963	75
Starzl	Hyperacute rejection of ABO-incompatible kidneys (from host isoagglutinins) and rules to prevent it.	1964	76
Terasaki	Description of microcytoxicity test, critical for further development and practical use of HLA typing.	1964	77
Bach	Independently described mixed lymphocyte culture (MLC) test	1964	78
Bain	of histocompatibility.	1964	79
Payne	Defined allelic system now known as HLA-A 1, 2, and 3.	1964	80
Van Rood	Described antigens now known as HLA-B7+B27 and HLA-B8 as part of a closely associated system.	1965	81
Dausset	Proposed single locus for the HLA system, analogous to the mouse H-2 system.	1965	82
Terasaki	Description of hyperacute kidney rejection associated with	1965	83
Kissmeyer-Nielsen	antigraft lymphocytotoxic antibodies and proposed prevention with cytotoxic crossmatch (Terasaki), confirmed and extended the following year with the leukoagglutinin test (Kissmeyer-Nielsen).	1966	84
Terasaki	First prospective trial of HLA matching for donor selection.	1966	85
Van Rood	Proposal that initiated the first international organ exchange organization.	1967	86
Ceppellini	Coined the term "haplotype" to indicate the chromosomal combination of HLA alleles.	1967	87
Amos	Showed that the MLC reaction was detecting the HLA-D locus.	1968	88
Kissmeyer-Nielsen	Described the first crossover between HLA-A and HLA-B, proving that HLA identified a chromosomal region and not a single locus.	1969	89
Dausset	Demonstrated the importance of HLA compatibility for the survival of skin grafts in unmodified human volunteers.	1970	90
Starzl	Long survival frequently achieved at all levels of HLA	1970–1	91
Mickey	mismatch using a living donor and cadaveric kidneys. However, the best function, histologic appearance of allografts, and survival as well as the least dependence on immunosuppression was with zero-HLA mismatched kidney allografts.		92
Terasaki	Identification of presensitized patients at high immunologic risk using the panel reactive antibody (PRA test).	1971	93
Van Leeuwen	Identified the first sera that could be used for HLA-DR typing. This formed the basis on which HLA-DR serology was developed.	1973	94

and to the more complex multiple abdominal visceral grafts [102]; in the end it resulted in successful engraftment of the small bowel alone [103]. Tacrolimus played a crucial role in making the abdominal procedures involving intestine clinically applicable.

Although pancreas transplantation was offered at first only to diabetic patients who also were undergoing kidney transplantation for diabetes-associated end-stage renal disease [104], pancreas transplantation alone has been performed more recently in nonuremic diabetics [105]. The alternative appealing approach of transplanting the isolated islets of Langerhans only was attempted during the 1970s but did not result in success (defined as insulin independence) until 1990 in a patient with postpancreatectomy diabetes [106] and 1991 in a patient with type I diabetes [107]. Success with this procedure still is achieved only in occasional cases.

Résumé

La transplantation d'organes, de cellules et de tissus a littéralement explosée dans ce dernier quart de siècle, avec le développement d'une multitude de nouvelle spécialités. Cependant, les principes de base qui ont rendu ceci possible ont été établi sur trois décennies, commençant pendant la deuxième guerre mondiale et terminant en 1974. Pendant la conférence de consensus historique tenu à l'UCLA du 25 au 27 mars, 1999, 11 chercheurs sur la transplantation travaillant en sciences

fondamentales et/ou en clinique se sont mis d'accord sur les contributions les plus significatives de cette période et ont donné à la discipline de transplantation sa crédibilité présente. Ces découvertes et accomplissements ont été résumés en six tableaux, dotées de 93 références.

Resumen

En los últimos 25 años se ha producido un auténtico renacimiento por lo que a trasplantes de órganos, células y tejidos se refiere, lo que ha propiciado el desarrollo de múltiples áreas nuevas de especialización. Sin embargo, los principios que hicieron posible los trasplantes se establecieron hace más de 3 décadas, ya que las investigaciones al respecto se realizaron en el periodo de tiempo comprendido desde los comienzos de la 2ª Guerra Mundial al final de 1974. En la histórica conferencia de consenso, celebrada en UCLA, del 25 al 27 de marzo de 1999, 11 investigadores pioneros, procedentes tanto de las ciencias básicas como de la clínica y del tratamiento mediante trasplantes, alcanzaron un acuerdo sobre, cuáles fueron los hitos más importantes de este periodo, que permitieron que la técnica de los trasplantes sea hoy una especialidad clínica bien definida y en continua expansión. Estos descubrimientos y realizaciones se resumen en 6 tablas y 93 referencias bibliográficas.

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Trends in Organ Donation and Transplantation in the United States, 1998–2007

R. A. Wolfe[a,*], R. M. Merion[b], E. C. Roys[a] and F. K. Port[a]

[a] *Scientific Registry of Transplant Recipients, Arbor Research Collaborative for Health, Ann Arbor, MI*
[b] *Scientific Registry of Transplant Recipients, University of Michigan, Ann Arbor, MI*
* *Corresponding author: Robert A. Wolfe, robert.wolfe@arborresearch.org*

The articles in this report are based on the reference tables in the 2008 OPTN/SRTR Annual Report. Table numbers are noted in brackets and may be found online at: http://www.ustransplant.org.

Key words: Allocation, graft survival, OPTN, organ donation, patient survival, SRTR, transplantation, waiting list

Introduction

This article presents an overview of solid organ transplantation in the United States and is produced as part of the 2008 *OPTN/SRTR Annual Report*. The *Annual Report* is prepared by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN) under contract with the Health Resources and Services Administration (HRSA). The report reviews many aspects of solid organ transplantation and is a valuable resource for patients, the transplant community, the public and the federal government.

This report includes eight articles focused on specific topics in solid organ transplantation. Each article was written by experts in the field of transplantation and provides a comprehensive look at the current state of transplantation and trends over the past 10 years. The text and figures in these articles are drawn from recent SRTR analyses and the extensive reference tables of the 2008 *Annual Report*. This report was prepared by the Arbor Research Collaborative for Health, which, with the University of Michigan, has been the contractor for the SRTR since October 2000. These eight articles and the 2008 *Annual Report* reference tables are available online, at the websites of the SRTR and OPTN (www.ustransplant.org and www.optn.org).

Summary Statistics on Organ Transplantation in the United States

At the close of 2006 there were 173 339 persons recorded in available OPTN data, who were living with a functioning organ transplant *[Table 1.14]*. This number reflects an increase of 1.6% over 2005 and a 60% increase since 1998.

The total number of organs transplanted decreased from 28 291 in 2006 to 27 578 in 2007; this was an overall decrease of 713 organs transplanted (2.5%) and a decrease of 423 (6.3%) in living donor transplants (Table 1). Deceased donor kidney transplants decreased by 1.3%, and living donor kidney transplants dropped by 6.1%. A decrease of 3.8% was observed in deceased donor liver transplants in 2007.

The number of lung transplants increased 4.3% while heart, deceased donor intestine, pancreas and heart–lung transplantation changed little. The 27 578 organs transplanted in 2007 came from 14 399 organ donors, 357 fewer donors than there were in 2006 (2.4% decrease) *[Table 1.1]*.

The total number of transplants in the United States increased on average by 872 transplants per year between 1998 and 2006 *[Table 1.7]*. Thus, the decrease of 713 transplants in 2007 represents a substantial divergence from the longstanding trend. This drop was largely due to decreases in donation, particularly by living donors. There were 423 (6.3%) fewer living donors in 2007 than in 2006. Living donation has been decreasing since 2004 *[Table 1.1]*.

The number of organs recovered for transplant from deceased donors has similarly departed from the recent trend. In 2007, there were 28 409 organs recovered compared with 28 322 in 2006 (Table 2). This increase of 87 organs is the smallest in 10 years. An average increase of 930 organs per year was seen between 1998 and 2006 *[Table 1.2]*.

More multiorgan transplants (97) were performed in 2007 than in 2006, the biggest increase in the number of multiorgan transplants in 10 years. Those 97 transplants involved 229 total organs *[Table 1.8]*.

The percentage of kidneys recovered but not used for transplant was the highest in 10 years. In 2007, there were

Table 1: Change in number of transplanted organs, 2006–2007

Transplanted organs	2006	2007	Percent change
Total	28 291	27 578	−2.5
Deceased donor	21 562	21 272	−1.3
Living donor	6729	6306	−6.3
Kidney	16 644	16 119	−3.2
Deceased donor	10 212	10 082	−1.3
Living donor	6432	6037	−6.1
Pancreas	1368	1304	−4.7
PTA	98	110	12.2
PAK	292	259	−11.3
Kidney–pancreas	914	848	−7.2
Liver	6136	5890	−4.0
Deceased donor	5849	5625	−3.8
Living donor	287	265	−7.7
Intestine	60	57	−5.0
Deceased donor	57	57	0.0
Living donor	3	—	n/a
Heart	2148	2141	−0.3
Deceased donor	2147	2141	−0.3
Living donor	1	—	n/a
Lung	1401	1461	4.3
Deceased donor	1397	1458	4.4
Living donor	4	3	−25.0
Heart–lung	31	29	−6.5

Source: 2008 *OPTN/SRTR Annual Report*, Table 1.7.

Table 2: Growth in number of recovered organs, 2006–2007

Recovered organs	2006	2007	Percent change
Total	28 322	28 409	0.31
Kidney	14 284	14 384	0.70
Pancreas–all	2032	1927	−5.17
Liver	7084	7029	−0.78
Intestine	185	205	10.81
Heart	2276	2289	0.57
Lung	2461	2575	4.63

Source: 2008 *OPTN/SRTR Annual Report*, Table 1.2.

Table 3: Patients on waiting lists, 2006–2007

	End of year		
Organs	2006	2007	Percent change
Total	92 845	97 248	4.7
Kidney	66 352	71 862	8.3
PTA	598	585	−2.2
PAK	988	918	−7.1
Kidney–pancreas	2326	2242	−3.6
Liver	16 623	16 438	−1.1
Intestine	234	222	−5.1
Heart	2769	2659	−4.0
Lung	2822	2217	−21.4
Heart–lung	133	105	−21.1

Source: 2008 *OPTN/SRTR Annual Report*, Table 1.3. PTA = pancreas transplant alone; PAK = pancreas after kidney.

Table 4: Unadjusted 1- and 5-year patient survival by organ

Organ transplanted	One-year survival (%)	Five-year survival (%)
Kidney		
Deceased donor	95.0	81.0
Living donor	98.2	90.6
Pancreas alone	97.9	88.7
Pancreas after kidney	97.3	83.9
Kidney–pancreas	95.1	86.6
Liver		
Deceased donor	87.1	73.3
Living donor	89.9	77.3
Intestine	81.4	56.2
Heart	87.6	73.9
Lung	83.6	53.4
Heart–lung	73.8	46.5

Source: 2008 *OPTN/SRTR Annual Report*, Table 1.13.

2389 kidneys (16.6% of kidneys recovered that year) that were discarded compared with the 2129 (14.9%) kidneys discarded in 2006 *[Table 3.1]*.

At the end of 2007, there were 97 248 people registered on organ waiting lists (65 411 active, 31 821 inactive and 16 of unknown status); this reflects a 4.7% increase over the number of people waiting for an organ at the end of 2006 *[Table 1.4]*. The percentage of patients who were inactive on the kidney waiting list at the end of each year has increased from 15% to 32% from 2003 to 2007, with 23 089 patients listed as inactive status in 2007 *[Tables 5.1a and b]*. This increase is presumably due to policy implemented in 2003 that allows accrual of waiting time during inactive status.

Table 3 shows the 1-year change in the number of patients on the waiting list for each organ and includes patients listed at both active and inactive status. The kidney waiting list grew by 8.3% while the list for kidney–pancreas

Table 5: Unadjusted 1- and 5-year graft survival by organ

Organ transplanted	One-year survival (%)	Five-year survival (%)
Kidney		
Deceased donor	90.4	68.2
Living donor	95.6	80.7
Pancreas alone	81.2	51.3
Pancreas after kidney	77.2	53.6
Kidney–pancreas (kidney)	92.8	78.5
Kidney–pancreas (pancreas)	86.0	72.6
Liver		
Deceased donor	82.4	67.6
Living donor	84.8	70.9
Intestine	68.3	36.3
Heart	87.2	72.8
Lung	82.2	50.5
Heart–lung	73.7	45.2

Source: 2008 *OPTN/SRTR Annual Report*, Table 1.13.

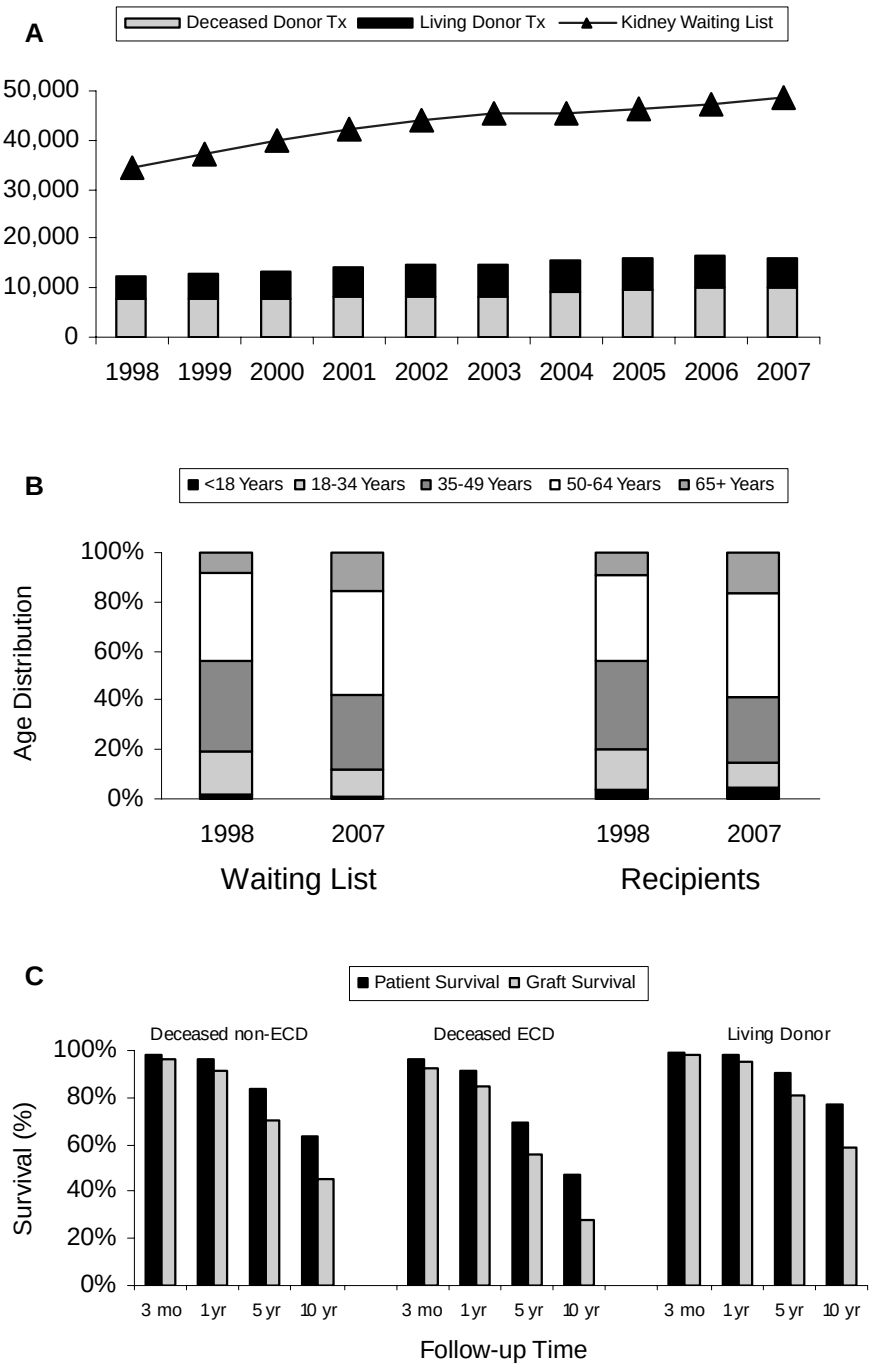

Figure 1: Kidney transplantation at a glance. (A) Number of transplants and size of active waiting list: There was a very large gap between the number of patients waiting for a transplant and the number receiving a transplant. This gap widened over the decade, meaning that the waiting times from listing to transplant continued to increase. The number of living donor transplants grew until 2004, while the number of deceased donor transplants continued to rise gradually. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 5.1a. (B) Age distribution of recipients and active waiting list: In 2007, older candidates (age > 50 years) made up a much larger fraction of patients actively awaiting an organ than a decade earlier. The same pattern was observed for transplant recipients, except that young patients (age < 35 years) showed a greater representation among recipients than on the waiting list. Source: *2008 OPTN/SRTR Annual Report*, Tables 5.1a, 5.4a, 5.4b, 5.4c. (C) Unadjusted patient and graft survival: Five-year patient survival percentages (based on transplants during 2001–2006) and 10-year patient survival (based on transplants during 1996–2006) were clearly higher for recipients of living donor organs than for those of deceased donor organs. Similarly, living donor organs had the highest 5- and 10-year graft survival. Source: *2008 OPTN/SRTR Annual Report*, Tables 5.10a, 5.10b, 5.10d, 5.14a, 5.14b, 5.14d.

shrank by 3.6%. Other modest declines were seen on the liver and heart lists; the largest decline (21.4%) was seen on the lung waiting list. Dramatic changes regarding the lung waiting list in 2005 and 2006 might be largely caused by changes in the deceased donor lung allocation policy that were implemented in May 2005. Changes in the solitary pancreas (pancreas transplant alone; PTA), pancreas after kidney (PAK), intestine and heart–lung waiting lists all reflect relatively small numbers of patients.

Patient survival after transplant is an important metric for evaluating the success of transplantation. Table 4 shows the percentage of transplant recipients still alive 1 and 5 years after transplantation, by organ. The cohort used to compute 1-year survival consists of recipients transplanted in 2005–2006, while the cohort for 5-year survival is based on recipients transplanted in 2001–2006. These are the most recent cohorts for which adequate follow-up data have been collected. Kidney recipients and pancreas

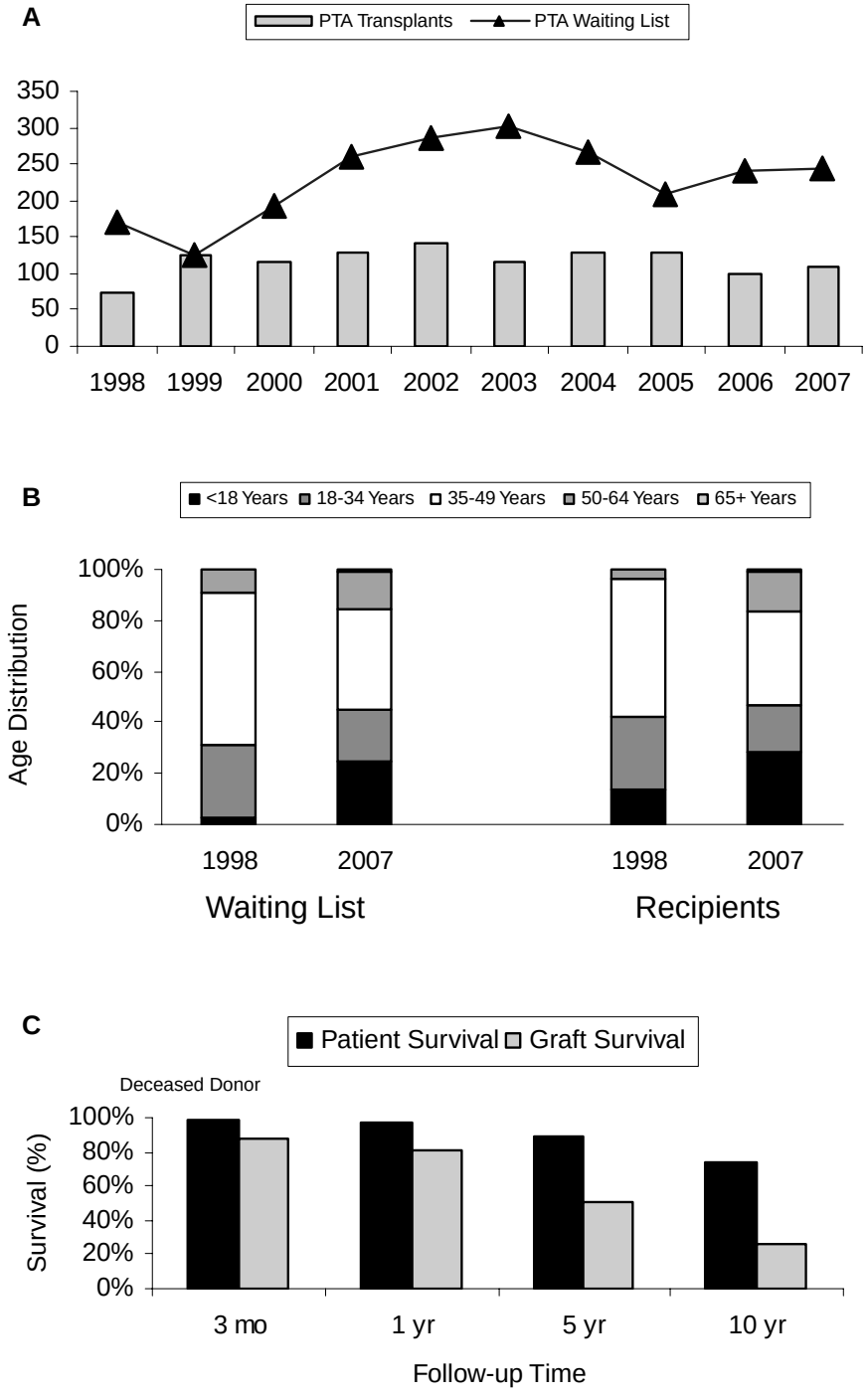

Figure 2: Pancreas transplantation alone (PTA) at a glance. (A) Number of transplants and size of active waiting list: The number of patients on the waiting list for a pancreas transplant alone had been decreasing since 2003, but it rose slightly in 2006 and 2007. The number of PTA transplants per year was relatively stable. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 6.1a. (B) Age distribution of recipients and active waiting list: For PTA, more pediatric candidates were wait-listed and more received a transplant in 2007 than in 1998, although the absolute numbers are small. At the same time, the fraction of recipients over age 50 years grew. Pediatric diabetic patients rarely have kidney failure before age 18 years, but they are candidates for PTA. Source: *2008 OPTN/SRTR Annual Report*, Tables 6.1a, 6.4. (C) Unadjusted patient and graft survival: For PTA transplants, patient survival has been excellent. The 5-year patient survival rate was 89%. Graft survival was considerably lower, especially at 5 and 10 years post-transplant. Source: *2008 OPTN/SRTR Annual Report*, Tables 6.10, 6.14.

recipients had the highest 1-year patient survival rates, ranging from about 95% to 98%. One-year survival for liver, intestine, lung and heart recipients was approximately 81–90%. Survival was lowest for the small number of heart–lung recipients: approximately 74% survived 1 year.

Table 5 shows graft survival by organ, that is, the percentage of transplanted organs that were still functioning 1 and 5 years after transplantation. Graft survival was calculated using the same cohorts as patient survival (Table 4); these groups represent the most recent cohorts available with adequate follow-up data. Over 90% of kidneys transplanted alone or as part of a kidney–pancreas transplant were functioning 1 year after transplantation. Graft survival rates were lower than corresponding patient survival rates because some patients survived organ failure by receiving

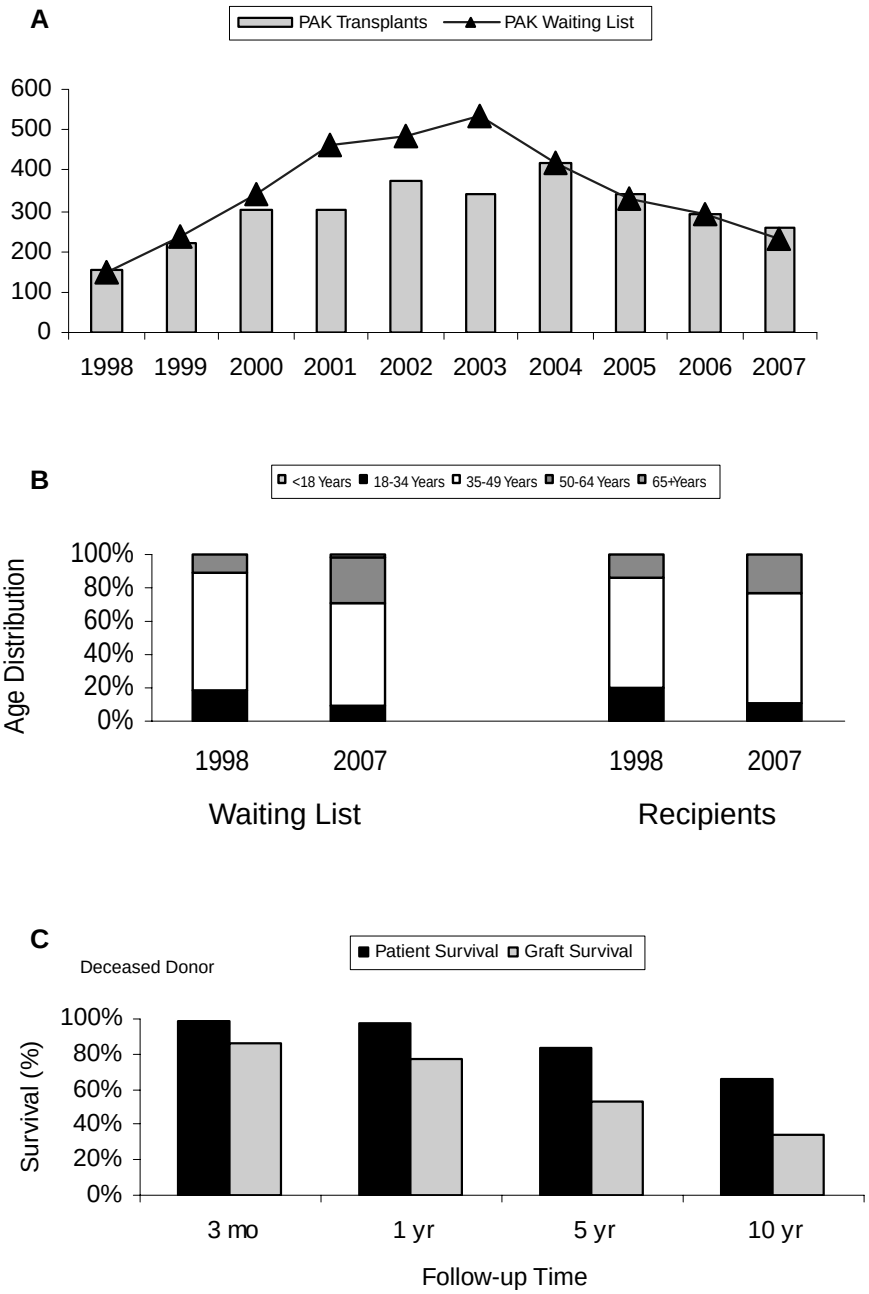

Figure 3: Pancreas after kidney (PAK) transplantation at a glance. (A) Number of transplants and size of active waiting list: As with PTA, the number of patients on the waiting list for a PAK transplant has decreased since 2003. The number who received a transplant has matched the number of candidates each year since 2004. The number of PAK transplants has decreased from its highest level of the decade in 2004. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 7.1a. (B) Age distribution of recipients and active waiting list: For PAK, a higher proportion of wait-listed and transplanted patients were over 50 years old in 2007 than in 1998. At the same, time, a smaller proportion of candidates and recipients were in the 18-34 year age group. (Since recipients were mostly type 1 diabetics, the ages below 18 and above 65 years were virtually unrepresented.) Source: *2008 OPTN/SRTR Annual Report*, Tables 7.1a, 7.4. (C) Unadjusted patient and graft survival: For PAK transplants, patient survival was similar to that seen for simultaneous kidney-pancreas transplant recipients. Five-year patient survival was 84%. Pancreas graft survival after PAK was considerably lower. Source: *2008 OPTN/SRTR Annual Report*, Tables 7.10, 7.14.

a subsequent transplant or alternative therapy such as dialysis or insulin therapy.

Transplantation at a Glance

The figure sets accompanying this article (Figures 1–8) provide overviews of the state of transplantation for different organs. These summary graphics are included for six organs: kidney, pancreas (as PTA or PAK transplant, liver, intestine, heart and lung), as well as the most common multiorgan procedure, simultaneous pancreas–kidney transplantation. Other multiorgan procedures are excluded from the counts presented here (e.g. heart–lung transplants) because of the small numbers of these procedures. Below we describe the three types of graph shown for each organ.

Number of transplants and size of active waiting list

These figures compare, for each of the past 10 years, the size of the active waiting list and the number of transplants

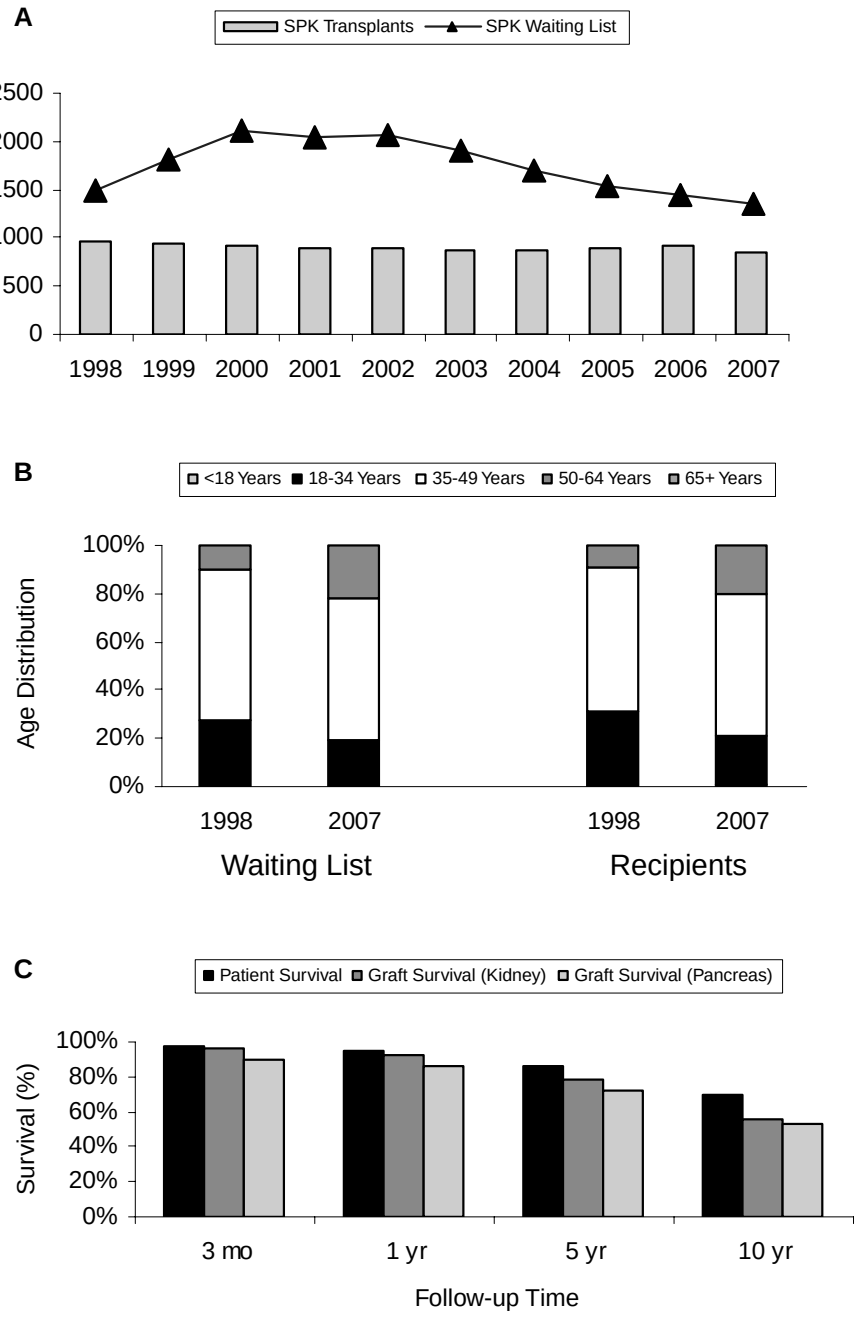

Figure 4: Simultaneous pancreas–kidney (SPK) transplantation at a glance. (A) Number of transplants and size of active waiting list: SPK accounts for the majority of all pancreas transplants. Numbers of this procedure were stable over the decade. The gap between the number of patients waiting for a transplant and the number receiving a transplant has dropped substantially since 2000. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 8.1a. (B) Age distribution of recipients and active waiting list: For SPK transplantation, patients over age 50 years made up greater fractions of both candidates and recipients in 2007 than in 1998. At the same time, smaller proportions of candidates and recipients were in the 18–34 year age group. (Since recipients were mostly type 1 diabetics, the ages below 18 and above 65 years were virtually unrepresented.) Source: *2008 OPTN/SRTR Annual Report*, Tables 8.1a, 8.4. (C) Unadjusted patient and graft survival: Patient survival has improved for SPK recipients in recent years. Five- and 10-year patient survival was 87% and 70%, respectively. Graft survival is shown separately for the pancreas graft and the kidney graft of each SPK transplant. Source: *2008 OPTN/SRTR Annual Report*, Tables 8.10, 8.14.

performed. The size of the waiting list is a snapshot of the number of candidates active on the waiting list on December 31 of each year, and does not count patients who were transplanted, listed or removed during the preceding 12 months. The number of transplants includes all transplants performed over the year. This difference in methods of counting explains why for some organs (e.g. lung), the number of transplants performed during a certain year may exceed the number of people awaiting a transplant on the last day of the same year. In other cases, changes in allocation policy and wait-listing practices help explain the narrowing gap between waiting list size and number of transplants.

Unadjusted patient and graft survival

These summary figures show survival of transplant recipients (patient survival) and continued function of the transplanted organ (graft survival) at 3 months, 1, 5 and 10 years following transplantation. The results for each follow-up

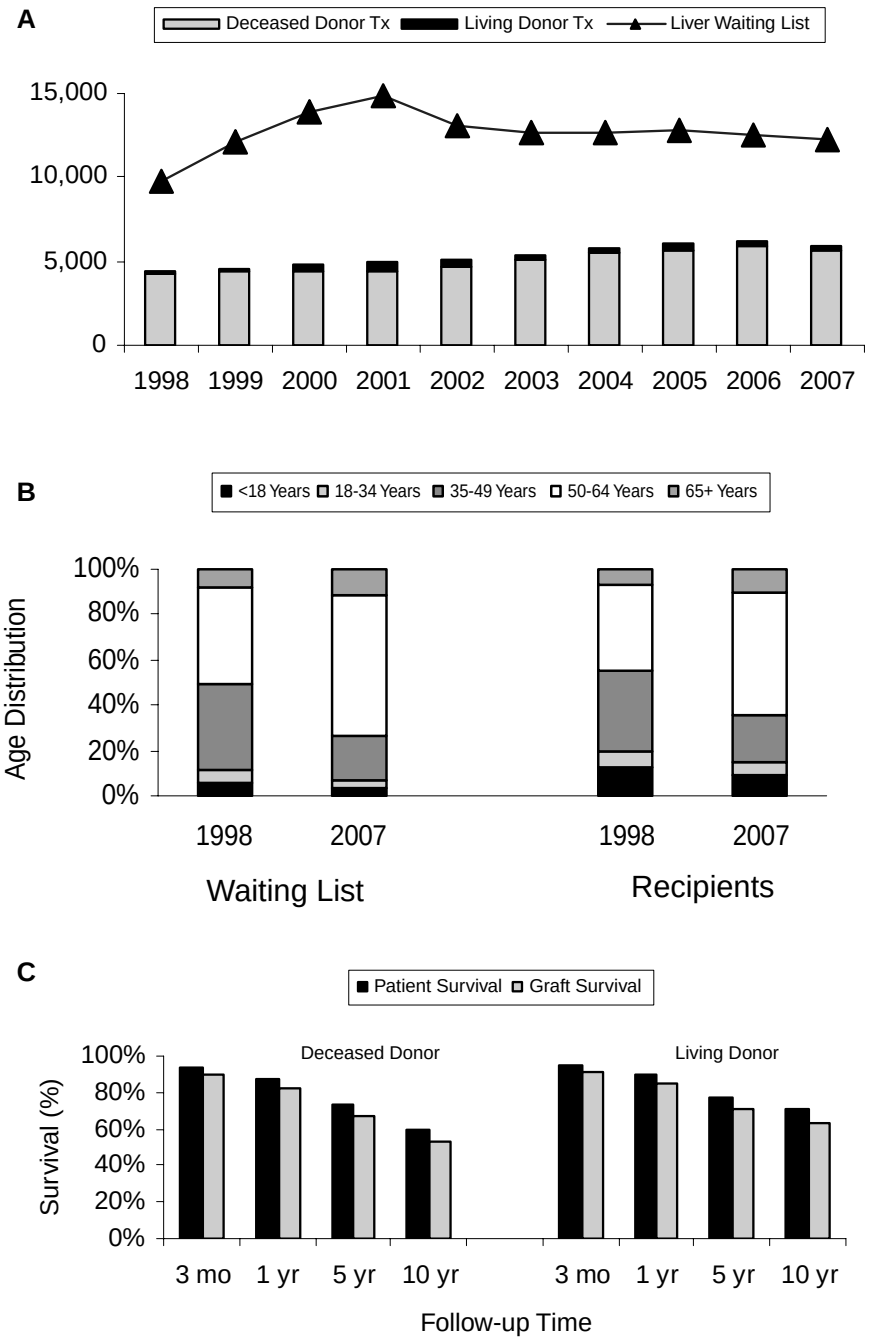

Figure 5: Liver transplantation at a glance. (A) Number of transplants and size of active waiting list: The number of patients awaiting a liver transplant at year-end peaked in 2001; this is clearly related to the introduction of the MELD/PELD allocation system in 2002. The number who received a deceased donor liver transplant has gradually increased, reaching a peak in 2006. The gap between the numbers of candidates and recipients has been slowly shrinking since 2002. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 9.1a, 9.1b. (B) Age distribution of recipients and active waiting list: The numbers of candidates and recipients age 35–49 years remained fairly constant over the decade, but the age group's proportion by both measures declined. Recipients included transplants from both living and deceased donors. Source: *2008 OPTN/SRTR Annual Report*, Tables 9.1a, 9.4a, 9.4b. (C) Unadjusted patient and graft survival: Patient survival in recent years has been improving for both deceased donors and living donors, with 73% and 77% of patients, respectively, alive 5 years following transplantation. Patient survival was higher than graft survival because of the opportunity for repeat liver transplantation in the event of graft failure. Source: *2008 OPTN/SRTR Annual Report*, Tables 9.10a, 9.10b, 9.14a, and 9.14b.

time are based on information about the most recent cohorts that allow sufficient follow-up time for data collection and ascertainment of events.

The Articles in the 2008 Report on the State of Transplantation

The articles in this report begin with a review of trends in organ donation and utilization (1). Following are four organ-specific articles covering kidney and pancreas (2), liver and intestine (3), heart (4) and lung transplantation (5); these provide detailed trends in donation, waiting time, allocation, posttransplant outcomes and the demographics of both candidates and recipients. Additionally, these articles supplement the reporting of 10-year trends with updates on recent changes in allocation policy, clinical practice and other areas relevant to the transplantation of different organ types.

This year's report concludes with two special-focus articles that look closely at issues of recent interest to

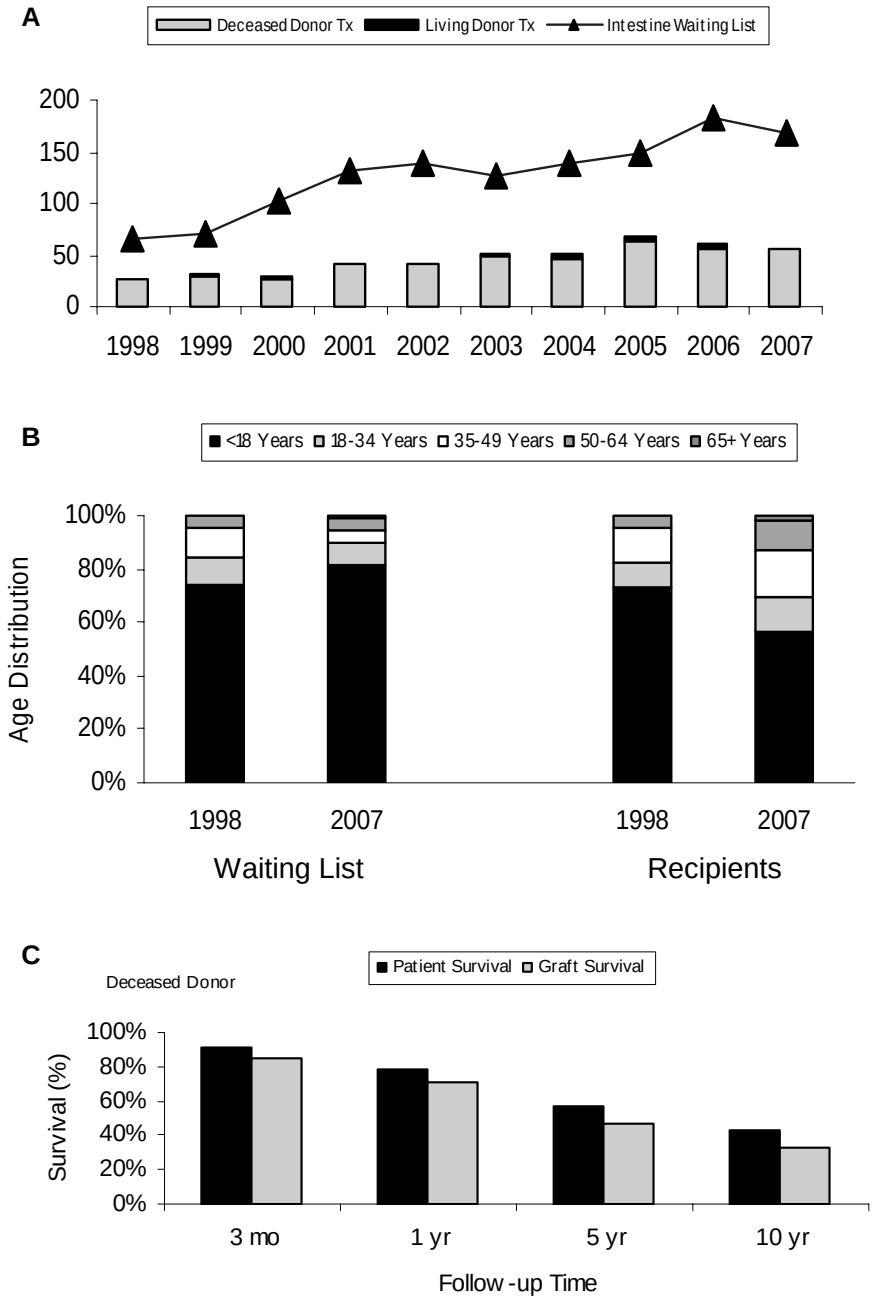

Figure 6: Intestine transplantation at a glance. (A) Number of transplants and size of active waiting list: The numbers of patients on the intestine waiting list and the number receiving a transplant both more than doubled between 1998 and 2007. The difference between the number of candidates and transplant recipients increased through the second half of the decade. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 10.1a. (B) Age distribution of recipients and active waiting list: About 74% of intestine candidates were in the pediatric age group in 1998 compared with 81% in 2007. The small group of candidates and recipients in the age group > 50 years doubled during the decade. Adults made up a greater portion of recipients than candidates. Source: *2008 OPTN/SRTR Annual Report*, Tables 10.1a, 10.4. (C) Unadjusted patient and graft survival: One-year patient survival was 79% in 2007. Survival at 5 years was 57%. Graft survival was lower, since recipients may receive parenteral alimentation or retransplantation after graft failure. Source: *2008 OPTN/SRTR Annual Report*, Tables 10.10, 10.14.

the transplant community. 'Innovation in Outcomes Assessment Follow Transplantation' (6) details the methodology used in recent SRTR work that focuses on providing better quality assessment and improvement tools for transplant programs. 'Survival Benefit-Based Deceased Donor Liver Allocation' (7) discusses ongoing methodological approaches developed by the SRTR to calculate the incremental years of life attributable to liver transplantation. This concept is central to a revision of deceased donor liver allocation policy currently under consideration. These articles all include special analyses conducted by the SRTR and touch on topics that are both timely and pertinent because of their implications for policy and clinical practice.

Acknowledgments

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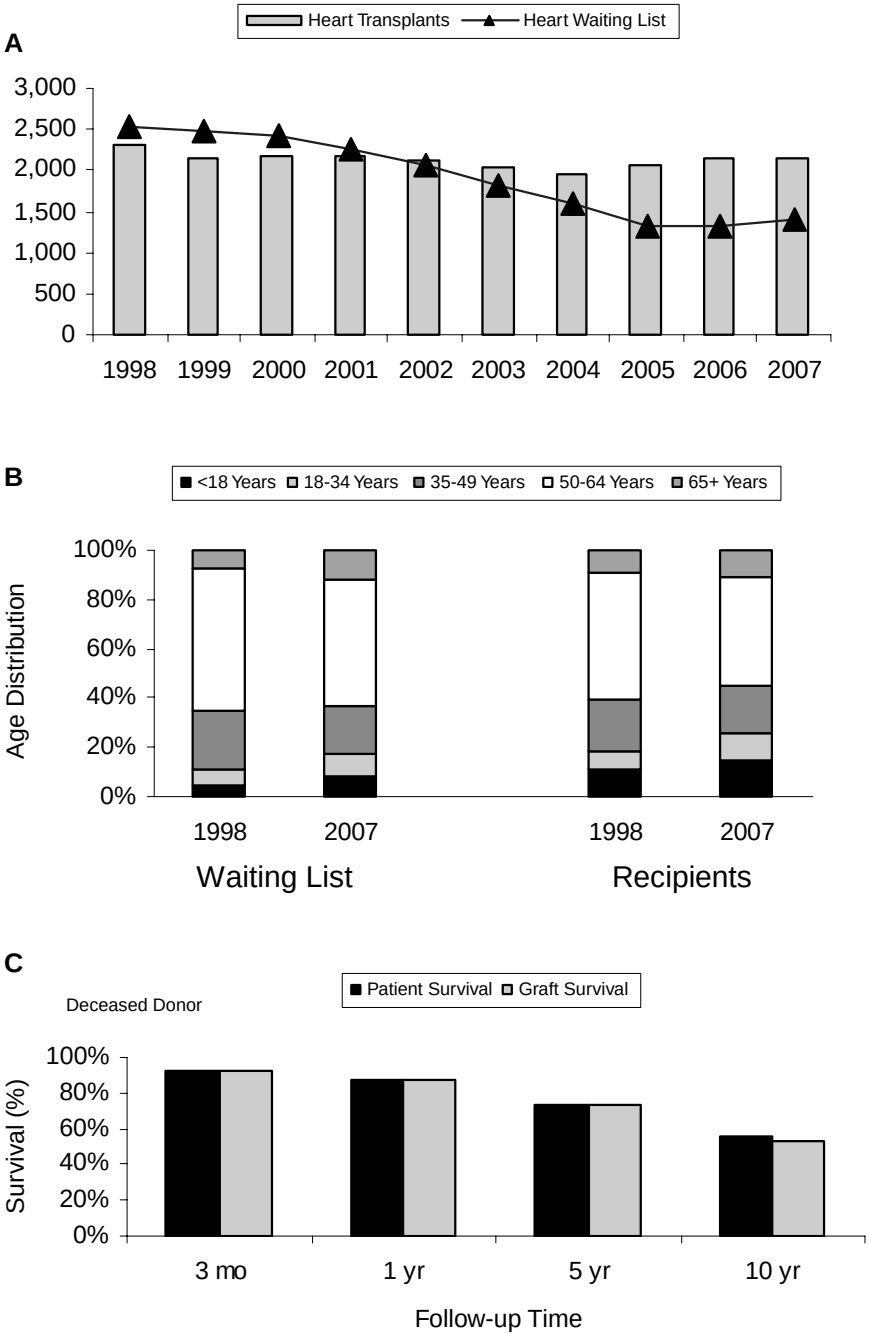

Figure 7: Heart transplantation at a glance. (A) Number of transplants and size of active waiting list: The number of heart transplants has increased since 2005 following several years of gradual reduction. The number of patients awaiting a heart decreased steeply from 2000 to 2005, likely reflecting improvements in medical and surgical therapy for end-stage heart failure. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 11.1a. (B) Age distribution of recipients and active waiting list: Trends in the age distribution of wait-listed candidates show that the proportions (and absolute numbers) of patients younger than 35 and older than 64 years increased, while the age group 35–64 years was less represented. The trend in transplant recipient age showed a similar pattern, although the ages below 35 years had greater representation than on the waiting list. Source: *2008 OPTN/SRTR Annual Report*, Tables 11.1a, 11.4. (C) Unadjusted patient and graft survival: Patient survival improved in recent years for heart recipients. At 1, 5, and 10 years following heart transplantation, 88%, 74%, and 55% of patients, respectively, were alive. Graft survival was very similar to patient survival because very few patients receive a second heart transplant. Source: *2008 OPTN/SRTR Annual Report*, Tables 11.10, 11.14.

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This study was approved by HRSA's SRTR project officer. HRSA has determined that this study satisfies the criteria for the IRB exemption described in the 'Public Benefit and Service Program' provisions of 45 CFR 46.101(b)(5) and HRSA Circular 03.

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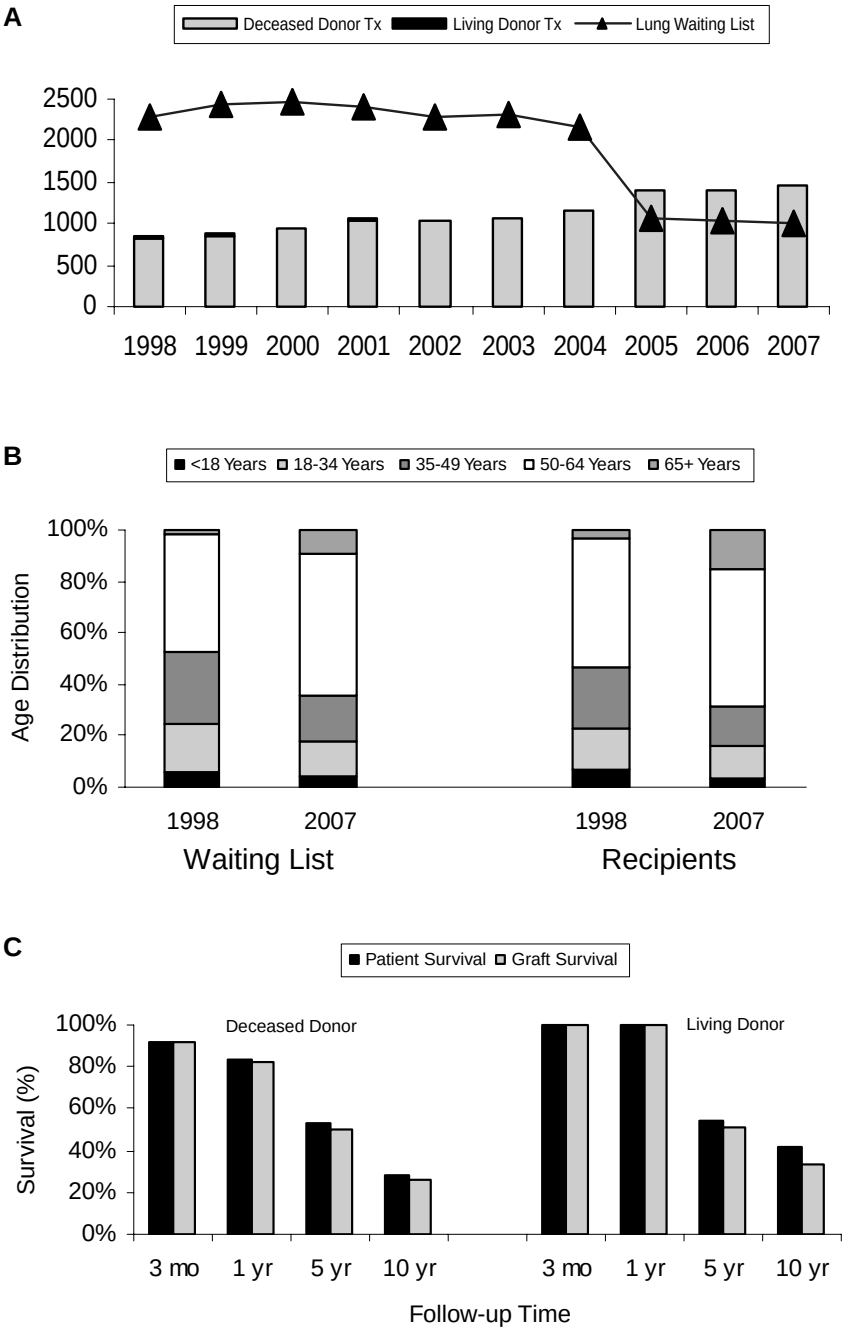

Figure 8: Lung transplantation at a glance. (A) Number of transplants and size of active waiting list: The number of lung transplants has increased in the last two years. The number of patients awaiting a transplant dropped steeply in 2005 after a stable pattern during the prior 7 years. This sharp reduction is largely attributable to a major changes in allocation policy, which is now based on medical urgency and calculated transplant benefit rather than waiting time. Source: *2008 OPTN/SRTR Annual Report*, Tables 1.7, 12.1a. (B) Age distribution of recipients and active waiting list: The lung waiting list showed a mixed trend in age distribution, with increasing percentages of candidates older than 50 years and decreasing percentages younger than 18 years. Candidates 18–49 years old showed a corresponding reduction in the percentage of the waiting list. The pattern for transplant recipients showed a similarly strong increase for ages 50 years and above, but a decrease in percentages for younger ages, including children. Source: *2008 OPTN/SRTR Annual Report*, Tables 12.1a, 12.4a, 12.4b. (C) Unadjusted patient and graft survival: Patient survival has been improving in recent years for recipients of deceased and (very small numbers of) living donor lung transplants. At 1 year following deceased donor and living donor lung transplantation, 84% and 100% of patients, respectively, were alive. Graft survival was very similar to patient survival because very few lung re-transplants are performed. Source: *2008 OPTN/SRTR Annual Report*, Tables 12.10a, 12.10b, 12.14a, 12.14b.

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Perspectives in Organ Preservation

Mark-Hugo J. Maathuis, Henri G. D. Leuvenink, and Rutger J. Ploeg

Maintaining organ viability after donation until transplantation is critically important for optimal graft function and survival. To date, static cold storage is the most widely used form of preservation in every day clinical practice. Although simple and effective, it is questionable whether this method is able to prevent deterioration of organ quality in the present era with increasing numbers of organs retrieved from older, more marginal, and even non-heart-beating donors. This review describes principles involved in effective preservation and focuses on some basic components and methods of abdominal organ preservation in clinical and experimental transplantation. Concepts and developments to reduce ischemia related injury are discussed, including hypothermic machine perfusion. Despite the fact that hypothermic machine perfusion might be superior to static cold storage preservation, organs are still exposed to hypothermia induced damage. Therefore, recently some groups have pointed at the beneficial effects of normothermic machine perfusion as a new perspective in organ preservation and transplantation.

Keywords: Transplantation, Ischemia-reperfusion, Preservation, Hypothermic machine perfusion, Normothermic machine perfusion.

(*Transplantation* 2007;83: 1289–1298)

Despite better insights in surgical technique, immunosuppressive agents, and treatment of postoperative complications, 5- and 10-year results in organ transplantation have only moderately improved in the past decades (*1*). One explanation for this slightly disappointing fact is that more experience has led to an increased acceptance of older and more complex recipient candidates. Another reason is the fact that due to the persistent shortage, criteria for inclusion of deceased donors have been extended. Organs are nowadays more often retrieved from older, more marginal, and sometimes non-heart-beating (NHB) donors than 10 years ago. Between 1988 and 1995, the United Network of Organ Sharing (UNOS) registered a 170% increase in the number of deceased donors more than 50 years of age (*2*, *3*). The use of older donor kidneys, livers, and pancreata has resulted in a decrease in graft function and survival compared to grafts retrieved from young donors (*4–7*). Marginal and NHB donor organs suffer from additional warm ischemic injury. As a result these organs have higher primary nonfunction (PNF) and delayed graft function (DGF) rates compared to heart-beating deceased donors (*8–11*).

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Department of Surgery, Surgical Research Laboratory, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.

Address correspondence to: R.J. Ploeg, M.D., Ph.D., Department of General Surgery, Transplantation, and Organ Donation, Surgical Research Laboratory, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, the Netherlands.

E-mail: r.j.ploeg@chir.umcg.nl

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Maintaining organ viability during preservation is an important prerequisite for successful outcome after transplantation. With the current practice to accept older and more injured donor organs, improvement of preservation techniques has now become a must. To date, most centers use static cold storage (CS) to preserve organs. This preservation method, however, was developed in an era with younger donors with good-quality organs (*12*). With the introduction of extended donor criteria the limitations of CS have probably been reached.

This review aims to describe a number of principles and pathophysiological mechanisms as well as current techniques in abdominal organ preservation.

Organ Preservation by Static Cold Storage

Currently, CS is the preferred organ preservation method in most centers. Simple cold storage starts with a rapid vascular washout to allow cooling of the organ, removal of blood components, and equilibrate the CS solution with the tissue (*13*, *14*).

Hypothermia

The principle of CS preservation is based on suppression of metabolism and catabolic enzymes by hypothermia (4°C). Metabolic rate is halved with each 10°C drop in temperature resulting in a remaining 10–12% metabolism at 4°C (*15*). Already in the early 1960s, it was shown that cooling by itself was able to improve preservation of small bowel, kidney, and liver: the so-called temperature effect (*16–18*). To further extend cold ischemic time (CIT) and counteract the detrimental side effects of the required hypothermia, special preservation solutions are necessary: the solution effect (*19*). Cell swelling, acidosis, and the production of radical oxygen spe-

cies (ROS) upon reperfusion are important side effects of hypothermia. To reduce these undesirable effects CS solutions include a number of specific compounds (*20*, *21*). The composition of several preservation solutions is illustrated in Table 1.

Cell Swelling

A very prominent alteration in the cellular structure during hypothermia is the formation of edema (*22*). The responsible mechanism is an impaired activity of Na^+/K^+ ATPase. As a result, sodium is no longer extruded but passively enters the cell. This creates a hyperosmolar intracellular environment and subsequently an influx of water. To prevent cell swelling, impermeants and colloids are added to preservation solutions.

Effective impermeants are saccharides and nonsaccharide anions. Molecular weight (MW) determines the effectiveness of saccharides to prevent cell swelling, with larger saccharides being more effective (*23–25*).

Glucose (MW 180) is a monosaccharide and was used in early CS solutions such as EuroCollins solution. When it became evident that glucose passes the cell membrane and becomes a source of lactate in an anaerobic environment, it was no longer considered as an effective impermeant (*26*). The slightly larger monosaccharide mannitol (MW 182) is not a source of lactate since it is not metabolisable and will not enter the cell through

TABLE 1. Composition of organ preservation solutions

	EC (76)	HOC (15)	PBS (28)	UW (80)	HTK (86)	CEL (90)	IGL-1 (35)
Colloids (g/L)							
HES	—	—	—	50	—	—	—
PEG-35	—	—	—	—	—	—	1
Impermeants (mM)							
Citrate	—	80	—	—	—	—	—
Glucose	195	—	—	—	—	—	—
Histidine	—	—	—	—	198	30	—
Lactobionate	—	—	—	100	—	80	100
Mannitol	—	185	—	—	38	60	—
Raffinose	—	—	—	30	—	—	30
Sucrose	—	—	140	—	—	—	—
Buffers (mM)							
Citrate	—	80	—	—	—	—	—
Histidine	—	—	—	—	198	30	—
K_2HPO_4	15	—	—	—	—	—	—
KH_2PO_4	43	—	—	25	—	—	25
$NaHCO_3$	10	—	—	—	—	—	—
NaH_2PO_4	—	—	13	—	—	—	—
Na_2HPO_4	—	—	56	—	—	—	—
Electrolytes (mM)							
Calcium	—	—	—	—	0.0015	0.25	0.5
Chloride	15	—	—	20	32	42	—
Magnesium	—	—	—	—	4	13	—
Magnesium sulphate	—	40	—	5	—	—	5
Potassium	115	79	—	120	9	15	25
Sodium	10	84	125	25	15	100	120
ROS scavengers (mM)							
Allopurinol	—	—	—	1	—	—	1
Glutathione	—	—	—	3	—	3	3
Mannitol	—	185	—	—	38	60	—
Tryptophan	—	—	—	—	2	—	—
Additives (mM)							
Adenosine	—	—	—	5	—	—	5
Glutamic acid	—	—	—	—	—	20	—
Ketoglutarate	—	—	—	—	1	—	—

EC, EuroCollins; HOC, hypertonic citrate/Marshalls solution; PBS, phosphate-buffered sucrose; UW, University of Wisconsin cold storage solution; CEL, Celsior; HTK, histidine-tryptophan-ketoglutarate; IGL-1, Institut George Lopez; HES, hydroxyethyl starch; PEG-35, polyethylene glycol with an average MW of 35 kDa; ROS, reactive oxygen species.

facilitated transport. In addition, mannitol has a beneficial effect as a scavenger of reactive oxygen species, and was therefore added in Marshalls, Bretschneider's histidine-tryptophan-ketoglutarate (HTK), and Celsior solutions. Sucrose (MW 342) is a disaccharide and is used in the renal preservation solution phosphate-buffered sucrose (*27*, *28*). Raffinose (MW 504) is the largest one and a trisaccharide. It was added as an impermeant in the University of Wisconsin (UW) CS solution (UW-CSS) developed by Belzer and Southard.

Nonsaccharide impermeants such as negatively charged gluconate, citrate, and lactobionate limit cell swelling by electrochemical forces. Effectiveness of these anions is determined by molecular weight as well as charge. Although hypertonic citrate (HOC) contains citrate, both UW-CSS and Celsior use the anion lactobionate.

As impermeants are predominantly effective at the level of cell membranes and the interstitial compartment, colloids are used for the intravascular compartment. These macromolecules are retained in the vascular spaces and act by imparting colloid osmotic pressure. Colloids were originally added to hypothermic machine preservation solutions (MPS) to prevent tissue edema due to hydrostatic pressure. Belzer and his group first used cryoprecipitated plasma, then albumin, and finally diafiltrated hydroxyethyl starch (HES) as they aimed at developing one solution suitable for both CS and hypothermic machine perfusion (HMP). The feasibility of HES as a colloid in UW-CSS has been extensively debated. HES prevents interstitial edema but also increases viscosity (*29*, *30*). For short preservation times, addition of a colloid has been doubted, although some organs such as the pancreas appeared to be more susceptible to edema when HES is omitted (*31*). Analyzing the effect of HES on red blood cells (RBCs), several authors have shown an increased RBC aggregability in both human and rat whole blood when large molecular sized HES is present (*30*, *32*). This effect could partially explain the frequently slower washout of blood and initially patchy reperfusion of organs when UW-CSS is used in clinical practice (*33*).

The HES controversy initiated a search for other colloids, such as dextran and polyethylene-glycol (PEG) (*34–36*). In this respect, UW-PEG preserved rat livers have shown lower transaminase levels, higher bile flow, and higher urea synthesis rate after transplantation (*37*). Several experimental studies have now confirmed the efficacy of PEG for liver as well as for kidney, pancreas, and small bowel preservation (*38–41*).

In contrast to UW-CSS, both HTK and Celsior do not contain a colloid. In a prospective study with short CIT, both solutions showed equal efficacy compared to UW-CSS for the preservation of kidney and liver grafts (*42*). With prolongation of preservation times beyond 24 hours, the presence of a colloid does appear to be important to maintain organ viability (*43*).

Energy and Acidosis

At a temperature of 0–4°C, cold storage results in a rapid depletion of cellular adenosine triphosphate (ATP). Within 4 hours, nearly 95% of ATP has disappeared with a shift to adenosine monophosphate as the predominant nucleotide. During CS, anaerobic metabolization of 1 mol glucose, however, only yields 2 mol ATP versus a maximum of 38 mol in aerobic glycolysis. Moreover, two lactic acid molecules are formed leading to acidosis (*13*, *44*).

The contribution of acidosis to ischemic injury is pH dependent. Severe acidosis activates phospholipases and proteases causing lysosomal damage and eventually cell death (*45*). Mild acidosis (pH 6.9–7.0), however, has been suggested to have a protective effect by inhibiting phosphofructokinase as the rate-limiting step in glycolysis (*45*, *46*). Adequate control of pH is therefore an important function of preservation solutions. UW-CSS uses phosphate as a buffer, while Celsior and HTK use histidine. Of those two solutions, HTK has the highest buffering capacity due to a high concentration of histidine (*21*).

Reactive Oxygen Species

Reactive oxygen species (ROS) are widely recognized as important mediators of postreperfusion induced organ injury (*47*). CS per se, however, has also been shown to promote ROS production, probably due to mitochondrial damage (*48*, *49*). An extensively studied generator of ROS is xanthine oxidase, which simultaneously produces hydrogen peroxide (H_2O_2) and the superoxide anion (O_2^-) (*50*, *51*). The subsequent reduction of H_2O_2, catalyzed by iron, leads to hydroxyl radical formation (·OH). Free or chelatable iron is not only a catalyst of ROS formation but also contributes directly to hypothermia induced injury by mediating mitochondrial damage and induction of apoptosis (*52–54*). ROS react rapidly with other molecules which will result in severe damage to lipids, nucleic acids, and proteins (*55*, *56*). The subsequent cell death mechanism appears to be ATP dependent. ATP is required for the execution of the apoptotic cell death program whereas complete ATP depletion will lead to necrosis (*57*, *58*). As free radical–mediated injury during preservation is strongly correlated with the absence of immediate and reduced long-term kidney function (*56*), preservation solutions aim to counteract ROS mediated injury during preservation and especially at time of reperfusion.

In UW-CSS, the compounds allopurinol and glutathione (GSH) were included to prevent formation of ROS. Allopurinol inhibits xanthine oxidase, which improved kidney preservation, whereas liver or pancreas preservation remain almost unaffected (*59*).

GSH is a tripeptide that is oxidized to glutathione disulphide together with converting peroxides. Experimental studies have shown the importance of GSH in an isolated perfused rabbit liver model. In the absence of GSH, more lactate dehydrogenase (LDH) was released into the perfusate (*60*), which was confirmed in the canine kidney transplant model. Subsequent studies have shown that GSH is especially important in long-term liver preservation (*61*).

GSH is also used in Celsior solution, whereas in HTK tryptophan might protect the organs against ROS-mediated damage. The antioxidative effects of tryptophan are controversial. Tryptophan can act as an antioxidant through its oxidative metabolites in the kyunerine pathway, such as 5-hydroxy-tryptophan (*62*). On the other hand, tryptophan can be pro-oxidant as well by presenting low molecular weight iron in a redox cycling event (*63*, *64*). In a cultured rat hepatocyte experiment, the amount of thiobarbituric acid reactive substances (TBARS), as a marker for ROS mediated injury, was measured. After 24 hours of preservation, TBARS were

significantly higher in HTK-preserved hepatocytes compared to UW-CSS, suggesting a superior antioxidant capacity of UW-CSS (*65*).

Electrolyte Composition

During the pioneering years in organ preservation a high potassium/low sodium ratio of the solution (intracellular type) was assumed necessary to prevent cell swelling. It was hypothesized that due to the inactivity of Na^+/K^+ ATPase during hypothermia, an intracellular sodium/potassium ratio in the extracellular fluid compartment would prevent sodium and chloride from entering the cell (*66*). Balancing extracellular sodium ions and intracellular protein anions creates the so-called Donnan equilibrium, which prevents edema formation (*24*). Intracellular type solutions such as UW-CSS were long considered to be pivotal for preservation of cell viability (*66*, *67*). Recent work, however, has suggested equal or improved results of extracellular type solutions with a low potassium/high sodium ratio, such as Celsior and HTK (*68–73*). This clearly demonstrates that sodium/potassium ratios as such do not play a central role in preservation. Also, a low potassium content will facilitate the washout of blood during organ procurement as no potassium induced vasospasm will occur (*70*, *74*).

In summary, essential components of effective preservation solutions are impermeants or colloids, an adequate buffering capacity, and anti-oxidants. In the next section, the clinical merits of some prominent preservation solutions for abdominal organs will be discussed.

Current Cold Storage Solutions

The first static CS preservation solution was developed by G.M. Collins in 1969 (*75*), which was modified by the Eurotransplant Foundation in 1976 by eliminating magnesium (Table 1) (*76*). EuroCollins (EC) solution was a simple and cheap intracellular type preservation solution. Phosphate was used for pH buffering and glucose served as the osmotic agent. In the late 1970s, an Australian group developed a HOC solution, which is also known as Marshalls solution. This solution was effective for 72 hours of canine kidney preservation and is still in clinical use (*77*). Another simple solution is phosphate-buffered sucrose, developed by Coffey and Andrews in the early 1980s. Phosphate-buffered sucrose was shown to be effective in kidney preservation confirming the hypothesis that high concentrations of impermeant saccharides suppress hypothermic cell swelling (*27*, *78*). When UW-CSS became available, a randomized clinical trial comparing EuroCollins with UW-CSS in kidney preservation showed that DGF was significantly lower in the UW-CSS group (23% vs. 33%). Also, 1-year graft survival was found to be significantly higher in the UW-CSS group. As a result of this study, EC was no longer the preferred solution for clinical abdominal organ preservation in Europe (Fig. 1A).

University of Wisconsin Solution

Continuous and systematic research by Belzer and Southard led to the development of the University of Wisconsin Solution in 1987. Metabolic inert substrates such as lactobionate and raffinose served as osmotic agents. HES was used as a colloid. Scavengers (glutathione, allopurinol) and an ATP precursor (adenosine) were added to the solution. Today, UW-CSS is still considered the gold standard preservation solution for kidney, liver, pancreas, and small bowel (Fig. 1) (*29*, *79–85*).

Histidine-Tryptophan-Ketoglutarate Solution

HTK solution was initially introduced as a cardioplegic solution in open-heart surgery by Bretschneider in the 1970s but was also tested in kidney, liver, and pancreas transplantation (*86*). The basic design of the solution consists of histidine, a very potent buffer, combined with two amino acids. Tryptophan serves as membrane stabilizer while ketoglutarate acts as substrate for anaerobic metabolism during preservation. HTK has a low viscosity and, to achieve complete tissue equilibration, high volumes (~15 l) have to be rinsed through the organs at low flow rates. A multicenter randomized prospective trial comparing UW-CSS versus HTK in kidney preservation showed equal results in terms of incidence of DGF (33% vs. 33%) (*79*). For prolonged cold storage times (>24 hours) little data is available. One single center study reported a twofold increase in incidence of DGF after HTK kidney preservation compared to UW-CSS when CIT was longer than 24 hours (*87*). The opposite was shown in another study with a DGF rate of 16% after HTK preservation versus 56% after UW-CSS (*88*). Direct comparison of these conflicting findings, however, is impossible due to a different definition of DGF in both studies.

In liver preservation, it has been suggested that HTK could be advantageous due to its low potassium concentration. Therefore, the need to flush out the potassium-rich

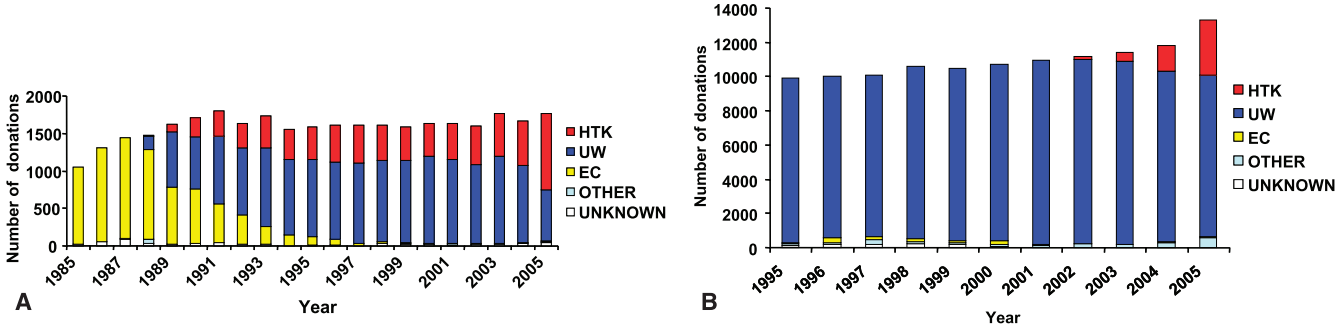

FIGURE 1. (A) Use of cold storage solutions in Eurotransplant region in deceased donors from 1985–2005 (based on Eurotransplant data of October 2006). (B) Use of cold storage solution in the United States in deceased donors from 1995–2005 (based on Organ Procurement and Transplantation Network data of October 2006).

UW-CSS from the organ prior to reperfusion would be limited. Although patient numbers were relatively small and cold ischemic times short, two studies using HTK in liver preservation showed equality of HTK and UW-CSS for short-term preservation (*81*, *89*). Despite the lack of a proper randomized and controlled trial, HTK is currently used by many centers as a preservation solution for all abdominal organs retrieved for transplantation (Fig. 1) (*88*).

Celsior Solution

Celsior is an extracellular type preservation solution developed in 1994 for CS preservation of cardiac grafts (*90*). This solution, however, proved to be effective in preserving abdominal organs as well (*42*, *83*, *84*). It combines the inert osmotic agent philosophy of UW-CSS with the strong buffering capacity of HTK. Reduced glutathione is added as a free-radical scavenger. Currently, it has been successfully used in clinical heart, lung, liver, pancreas, kidney, and small bowel preservation (*91*, *92*). The likelihood whether Celsior will eventually replace UW-CSS may depend on the results of a sufficiently powered multicenter trial.

New Solutions

The increasing awareness that ischemia/reperfusion injury does determine a significant part of posttransplant outcome has stimulated research in the field of preservation injury and the development of new preservation solutions. A relatively new preservation solution developed at the University of Amsterdam is Polysol. Its composition is based on the fact that metabolism is still present at 4°C. Polysol has been tested both as an experimental CS solution and as HMP solution (*93*, *94*). It is a classic preservation solution enriched with amino acids, vitamins, and antioxidants (*95*). Many components in Polysol, however, have not yet been evaluated separately. In experimental liver preservation studies, superiority over HTK was seen in CS preservation of steatotic livers showing improved functional parameters, such as oxygen consumption, bile production, and damage markers (*93*). Transplantation data in experimental and clinical preservation are now required to demonstrate the efficacy of Polysol. Based on its "metabolic support" design, however, beneficial effects of Polysol can be expected.

Another new and now clinically available preservation solution is IGL-1 (Institut George Lopez), developed by the Lyon group in France. IGL-1 builds on the heritage of both UW-CSS and Celsior (*35*, *93*, *96*). It combines the extracellular composition of Celsior with the colloidal support of UW-CSS using polyethylene glycol (PEG) instead of HES. In a porcine kidney autotransplantation model with IGL-1, PEG was found to limit influx of macrophages by approximately 50% (*97*). Polymers, such as PEG, spontaneously bind to cell and tissues surfaces and sterically stabilize the underlying surface from interactions with other components. The main advantage of this "immunocamouflage" is that it directly modifies inherent immunogenicity of donor tissue (*98*, *99*). PEG does not exert any aggregating effects on RBCs and in combination with the extracellular composition of IGL-1, washout of blood during the donor operation should be superior to UW-CSS (*30*, *32*, *100*).

Both rat and porcine transplantation studies of liver and kidney have shown encouraging results in terms of organ function after transplantation following preservation with IGL-1 (*41*, *69*, *101*). The first preliminary clinical results in renal transplantation with IGL-1 demonstrated a reduction in DGF compared to kidneys preserved with UW-CSS (5.7% vs. 13.8%, respectively). Also, less apoptosis was seen in IGL-1 preserved kidneys (*102*). Until now, however, patient numbers have been too small to draw clinically relevant conclusions and a randomized controlled multicenter study will have to confirm the initial results. Given its extracellular composition and the beneficial effects of PEG, IGL-1 could be considered a promising successor to UW-CSS.

Despite the fact that CS preservation methods have facilitated many transplant programs all over the world, it appears that the increasing challenge to maintain viability in extended-criteria donor organs is touching the limits of CS preservation. Even with beneficial additives and enriched compositions, static CS, at best, slows down ischemic damage. Furthermore, pretransplant viability testing is limited and preservation time is still counted in hours rather than in days. To further improve organ viability, a more dynamic preservation method is needed to better fulfil the metabolic demands of damaged organs. Therefore, many groups have recently switched gears and are revisiting the possibilities of hypothermic machine perfusion (HMP) (*103–105*).

Preservation by Hypothermic Machine Perfusion

In the early 1970s, hypothermic machine perfusion (HMP) was used by many centers in the United States and Europe to preserve kidneys, allowing transportation to a transplant center (*106–109*). Although modern HMP systems are smaller, lighter, and more sophisticated than the original machine used by Belzer and coworkers, the principles of HMP have not changed.

Machine perfusion generates a controlled continuous or pulsatile recirculating flow of preservation solution at 0–4°C. This continuous flow allows complete perfusion of the organ promoting a thorough washout of blood and subsequent tissue equilibration with the preservation solution. Beneficial effects claimed on behalf of machine perfusion are a low incidence of DGF, the possibility of real-time viability assessment, the ability to provide metabolic support during perfusion, and the potential to add pharmacologic agents to the perfusate.

In kidney preservation, both in animal experiments and in historical controlled retrospective clinical studies, HMP has been demonstrated to provide better early graft function compared to CS (*110*, *111*). In addition, when kidneys retrieved from extended-criteria, marginal, or NHB donors were analyzed, HMP was found to be superior to CS (*112–116*). Unfortunately, in most studies no prospective randomization was performed and patient numbers were not large enough to allow extrapolation of the results. Recently, Wight et al. reported an excellent meta-analysis based on aggregated results of the current literature concerning HMP versus CS, clearly demonstrating a 20% reduction in DGF with HMP (*117*). DGF reflects a compilation of accumulated risk factors and depends on the presence or absence of independent donor, preservation, and recipient characteristics (*29*). Possibly, some of the detrimental effects caused by these risk factors can be reduced with HMP. The occurrence of DGF requires continuation of dialysis and is associated with

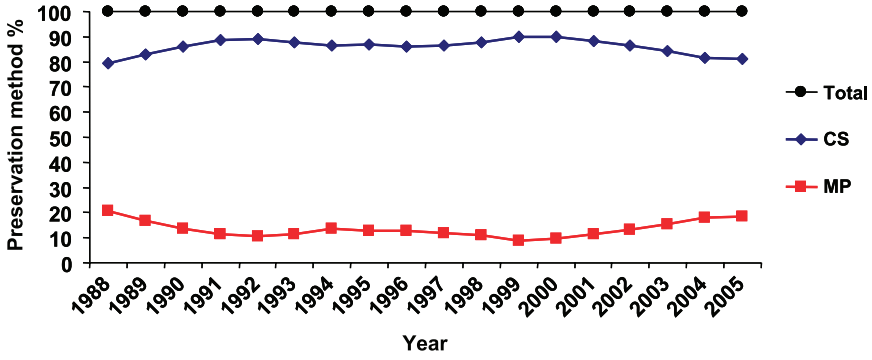

FIGURE 2. Relative amount of renal machine (MP) and cold storage preservation (CS) in the United States from 1988–2005 (based on Organ Procurement and Transplantation Network data of October 2006).

an increased incidence of acute rejection and inferior long-term outcome (*118*, *119*). While individual studies suggest potential benefits of HMP such as reduced DGF rates, less acute rejection, and improved short- and long-term function, no comparative study of these modalities has been performed under strict conditions (*120*). For this reason, recently a European multicenter prospective randomized clinical trial has been conducted in the Netherlands, Belgium, and Germany comparing HMP versus CS in a consecutive series of more than 300 donors and 600 kidney transplants (*121*).

Most experience with HMP concerns the kidney (Fig. 2). Only scarce experimental data exist in experimental liver transplantation by the groups of Belzer, Slapak, and Brettschneider (*122–124*). Several strategies regarding perfusion of portal vein and/or hepatic artery have been applied. In 1986, D'Alessandro, and later Pienaar, from the Madison group managed to successfully transplant canine livers after 72 hours HMP (*125*, *126*). Clinical application of HMP in liver transplantation, however, has been limited to recent pioneering work of Guarrera et al. (*103*).

Overall, both experimental and clinical data suggest that HMP improves kidney and liver preservation. Modern, portable, and stand-alone HMP systems for kidney preservation are now available, allowing user-friendly transportation within an international organ sharing system. Therefore, a broader clinical application of HMP should be considered to reduce the impressively high DGF rate of 60–85% in NHB kidneys and possibly reduce the Achilles heel in liver transplantation: ischemic type biliary lesions (*127–130*).

New Approaches in Organ Preservation

Apart from HMP, several other concepts have been developed to allow expansion of the donor pool. During the past decades, not only age but also the type of organ donors has changed. The cause of death has shifted from a relative healthy donor with cerebral trauma to older patients suffering from cerebral hemorrhage. As a result, average donor organ quality has decreased and the task to at least maintain the quality of the graft before transplantation has become much more important.

A rather unusual but attractive technique to resuscitate damaged kidneys and livers is the perfusion of gas through the vasculature. This concept was initially described by Bunzl in 1954 and named "persufflation" by Isselhard in 1972. It consists of retrograde venous application of humidified pure oxygen (O_2) at 13–18 mmHg during CS (*131*, *132*). Renal persufflation preservation has been applied clinically in a small pilot study including 10 paired kidneys. Although numbers were small, persufflated kidneys did show improved initial function compared to CS (*133*). Its application in liver preservation was extensively studied by the group of Minor. In several experiments, they showed that gaseous oxygenation during CS was highly effective in improving liver graft viability (*134–136*). Using this method, survival after 45 minutes of warm ischemia in a NHB liver transplant model was 100%, compared to 0% in the CS group (*136*).

Another, more static, way to deliver O_2 to CS grafts is the dual-layer perfluorocarbon technique. Perfluorocarbons (PFC) are hydrocarbons in which most of the hydrogen atoms have been replaced with fluorine. The attractive property of PFC is a very high capacity for dissolving O_2. PFC liquids can store 20–25 times greater amounts of O_2 than water or blood. In addition, the very low O_2 binding constant of PFC allows a more effective release of O_2 in tissue than hemoglobin does. These properties make PFC-based solutions interesting for organ preservation (*137*).

PFC was first used in organ preservation as a component of the two-layer method (TLM) (*138*). The TLM is comprised of UW-CSS and oxygenated PFC for pancreas preservation. During preservation by TLM, canine pancreas grafts continuously generated ATP up to 96 hours (*139*). In animal models, TLM appeared to be useful not only for pancreas but also for small bowel preservation (*140*). In the clinical setting, however, TLM remains controversial because it did not improve whole pancreas transplantation (*141*). Furthermore, there is debate about its effects on islet isolation. Although some small clinical trials have reported beneficial effects, the largest and most recent survey did not demonstrate superiority over UW-CSS in the field of human islet isolation (*142–144*).

The latter alternative approaches all have in common that they take advantage of the beneficial effect of O_2 during hypothermic preservation. Improving the energy status of organs during preservation leads to earlier recovery, especially in ischemically damaged organs. Whereas O_2 supports metabolism, various other gaseous compounds that act on signal transduction have also proven their efficacy to improve graft viability in the experimental setting. Donor pretreatment with carbon monoxide (CO) at low concentrations in a rat small intestine transplant model reduced pro-inflammatory interleukins and improved survival to 100% compared to 58% in air-treated controls (*145*). Similarly, in rat liver transplants, exposure of the recipient to CO-suppressed induction of tumor necrosis factor-α, inducible nitric oxide synthase,

and intercellular adhesion molecule-1. Liver grafts showed improved liver function and less neutrophil infiltration after CO exposure (*146*). Nitric oxide (NO), the radical produced from L-arginine by the enzyme NO synthase (NOS), is a potent vasodilatator that inhibits platelet and neutrophil aggregation and adhesion (*147*, *148*). This effect is potentially beneficial for preservation. Vasodilatation will improve organ washout during procurement, whereas the immunological effects of NO may limit reperfusion damage. Adding NO during cold ischemia improved small bowel viability in both rat and pig autotransplantation models (*149*). In addition, topical exposure of rat kidneys with NO significantly reduced the effects of 60 minutes of warm ischemia (*150*).

These experiments suggest that exposure of the graft to CO and/or NO during preservation might induce a protective effect before reperfusion. HMP devices could thus enable administration of these compounds, either via an oxygenator as a gas or by pharmacological donors in the solution.

Outlook

As often before in transplant history, major improvements in preservation will probably be derived from new philosophies instead of adaptations of current strategies. Ideally, good preservation should facilitate the use of marginal and older organs and provide real-time viability assessment before transplantation. Normothermic (37°C) or subnormothermic (25–32°C) perfusion is becoming popular as a preservation alternative that may indeed achieve these goals (*151*). In canine kidney transplantation after 120 minutes of warm ischemia, 18 hours of normothermic perfusion allowed eventual recovery of normal renal function, whereas primary nonfunction occurred in all kidneys preserved for 18 hours with static CS (*152*). Raising the temperature during preservation provides more adequate ways to test and optimize graft viability and allows elimination of hypothermia induced injury (*13*, *153*).

Normothermic perfusion of the abdominal organs using a cardiopulmonary bypass system followed by CS has already been applied in human kidney transplantation. This so-called normothermic recirculation protocol showed significant improvements in a group of 44 NHB kidneys. PNF and DGF rates were 0% and 12.5%, respectively, compared to 22.5% and 55% for conventional preservation techniques. Despite the fact that this study was retrospective and included patients over a 12-year period, it suggests a potential benefit for clinical application of normothermic techniques (*154*).

In liver preservation, normothermic perfusion of porcine livers subjected to 60 minutes of warm ischemia resulted in functioning liver grafts, whereas the animals transplanted with CS livers all died. Normothermic perfused livers demonstrated stable metabolic function with adequate production of coagulation factors, hyaluronic acid clearance, glucose metabolism, and significantly lower transaminases compared to CS grafts (*151*, *155*, *156*).

The voluminous perfusion setup, necessity of continuous monitoring during perfusion, and technical complexity, however, have limited clinical application of normothermic machine perfusion (NMP) so far (*153*, *157*). To introduce NMP as a feasible option in clinical practice a combination of techniques has to be used. After an initial period of conventional hypothermic preservation, allowing transportation to a specialized facility, NMP can be started. In kidney preservation it has been shown that HMP with intermittent NMP improves graft survival of canine kidneys after 30 min warm ischemia (*158*). The liver, however, is more vulnerable. Recently, initial NMP for 24 hours was compared to 4 hours of CS followed by 20 hours of NMP in a porcine NHB model with 60 minutes of WIT. The latter combination, however, was ineffective as the benefits of NMP were lost due to the short CS period (*159*).

Overall, NMP offers several advantages over conventional preservation techniques. Therefore, the development of a portable and easy-to-handle stand-alone device is crucial for the introduction of NMP into day-to-day practice for kidney and liver preservation.

CONCLUSION

Organ preservation has always been crucial for transplant outcome, but will become even more important in the present era with increasing numbers of older, more marginal, and NHB donors. Although CS has proven its efficacy in the past, it seems that the limitations of this technique have been reached. To maintain organ viability, more efforts are necessary to reduce ischemia/reperfusion injury and initiate repair. Awaiting the results of several clinical trials, hypothermic machine perfusion, or even normothermic machine perfusion may be (re)introduced in clinical preservation in general or for special categories of donor organs.

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Donor-Derived Disease Transmission Events in the United States: Data Reviewed by the OPTN/UNOS Disease Transmission Advisory Committee

M. G. Ison[a,*], J. Hager[b], E. Blumberg[c], J. Burdick[d], K. Carney[e], J. Cutler[f], J. M. DiMaio[g], R. Hasz[h], M. J. Kuehnert[i], E. Ortiz-Rios[j], L. Teperman[k] and M. Nalesnik[l]

[a] *Divisions of Infectious Diseases and Organ Transplantation, Northwestern University Feinberg School of Medicine, Chicago, IL*
[b] *United Network for Organ Sharing (UNOS), Richmond, VA*
[c] *Division of Infectious Diseases, University of Pennsylvania, Philadelphia, PA*
[d] *Health Resources and Services Administration (HRSA), US Department of Health and Human Services (HHS), Rockville, MD*
[e] *Department of Lung Transplantation, University of Pennsylvania, Philadelphia, PA*
[f] *Southwest Transplant Alliance, Dallas TX*
[g] *Department of Cardiothoracic Surgery, UT Southwestern, Dallas, TX*
[h] *Gift of Life Donor Program, Philadelphia, PA*
[i] *Centers for Disease Control and Prevention (CDC), Office of Blood, Organ, and Other Tissue Safety Atlanta, GA*
[j] *HRSA, HHS Rockville, MD*
[k] *Department of Transplantation, New York University, New York, NY*
[l] *Department of Pathology, University of Pittsburgh, Pittsburgh, PA*
* *Corresponding author: Michael G. Ison, mgison@northwestern.edu*

Donor-derived disease transmission is increasingly recognized as a source of morbidity and mortality among transplant recipients. Policy 4.7 of the Organ Procurement and Transplantation Network (OPTN) currently requires reporting of donor-derived events. All potential donor-derived transmission events (PDDTE) reported to OPTN/UNOS were reviewed by the Disease Transmission Advisory Committee (DTAC). Summary data from January 1, 2005–December 31, 2007, were prepared for presentation. Reports of PDDTE have increased from 7 in 2005, the first full year data were collected, to 60 in 2006 and to 97 in 2007. More detailed information is available for 2007; a classification system for determining likelihood of donor-derived transmission was utilized. In 2007, there were four proven and one possible donor-derived malignancy transmissions and four proven, two probable and six possible donor-derived infectious diseases transmissions. There were nine reported recipient deaths attributable to proven donor transmissions events arising from eight donors during 2007. Although recognized transmission events resulted in significant morbidity and mortality, transmission was reported in only 0.96% of deceased donor donations overall. Improved reporting, through enhanced recognition and communication, will be critical to better estimate the transmission risk of infection and malignancy through organ transplantation.

Key words: Donor risk, donor-to-host transmission, infectious diseases, malignancy

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Introduction

Solid organ transplantation has given life-extending benefit to many patients with end-stage organ failure and a large majority of patients have a functioning organ at the end of 1 year (1). But, as with most medical interventions, there is a risk of serious complications. One potential complication is disease transmission (e.g. infection, malignancy) from the donor to the recipient(s). Donor-transmitted diseases are typically 'expected' when routine testing shows donor infection (e.g. seropositivity) and recipient susceptibility (e.g. seronegativity) before transplantation. When transplants are performed under these circumstances, the urgent need may be deemed to outweigh the expected risk and are considered acceptable medical practice. Careful monitoring of these patients and the use of prophylaxis or treatment may reduce the frequency or severity of these transmitted diseases (e.g. cytomegalovirus [CMV], Epstein–Barr virus [EBV] and hepatitis B virus [HBV]) and may permit the use of these organs.

Unfortunately, diseases that are not expected or are not identified in the donor can also be transmitted to the recipient(s) with potentially fatal results. Recent high profile transmission events include human immunodeficiency virus (HIV), hepatitis C virus (HCV), lymphocytic

Table 1: Known conditions that may be transmitted by the donor organ that must be communicated to the transplant center prior to transplantation (13)

• Unknown infection of central nervous system (encephalitis, meningitis)
• Suspected encephalitis
• Hepatitis C
• Herpes simplex encephalitis or other encephalitis
• History of JC virus infection (causes progressive multifocal leukoencephalopathy)
• West Nile virus infection
• Cryptococcal infection of any site
• Rabies
• Creutzfeldt–Jacob disease
• Other fungal or viral encephalitis
• Bacterial meningitis
• Infection with HIV (serologic or molecular)
• Active viremia: herpes, acute EBV (mononucleosis)
• Serologic (with molecular confirmation) evidence of HTLV-I/II
• Active hepatitis A or B
• Infection by: Trypanosoma cruzi, Leishmania, Strongyloides, Toxoplasmosis
• Active Tuberculosis
• SARS
• Pneumonia
• Bacterial or fungal sepsis (e.g. candidemia)
• Syphilis
• Multisystem organ failure due to overwhelming sepsis, such as gangrenous bowel
• Malignancies-other active malignant neoplasms,
• Melanoma, Merkel cell, including Kaposi's
• Hodgkins' disease and non-Hodgkin's lymphoma
• Multiple myeloma
• Leukemia
• Aplastic anemia agranulocytosis
• Miscellaneous carcinomas
• Any new conditions identified by the CDC as being a potentially communicable disease

choriomeningitis virus (LCMV) and a related arenavirus, tuberculosis (TB), West Nile Virus, rabies, Chagas disease, leukemia and lymphoma (2–11). Several OPTN policies have been enacted with the goal of reducing the risk of potential disease transmission. Current OPTN policy mandates the pathogens for which potential donors must be routinely screened (i.e. HIV, HBV, HCV, syphilis, human t-lymphotropic virus (HTLV), CMV, EBV; in addition blood and urine must be cultured for bacteria in donors hospitalized $\geq$72 h) (12). Policy also requires acquisition of the donor's medical and social history; unfortunately the quality of these data is highly variable and dependent on how familiar the data source is with the donor. Other known conditions that may be transmitted by the donor organ must also be communicated to recipient transplant center personnel, who may accept the organs for transplant as appropriate with the informed consent of the recipient(s) (Table 1) (13). Further, OPTN policy requires informed consent of the recipient of organs from donors at 'high risk' for HIV transmission based on the Public Health Service (PHS) definition (14).

The Disease Transmission Advisory Committee (DTAC) was first established in 2005 as an advisory group to the OPTN/UNOS Operations Committee to identify and review potential donor-derived disease transmission events. The DTACs core tasks include:

(i) Estimating the risk within the OPTN of donor-derived disease transmission.
(ii) Accumulating evidence necessary for this estimation through review of cases reported to UNOS as the OPTN contractor.
(iii) Providing initial notification to public health agencies when there are suspected transmission of reportable diseases.
(iv) Reporting the Committee's aggregate findings and observations to the transplant community.
(v) Providing recommendations to the OPTN on policy with the goal of reducing donor-derived transmission events.

The Committee comprises a broad representation of the transplant and organ procurement communities, as well as *ex officio* representatives from the Centers for Disease Control and Prevention and the Health Resources and Services Administration's Division of Transplantation. It includes experts representing the fields of infectious diseases, pathology, donor evaluation and management and organ transplantation. The authors of this article were members of the Committee at the time that the article written.

OPTN Policy 4.7 requires that 'when a transplant program is informed that an organ recipient at that program is confirmed positive for or has died from a transmissible disease or medical condition for which there is substantial concern that it could be from donor origin, the transplant program must notify by phone and provide available documentation, as soon as possible and not to exceed one complete working day, to the procuring Organ Procurement Organization (OPO)' which then must submit the finding to the OPTN (15). These reports are submitted through the OPTN Patient Safety System (https://portal.unos.org/index.aspx). When these notifications are received by UNOS staff, redacted initial reports and supporting data are prepared that remove all patient-, OPO- and transplant center-identified information. These reports are then uploaded onto a password-protected secure web site accessible to the members of the DTAC, and an e-mail is sent to the DTAC members alerting them of the new report. The Committee then engages in an e-mail-based confidential medical peer review process. DTAC recommendations about additional information that may be needed to determine if a transmission has occurred are made within 24–48 h of the initial report. Further rounds of e-mail communication occur as additional case details are received from the OPO or recipient transplant centers. If warranted

Table 2: Expected donor-derived disease

Any case in which information about the potentially transmissible donor-derived disease was known before transplantation or for which there are recognized standard guidelines for routine prevention of the pathogen are available were excluded; examples include: • Cytomegalovirus (CMV) • Ebstein–Barr virus (EBV) • Toxoplasmosis • Known HBc Ab+ only

or required (e.g. nationally notifiable infectious diseases as defined by the CDC), local, state and federal (CDC) health authorities are involved. Similarly, case-specific conference calls are sometimes initiated to facilitate communication among the transplant centers, OPOs, health authorities and DTAC. OPOs are required to submit a report 45 days after the initial report that summarizes the findings of their investigations of each reported cases. These 45-day reports are reviewed by the DTAC as well to determine if additional information is needed. All communication between the OPOs, transplant centers and DTAC is coordinated by the DTAC's UNOS staff liaisons. The Committee is currently developing a process to ensure that the OPOs and transplant centers are made aware of the determination of the Committee once the 45-day report has been reviewed. The Committee meets at least monthly via conference call to discuss cases currently under review. Additionally, twice-yearly in-person meetings are convened to further discuss events, tasks and policy issues assigned to the DTAC by the OPTN/UNOS Board of Directors.

The DTAC began reviewing reports of potential donor-derived transmission events (PDDTE) in late 2004. This report summarizes the results of reviews since that time, with an emphasis on cases reported in 2007 when more detailed reviews by the Committee began.

Materials and Methods

All PDDTE that were reported to the OPTN via the electronic Patient Safety System have been reviewed by the DTAC. An event is defined as all potential disease or malignancy transmissions from one donor to one or more recipients. All of the available data were reviewed in detail and events were categorized first as either 'expected' (i.e. transmission would be expected as transplantation is conducted when there were known risks) or 'unexpected' (see Table 2).

The Committee devised a classification scheme for the events reported in 2007 and since then have further classified events as to the likelihood of being transmitted from the donor with the classifications of proven, probable, possible or excluded. (defined in Table 3). Each case was discussed by the entire Committee until a consensus classification was made. Essentially, proven transmission required identification of the same pathogen in the donor and recipient or a malignancy of documented donor origin; probable transmission was used when there was strong evidence to suggest that the pathogen or malignancy was of donor origin, but there was no documentation of donor origin and possible transmission was used when the Committee felt that the evidences suggested a transmission event but for which clear evidence of donor origin could not be determined. These categories have evolved over the period of time that the DTAC has been in existence, are working designations, and have not been formally ratified by the OPTN. The Committee has great confidence that transmission has clearly occurred in all proven cases; classification of cases as other than proven is dependent on the available data. The DTAC provides suggestions regarding additional testing to the OPO and transplant centers, but current OPTN policy does not require these groups to conduct the recommended evaluation. As such, there is no way to validate or definitively document the likelihood of transmission in every case. Last, appropriate specimens were not always available, further limiting appropriate testing required to document transmission. Descriptive statistics were calculated.

Table 3: Classification system for determining likelihood of the transmission event being donor-derived

Definition	Criteria
Proven	All of the following conditions must be met: • Suspected transmission event • Laboratory evidence of the suspected organism or malignancy in a recipient • Laboratory evidence of the same organism or malignancy in other recipients • Laboratory evidence of the same organism or malignancy in the donor • If there is pretransplant laboratory evidence, it must indicate that the same recipient was negative for this organism prior to transplantation
Probable	Both of the following two conditions must be met: • Suspected transmission event and • Laboratory evidence of the suspected organism or malignancy in a recipient *And at least one of the following criteria must also be met:* • Laboratory evidence of the same organism or malignancy in other recipients; • Laboratory evidence of the same organism or malignancy in the donor; If there is pretransplant laboratory evidence, it must indicate that the same recipient was negative for this organism prior to transplantation
Possible	Suspected transmission event and Laboratory evidence of the suspected organism or malignancy in a single recipient or No evidence of transmission in the setting of active prophylaxis or treatment for the infection or Data that strongly suggests but does not prove a transmission event
Excluded	Suspected transmission event *and at least one of the following conditions is met:* • There is clear evidence for an alternative reason for the event • Lack of infection with the same organism in any other recipients, from the same donor, given appropriate testing • Laboratory evidence that the recipient had infection with this organism or malignancy prior to transplantation
Confirmed	Any case that is classified as proven, probable or possible.

Table 4: Reports received by the OPTN between 2005 and 2007 regarding a potential donor-derived infectious disease transmission

Infections	Donor reports[2]	Confirmed recipients[3]	Recipient deaths[4]
Hepatitis C virus	9	4[5]	1[5]
Tuberculosis	8	3	2
HIV	7	4[5]	1[5]
Chagas	6	3	2
Hepatitis B virus	6	0	0
Toxoplasmosis[1]	6	4	0
West Nile virus	6	2	0
Histoplasmosis	4	2	0
Bacteremias	3	2	2
Candidemia	3	3	2
EBV	3	0	0
Cryptococcus	2	1	0
Schistosomiasis	2	1	0
Strongyloides	2	1	1
Syphilis	2	0	0
Bacterial meningitis	1	0	0
Cytomegalovirus	1	0	0
HTLV	1	0	0
Influenza A	1	0	0
LCMV	1	4	3
Legionella	1	1	0
Listeria	1	0	0
Mycotic Aneurysm	1	0	0
RMSF	1	0	0
S. aureus in transport fluid	1	0	0
Zygomycetes	1	0	0
Totals	80	30	14

[1]Expected events, based solely on positive serology of the donor.
[2]Number of donors with reported possible donor-derived disease transmission.
[3]Number of recipients with confirmed (proven, probable or possible) donor-derived disease.
[4]Number of recipients who died as the result of a donor-derived disease transmission.
[5]These are four recipient transmissions from a single donor of two different infectious agents.

Results

A total of 164 events have been reported through the Patient Safety System between 2005 and 2007. There were 7 reports of PDDTE reported in 2005, 60 in 2006 and 97 in 2007. Of these reports, 10 (0 in 2005, 0 in 2006, 10 in 2007) were classified as 'expected' and the remainder were 'unexpected'. Eighty-nine of the unexpected events reported were related to a potential infectious disease transmission (Table 4). This accounted for over half of all unexpected events reported (58% of 154 events: 4 in 2005, 31 in 2006 and 54 in 2007). The most commonly reported potentially transmitted infections included HCV, TB, HIV, Chagas, HBV, toxoplasmosis and West Nile Virus. There were 65 reports (42% of 154 events; 3 in 2005, 29 in 2006 and 33 in 2007) of unexpected malignancy related events made to the OPTN (Table 5). The most frequently reported potential transmissions of cancers involved renal cell carcinoma, lung cancer, glioblastoma multiforme and lymphoma.

Table 5: Reports made to DTAC regarding a potential donor-derived malignancy transmission 2005–2007

Malignancies	Donor reports[1]	Confirmed recipients[2]	Recipient deaths[3]
Renal cell carcinoma	25	3	0
Lung—adenocarcinoma	5	2	2
Glioblastoma multiforme	4	1	1
Lymphoma	3	4	2
Metastatic melanoma	3	2	1
Prostate adenocarcinoma	2	0	0
Thyroid cancer	2	0	0
Breast cancer	1	0	0
Colon cancer	1	0	0
Hepatocellular cancer	1	1	0
Kaposi's sarcoma	1	0	0
Leukemia	1	0	0
Lung—bronchoalveolar cancer	1	0	0
Lung—small cell cancer	1	1	0
Myeloid sarcoma	1	0	0
Ovarian carcinoma	1	1	0
Pancreatic adenocarcinoma	1	0	0
Renal Papillary adenocarcinoma	1	0	0
Totals	55	15	6

[1]Number of donors with reported possible donor-derived disease transmission.
[2]Number of recipients with confirmed (proven, probable or possible) donor-derived disease.
[3]Number of recipients who died as the result of a donor-derived disease transmission.

Since cases reported during 2007 received greater review, the Committee was better able to classify the likelihood of transmission of these events (see Table 6). Half of the infectious disease reports (27) and 30% of the malignancy reports (10) were classified by the Committee as excluded as no transmission could be documented. Most of the excluded cases had no clear associated disease (19 cases) or had insufficient data (13 cases; most were seroconversions of single recipients with no data from the other recipients); and nine were excluded because they were reports of false-positive testing.

Of the 2007 infectious diseases reports, five were ultimately determined to be proven, two were deemed probable based on available information and six were deemed possible (Table 6). In all proven cases, the pathogen was clearly documented in both the donor and at least one of the recipients. In one of the probable TB transmissions, the infection was diagnosed in the transplanted organ shortly after transplantation and typing suggests that the TB may have originated from the donor. In the second probable TB transmission, the donor was recognized, as part of a look-back investigation, to have been diagnosed but not treated for latent TB. In both cases, predonation cultures were not available in the donor. In many of the cases of

Table 6: 2007 confirmed transmission events

Type	Status	Disease	Reported (time posttransplant)	No. of recipients affected[1]	Organs affected	No. of recipients who died
Malignancy	Proven	Hepatocellular CA	5 months	1/3	Liver	0
		Glioblastoma multiforme	2 months	1/4	Lung × 2	1 Double lung
		Lymphoma	1.5 months	4/4	Liver pancreas kidney × 2	4 Liver, pancreas, kidney × 2
		Small cell lung cancer	10 months	1/1	Liver	0
	Possible	Melanoma	6 months	1/2	Liver–kidney	1 Liver–kidney
Infection	Proven	Strongyloides	3 months	1/3	Kidney	1 Kidney
		C. albicans	4.5 months	2/3	Kidney × 2	1 Kidney
		Tuberculosis	7 weeks	2/3	Kidney × 2	1 Kidney
		HIV + HCV	10 months	4/4	Heart kidney × 2 Liver	1 Liver
	Probable	Tuberculosis	3 months	1/5	Lung	0
		Tuberculosis[2]	3 months	1/6	Lung	1 Lung
	Possible	Legionella	12 days	1/6	Lung	0
		Syphilis	4 days	0/3[3]	–	0
		C. albicans	6 weeks	1/4	Heart	1 Heart
		Schistosomiasis	1 day	0/6[4]	–	0
		Histoplasmosis	4 months	1/4	Liver	0
		Histoplasmosis	11 months	1/1	Liver	0

[1]No. with confirmed disease/no. of recipients from the same donor.
[2]Donor had documented untreated latent tuberculosis pretransplant.
[3]Donor initially had negative RPR; subsequent testing was repeatedly positive for RPR and FTA Abs; early treatment given and no follow-up testing on treated patients.
[4]Donor noted to have colitis at procurement and biopsy demonstrated agents consistent with *S. mansoni*. Two recipients received praziquantel and no other testing results are available.

possible transmissions, all or most of the recipients received antimicrobial therapy directed at the detected pathogen before testing could be completed. As a result, infection could not be documented in more than one recipient. In the case of the syphilis and schistosomiasis cases, donor testing suggested active infection and early therapy was provided; none of the recipients had documented infection but the evidence available to the Committee suggested that transmission could have occurred without antimicrobial intervention. There were seven deaths, and all events with proven transmission of a donor-derived infection had at least one fatality. Death occurred in 40% of those recipients with documented infection, while 12% of all recipients of organs with proven, probable or possible donor-derived infection transmission died.

In 2007, definitive transmission of a donor-derived tumor could be documented in seven recipients from four donors; in another event, a recipient developed melanoma in the transplanted organ (liver) 6 months after transplant but the Committee was unable to determine if the tumor was of donor or recipient origin**.** Transmission occurred in 57% of the recipients of proven or possible donor-derived malignancy events in 2007. There were five deaths attributable to donor-derived malignancy transmissions. This represents deaths in 40% of reported donor-derived malignancy events and 63% of recipients with confirmed transmission of malignancy.

Discussion

Over the past 3 years, the number of potential donor-derived infections and malignancies reported to the OPTN has increased from 7 to 97 per year. Transmission of infection was documented in 24% of reports while malignancy transmission was documented in 22% of reports. When transmission occurred, there was substantial morbidity and mortality among affected recipients. Donor-derived disease transmission, though, remains a rarely recognized complication of solid organ transplantation with a reported incidence of 0.96% of deceased donor donations in 2007; documented incidence has increased every year since reporting has been required. Most of this increase is likely the result of improved recognition and the development of a formalized reporting process. The true incidence is not known but will be clarified over time through enhanced reporting by OPOs and transplant centers and improved evaluation of cases by DTAC. When transmission occurred, significant morbidity and mortality, around 40%, was observed. A better understanding of these risks will be important to better inform patients and to provide advice on how to minimize transmissions in the future.

The work of the DTAC allows the OPTN to better understand the risks associated with infection and malignancy transmission from a donor to transplant recipients. Although reports of potential transmissions of HIV, HCV and

HBV were not uncommon, most of these reports were attributable to false positive laboratory testing, typically stemming from additional testing required for tissue donated from the same donor. Similarly, several of the Chagas and toxoplasmosis events represented positive donor serology without evidence of transmission to the recipients. Many of the reported malignancies, especially renal cell carcinomas, were of limited disease in the donors and do not appear to have been readily transmitted to the recipients (16); follow-up on these malignancy cases is still ongoing and a more comprehensive review of this issue is needed. As more data are accrued, better inferences into the relationship between tumor stage and risk of transmission may be possible. From such data, it may be possible, in the future, to identify situations where risk of malignancy transmission from donor to recipient is low and may be used more consistently and safely. A working group has recently been formed to review the available data and provide recommendations along these lines.

These data must be interpreted with great caution because of the intrinsic limitations involved in the OPTN reporting process. First, there is underreporting of events. Reporting of potential donor-derived disease transmission events to the OPTN is a subjective process. Providers may have varying interpretations of the clinical scenarios that present themselves in transplant recipients. If the clinician does not consider the possibility that an infection or malignancy is donor-derived, a report from the transplanting institution will not be submitted. Although reporting is required by OPTN policy, it is likely that events are underreported. There are increasing numbers of events reported each year, most likely due to increased awareness of the responsibility of OPTN members to report these cases, rather than a true increase in the underlying incidence. Additionally, we are aware of cases that have been published in the literature but have not been reported to UNOS (17–19). Second, there may be significant delays between the transmission event and reporting to the OPTN. Several events were reported well after the potential transmission had occurred allowing for limited input from DTAC in the evaluation and management of events. There are also challenges to recognizing donor-derived disease transmissions. If organs from the same donor are transplanted into recipients at different centers, clinical features seen in all or most of the recipients may not be recognized as occurring because of limited communications between centers post transplant. In most events, discussion between centers spontaneously occurred when a patient was significantly ill or had expired.

Third, there is no standardized cluster analysis of recipient outcomes to determine if similar disease occurred in recipients of organs from the same recipient—it is currently possible to report two or more recipients as having the same rare malignancy without linking these findings in the already established data bases.

Fourth, the follow-up on these events is very limited. Testing recommendations provided by the DTAC during the course of determining whether transmission has occurred is entirely voluntary; although the Committee often suggests additional testing that may assist in proving or disproving the possibility of donor-derived transmission, follow through with additional testing is highly variable. Similarly, detailed follow-up information may be limited, particularly in events with the threat of litigation. Finally, by current OPTN policy, the DTAC can only obtain direct follow-up specific to the event reported in the Patient Safety system through 45 days after the first report. For many potential donor-derived diseases, particularly malignancies, the ability to identify potential transmission at 45 days is limited. Longer-term detailed follow-up to the DTAC is clearly needed to document potential transmission as well as recipient outcomes.

Fifth, the evaluation of cases and review of data by DTAC has been evolving over the past several years. Although there was informal discussion of cases reported during 2005 and 2006, DTAC met in person for the first time in 2007 and developed a more formalized process for reviewing cases. During this meeting, the Committee reviewed the details of the 2007 cases in far greater detail than was done in previous years. As a result, the Committee feels that it can more accurately proscribe the likelihood of transmission of cases from 2007 than those reported earlier. We present the unaudited cases reported during the first 2 years both because reporting was required during the first 2 years and also to give a clearer sense of the types of reports made to the group.

The Committee is working to address some of these limitations. First, we are attempting to educate the transplant community about the reported donor-derived infections and to encourage enhanced identification and reporting of events. We are accomplishing this through presentation at national and international scientific meetings, presentation at the OPTN/UNOS regional meetings, and publications, such as this one. Second, we are going to attempt to utilize existing data bases to better identify disease transmissions. We are crafting a data request to look at mortality and malignancy data of all recipients from the donors that have had reports of potential disease transmission. Hopefully, this will allow us to identify information that comes in beyond the day 45 report, particularly in the setting of malignancies, or transmissions that are not recognized locally. Additionally, we are looking into the feasibility of attempting to do a cluster analysis of all donor and recipient data in the OPTN and Scientific Registry of Transplant Recipients (SRTR) data bases to identify unreported or unidentified transmissions. Third, the Committee is continuing to revise how we collect, manage and evaluate the data that is provided to us. Hopefully, this will refine our results as we move forward. Fourth, we are in the process of considering recommending revisions to current OPTN policy to

streamline and enhance the current reporting of potential and proven transmissions.

This represents the first formal DTAC report of potential disease transmission cases reported to the OPTN. The DTAC is refining the process by which collection and review of the available data enable a better understanding of the changing trends in donor-derived disease transmission. Additionally, there are similar efforts to identify potential donor-derived infections in Europe, Australia and New Zealand that may further our ability to more accurately estimate the true risk of donor-disease transmission through transplantation. Despite the limitations of the current data, it helps to advance our understanding of donor-derived disease transmission. Without an OPTN system of reporting and review in place similar to what is currently in use, it would not be possible to catalogue transmission events and potential near misses to allow changes in policy and practice to improve patient safety. Clinicians should be constantly aware of the possibility of donor-derived disease. Enhanced communications between clinicians caring for transplant recipients with infections and malignancies is crucial and may lead to the earlier identification of transmission events and reduced morbidity and mortality through earlier intervention. Similarly, early involvement of the local OPO to facilitate communication and reporting of potential events is essential. With enhanced recognition of donor-derived disease transmission, increased reporting to the OPTN and review by the DTAC should occur. As more is learned from reviewing these transmission events, the DTAC can advocate for modifications to OPTN policies and develop practices to reduce the likelihood of these transmissions. As a public health issue the dominant risk is that 6000 patients a year die waiting on the list. Although the disease transmission rate is comparatively small, this OPTN effort in DTAC is critical for minimizing the chances that disease transmission will negate the otherwise lifesaving potential of each transplanted organ.

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REVIEW

Monitoring of human liver and kidney allograft tolerance: a tissue/histopathology perspective

Anthony J. Demetris,[1,2] John G. Lunz III,[1,2] Parmjeet Randhawa,[1,2] Tong Wu,[1,2] Michael Nalesnik[1,2] and Angus W. Thomson[1,3]

1 Division of Transplantation, Thomas E Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
2 Division of Transplantation, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
3 Division of Transplantation, Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

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Correspondence
Anthony J. Demetris MD, UPMC-Montefiore, Room E741, 3459 5th Avenue, Pittsburgh, PA 15213, USA. Tel.: 412-647-2072; fax: 412-647-2084; e-mail: demetrisaj@upmc.edu

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Summary

Several factors acting together have recently enabled clinicians to seriously consider whether chronic immunosuppression is needed in all solid organ allograft recipients. This has prompted a dozen or so centers throughout the world to prospectively wean immunosuppression from conventionally treated liver allograft recipients. The goal is to lessen the impact of chronic immunosuppression and empirically identify occasional recipients who show operational tolerance, defined as gross phenotype of tolerance in the presence of an immune response and/or immune deficit that has little or no significant clinical impact. Rare operationally tolerant kidney allograft recipients have also been identified, usually by single case reports, but only a couple of prospective weaning trials in conventionally treated kidney allograft recipients have been attempted and reported. Pre- and postweaning allograft biopsy monitoring of recipients adds a critical dimension to these trials, not only for patient safety but also for determining whether events in the allografts can contribute to a mechanistic understanding of allograft acceptance. The following is based on a literature review and personal experience regarding the practical and scientific aspects of biopsy monitoring of potential or actual operationally tolerant human liver and kidney allograft recipients where the goal, intended or attained, was complete withdrawal of immunosuppression.

Introduction

Allograft biopsy evaluation plays a critical role in the emerging field devoted to minimization or complete weaning of immunosuppression from human solid organ allograft recipients. The immediate practical goal of this field is to improve the quality of life and outcomes for allograft recipients by minimizing exposure to the high cost and serious side-effects of chronic immunosuppression, such as hypertension, diabetes, hyperlipidemia, kidney damage, and increased susceptibility to malignancies. Presumably, this can be achieved without sacrificing allograft structure and function consequent to uncontrollable acute or even indolent chronic rejection. A secondary, but equally important, goal is to use allografts as probes to understand cellular and molecular mechanisms associated with immunologic tolerance. The hope is that treatment algorithms might then be devised to routinely induce tolerance to allografts in a large percentage of recipients. These concepts might also be transferable to the related fields of autoimmunity and cancer immunosurveillance.

Since the advent of solid organ transplantation, two general approaches have been used to study clinical allograft acceptance/tolerance: (i) so-called 'spontaneous operational tolerance (SOT)' is a term borrowed from Ashton-Chess *et al.* [1]. It refers to rare noncompliant recipients and others deliberately removed from immunosuppression who do not develop rejection even long after

the event. SOT recipients are usually identified by trial and error. This approach was pioneered by Starzl who realized that acute rejection was reversible with temporarily increased immunosuppression, with the need for such immunosuppression significantly diminished afterward [2] and (ii) tolerance can also be induced intentionally via hematopoietic macrochimerism, using bone marrow or hematopoietic stem cell transplantation combined with simultaneous [3–5] or delayed kidney transplantation [6–10]. The hematopoietic chimerism approach was based on the original experimental animal observations of Billingham *et al.* [11]. It was matured in further experimental animal studies and then successfully applied to humans by Sachs, Sykes, and Cosimi, using preconditioning with a nonmyeloablative regimen and major histocompatibility complex (MHC)-matched [3–5] or -mismatched [12] simultaneous bone marrow and living-donor kidney transplantation.

Distinguishing between these approaches has meaning beyond the purpose of understanding how the histopathology literature developed in this field. It also provides insights about the predominant immunologic mechanisms involved in allograft acceptance/tolerance. In experimental animals, tolerance achieved through hematopoietic chimerism is robust, mediated predominantly by deletion [13], and organ-independent. In the first author's experience as a clinical and experimental transplant pathologist, this approach leads to the 'cleanest', or the most normal-appearing allografts. Stable macrochimerism, however, is very difficult to achieve in mismatched humans without graft-versus-host disease [12]. Comparatively, SOT is meta-stable and probably mediated by a combination of deletion, ignorance, and regulation and is organ-dependent. Liver allografts exhibit SOT more frequently than other allografts (see below). SOT allografts are usually not as clean or free from inflammation as allografts in chimerically tolerant recipients. Rather than being totally different, however, the two approaches are qualitatively similar, but differ quantitatively in reference to underlying mechanisms, such as deletion and (micro-)chimerism, that contribute to long-term allograft survival [12,14].

These approaches also fit well with tolerance as recently defined by Girlanda and Kirk [15]. 'True tolerance' refers to the absence of any detectable detrimental immune response as well as the absence of immunocompromise. 'Operational tolerance' refers to the gross phenotype of tolerance in the presence of an immune response and/or immune deficit that has no significant clinical impact. For the histopathologist, the difficult phrase in the operational tolerance definition is, '…that has no significant clinical impact'. This is not so easily determined and will be discussed in greater detail subsequently. For this review, we excluded an evaluation of pathology material from so-called prope tolerance studies [16,17] and reports of late rejection occurring in patients with low immunosuppression levels because it was difficult to determine what exactly constituted low-level or minimal immunosuppression.

Instead, this review is based on a literature survey and on personal observations from studies in which complete weaning from immunosuppression was the intended goal or provided some insight into the weaning process. It focuses primarily on studies of human SOT; detailed mechanistic studies in experimental animals are beyond the intended scope, except where they serve to illustrate a point relevant to human material. We apologize in advance because many of the histopathologic observations discussed are, because of trial design and material available, anecdotal and descriptive. But currently, that is the state of the field.

All allografts are not created equal

Spontaneous operational tolerance in conventionally treated recipients is, by far, most commonly observed in liver allograft recipients. Most clinical trials that attempt to prospectively wean human recipients from immunosuppression are conducted in liver allograft recipients and these also show the highest rate of success (see below). The traditional and probably the most accurate and authoritative reason given for this success is the so-called 'hepatic tolerogenicity' (reviewed in Benseler *et al.* [18] and Crispe *et al.* [19]). This refers to the liver's unique role as an immunologic organ. Examples include: (i) oral tolerance, or the observation that systemic immune responses to any particular antigen are significantly less robust if the antigen is fed orally beforehand; (ii) spontaneous acceptance of fully MHC-mismatched liver allografts without immunosuppression in many animal species, excepting humans and primates; (iii) ability of liver allografts to protect other, extrahepatic, allografts from rejection if the latter are derived from the same donor; and (iv) ability of the liver to protect central immune organs from overstimulation by gut bacteria, bacterial products, and other antigens that normally leak through the intestinal barrier. A detailed discussion of the various mechanisms of hepatic tolerogenicity is beyond the scope of this review. Included are the release of soluble MHC antigens, migratory passenger leukocytes and activation of recipient lymphocytes in secondary lymphoid tissues, microchimerism, hepatic dendritic cell immaturity, activation of naïve T cells and purging of cytotoxic cells within the liver, and stimulation of regulatory T cells (reviewed in Benseler *et al.* [18] and Crispe *et al.* [19]).

There are, however, other reasons as to why the liver allograft recipients are more ideal candidates for weaning studies than other conventionally treated solid organ

allograft recipients. First, the vast majority of acute cellular rejection episodes, regardless of severity, are not life- or allograft-threatening, do not produce significant morbidity, and are easily reversible with current immunosuppressive medications [20,21]. Second, reversal of rejection is usually complete: the allografts heal without significant fibrosis, architectural distortion, or loss of function because of robust hepatic regeneration [21–23]. Even the early phases of chronic rejection are reversible in the liver [24]. Therefore, if a liver allograft recipient develops acute or the early chronic rejection during or after weaning, the process is likely to be completely reversible without significant sequelae [21,24]. But there are exceptions and weaning is not risk-free (Table 1).

Table 1. Pre- and postweaning duration, and histopathologic diagnosis in follow-up liver tissue samples from liver allograft recipients withdrawn from immunosuppression.

				Histopathologic diagnoses in postweaning biopsy specimens						
Study	No. Pts off IS/ attempted	IS time	IS-free time	NS* changes	NRH	AR/ CR†	CH	PBC	AIH	Biliary
Starzl *et al.* [14]	6‡	15 years	8.8 years	2		NA	2			
Sandborn *et al.* [29]	0/12	>1 years	0			6/3				
Ramos *et al.* [23]	16/59§ 27.1%	>5 years	3–19 months	7 12%	1 2%	15/0 25.4%	2 3.4%			1 2%
Mazariegos *et al.*¶ [22]; updated in [84]	18/95§ 19%	ca. 8 years 1.7–25 years	0.6–3.5 years	10 11%		21**/3 26%	3 3.1%	2 2%		3 3.1%
Devlin *et al.* [57] and Girlanda *et al.* [61]	5–3/18 28%	>5 years 5–11 years	8–24 months	7	3	4–13/1 22–66%				2
Takatsuki *et al.* [52]	24/63 38.1%	Most >2 years	Mean = 23.5 months			16/0 (25.4%)				
Pons *et al.* [58]	3/9 33%	62.5 months 24–105	17–24 months			2–6†† 22–66%				
Tryphonopoulos *et al.* [102]	20/104 19%	>3 years	0.9–3.3 years			70‡‡/2 69.2%	3 3%			
Eason *et al.* [103]	1/18 5.5%	>6 months	<1 year			11/0 61%	4 22%			
Eghtesad *et al.* [30]	NR/23					13	19			
Tisone *et al.* [31] and Martinez-Llordella *et al.* [33]§§	8+8=16/>34 23.4%	4.5–5 years average >1 year.	Yearly Bxs Av. = 45.5 months			26/0*** 76.5%	34/34 100%			
Koshiba *et al.* [32] and Yoshitomi *et al.* [51]	87/581 15%	Most >2 years	>5 years			8/25†††				

AIH, autoimmune hepatitis; AR, acute rejection; CH, chronic hepatitis; CR, chronic rejection; IS, immunosuppression; NR, not reported; NRH, nodular regenerative hyperplasia; NS, nonspecific; PBC, primary biliary cirrhosis.

*Most frequently consists of mild 'nonspecific' portal inflammation and steatosis.

†Vast majority of acute rejection episodes were Banff [104] mild to moderate. Two patients from the Tryphonopoulos *et al.* [102] study developed chronic rejection; one required re-transplantation; three patients in the Mazariegos *et al.* [22] study developed early chronic rejection stabilized by a return to immunosuppression; three patients in the Sandborn *et al.*'s study [29] developed CR and two died; one patient in the study of Devlin [57] and Girlanda *et al.* [61] required re-transplantation because of CR.

‡Pathology results were not available for all patients in this study because some samples were submitted for immunofluorescence and PCR analysis.

§Not all patients were routinely subjected to follow-up biopsies after withdrawal of immunosuppression.

¶Overlaps with the study of Ramos *et al.* [23], but with longer follow-up.

**Seven patients were treated for rejection without biopsy.

††Four patients developed 'portal inflammation' with elevated liver injury test parameters, not necessarily diagnostic of rejection, but were returned to immunosuppression.

‡‡Forty rejection episodes were clinically suspected and 30 were biopsy-proven.

§§Overlapping patient populations.

***Focal ductopenia involving <20% of portal tracts was observed in occasional recipients, but criteria for chronic rejection were felt not to be present.

†††Yoshitomi *et al.* [51] reported decrease in size and increase in number of bile duct and fibrosis in patients, which they attributed to possibly a variant of chronic rejection.

This is in contrast to cardiac allografts, where severe acute cellular rejection might be lethal. In pancreas, lung, and renal allografts significant acute rejection more frequently results in irreversible scarring, architectural distortion, and permanent loss of function. Intestinal allografts can also heal without significant fibrosis, but severe acute rejection is usually more difficult to reverse and accompanied by significant morbidity. Third, liver injury test parameters are more sensitive indicators of injury than are standard function tests for other organs, for example, serum creatinine in kidneys, pulmonary function tests in lung allografts, or symptoms of decreased cardiac output in heart allografts. Finally, liver allografts are more resistant to antibody-mediated rejection than are other solid organ allografts [25].

The reported experience of SOT in conventionally treated liver- and kidney allograft recipients is shown in Tables 1–3. One study from our center [26] that included 50 kidney-, 17 liver-, 14 pancreas-, and 11 intestinal allograft recipients treated with leukocyte-depleting antibodies was not included in these tables because long-term follow-up has not yet been tabulated and the patients were not entirely immunosuppression-free at the time of publication. Spaced weaning leading to a significant reduction in immunosuppression, however, was achievable in a majority of surviving recipients [26]. The kidney cohort in that study overlaps with a series reported subsequently by Shapiro *et al.* [27] with longer follow-up.

Much of the data used to construct these tables are difficult to verify because individually tolerant recipients are often reported more than once and the same patients are not easily traced among the studies. But, even so, the relative ease with which liver allograft recipients can be completely weaned from immunosuppression as compared with kidney allografts recipients is obvious, especially if one compares the ratio of SOT/total transplants. SOT has been reported in the global literature in at least 49 kidney allograft recipients versus 148 liver allograft recipients (Tables 1–3). These numbers, however, are probably significantly lower than the actual number of SOT recipients who are either unknown and/or unreported.

Weaning trial designs

Most prospective 'weaning trials' have been conducted in liver allograft recipients. The various trials were similar in design and comprised primarily of conventionally treated and immunologically stable liver allograft recipients more than 2 years after transplantation, without technical complications, evidence of rejection or significant allograft pathology (Table 4). The clinical perspective, including the details of initial immunosuppression, which differed somewhat among the studies, has been expertly reviewed elsewhere [28]. Most trials weaned immunosuppression slowly over a period of months. Attempts at weaning earlier after transplantation were reported in recipients treated with lymphocyte-depleting antibodies at the time of transplantation [26].

It is difficult to contest the premise of weaning trials that less immunosuppression without rejection is desirable. But only the study of Sandborn *et al.* [29], who attempted to wean Cyclosporine, included contemporaneous matched controls maintained on conventional immunosuppression to determine whether the withdrawal from immunosuppression was indeed beneficial overall. One study from our center included comparison to a historic control group [30]. Other studies compared immunosuppressant-dependent (failed weaning) with immunosuppressant-free (successful weaning) recipients [31–33]. In general, no specific molecular mechanistic hypothesis was being tested in these weaning trials other than the one that microchimerism and long-term allograft acceptance under immunosuppression are conducive to immunosuppression-free allograft acceptance [34]. Therefore, the data collected differed somewhat among the studies. It would be more ideal, in conventionally treated recipients, to compare a 'weaning' group with a maintenance immunosuppressive therapy group and include both potentially positive and negative endpoints, such as incidence of acute and chronic rejection and development of graft fibrosis over a period of time, incidence and severity of immunosuppression-related complications (renal failure, diabetes, cardiovascular disease, malignancies) and cost of medications.

The data on majority of the SOT kidney allograft recipients have been derived from anecdotal reports based on individual patients that were either noncompliant or who had anti-rejection medication withdrawn because of immunosuppression-related complications (Table 2). Again, no specific hypothesis was being tested in these reports other than the possibility that immunosuppression weaning might be possible. In contrast, studies attempting to induce tolerance through macrochimerism were all conducted prospectively and tested the hypothesis that hematopoietic chimerism would lead to allograft tolerance in outbred humans (Table 3). The approaches included: (i) using a nonmyeloablative preparatory regimen and simultaneous MHC-matched [3–5] or -mismatched [12] bone marrow- and living-related kidney transplants; (ii) delayed renal transplantation after successful bone marrow transplantation from the same living-related donor using myeloablative therapy [6–10]; and (iii) MHC-mismatched renal transplantation after total lymphoid irradiation, lymphoid depletion, and donor hematopoietic stem cell infusion [35,36].

Table 2. Retrospective studies of 'spontaneous' kidney allograft acceptance/tolerance.

Study	Pts	IS time	IS free	Graft routine histopathology follow-up	IPEX and other allograft tissue studies
Owens *et al.* [54]	4/6	3–180 months	8–52 months	Acute rejection occurred in 2/6 patients 8–18 months after weaning from IS; no histopathology descriptions of rejecting or stable allografts	NA
Uehling *et al.* [55]	1/5	0.2–1	0.5–2.5	Two patients developed severe rejection after several months off IS, two returned to IS and one remained off IS; no histopathology description of any rejecting or stable allografts	Not done
Hussey [105]	1/8	NA	40 months	Most patients either developed severe rejection or returned to immunosuppression; no histopathology description of any rejecting or stable allografts	
Zoller *et al.* [56]	6/48	958 ± 792 days	394 ± 645 days	21/48 experienced graft failure (most within a few months) and only six continued to do well after IS cessation; no histopathology description of any rejecting or stable allografts	NA
Starzl *et al.* [64,66] and Randhawa *et al.** [63]	7	2–36 years	0.3–>12 years	Three patients subjected to Bx: one tolerant and two patients on minimal steroids showed mild patchy interstitial lymphocytic inflammation forming occasional small clusters, but without tubulitis or vascular injury. Mild arterial nephrosclerosis, focal glomerular lobular accentuation and global glomerulosclerosis involving up to 10% of glomeruli	In testable cases, lymphoid infiltrates were of recipient origin; endothelium and tubules were primarily of donor origin; one case showed recipient-type cells in the mesangium
Fischer *et al.* [106] associated with pregnancy	1	9 years	9 years	No pathology	NA
Burlingham *et al.* [68,69]	1	2 years	7–8 years	Pre weaning biopsy showed focal infiltrate and fibrosis, but no tissue damage; F/U biopsy 7 years subsequently showed focal infiltrates but no tubulitis or evidence of acute rejection. Biopsy at ca. 8 years showed acute rejection; details not given	Not done
Christensen *et al.* [107]	1	3 years	3 years	No pathology	NA
VanBuskirk *et al.* [108], Xu *et al.* [67]	2	>1.5 years	10.3 and 18 years	Biopsies obtained from two SOT patients after F/U of 10.3–18 years. Both showed lymphoid aggregates and scattered interstitial mononuclear cell infiltrates without tubulitis, allograft vasculopathy, interstitial fibrosis, or tubular atrophy	Numerous $CD4^+/TGF\text{-}\beta1^+/CD25^{+/-}/FoxP3^-$ in the interstitium; $TGF\text{-}\beta1\text{-}/FoxP3^+/CD25^+$ cells mainly in lymphoid aggregates
Roussey-Kesler *et al.* [53] and Brouard *et al.* [109]	10	1–13 years	1–20 years	Biopsies from two recipients: (i) after 13 years of SOT renal dysfunction prompted a biopsy that showed grade I CAN with mild nephroangiosclerosis without significant lymphoid infiltration or specific changes suggestive of chronic rejection (C4d-) and (ii) after 7 years of SOT renal dysfunction prompted dialysis and a biopsy that was negative for acute rejection, but showed focal fibro-edema associated with mild mononuclear infiltration, and double contours in GBM and moderate arteriolar hyalinosis (not shown) diagnosed as grade Ib CAN with allograft glomerulopathy (C4d-)	Not done
Newell *et al.* [110]	16	13 ± 10 years	>1 year	No pathology	NA

CAN, chronic allograft nephropathy; F/U, follow-up; GBM, glomerular basement membrane; IPEX, immunoperoxidase tissue staining; IS, immunosuppression.
*Two of the three patients were maintained on very low (7.5 and 10 mg, respectively) of prednisone daily.

Table 3. Prospective studies of 'induced' kidney allograft acceptance/tolerance.

Study	Pts	IS time	IS free	Allograft findings/histology, if available	IPEX and other allograft tissue studies
MHC-mismatched cadaveric renal Tx (Strober *et al.* [35]) or living-related kidney Tx (Scandling *et al.* [36]) after TLI, lymphoid depletion and donor hematopoietic cell infusion	4	ca. 3 years	2–5.8 years	Obstructed ureter prompted a Bx at 10 m after IS withdrawal showed normal glomeruli and blood vessels and an occasional focus/cluster of interstitial mononuclear cells. A subsequent episode of obstruction was followed by increased creatinine and a second biopsy showed diffuse mononuclear-cell infiltrate consistent with either chronic obstruction or rejection. Another patient had a 'normal' Bx 20 m after withdrawal of IS. Most recent patient [36] had normal function at 28 months, but no biopsy was reported	Not done
Simultaneous MHC matched (Spitzer *et al.* [3], Buhler *et al.* [4], Fudaba *et al.* [5]) and MHC mis-matched (Kawai *et al.* [12]) living-related renal and BMTx using non-myeloablative regimen	6 m + 4/5 mm	9–14 months	2–5.3 years	MHC matched donors: not reported in any detail MHC mismatched donors: one allograft lost to antibody-mediated rejection. One developed anti-donor HLA class II antibodies 2 months after complete IS withdrawal with C4d deposits in the allograft and segmental duplication of the GBM in some glomeruli. Biopsies from the three other grafts reported as normal and/or showing transient mononuclear cell infiltrates after IS withdrawal	MHC mismatched donors: intragraft levels of FoxP3 mRNA were about six times higher in the stable IS-free group than in the stable-with-IS group, whereas the granzyme B mRNA levels were similar. Therefore, the ratio of FoxP3:granzyme B might be important
Delayed living-related renal Tx after successful BMTx from the same donor: Sayegh *et al.** [6], Butcher *et al.** [7] Helg *et al.** [8], Jacobsen *et al.*† [9], Sorof *et al.*† [10]	8	0–2 years	0–2.5 years	No pathology	NA
alemtuzumab depletion without (Kirk *et al.* [37])or with deoxyspergualin (Kirk *et al.* [38]) in living donor Tx	7 + 5	0	18–32 days	Atypical rejection developed in all 12 patients in both studies characterized by macrophages-rich infiltrates before T-cell extravasation, which initially distended interstitial capillaries, but subsequently became diffuse involving most of the interstitial capillaries, the interstitium, and tubules during clinical during rejection	IPEX staining for CD3, CD4 CD8, CD20, CD45RO CD68, CD56, perforin, granzymeB, and HLA-DR showed macrophage-rich infiltrates during rejection with few $CD45RO^{+}$ cells, no increased NK cells, and upregulation of HLA-DR on tubules; increased transcripts associated with macrophage/monocyte function and chemokines. C4d staining was negative in the second study [38]

Table 3. continued

Study	Pts	IS time	IS free	Allograft findings/histology, if available	IPEX and other allograft tissue studies
Thymoglobulin or Campath pretreatment followed by Post-Tx tacrolimus monotherapy and spaced weaning (Shapiro *et al.* [27]) in cadaveric Tx	191	3–6 months	NA	45% of recipients developed rejection during weaning. Weaning was unsuccessful in about one-third of Thymoglobulin-treated recipients. Two-thirds were on reduced/spaced tacrolimus therapy after a follow-up of 24–39 months. Seventy-four per cent of the Campath-treated patients were on spaced weaning after 12–18 months of follow-up. Protocol biopsies were not performed, but patient and graft survival and the rate of CAN progression was similar to historic, conventionally treated controls	NA

BMTx, bone marrow transplant; GBM, glomerular basement membrane; IPEX, immunoperoxidase tissue staining; IS, immunosuppression; MHC, major histocompatibility complex; TLI, total lymphoid irradiation; Tx, transplant; m, MHC matched donors; mm, MHC mismatched donors.
Kidney transplants after MHC-identical* or parent to offspring (haploidentical)† BMT from the same donor.

Three other prospective kidney trials are included in Table 3. Two by Kirk *et al.* [37,38] used alemtuzumab leukocyte depletion both without [37] and with coexistent deoxyspergualin therapy [38], but without other immunosuppressants in related and unrelated living donors. Shapiro *et al.* [27] used thymoglobulin or alemtuzumab depletion plus tacrolimus monotherapy with fully mismatched cadaveric donors. These studies differed in the timing and dosage of alemtuzumab. Kirk *et al.* [37,38] did not use any baseline immunosuppression except deoxyspergualin in his second study [38], whereas Shapiro *et al.* [27] relied on tacrolimus monotherapy, which was weaned shortly after transplantation. They were also, however, testing the hypothesis that depletion of the recipient immune system would create favorable conditions for the development of tolerance [37,38] and donor leukocyte migration might positively contribute to this process [34] through the induction of chimerism.

Clinical and detailed histopathologic observations

This section will follow the observations during enrollment and follow-up of patients participating in immunosuppression minimization trials.

Pre weaning clinical profiles and biopsies

In most, but not all, prospective SOT liver and kidney allograft immunosuppression minimization trials, 'pre weaning' biopsies are obtained. The liver injury test parameters and serum creatinine are usually normal or near-normal, but minor abnormalities are not uncommon. The purposes of the biopsy are to: (i) exclude any histopathologic rejection-related activities or other findings, such as significant fibrosis, that might exclude the patient from the trial and (ii) document any other baseline inflammatory and/or structural changes present before withdrawal so that they can be compared with findings in subsequent biopsies. The rationale for these biopsy-based exclusions is as follows. Low-level subclinical rejection is likely to significantly worsen after weaning and any additional insult on an already structurally compromised allograft would likely lead to failure. In addition, any changes to allograft structure might represent a heretofore unrecognized manifestation of rejection, or a beneficial effect of immunosuppression withdrawal.

Most 'pre weaning' liver allograft biopsies are obtained several years after transplantation and show changes that are typical of protocol biopsies obtained at that time. These biopsies are often difficult to interpret and the subject of a recent Banff consensus document [39]. Nearly 75% of biopsies obtained from adult recipients surviving more than 1 year 'with abnormal liver tests' will show

Table 4. Design of immunosuppression withdrawal trials after liver transplantation and time until rejection, if it occurred.

Study	Inclusions/exclusion criteria	Time until rejection
Sandborn *et al.* [29]	Adult, cadaveric donors >12 months s/p Tx Normal liver biopsy within 3 months of weaning Serum creatinine >2.1 mg/dl or creatinine clearance <35 ml/min	6 months (1–21 months)
Ramos *et al.* [23]	Adult, cadaveric donors, >5 years post-transplant; >2 years without rejection History of medical compliance Immunosuppression related complications Primary physician cooperation Absence of rejection or severe necro-inflammatory disease on liver biopsy	6.5–22.5 m Average = 15 months
Mazariegos *et al.** [22]	Same as above	0.2–42 months
Devlin *et al.* [57] and Girlanda *et al.* [61]	Adult, cadaveric donors Side effect of immunosuppression	(range, 10–176 days) Three weeks after withdrawal of immunosuppression
Takatsuki *et al.* [52]	Pediatric, living-related donor ≥2 year post-Tx; nl. graft function; ≥1 year rejection-free Evidence of medical compliance Cooperative local physician for follow-up	Median = 9.5 months (1–63 months)
Pons *et al.* [58]	Adult cadaveric donors >2 years after Tx	NA
Tryphonopoulos *et al.* [102]	Adult, cadaveric donors Compared BM infusion group to controls to determine if infusion augmented chimerism and whether augmented chimerism increased tolerance >3 years post-Tx; stable liver function tests; rejection-free 12 months Recipients with autoimmune disorders excluded	17.8 (no BM)–23.9 (BM) months
Eason *et al.* [103]	Adult and pediatric, presumed cadaveric >6 months post-transplant without rejection Tacrolimus monotherapy with trough levels <5 ng/ml Normal liver function tests; no recurrent disease	NA
Tisone *et al.* [31]	Adult, cadaveric donors HCV RNA serum positivity 12 months after transplant; absence of advanced disease Biopsy proven HCV recurrence with normal graft function Treatment compliance	0.5–8 months, except for one patient at month 43
Koshiba *et al.*† [32]	Pediatric, living-related donor Pediatric patients; >2 years s/p Tx; >1 year rejection-free Normal liver function Parental permission	NA

*Overlaps with the population of Ramos *et al.* [23].
†Overlaps with the population of Takatsuki *et al.* [52].

histopathologically significant abnormalities [40–45], which are usually attributable to recurrent disease or biliary tract strictures [40–45]. The percentage is significantly less in pediatric recipients because recurrent disease is much less common. However, unexplained chronic hepatitis/inflammation is seen in a high percentage of pediatric recipients at some centers and this might represent a form of late rejection [46,47]. In addition, nearly 25% of biopsies from long-surviving 'asymptomatic adult recipients with normal liver tests' will show significant abnormalities if the original disease is one that commonly recurs, such as hepatitis C virus(HCV), steatohepatitis, primary biliary cirrhosis, or autoimmune hepatitis [40–45] and in up to 11% of recipients the pathology findings were judged to be of clinical significance [48].

Other minor histopathologic abnormalities occur in about two-thirds of long-term biopsies, even without recurrent disease, in asymptomatic recipients with normal or near-normal liver tests [40–45]. Common findings are portal venopathy and nodular regenerative hyperplasia; thickening and hyalinization of small hepatic artery branches [43,49], 'nonspecific' portal and lobular inflam-

mation [43–45,50], and Ito cell hyperplasia [48]. A higher percentage of split and liver donor allografts also show architectural changes compared to whole cadaveric organs (A. Demetris, unpublished observation). The pathogenesis, significance, long-term consequences, and impact of weaning on these otherwise unexplained, long-term histopathologic findings are in need of further study. It is important in drug minimization trials that changes associated with long-term engraftment are not confused with variants of rejection after weaning, which reinforces the need for pre weaning biopsies.

It is worth emphasizing that original disease recurrence is a significant problem in adult liver transplantation [39], accounting for about 50% of all episodes of allograft dysfunction occurring more than 1 year after transplantation. In contrast, biliary atresia is the indication for the vast majority of pediatric liver transplants. As this disease does not recur after transplantation, interpretation of the results of pediatric weaning studies, such as those from the Kyoto group [32,51,52], are less complicated from the perspective of recurrent disease.

In kidney allografts, interpretation of baseline pathology changes is generally much less complicated and the findings are usually attributable to age and hypertension, calcineurin toxicity, and/or diabetes-related changes, such as patchy interstitial fibrosis/tubular atrophy, mild mononuclear interstitial inflammation, and arterial and arteriolonephrosclerosis of varying severity. In contrast to liver allografts, recurrence of the original disease is much less common and rarely impacts the decision to continue with weaning. For weaning studies, however, theoretically the pre weaning biopsy should show no evidence of active tubulitis or other obvious rejection-related changes and negative C4d stains [26]. Weaning has been attempted, however, in recipients showing borderline changes when the serum creatinine levels were near-normal and/or stable [26]. It is very difficult, however, on the basis of routine light microscopy to absolutely and reliably distinguish between borderline changes and nonspecific inflammation associated with aging and arterial and arteriolonephrosclerosis. Some of these patients did not develop more severe rejection after weaning [26], but characterization of the infiltrates with formal long-term follow-up studies are needed to determine the impact of this decision.

Clinicopathologic observations during and shortly after weaning

Allograft biopsies were obtained in most weaning trials only when there was an elevation in liver injury test parameters for liver allografts or serum creatinine for kidney allografts. However, at the Liver Sessions 2007 Banff consensus meeting, which was devoted to late allograft dysfunction and weaning of immunosuppression, all participating hepatologists and surgeons agreed that protocol follow-up biopsies should be mandatory, or at least strongly encouraged, in such trials. Arguments raised by Roussey-Kesler *et al.* [53] not to conduct protocol biopsies in stable SOT kidney allograft recipients included the possibility that minimal histopathology findings might be misleading and result in an unjustified return to immunosuppression. In addition, the need to conduct serial biopsies to monitor possible progression of subtle findings carries a risk of morbidity. And weaned stable allograft recipients might not want to undergo biopsy evaluation. Counter-arguments to these reasonable points of concern are that the biopsy findings and interpretation should be viewed similar to any other laboratory test result and incorporated into the entire clinicopathologic profile. In addition, much can be learned from the biopsy material, particularly as there is evidence to suggest that in humans the allograft plays an important role in the maintenance of tolerance.

Elevation of the liver injury test parameters in liver allograft recipients or serum creatinine in kidney allograft recipients is not uncommon and usually occurs within the first several months during or after drug withdrawal [22,23,27,52,54–57]. In liver allograft recipients, however, elevated liver injury test parameters did not distinguish between those who developed acute rejection and those who did not [22,23,57]. This is because biopsies obtained for elevated liver injury test parameters showed a variety of changes, including recurrence and/or exacerbation of underlying chronic viral or autoimmune hepatitis, primary biliary cirrhosis, biliary tract complications, steatohepatitis, nodular regenerative hyperplasia, and nonspecific 'lobular reactive changes' (Table 1). This illustrates that immunosuppression also prevents immunologically mediated liver injury other than rejection. In addition, mildly elevated liver injury test parameters occasionally returned to normal without therapeutic intervention after a biopsy had largely excluded acute rejection as the cause of the dysfunction [22,23].

Persistent significant elevations of liver injury test parameters (3X baseline values), however, usually signal the development of a clinically relevant problem. Of the various enzyme measurements comprising the standard liver injury test profile, gamma-glutamyl transpeptidase (GGTP) elevations were felt to be the most specific and sensitive for rejection in two studies from two different studies/centers [23,52]. In contrast to its value in the early post-transplant period, total serum bilirubin was a relatively insensitive marker for acute rejection developing after weaning [22,23,52,57].

When acute rejection was identified as a cause of liver allograft dysfunction during or after weaning, in our experience, the histopathologic appearance of most, but not all cases, was typical of that reported for late onset acute rejection (reviewed in [39]). Most of these episodes were graded as mild, but occasional cases of moderate to severe symptoms were reported (Table 1). In several liver studies, however, even when biopsy analysis had excluded other causes of allograft dysfunction associated with a clinical diagnosis of acute rejection, the histopathologic findings were not always typical of those reported for acute rejection [22,23,57,58]. There are at least four possible reasons for this observation: (i) acute rejection occurring late after transplantation (>1 year) in liver allografts differs from that occurring earlier within the first several months of transplantation, even in conventionally immunosuppressed recipients (reviewed in [39]); (ii) the composition of both the allograft and the recipient immune system are different early versus late time points after transplantation; (iii) immunosuppressive regimens used before weaning, such as lymphocyte-depleting antibodies, can alter the histopathologic appearance of acute rejection after weaning; and (iv) understandable clinical anxiety about elevated liver injury test parameters occurring in conjunction with weaning might trigger therapeutic intervention before characteristic histopathologic changes have time to develop.

Portal and/or perivenular inflammation is almost invariably observed. The major histopathologic differences between acute liver allograft rejection occurring after weaning versus 'typical early acute rejection' include less inflammatory bile-duct damage and more interface and lobular necro-inflammatory activity in the former. These differences cause the biopsies obtained after weaning to resemble hepatitis, which in turn, results in some diagnostic difficulties for the pathologist [22,23,57,58]. Increased interface and disease activity is also seen after weaning in patients with an original disease of autoimmune hepatitis [57] and primary biliary cirrhosis [23], some of whom develop new onset autoimmune hepatitis. In addition, early and rapid weaning of immunosuppression in HCV^+ recipients treated with lymphocyte-depleting antibodies can 're-arm' the immune system [30]. This manifests histopathologically as an aggressive hepatitis with rapid progression of fibrosis [30]. The important message from the above observations is that rejection is not the only cause of allograft dysfunction that occurs after weaning (Table 1).

No standardized reliability studies have been conducted on biopsy samples obtained in the setting of immunosuppression weaning to determine if pathologists agree in their interpretation. This is because of the rarity of such samples and the anxiety associated with clinical decision-making process. Such studies, however, are important and will be needed, particularly to define changes that might signal a need to return to immunosuppression versus ones that are probably benign and nonprogressive. In the meanwhile, in the first author's experience, reliance on standardized criteria is suggested [39].

Acute and chronic kidney allograft rejection occurring during or after weaning, in our experience, has not differed significantly enough from that usually seen in conventionally treated immunosuppression patients to cause diagnostic difficulties for the histopathologist. Development of anti-donor antibodies in the peripheral circulation and C4d deposits in the kidney, however, usually signals a need for returning to immunosuppression if the deposits are accompanied by histopathological evidence of significant tissue injury. Recurrence of the original disease can also be the primary cause of allograft kidney dysfunction after weaning, but the incidence is much less common than in liver allografts (unpublished observation).

The leukocyte-depleting alemtuzumab studies of Kirk *et al.* [37,38] nicely illustrate how the treatment strategy can influence the histopathologic findings. In the first study, no other baseline immunosuppression was used [37]. The histopathologic findings during clinical rejection that subsequently developed in all recipients, several weeks after transplantation, were not typical of early acute cellular rejection in conventionally treated renal allograft recipients. Instead, chemokine and macrophage function-rich transcripts were detected in needle biopsies early after transplantation, accompanied by margination of macrophages in interstitial capillaries on day 14. This occurred before the onset of significant T-cell infiltration and clinically evident rejection. Macrophage infiltrates became more diffuse at the onset of clinical dysfunction involving most of the interstitium, capillaries, and tubules. Small numbers of $CD45RO^+$ (memory) T cells were limited to the areas of macrophage infiltration, and tubulitis, when seen, was macrophage-predominant. Treatment with corticosteroids, OKT3, and sirolimus monotherapy reversed these episodes. A follow-up study added deoxyspergualin to the regimen in an attempt to inhibit macrophage function [38]. However, neither the clinical results nor the histopathologic features of rejection were significantly different from the study using alemtuzumab alone [37] and C4d stains to monitor for antibody-mediated rejection were negative in the second study [38].

A general consensus in most kidney- and liver-weaning studies is that biopsy monitoring to determine the cause of dysfunction after weaning is an absolute necessity. Close clinicopathologic correlation, however, is even more important. In our experience, unbalanced emphasis on either the histopathologic findings or clinical profile can

adversely impact either the scientific validity of the study and/or patient safety. The usual situation is that the pathologist is the worrier, whereas the clinicians are more reassured by stable liver injury test parameters or creatinine, although the reverse can also occur. As the truth is often somewhere in between the two viewpoints, one should override the other only when findings are obvious, or there is evidence of a clear trend over a period of time. Long-term follow-up often provides a clear indication of whether the chosen approach was correct or not.

Correlation between preweaning biopsy findings and outcome

Baseline biopsy findings in some liver studies proved to be associated with successful weaning when compared with biopsies from unsuccessfully weaned patients. Significant variables included: (i) less portal inflammation, overall; (ii) less $CD3^+$ and $CD8^+$ but more $CD45RO^+$ lymphocytes within the lobules [59]; (iii) more advanced portal fibrosis in HCV^+ recipients [31]; and (iv) an increase of potentially regulatory $FoxP3^+$ T cells within the allografts of pediatric recipients [32,60]. Unsuccessful weaning, conversely, was associated with significantly more chronic portal inflammation on hematoxylin and eosin (H&E) stains and decreased $CD45RO^+$ as well as increased $CD8^+$ lymphocytes in the lobules [59]. These observations suggested that chronic portal inflammation and lobular $CD8^+$ cells might represent a latent form of rejection held in check by medications, which manifests itself clinically after the removal of immunosuppression.

A worry is that seemingly 'tolerant' patients might actually be experiencing low-grade chronic rejection. For example, in one liver series, five patients were categorized initially as showing SOT [57]. During longer follow-up, however, one recipient developed acute rejection requiring reinstitution of immunosuppression, another required retransplantation for chronic rejection, and a third resumed immunosuppression because of a kidney transplant [61]. We appear to have observed similar occurrences in liver allograft recipients, but more characterization of tissue samples is needed (A. Demetris, unpublished observations). Similar findings have been reported in SOT kidney allograft recipients, so it is prudent to continue to closely follow seemingly tolerant patients.

Studies examining associations between pre weaning kidney biopsy findings and postweaning outcome have not been reported.

Clinicopathologic observations in stable SOT recipients

Many centers do not sample the allografts of SOT recipients if they are 'clinically stable'. Thus the number of tissue samples from SOT liver and kidney allografts who remain off of immunosuppression after the biopsy, is considerably smaller than the total number of SOT recipients. Instead, biopsies are obtained only when indicated by elevated liver injury test parameters or serum creatinine. Consequently, the number of reported protocol tissue samplings in stable SOT recipients, whom remain immunosuppression-free after biopsy, is exceeding small. In total, 'more or less' protocol biopsies were obtained from eight SOT kidney recipients and from six chimeric-bone marrow plus kidney recipients and reported in the literature (Tables 2 and 3). The total number of liver allograft biopsies from SOT is more difficult to tabulate because different reports frequently contain overlapping patient populations (Table 1). The number appears to be between 100 and 200. This is somewhat disappointing because protocol biopsies from SOT patients can provide clinically and scientifically useful information.

There have been three studies, two liver- [14,58,62] and one kidney transplant [63,64] that have characterized the donor/recipient phenotype of cells infiltrating and comprising SOT allografts. In liver allografts, the vast majority of hepatocytes, bile ducts cells, and large vessel endothelia remain of donor origin, as do the tubular epithelial cells and endothelial cells of kidney allografts [63,64]. A majority of infiltrating leukocytes, however, were of recipient origin [14,58,62–64]. But donor hematopoietic cells can also be detected amidst the interstitial inflammation in some nearly SOT kidney allografts on low-dose immunosuppression [26]. The significance of persistent donor hematopoietic cells within the allograft and whether it predicts subsequent acceptance has not been studied in any detail. In SOT liver allografts, some replacement of sinusoidal lining cells can be seen. But it is difficult to distinguish between Kupffer's cells and endothelial cells and the level of sinusoidal cell replacement did not correlate with the ability to wean immunosuppression [58].

No long-term follow-up biopsies were conducted in the SOT kidney allografts, and because of the small numbers, it is difficult to draw any conclusions regarding any association with weaning. At this time, however, the evidence suggests that recipient replacement of donor epithelial or endothelial cells within the allograft is not a substantial mechanistic contributor to the development of SOT. Whether persistence of donor hematopoietic cells, including dendritic cells (DC) in the interstitium of allografts is associated with acceptance, as in experimental animals [65] is being actively investigated in our SOT tissue samples.

The Kyoto group conducted protocol biopsies in 14 pediatric living-donor liver allograft recipients who had been weaned from all immunosuppression. These biopsies

were compared with biopsies from control liver allograft recipients maintained on chronic immunosuppression [51]. There was more extensive portal fibrosis, ductular reactions, more $CD8^+$ cells and decreased luminal diameter of bile ducts in SOT immunosuppression-free recipients [32,51]. The authors worried that the changes observed in the SOT recipients might represent a subtle, heretofore unrecognized, variant of chronic rejection [32,51]. Some of their concern is warranted because of the significant fibrosis and increased $CD8^+$ lymphocytes is similar to that reported by Wong *et al.* [59], above. The mean follow-up in the Kyoto SOT group, however, was several years longer than that in their control group. This raises some questions about the etiology of these changes, which are not entirely typical of either early or late, acute or indolent chronic rejection. As the influence of longer term engraftment, regardless of immunosuppression, needs to be considered, more follow-up and detailed characterization of the changes are needed in this cohort.

Tisone *et al.* [31] studied the effect of immunosuppression weaning in HCV^+ recipients and conducted protocol biopsies at 1 month after completion of weaning and yearly thereafter. Interestingly, successfully weaned patients initially showed more advanced fibrosis in baseline biopsies than immunosuppression-dependent HCV^+ recipients. After weaning, fibrosis failed to progress significantly, or actually regressed, in patients removed from immunosuppression [31]. In contrast, the immunosuppression-dependent HCV^+ recipients showed fibrosis progression typical of conventionally treated HCV^+ recipients. Thus, complete weaning of immunosuppression showed a beneficial effect on HCV-induced fibrosis progression in one patient subset [31]. They also mentioned that focal ductopenia, a histopathologic finding of concern for early chronic rejection, was occasionally observed in protocol biopsies from the SOT patients. It was, however, always limited to less than 20% of the portal tracts, which is of uncertain significance. Once again, however, longer follow-up is needed in this cohort to make sure that early chronic rejection does not occur. But it is reassuring that this group did not show significantly elevated GGTP levels (a biochemical marker of ductopenia) as compared with the immunosuppression-dependent controls [31].

A common finding reported in SOT kidney allografts is that of patchy interstitial inflammation that is often arranged into small nodular aggregates [63,64,66,67] (Tables 2 and 3). Some biopsies have been reported as normal. Other findings include mild arterial nephrosclerosis, focal global glomerulosclerosis, grade 1 chronic allograft nephropathy with mild 'nephroangiosclerosis', moderate arteriolar hyalinosis and double contours of the glomerular basement membrane indicative of transplant glomerulopathy (Table 2). Most of these findings, however, are largely nonspecific from a light microscopic perspective and are commonly encountered in aged and/or hypertensive or diabetic kidneys and those with calcineurin toxicity. A possible exception is some of the transplant glomerulopathic changes, which might signal a form of antibody-mediated injury.

Xu *et al.* [67] characterized the patchy tubulointerstitial lymphocytic infiltrates in two SOT kidney allografts after 10.3 and 18 immunosuppression-free years. They found the interstitial infiltrates to be enriched with $CD4^+$/transforming growth factor (TGF)-$\beta1^+$/$CD25^{\pm}$/$FoxP3^-$ adaptive regulatory T cell (T_{reg}) and lymphoid aggregates enriched with TGF-$\beta1$-/$FoxP3^+$/$CD25^+$ natural T_{reg}. Several years earlier, Burlingham *et al.* [68] reported a SOT kidney allograft recipient that showed similar findings (i.e. patchy interstitial infiltrates without damage) in a pre weaning biopsy. The patient remained stable during follow-up and a biopsy after 7 immunosuppression-free years was unchanged. The serum creatinine, however, gradually increased from 1.6 to 1.8 to 2.0 mg/dl and the patient eventually developed biopsy-confirmed acute rejection 9.7 years after transplantation [68,69].

Roussey-Kesler *et al.* [53] reported 10 SOT kidney allograft recipients after 9.4 ± 5.2 immunosuppression-free years. Most of these patients had interrupted weaning of immunosuppression over a long period of time and donor age was younger than donors used in the general transplant population. One patient, after 13 years of SOT, developed renal dysfunction. A biopsy showed grade I chronic allograft nephropathy with mild nephroangiosclerosis without significant lymphoid infiltration or specific changes suggestive of chronic rejection. C4d staining was negative and no anti-HLA antibodies were detected in the circulation. Renal function also deteriorated progressively in another patient, requiring dialysis. An allograft biopsy in this patient performed after 7 immunosuppression-free years, showed grade Ib chronic allograft nephropathy with allograft glomerulopathy, but without C4d staining.

The most impressive and carefully documented series of biopsies from tolerant kidney allograft recipients were reported by Kawai *et al.* [12]. They induced tolerance using combined bone marrow and kidney transplants from MHC single-haplotype mismatched living-related donors with a nonmyeloablative preparative regimen. Of the five patients enrolled in that trial, one allograft was lost to antibody-mediated rejection. One other developed anti-donor HLA class II antibodies 2 months after complete immunosuppression withdrawal. Biopsies from this patient showed C4d deposits and segmental duplication of the glomerular basement membrane in some glomeruli. The patient was not returned to immunosuppression because of uncertainty about the significance of the

relatively minor changes that did not worsen over a period of time. Protocol biopsies from the three other grafts obtained from between 666 and 1135 days after transplantation and from about 400 to 1000 days after withdrawal of all immunosuppression were reported as normal and/or showing transient mononuclear cell infiltrates; C4d stains were negative. Intra-graft levels of FoxP3 mRNA were about six times higher in the stable immunosuppression-free group than in the stable-with-immunosuppression group, whereas the granzyme B mRNA levels were similar. Therefore, the ratio of FoxP3:granzyme B might be an important marker of a favorable T_{reg}–$T_{effector}$ ratio and allograft acceptance.

Lessons learned and common characteristics of spontaneously/operationally tolerant allografts

Common clinical characteristics of successful weaning that emerge from the review of SOT liver- and kidney allografts include living-related allografts, immunologically stable/noninflamed allografts, and long survival, in situ, under conventional immunosuppression with gradual weaning of immunosuppression over months to years (Tables 1–3). Conversely, early and abrupt weaning of immunosuppression, nonrelated cadaveric allografts, or previously inflamed allografts are more likely to experience rejection after weaning. The 'take home' messages reported in the liver trials are shown in Table 5. These observations/lessons are beginning to point toward immunologic processes associated with graft acceptance, and eventually, these will translate into molecular pathways. But currently, the field is in its infancy.

Problems with early abrupt weaning and the advantage of relatively long allograft residence under immunosuppression and slow weaning are all probably related to the immunologic interface between the donor and recipient. Early after transplantation, in conventionally treated recipients, this interface is an activated and contentious one because: (i) the massive migration of donor hematolymphoid cells and cellular debris (danger-associated molecules) from the allograft floods the recipient lymphoid tissues [70,71] and (ii) tissue damage from preservation-related injury [72] fosters recipient leukocyte migration and retention within the allograft. The migration of donor leukocytes and debris, particularly from liver allografts, floods recipient lymphoid tissues with innate activation signals that can have both positive and negative effects, such as activation and partial deletion of donor-reactive lymphocytes and/or development of allospecific memory cells [14,34,73,74]. This probably explains why more immunosuppression is needed to prevent rejection early after transplantation and why it is more difficult to wean immunosuppression at this time [2,14].

The immunologic barrier is overcome or subverted, however, while using the combined bone marrow or hematopoietic stem cell and kidney transplant approach [12]. Part of the early success in this pioneering trial is likely related to the relatively harsh conditioning regimen; but it is also nonmyeloablative, and weaning from immunosuppression has been rapid and deliberate. As compared with other trials using more conventional immunosuppression, this approach also shows a higher overall rate of success, but currently it can be applied to only a limited subset of patients. Nevertheless, the high rate of success, convincing demonstration of donor-specific nonreactivity, and 'cleanliness' of the allografts [12], in our opinion, suggest that deletion has occurred in these patients, at least early after transplantation. And deletion results in more robust tolerance. As macrochimerism was only observed transiently in these patients [12], it will be interesting to determine whether the deletion, donor-specific nonreactivity and allograft cleanliness persist long-term.

Preservation injury eventually heals, donor passenger leukocyte migration diminishes, and most, but not all, hematolymphoid cells within the allograft are eventually replaced by recipient ones. And the recipient immune system is no longer the same as is was before transplantation. In SOT, however, the allograft also contributes significantly to acceptance because the organ (liver versus kidney) and prolonged exposure under treatment enhances the ability to ultimately wean immunosuppression. However, the role of the allograft in SOT is not well understood and is evolving. Speculations include: (i) provision of a stromal niche for donor hematopoietic stem cells [75] and maintenance of microchimerism [65]; (ii) provision of donor antigen needed to stimulate adaptive T_{reg} cells, which causes them to locate there [67,76]; (iii) a unique micro-environment in the case of the liver variably dampens a number of different immune responses [18,19,77]; (iv) a sink for alloreactive cells slowly mediating chronic rejection; or (v) some combination of the above.

Other nonrejection-related insults, such as recurrence of the original disease and technical complications associated with inflammation, can either sustain or re-activate the contentious allograft/recipient immunologic interface. This, in turn, can predispose to rejection, even in seemingly SOT allografts. Examples include diminished ability to wean immunosuppression in patients with autoimmune hepatitis or primary biliary cirrhosis in liver allografts [22,23] and triggering of apparent rejection after an episode of obstructive uropathy [35] (Table 3). Also, HCV-negative liver allografts that are inflamed at the time of weaning are more prone to rejection. This is probably related to the alterations of leukocyte trafficking

Table 5. 'Take home' messages of the liver immunosuppression minimization trials.

Study	Take home messages
Starzl *et al.* [14]	Micro-chimerism is frequently observed in long-term liver allograft survivors Not all recipients require long-term maintenance immunosuppression 'Tolerant' recipients/accepted allografts and show inflammation/hepatitis, not attributable to rejection
Sandborn *et al.* [29]	Renal toxicity of cyclosporine is improved High percentage (ca. 50%) developed acute rejection; 2/12 (17%) developed chronic rejection
Ramos *et al.* [23]	Enzyme elevations typically occurred about 150 days into the weaning process, but not all associated with rejection Close monitoring needed; liver injury test parameters not adequate monitor, but weaning is safe: no allografts failures or permanent damage Weaning should not be attempted until 5–10 years after transplantation; micro-chimerism not necessarily associated with acceptance Acute/chronic rejection had typical presentation; sometimes preceded by 'nonspecific lobular changes'
Mazariegos *et al.* [22]	Close physician surveillance during weaning with frequent assessment of liver function; weaning should not be abrupt/quick LFTs not a good discriminator of rejectors versus tolerant; but patient should be biopsied and returned to immunosuppression, if needed Cyclosporine-treated recipients more resistant to weaning than those treated with tacrolimus or azathioprine
Devlin *et al.* [57], Wong *et al.* [59], and Girlanda *et al.* [61]	Close physician surveillance as required; transient rise in liver injury test parameters not always indicative of rejection – can spontaneously resolve Acute rejection that develops does not always show histopathologic features of 'classic' acute cellular rejection Microchimerism not statistically associated with graft acceptance Successful drug withdrawal correlated with nonimmune mediated liver diseases, HLA matching, low incidence of early rejection Ability to wean associated with less portal inflammation, less $CD8^+$ lymphocytes and more lobular $CD45RO^+$ lymphocytes
Takatsuki *et al.* [52]	Weaning can be attempted in a majority of recipients; successful in up 38.1% of living-related donor liver recipients Liver injury test parameters were not significantly different in the rejection versus weaned groups Mechanisms of graft acceptance unclear
Pons *et al.* [58]	'Tolerance'/graft acceptance observed in 33% of recipients Sinusoidal endothelial cell chimerism was frequent, but not necessary for graft acceptance Portal inflammation without endothelialitis or bile duct damage might represent either 'latent' rejection or 'immunologic activation' associated with graft acceptance
Tryphonopoulos *et al.* [102]	Bone marrow infusion increases the level of microchimerism, but does not significantly increase the percentage of patients that can be weaned from immunosuppression About 20% of stable liver allograft recipients can be weaned from all immunosuppression
Eason *et al.* [103]	Clinical 'tolerance'/graft acceptance can be achieved in a minority of recipients Weaning from immunosuppression can be risky
Tisone *et al.* [31] and Martinez-Llordella *et al.* [33]	Univariant analysis: longer F/U after Tx, treatment with ribavirin, less steroids, more advanced architectural distortion/fibrosis on entry biopsy, and lower first week cyclosporin blood levels associated with ability to wean. Multivariate analysis: low cyclosporine trough levels during those first post-transplant week and initial steroid free immunosuppression independently associated with ability to wean 'Tolerance'/graft acceptance associated with lower fibrosis progression/regression after weaning Differential expression of genes in circulating blood mononuclear cells associated with: (i) IL-2 signaling; (ii) pro-inflammatory, oxidative stress, apoptosis, etc. associated with HCV; (iii) upregulation of $V\delta1\gamma\delta$, NK receptors and TGF-β signaling; and (iv) increased percentage of $FoxP3^+$, increased $V\delta1\gamma\delta$: $V\delta2\gamma\delta$ ratio $CD4^+$/$CD25^+$/$CD62L^{high}$
Koshiba *et al.* [32], Yoshitomi *et al.* [51], and Li *et al.* [60]	Recipients of living-related donors can be successfully weaned more frequently than mismatched cadaveric allografts Baseline biopsies show increased infiltration by $CD4^+$/$CD25^{high}$ and peripheral blood shows increased ratio of $V\delta1$/$V\delta2$ ratio as compared with normal individuals Graft acceptance resembles successful pregnancy in that $V\delta1\gamma\delta$ T cells express very high IL-10 levels Tolerant grafts showed more portal fibrosis, ductular reactions, and decreased luminal diameter of bile ducts as compared with those maintained on immunosuppression; might be a variant of late onset rejection

through the organ, which diminishes immunologic ignorance.

It is not surprising that recipients of living-related allografts are more easily weaned from immunosuppression than nonrelated cadaveric organs. They are usually better MHC-matched than cadaveric organs and generally experience less severe ischemic/preservation-related injury. And if the donor is the mother, oral exposure to maternal antigens through breast feeding might positively contribute to tolerance induction. Clearly, more work is needed in studying the relationship between innate and adaptive immunity in triggering rejection in stable SOT allografts.

Several studies showed the presence of mononuclear infiltrates in SOT kidney and liver allografts (Tables 1–3). Many completely normal nonallograft kidney and livers show similar findings. But most transplant pathologists intuitively react with some level of concern because inflammation is so frequently associated with tissue damage and formation of aggregates and/or germinal centers in tissues is a time-tested marker of chronic inflammation. Yet Xu and Burlingham [67] have reported, in humans, how these infiltrates might represent a 'protective' response in the allograft. Their observation of a T_{reg}-rich infiltrate supports the hypothesis that peripheral allograft tolerance involves T_{reg}-dominance in the T_{reg}–$T_{effector}$ ratio homeostasis, as in experimental animals [76,78,79]. Their observation is also consistent with the finding that T_{reg} localize in allograft tissue and at sites of inflammation [76]. A higher T_{reg}–$T_{effector}$ ratio was also observed in tolerant kidney allografts studied by Kawai *et al.* [12] and increased T_{reg} were noted in the liver allografts of tolerant pediatric recipients, but a T_{reg}–$T_{effector}$ ratio was not reported [32].

It should also be noted, however, that nodular lymphoid aggregates have also been used to distinguish chronically rejecting organs from seemingly tolerant ones in experimental animal studies [65,80]. But perhaps the quantity, composition or function of the lymphocytes/nodules differ between tolerance and chronic rejection, as in the peripheral circulation [81,82]. Or perhaps the two processes, tolerance and chronic rejection, are closely related and differ only in the severity and pace of the response in relationship to the lifespan of the recipient: A 65-year-old liver allograft recipient that is slowly developing chronic rejection over a period of 20 years might be better off considered tolerant rather than returned to maintenance immunosuppression. Regardless, better characterization and comparison to similar infiltrates in normal nontransplant tissues and stable allograft recipients on immunosuppression is needed. These seemingly benign infiltrates in tolerated organs appear to be related to the well-recognized affinity of adaptive T_{reg} for allograft tissue and sites of inflammation [76,78,79]. But as TGF-β secretion plays an important role in their function [76] it will be important to determine whether regulation itself might produce pathology/fibrosis. T lymphocytes showing a regulatory phenotype, and producing significant TGF-β, were recently shown to be associated with IgG4-cholangiopathy, a fibrosing condition of bile ducts [83] that can affect other organs.

Another common characteristic of SOT in liver- and kidney allografts is that it appears to be meta-stable and to evolve over a period of time. Seemingly minor perturbations can trigger clinically significant acute rejection episodes, even in patients who have been off all suppression for many years. At least one study, however, suggests that the instability decreases with time [84]. In addition, it is not entirely surprising that some apparently well-tolerated human allografts show features of chronic rejection after longer follow-up. This occurred in several renal allografts and at least one liver allograft recipient (Tables 1–3). And as we already know that liver injury test parameters and serum creatinine are not sensitive markers of tissue injury, some method of follow-up by protocol will benefit patient management and contribute to an understanding of mechanisms associated with allograft acceptance. The first author would certainly advocate for protocol biopsies, even in stable SOT patients, at least until we understand the process better.

Roles of the pathologist, features of interest within tolerated allografts, and sampling/testing recommendations

The pathologist will be asked to play two roles in this emerging field of immunosuppression minimization. The first, and most important, will be a clinical one in monitoring allograft acceptance and 'helping in decision-making, but not unilaterally deciding,' as to whether a particular recipient needs to be returned to immunosuppression or not. To successfully play this role, the pathologist has to be able to distinguish all of variants of antibody- and cell-mediated rejection that might require a return to immunosuppression from changes associated with long-term engraftment, recurrent disease, and technical complication where immunosuppression might not be indicated. Furthermore, there are likely to be findings of uncertain significance and these will require follow-up over a period of time. As with any new pathology endeavor, limiting biopsy analysis and interpretation to one or a small group of pathologists with a specific interest in immunosuppression minimization will decrease observer variation.

Thus, at a minimum, samples that should be obtained in any weaning study include: (i) indicated biopsies to

determine the cause of any allograft dysfunction before weaning; (ii) protocol biopsies immediately before weaning in stable recipients; (iii) indicated biopsies in recipients who develop any significant evidence of graft dysfunction after weaning; and (iv) protocol biopsies in stable recipients after weaning. The schedule for, and even whether to obtain, protocol biopsies in stable recipients off all immunosuppression is controversial. But at least one sample after 1, 3, and 5 immunosuppression-free years is reasonable, in the first author's opinion. But defensible arguments can be made for more or less frequent sampling. It is ideal to also have available donor and/or postreperfusion biopsies to determine whether early events, such as significant donor disease or preservation/reperfusion injury affect the ability to wean subsequently.

Routine light and histochemical microscopic examination, appropriate to the organ, is mandatory because it provides a plethora of information and because it is fast and inexpensive and based on abundant empirical data. We attempt to preserve as much tissue as possible for future experimental studies and routinely obtain H&E stains alone in liver allografts and H&E, Methenamine–silver–trichrome (MST) combination, PAS, and C4d stains in kidney allografts. Fibrosis can be reliably assessed by polarization microscopy. Beyond these tests, more sophisticated (experimental) analyses must balance the various limitations such as: sample size, potential yields of testing modalities; and research interests of the investigator and the field.

Beyond basic general diagnostic considerations, major histopathologic features of focus should include the overall tissue architecture, severity and composition of inflammatory infiltrates, development and/or progression of fibrosis and parenchymal cell atrophy and obliterative arteriopathy. The latter features are more easily followed in kidney than in liver allografts and are important, albeit not entirely specific, histopathologic markers of chronic allograft rejection. Routine tissue monitoring for C4d deposition in conjunction with circulating anti-donor antibodies is an absolute necessity in kidney allografts. In liver allografts, C4d deposits are infrequent and their clinical significance is much less certain unless there is sinusoidal deposition, which is rare. Most centers do not routinely obtain C4d stains for liver allograft recipients, but it is probably prudent to do so for any cause of unexplained allograft dysfunction or when anti-donor antibodies are detected in the circulation.

Any noticeable progression of routine histopathologic findings over a period of time, such as interstitial fibrosis and parenchymal cell atrophy, especially if it is accompanied by laboratory-validated deterioration of function, should prompt a thorough re-evaluation of the immunosuppression management policy. This recommendation includes caveats of intra- and inter-observer variation, sampling issues, and whether the rate of deterioration is relevant to the clinical setting. For example, a minimal or very slow progression of allograft fibrosis over a period of time without immunosuppression might be the result of sampling issues or be an acceptable alternative for a diabetic-prone elderly allograft recipient.

The second scientific role of the pathologist complements and extends the clinical one. Immunostaining, *in situ* hybridization, and various nucleic acid and protein expression arrays can be used to gain a functional understanding of the routine histopathology findings. Specific areas of interest would include evidence of injury and a response to injury in endothelial and parenchymal cells and the phenotype and activation/maturational status of various leukocyte populations, including organ-resident DC and various T-cell subsets. But assay selection should be balanced by considerations of tissue availability and potential significance and impact of any result(s). Recent development of multiplex staining in tissue sections has helped to conserve tissue by staining for multiple antigens in the same section (Fig. 1).

For example, livers and kidney (and allografts) usually show a relatively low rate of cellular stress and regeneration, as determined by immunohistochemical staining, and deviations from controls/normal might be a reason for concern. But any experimental result should not significantly influence the clinical decision-making process,

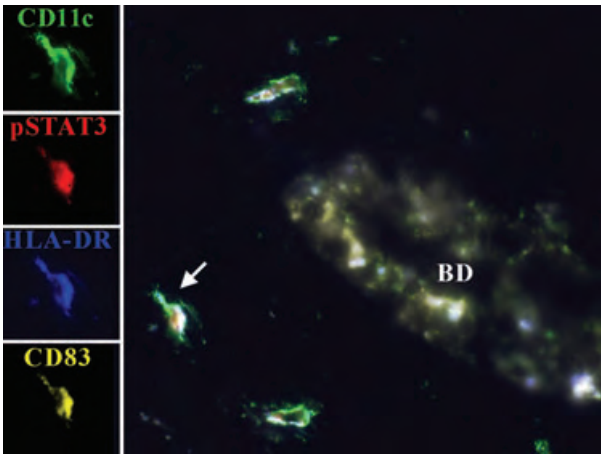

Figure 1 Nanoparticle colloidal semiconductor quantum dot (Qdots)-labeled monoclonal antibodies can be used for multiplex staining of tissue sections to monitor dendritic cell maturation status using markers such as CD11c (green; mDC), CD83 (yellow), HLA-DR (blue) and phosphorylated STAT3 (red; pSTAT3). This is an example from a 'tolerant' human liver allograft recipient. The individual colors of the cell highlighted by the arrow are shown in the left panels. Protein expression from Qdot immunofluorescence images is also quantifiable using NIH ImageJ analysis software. BD, bile duct.

unless scientifically validated. Evidence of injury and response to injury in endothelial and parenchymal cells might be monitored using markers of caspase activation, apoptosis, proliferation, and senescence-related changes, such as Ki67, PCNA, TUNEL, caspase 3, p16, p21, heme oxygenase-1, and increased expression of DNA repair enzymes. Beyond C4d deposits, one might look for immunohistochemical evidence of subtle endothelial injury. This might manifest as upregulation of anti-apoptotic molecules bcl-2, bcl-xl, or stress-induced hemoxygenase-1 HO-1 [85–89], CD46 [90,91], the complement regulatory proteins CD55 [92] and CD59 [93,94]; or pAKT [85,95,96] and Phospho-S6 Ribosomal Protein (Ser235/236) [86,97] or reduced expression of ICAM-1 and VCAM-1 [85–89], complement component 3 receptor-alpha [91,98], and complement component 5a receptor [91,98].

Our group is particularly interested in the donor versus recipient origin and activation/maturational status of organ-resident DC as they occupy an important niche within the immune system as monitors of the environment and translators of innate-into-adaptive immunity (Figs 1 and 2). In the first author's experience, well-tolerated allografts almost invariably contain residual donor DC [65]. And DC are especially good at triggering rejection [99] and tolerogenic pathways [100]. At a very basic level, however, we do not know whether the composition and maturational/activation status of interstitial leukocytes and DC in tolerated allografts resembles normal organs. And this will likely provide information about the mechanisms of allograft acceptance. Considering previous studies on the importance of naïve and memory T cells [59] and $\gamma\delta$-T cells [32,33] the composition of resident allograft leukocytes will certainly be of interest, as will expression of immunomodulatory cytokines such as TGF-β and interleukin (IL)-10.

The position of the liver within the body, immediately downstream of the intestines, also appears to be an important contributor to the tolerogenic properties [19] of the liver and might help explain why it is the liver allograft recipients who are able to be more easily withdrawn from immunosuppression and display SOT [77]. Our group has been interested in hepatic STAT3 activity (pSTAT3) [77], which is higher in the liver than in other commonly transplanted organs. Bacteria and bacterial products normally leak through the intestines into the portal venous blood and this stimulates Kupffer's cells to produce IL-6, which in turn, upregulates hepatic STAT3 activity. Activated or phosphorylated STAT3 inhibits hepatic myeloid and plasmacytoid dendritic cell maturation [77]. The critical role of IL-6 is illustrated in normal IL-6-deficient mice livers, which harbor DC that are significantly more mature than DC in normal wild-type mice livers. Depletion of gut commensal bacteria in the wild-type decreased hepatic pSTAT3 levels and caused hepatic dendritic cell maturation [77]. Activated STAT3 has also recently been recognized as a key modulator of tumor immunity [101] being involved in several aspects of tumor immunology including inhibition of DC maturation and expansion of T_{reg} within neoplasms (reviewed in [101]). Thus, the normal physiologic state of the liver might resemble a tumor microenvironment [77,101] and in turn, this might enable recipients to be weaned from immunosuppression without triggering a rejection reac-

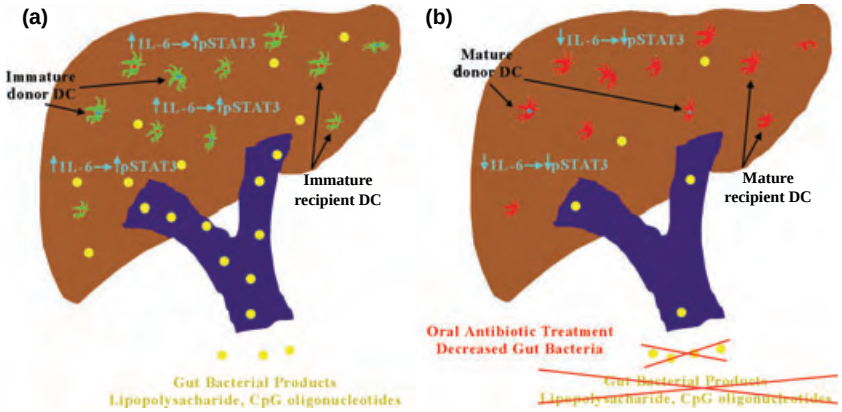

Figure 2 A model whereby intrahepatic IL-6-mediated activation of STAT3 (pSTAT3) stimulated by gut-derived bacterial products in the portal blood inhibits liver DC maturation. (a) In an allograft with gut-bacterial product stimulated IL-6/STAT3 activity, both passenger donor DC (green cells with blue nuclei) and infiltrating recipient DC (green cells with red nuclei) are immature with subsequent reduced migration to secondary lymphoid tissues. This might help explain the persistence of donor DC within tolerant allografts. (b) When hepatic IL-6/STAT3 activity is reduced, by decreasing gut bacteria with oral antibiotics, for example, the DC maturational threshold is reduced and both donor and recipient liver DC are more mature (red cells). These mature DC have increased migration from the organ and are more potent stimulators of allo-reactivity. Subsequently, the liver DC compartment would be repopulated with recipient DC.

tion. Clearly, molecular mechanisms beyond STAT3 are likely involved in the complex process of liver allograft acceptance, but pre-existing mechanisms to prevent an over-reaction to gut-derived antigens likely contribute significantly to the process.

Tolerance in humans induced via hematopoietic macrochimerism, even if transient, appears to be deletional and robust, at least early after transplantation [12], but might evolve towards relatively less stable regulatory pathways subsequently after transplantation [12]. SOT in conventionally treated allograft recipients can be studied in more patients and appears to rely less on deletion and more on active regulation. Therefore, study of the regulatory characteristics of lymphocytes within SOT allografts has, and will continue, to gain popularity. As many of these studies will likely involve study of FoxP3 expression it is worthwhile to note that most human T cells express FoxP3 during early stages of T-cell activation [76]. Therefore, studies using this marker alone to define T_{regs} should be interpreted with caution. Expression of the IL-7 receptor (CD127) might be helpful in this regard, as $CD4^+CD25^+CD127^{+low}$ cells include threefold more $FoxP3^+$ T cells than the classic $CD4^+CD25^{hi}$ subset, but show equivalent regulatory activity [76].

Perspective and future studies

One of the most remarkable observations made during compilation of this article was the realization that tissue samples from SOT liver- and kidney allograft recipients were scarce. This is not only attributable partially to the infrequency with which SOT patients are identified, but also to the fact that clinicians are hesitant to perform biopsies on otherwise seemingly stable SOT recipients. As mentioned before, clinicians might be misled by insignificant histopathologic curiosities. In addition, biopsies are invasive and not without risk of morbidity, and even mortality albeit rarely. In our opinion, the benefits of protocol biopsies in this situation outweigh their risks. It is crucial, however, that the tissue samples be used wisely. The issue of how to use them is not always an easy decision.

The choice of controls for SOT tissue studies can be problematic, especially for liver allografts because of the high incidence of recurrent disease. Normal age-matched control liver tissue, stable allograft recipients on immunosuppression, stable allograft recipients on immunosuppression with the same recurrent disease, and recipients that fail weaning attempts are possible comparison groups for SOT patients. Each one has advantages and drawbacks.

The advent of array technology and discovery science often pits those who practice 'discovery' science against those who practice 'mechanistic/hypothesis testing' science. Both have advantages and shortcomings. The essence of hypothesis testing is to associate a specific cell or pathway or system with a specific phenomenon. Key interventional experiments that change the potentially critical component are then conducted to determine whether the relationship holds up, as expected/hypothesized. The major problem, however, is how to identify the critical cell, pathway, or system that ultimately controls complex biologic phenomena like immunologic tolerance to human allografts. One could expend significant resources studying an unimportant cell, pathway, or system. In addition, interventional experiments in humans are usually delayed until the final stage of hypothesis testing. Moreover, they are expensive and often difficult to interpret.

'Discovery science', in contrast, has recognized that array technology and bioinformatics are reducing biologic phenomena to a 'closed', albeit very complex, systems. No assumptions are made about the particular importance of one cell, molecule, or signaling system over another. Instead, expression array analyses are conducted on populations that exhibit a phenomenon and prominent genes, proteins, pathways, or systems emerge [33]. Single nucleotide polymorphism arrays also have the potential to contribute significantly to our understanding of tolerance. Genetic tendencies certainly contribute to the development of certain original diseases that lead to transplantation and are likely to also contribute to the ability to wean immunosuppression. This discovery approach also has drawbacks. Not all components of biologic systems are amenable to array analyses. For example, mRNA expression arrays are only quantitative and some protein arrays do not account for the activation/phosphorylation status of proteins (e.g. STAT3), which can significantly affect function. It is not a trivial task to identify nodal points in complex systems that ultimately control or significantly influence the phenomenon being observed. A particular gene or protein might be one of the most up- or downregulated quantitatively during the process, but might not be an important nodal regulator.

In the end, it is our opinion, that the best approach to the study of tolerance in tissue samples will be a combination of both the approaches. The 'shotgun' criticism currently applied to many array studies will eventually give way to 'targeted' or focused arrays through hypothesis testing that measure only key parameters associated with the biologic process of interest.

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2005 Update of Banff 97 Diagnostic Categories for Renal Allograft Biopsies

1. Normal

2. Antibody mediated rejection-due to documented anti-donor antibody ('suspicious for' if antibody not demonstrated); may coincide with categories 3-6

a. Acute antibody-mediated rejection

Type (Grade)	Histopathological Findings
I	ATN-like; C4d positive, minimal inflammation
II	Capillary margination and/or thromboses, C4d positive
III	Arterial v3 changes, C4d positive

b. Chronic active antibody-mediated rejection

Grade	Histopathologic Findings
---	Glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries; C4d positive

3. Borderline Changes:"Suspicious" for acute T-cell-mediated rejection

Grade	Histopathological Findings
"Suspicious"	This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2 or t3 with i0 or i1) although the i2 t2 threshold for rejection diagnosis is not met (may coincide with categories 2, 5 and 6)

4. T-cell mediated rejection (may coincide with categories 2, 5 and 6)

a. Acute T-cell-mediated rejection

Type (Grade)	Histopathological Findings
IA	Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)
IB	Cases with significant interstitial infiltration (> 25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)
IIA	Cases with mild to moderate intimal arteritis (v1)
IIB	Cases with severe intimal arteritis comprising > 25% of the lumenal area (v2)
III	Cases with "transmural" arteritis and/or fibrinoid change and necrosis of medial smooth muscle cells with accompanying

	lymphocytic inflammation (v3)

b. Chronic active T-cell-mediated rejection

Type	Histopathological Findings
---	"Chronic allograft arteriopathy" (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)

5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology

Grade	Histopathological Findings
	Note: Grades I, II and III may include nonspecific vascular and glomerular sclerosis, but severity is graded by tubulointerstitial features
Grade I (mild)	Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)
Grade II (moderate)	Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area)
Grade III (severe)	Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)

6. Other: Changes not considered to be due to rejection

Diagnosis	Histopathological (and other) features
Chronic hypertension	Arterial/fibrointimal thickening with reduplication of elastica, usually with small artery and arteriolar hyaline changes
Calcineurin toxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cell injury with isometric vacuolization
Chronic obstruction	Marked tubular dilatation. Large Tamm-Horsfall protein casts with extravasation into interstitium, and/or lymphatics
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation
Viral infection	Viral inclusions on histology and immunohistology and/or electron microscopy

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Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

Specimen Adequacy and Lesion Scoring (Banff '97)

Specimen Adequacy (a necessary prerequisite for numeric coding)	
Unsatisfactory	Less than 7 glomeruli & no arteries
Marginal	7 glomeruli with one artery
Adequate	10 or more glomeruli with at least two arteries
Minimum Sampling	
7 slides	3 H&E, 3 PAS or silver stains, and 1 trichrome, section thickness 3-4 microns.

Quantitative Criteria for Tubulitis ("t") Score (assumes minimum sampling)	
t0	No mononuclear cells in tubules
t1	Foci with 1 to 4 cells/tubular cross section or 10 tubular cells
t2	Foci with 5 to 10 cells/tubular cross section
t3	Foci with >10 cells/tubular cross section, or the presence of at least two areas of tubular basement membrane destruction accompanied by i2/i3 inflammation and t2 tubulitis elsewhere in the biopsy.

Quantitative Criteria for Mononuclear Cell Interstitial Inflammation ("i")	
i0	No or trivial interstitial inflammation (<10% of unscarred parenchyma)
i1	10 to 25% of parenchyma inflamed cells
i2	26 to 50% of parenchyma inflamed
i3	>50% of parenchyma inflamed
Indicate presence of remarkable numbers (>10% of total cells) of eosinophils, polys, or plasma cells (specify which) with an asterisk on i	

Quantitative Criteria for the Early Type of Allograft Glomerulitis ("g")	
g0	No glomerulitis
g1	Glomerulitis in <25% of glomeruli
g2	Segmental or global glomerulitis in about 25 to 75% of glomeruli
g3	Glomerulitis (mostly global) in >75% glomeruli

Quantitative Criteria for Arteriolar Hyaline Thickening ("ah")	
ah0	No PAS-positive hyaline thickening
ah1	Mild-to-moderate PAS-positive hyaline thickening in at least one arteriole
ah2	Moderate-to-severe PAS-positive hyaline thickening in more than one arteriole
ah3	Severe PAS-positive hyaline thickening in many arterioles

Indicate arteriolitis (significance unknown) by an asterisk on ah

Quantitative Criteria for Intimal Arteritis ("v")	
v0	No arteritis
v1	Mild-to-moderate intimal arteritis in at least one arterial cross section
v2	Severe intimal arteritis with at least 25% luminal area lost in at least one arterial cross section
v3	Arterial fibrinoid change and/or transmural arteritis with medial smooth muscle necrosis with lymphocytic inflammation
Note number of arteries present and number affected. Indicate infarction and/or interstitial hemorrhage by an asterisk (with any level v score)	

Quantitative Criteria for Allograft Glomerulopathy ("cg")	
cg0	No glomerulopathy, double contours in <10% of peripheral capillary loops in most severely affected glomerulus
cg1	Double contours affecting up to 25% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg2	Double contours affecting 26 to 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg3	Double contours affecting more than 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
Note number of glomeruli and percentage sclerotic	

Quantitative Criteria for Interstitial Fibrosis ("ci")	
ci0	Interstitial fibrosis tissue in up to 5% of cortical area
ci1	Mild- Interstitial fibrosis tissue in 6 to 25% of cortical area
ci2	Moderate- interstitial fibrosis of 26 to 50% of cortical area
ci3	Severe interstitial fibrosis of >50% of cortical area

Quantitative Criteria for Tubular Atrophy ("ct")	
ct0	No tubular atrophy
ct1	Tubular atrophy in up to 25% of the area of cortical tubules
ct2	Tubular atrophy involving 26 to 50% of the area of cortical tubules
ct3	Tubular atrophy of >50% of the area of cortical tubules

Quantitative Criteria for Fibrous Intimal Thickening ("cv")	
cv0	No chronic vascular changes
cv1	Vascular narrowing of up to 25% lumenal area by fibrointimal thickening of arteries ± breach of internal elastic lamina or presence of foam cells or occasional mononuclear cells*
cv2	Increased severity of changes described above with 26 to 50% narrowing of vascular lumenal area*
cv3	Severe vascular changes with >50% narrowing of vascular lumenal area*
* in most severely affected vessel. Note if lesions characteristic of chronic rejection (elastica breaks, inflammatory cells in fibrosis, formation of neointima) are seen	

Quantitative Criteria for Mesangial Matrix Increase ("mm")*	
mm0	No mesangial matrix increase
mm1	Up to 25% of nonsclerotic glomeruli affected (at least moderate matrix increase)
mm2	26-50% of nonsclerotic glomeruli affected (at least moderate matrix increase)
mm3	>50% of nonsclerotic glomeruli affected (at least moderate matrix increase)

***** The threshold criterion for the moderately increased "mm" is the expanded mesangial interspace between adjacent capillaries. If the width of the interspace exceeds two mesangial cells on the average in at least two glomerular lobules the "mm" is moderately increased

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[FRAMES] [NO FRAMES]

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Meeting Report

Banff '05 Meeting Report: Differential Diagnosis of Chronic Allograft Injury and Elimination of Chronic Allograft Nephropathy ('CAN')

K. Solez[a,*], R. B. Colvin[b], L. C. Racusen[c], B. Sis[a], P. F. Halloran[a], P. E. Birk[d], P. M. Campbell[a], M. Cascalho[e], A. B. Collins[b], A. J. Demetris[f], C. B. Drachenberg[g], I. W. Gibson[d], P. C. Grimm[h], M. Haas[c], E. Lerut[i], H. Liapis[j], R. B. Mannon[k], P. B. Marcus[l], M. Mengel[m], M. J. Mihatsch[n], B. J. Nankivell[o], V. Nickeleit[p], J. C. Papadimitriou[g], J. L. Platt[e], P. Randhawa[f], I. Roberts[q], L. Salinas-Madriga[r], D. R. Salomon[s], D. Seron[t], M. Sheaff[u] and J. J. Weening[v]

[a] *University of Alberta, Edmonton, Alberta, Canada*
[b] *Massachusetts General Hospital, Boston, MA, USA*
[c] *John Hopkins University School of Medicine, Baltimore, MD, USA*
[d] *University of Manitoba, Winnipeg, MB, Canada*
[e] *Mayo Clinic, Rochester, MN, USA*
[f] *University of Pittsburg, Pittsburg, PA, USA*
[g] *University of Maryland, Baltimore, MD, USA*
[h] *University of California San Diego, La Jolla, CA, USA*
[i] *University Hospitals, Leuven, Belgium*
[j] *Washington University School of Medicine, St. Louis, MO, USA*
[k] *National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA*
[l] *Methodist Health System, Dallas, TX, USA*
[m] *Institut fuer Pathologie, Medizinische Hochschule Hannover, Germany*
[n] *University of Basel, Basel, Switzerland*
[o] *University of Sydney, Sydney, Australia*
[p] *University of North Carolina, Chapel Hill, NC, USA*
[q] *Department of Cellular Pathology, John Radcliffe Hospital, Headington, Oxford, UK*
[r] *Saint Louis University Health Sciences Center, St. Louis, MO, USA*
[s] *Scripps Institute, La Jolla, CA, USA*
[t] *University of Barcelona, Barcelona, Spain*
[u] *Royal London Hospital, London, UK*
[v] *University of Amsterdam, Amsterdam, The Netherlands*
* *Corresponding author: Kim Solez, Kim.Solez@ualberta.ca*

The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada, 15–21 July 2005. Major outcomes included the elimination of the non-specific term 'chronic allograft nephropathy' (CAN) from the Banff classification for kidney allograft pathology, and the recognition of the entity of chronic antibody-mediated rejection. Participation of B cells in allograft rejection and genomics markers of rejection were also major subjects addressed by the conference.

Key words: Banff classification, central slide review, scoring

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The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada from 15 to 21 July 2005. A large group of clinicians, pathologists, and researchers met in plenary and specialty sessions and participated in several active consensus discussions. A summary of major topics and results of consensus discussions are provided in this manuscript.

Allograft Fibrosis and Atrophy Revisited

A major topic discussed at the 8th Banff Conference was the elimination of the term 'chronic allograft nephropathy' or CAN from the Banff schema for diagnosis and grading of renal allograft rejection (1,2). Originally coined fifteen years ago in 1991 as a more generic alternative to the then popular and misleading term 'chronic rejection,' acceptance of 'CAN' did succeed in reversing the misconception that all late scarring of the graft was due to alloimmune injury/rejection. However, there are now over 550 PubMed citations using the term, many fostering the misconception that 'CAN' is a specific disease rather than just another term for non-specific parenchymal scarring. In this consensus report are outlined targeted alterations in the Banff schema replacing 'CAN' as a diagnostic term. The rationale for this update of the Banff schema is the misusage of 'CAN' as a generic term for all causes of chronic renal allograft dysfunction with fibrosis that inhibits the accurate diagnosis and appropriate therapy. In order to treat something, first you would need a definitive diagnosis, which is not artificial but rather specifies the underlying disease process(es). Thus there is an emerging need for an appropriate classification of chronic allograft injury. On the other hand, with the burgeoning recent literature, the role of alloantibody in chronic renal allograft deterioration and the corresponding morphological changes are increasingly recognized, making the identification of an antibody-mediated

component of chronic rejection reaction possible. The second part of the revisions on the Banff schema reflects the outlined pathological criteria for chronic antibody-mediated rejection (AMR) in kidney allografts which emerged from a consensus process after in-depth discussions at the 2005 Banff meeting.

Chronic Alloimmune Injury/Rejection versus Non-Immune Injury

Use of the non-specific term 'CAN' has tended to undermine recognition of morphological features enabling diagnosis of specific causes of chronic graft dysfunction. For example, many allograft recipients are hypertensive, which can lead to chronic allograft injury with fibrosis; pathological changes recognizable in the allograft include arterial fibrointimal thickening with duplication of internal elastica (fibroelastosis), arteriolar and small artery hyalinosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (IF/TA) (3). Chronic calcineurin inhibitor toxicity produces hyaline arteriolar changes, sometimes with peripheral hyaline nodules, and IF/TA either in 'striped' ischemic or diffuse form (4–6). Co-incident thrombotic microangiopathy and/or isometric vacuolization of tubular cells suggests ongoing toxic injury (7,8). Chronic obstruction in or extrinsic to the ureter can lead to IF/TA with relative glomerular sparing: dilated tubules, atubular glomeruli and intratubular Tamm–Horsfall protein casts with extravasation into the interstitium are pathological features suggestive of obstruction, which can be recognized in the allograft (9). Chronic polyomavirus infection can lead to IF/TA with chronic inflammation—intranuclear viral inclusions, highlighted on immunostaining for the SV40 large T antigen, are diagnostic of infection, though they may be sparse or even absent in very late fibrotic stages of polyoma virus nephropathy (10). Many recurrent and de novo glomerular or vascular diseases can also lead to glomerulosclerosis and IF/TA, both early and late post-transplant. In addition, de novo diabetic changes are becoming more common in allografts. All of these specific causes of IF/TA can and should be recognized by the pathologist (Table 1).

Table 1: Morphology of specific chronic diseases

Causes of IF/TA (non-rejection)	
Etiology	Morphology
Chronic hypertension	Arterial/fibrointimal thickening with reduplication of elastica, usually with small artery and arteriolar hyaline changes.
CNI[1] toxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cell injury with isometric vacuolization.
Chronic obstruction	Marked tubular dilation. Large Tamm–Horsfall protein casts with extravasation into interstitium, and/or lymphatics.
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation.
Viral infection	Viral inclusions on histology and immunohistology and/or electron microscopy.

[1] CNI, calcineurin inhibitor toxicity.

In addition, chronic alloimmune injury is an important cause of IF/TA in the graft. The Banff schema already mandates recognition and notation of morphological features of 'true' chronic rejection. Arterial and capillary changes have been emphasized as discriminating features (1). Recent data on alloantibodies and C4d in chronically failing renal allografts indicates a pathogenic role of humoral immunity in a subset of patients with chronic allograft dysfunction. There is strong evidence that anti-HLA antibodies participate in chronic rejection and previous studies have associated circulating anti-HLA antibodies with chronic vascular damage and late graft failure (11–13). In a large prospective trial, HLA antibodies were detected in 20.9% of 2278 renal allograft recipients, and graft failure at 1 year occurred more frequently in patients who developed de novo alloantibodies than in those who did not (8.6% vs. 3%) (14). De novo production of donor HLA-specific antibodies was shown in 51% of 112 renal transplant recipients with graft failure compared with 2% of 123 stable controls and the presence of alloantibodies predicted the subsequent development of chronic allograft rejection and graft loss (13). However, the majority of patients with anti-donor HLA antibodies do not demonstrate a progressive loss of transplant function within the follow-up periods. It is possible that the accumulation of antibody-mediated injury takes a longer time, or that only certain classes of anti-donor antibodies can mediate chronic injury or that cellular regulatory mechanisms are in play that counteract the injury mechanisms. Alternatively, the presence of anti-donor antibodies may not be sufficient to mediate the full spectrum of allograft injury without the concomitant activity of cell-mediated allograft immunity.

Recent reports have described morphologic features of chronic rejection in association with capillary-endothelial C4d deposits and concomitant circulating anti-donor antibodies (15–20). Mauiyyedi et al. (15) demonstrated deposition of C4d in peritubular capillaries (PTC) in 61% of 38 chronic rejection cases with chronic transplant glomerulopathy (TG) and/or 'chronic allograft arteriopathy' (arterial intimal fibrosis with intimal mononuclear inflammatory cell and/or foam cell infiltration) and most of the C4d positive chronic rejection cases had antidonor HLA antibody (88%). Regele et al. (16) detected C4d deposits in PTC in 34% of 213 renal allograft recipients with chronic allograft dysfunction. PTC C4d deposition was strongly associated with TG (53% of positive vs. 14% of negative biopsies) and severe PTC basement membrane multilayering (PTCBMML) (15 of 21 in positive vs. 3 of 22 in negative cases). Furthermore, C4d deposits in PTC preceded the development of

TG in follow-up biopsies. Vongwiwatana et al. (18) reported C4d deposition in PTC in 25% of 24 patients with TG but none with recurrent IgA nephropathy. PTCBMML was significantly increased in TG. Thus, the authors suggested that the association of TG with PTCBMML and C4d in PTC indicates a generalized disorder of the graft microcirculation and its basement membrane due to AMR in at least some cases. Sijpkens et al. (19) identified TG in 18 (1.6%) of 1111 kidney transplants with at least 6 months of graft function, and found C4d deposits in the glomerular capillary walls in 10/11 biopsies with TG. PTC C4d deposits were demonstrated in 4 and anti-HLA antibodies in 3 of the 10 biopsies with glomerular C4d deposits, suggesting that some of the glomerular staining was non-specific. Smavatkul et al. (21) reported increased graft loss over a 2-year period in patients with biopsy-proven graft fibrosis that were C4d positive (60%) compared to those that were negative (30%), and found TG and macrophage infiltrates as predictors of graft failure in grafts that were C4d positive.

The Diagnostic Triad of Late or Chronic Antibody-Mediated Rejection

Based on this accumulated literature, at the 2005 Banff meeting criteria for identification of late or chronic AMR were discussed and defined. The diagnostic criteria of late/chronic AMR include the following: (1) *morphological features* including TG (duplication or 'double contours' in glomerular basement membranes, Banff score cg1–3, see Figure 1) and/or PTCBMML (see Figure 2) and/or IF/TA with or without PTC loss, and/or fibrous intimal thickening in arteries without duplication of the internal elastica; (2) diffuse *C4d* deposition in PTC and (3) the presence of *donor specific antibody* (DSA) (Table 2). Diffuse C4d positivity has been defined as bright linear staining along PTC involving over half of sampled capillaries (2). The term 'late or chronic' means a slow but active process extending over some time (22). Indeed, the presence of C4d itself provides the best in situ evidence for an active humoral immunologic process (22,23). Other morphologic features that may accompany late AMR are aggregation of mononuclear inflammatory cells in PTC (16) (see Figure 3), transplant glomerulitis (19) (see Figure 4), and a plasma cell infiltrate in the interstitium (24). As with acute AMR, if only C4d deposits (with no DSA) or DSA (with no C4d) is present, with documented morphologic capillary changes, a diagnosis of 'suggestive of chronic AMR' can be made, although activity is more difficult to assess in the absence of C4d.

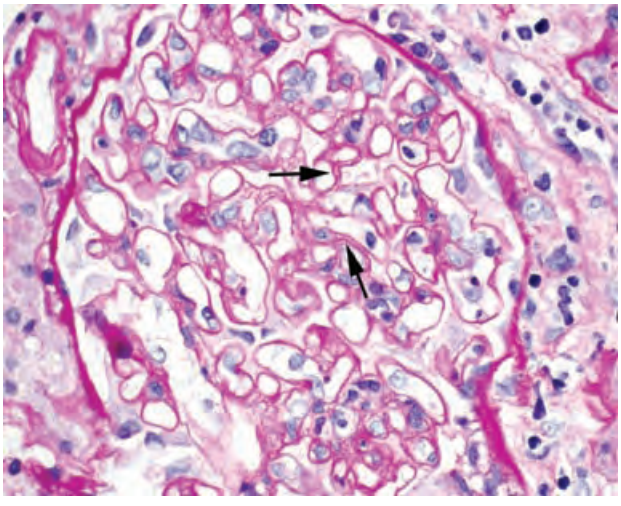

Figure 1: Chronic transplant glomerulopathy with numerous double contours (arrows) in glomerular basement membranes (PAS, original magnification ×600).

Figure 2: Marked splitting and multilayering in peritubular capillary basement membranes (arrows) in a renal allograft biopsy (uranyl acetate-lead citrate, original magnification ×5000).

Endothelial cells are thought to be the predominant target of antibody mediated injury (22,23). It has been suggested that the binding of complement-fixing alloantibody to endothelium induces tissue injury and acute rejection through the lysis of endothelial cells, coagulation (endothelial cell activation), complement activation and subsequent recruitment of macrophages and neutrophils. Recently, late/chronic AMR has been proposed as a partial accommodation (resistance of a graft to alloantibody-mediated injury) state which might be sufficient to prevent cell lysis through incomplete inhibition of complement but insufficient to prevent smoldering endothelial cell injury and activation (23). Dr. Jeffrey Platt emphasized accommodation as a possible contributor to chronic rejection in his presentation at the Banff meeting. Indeed, it has been shown that nucleated cells exposed to sublytic doses of the complement membrane attack complex become resistant to lytic complement doses (25). Dr. Platt suggested that accommodation may allow the allograft to survive long enough to acquire chronic rejection. Further studies are needed to determine whether true accommodation occurs, or whether

Table 2: Banff 97 diagnostic categories for renal allograft biopsies—Banff'05 update

1. Normal
2. Antibody-mediated rejection
Due to documented anti-donor antibody ('suspicious for' if antibody not demonstrated); (may coincide with categories 3–6)

Acute antibody-mediated rejection

Type (grade)

I. ATN-like – C4d+, minimal inflammation

II. Capillary-margination and/or thromboses, C4d+

III. Arterial – v3, C4d+

Chronic active antibody-mediated rejection[1]

Glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries, C4d+
3. Borderline changes: 'suspicious' for acute T-cell-mediated rejection
This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2 or t3 with i0 or i1) although the i2 t2 threshold for rejection diagnosis is not met (may coincide with categories 2, 5 and 6)
4. T-cell-mediated rejection[1] (may coincide with categories 2, 5 and 6)

Acute T-cell-mediated rejection

Type (grade)

IA. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)

IB. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)

IIA. Cases with mild to moderate intimal arteritis (v1)

IIB. Cases with severe intimal arteritis comprising >25% of the luminal area (v2)

III. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)

Chronic active T-cell-mediated rejection[1]

'Chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology[1]

Grade

I. Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)

II. Moderate interstitial fibrosis and tubular atrophy (26–50% of cortical area)

III. Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)
(may include non-specific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features)
6. Other: Changes not considered to be due to rejection-acute and/or chronic (the diagnoses given in Table I); may coincide with categories 2–5

[1]Indicates changes in the updated Banff'05 schema.

the presence of alloantibody and complement in the absence of classical histological changes simply reflects subtle allograft injury over a long time frame (26).

TG and PTCBMML tend to occur concomitantly, and both lesions show basement membrane thickening and multilayering, which are regarded as markers of past or recent

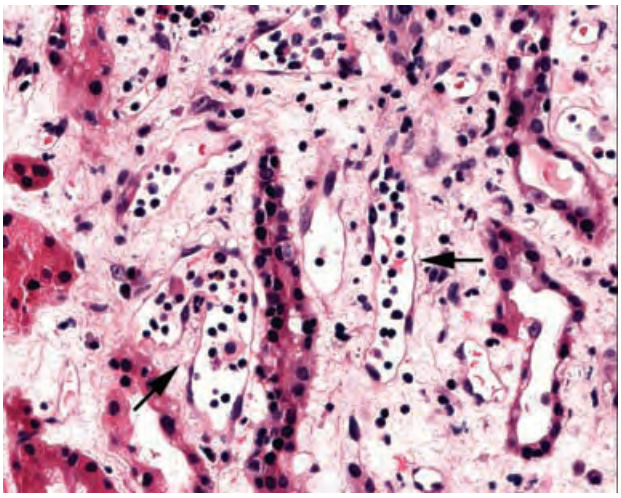

Figure 3: Aggregates of mononuclear inflammatory cells in dilated peritubular capillaries, scored as ptc3 (Hematoxylin and Eosin, original magnification ×265).

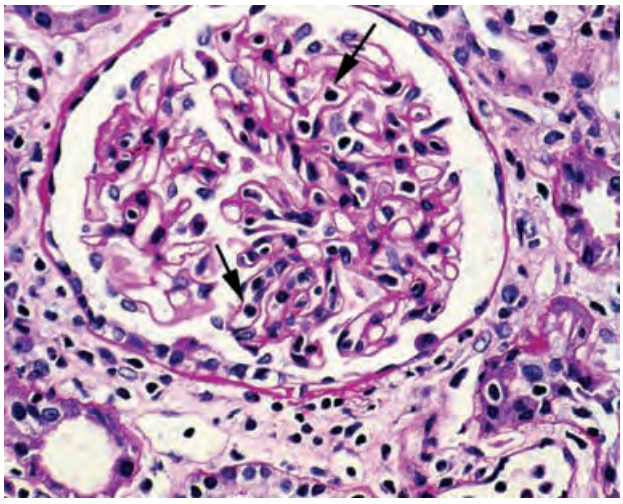

Figure 4: Transplant glomerulitis with infiltrating mononuclear inflammatory cells (arrows) within capillary loops (PAS, original magnification ×400).

endothelial cell injury and repair (15,17–22,27–32). Initially, Monga et al. (28,29) described splitting and multilayering of PTC basement membranes in renal allografts in association with TG. Ivanyi et al. (30) have reported moderate (5–6 layers) and severe (≥7 layers) PTCBMML in 16% and 12% of allograft biopsies and in 21% and 38% of failed transplant nephrectomy specimens with chronic rejection, respectively. Recently, Regele et al. (16) associated endothelial C4d deposition with TG, PTCBMML and accumulation of mononuclear inflammatory cells in PTC. Similarly, Mauiyyedi et al. (33) correlated marked PTCBMML with the presence of C4d in PTCs; they found 4.7 ± 1.8 layers in C4d+ cases versus 1.9 ± 1.2 layers in those that were C4d−. Thus the association of C4d deposition and alloantibody with TG and PTCBMML in some cases suggests AMR as a pathogenesis in at least a subset of patients. On the other hand, the precise definition of PTCBMML is critical when comparing studies describing associations with PTCBMML. For instance, Drachenberg et al. (34) showed that TG was mostly associated with severe PTCBMML (more than 6 layers), whereas lesser degrees of these changes (mostly 2–3 layers) were observed in transplants with other types of glomerulopathies and in native kidneys with various types of immune complex glomerulonephritis, diabetes, and hypertension. A representative picture of marked PTCBMML is shown in Figure 2.

The thickening and lamination of PTC basement membranes might be appreciated on periodic acid-Schiff or silver stains at least in advanced cases (15) and sometimes in Toluidine blue stained EM thick sections but would not allow one to define the severity of lesion, that is, count the layers. The question of whether electron microscopy should be routinely done on every or some subset of renal allograft biopsies remains open and should be addressed at the next Banff meeting along with the feedback from transplant physicians.

The pathogenesis of C4d negative TG and PTCBMML is unclear. In contradiction with the previous observations, three recent studies found no significant correlation between TG and C4d deposition in PTC (35–37) or in glomerular capillaries (36). Akalin et al. (36) showed glomerular infiltration by CXCR3+ ICOS+ activated T cells in grafts with TG/CAN, but not in CAN alone, suggesting an ongoing effector T-cell response to glomerular antigens can result in TG. At the 2005 Banff meeting, Dr. Colvin suggested possible causes of TG that is not associated with C4d staining: (1) technical/sampling error in AMR (e.g. PTC may disappear with allograft fibrosis); (2) residual injury from prior episodes of AMR; (3) T-cell-mediated TG or (4) non-alloimmune causes of TG (such as thrombotic microangiopathy). PTCBMML also appears to be a non-specific regenerative response to various types of injury both in transplants and native kidneys, including obstructive uropathy, thrombotic microangiopathy, analgesic nephropathy, various types of glomerulonephritis and radiation nephritis (30,32,34). Thus, definitive diagnosis of 'chronic' AMR requires a combination of morphologic changes (e.g. TG and/or PTCBMML and/or IF/TA and/or chronic arterial changes), with positive C4d immunostaining, and demonstration of DSA.

Category 5 in the Banff classification now includes only those cases for which no specific etiologic features can be defined (see Table 2). Quantitation of these changes is based on the percentage of cortex involved by IF/TA. Another change in the updated schema is the replacement of 'cellular rejection' with 'T-cell-mediated rejection'. Cellular rejection is associated with a primarily T-cell infiltrate, although the other inflammatory cells including

Table 3: Changes from Banff '97 and '01 diagnostic categories

Category 2. Antibody-mediated rejection now includes 2 subcategories:

Acute antibody-mediated rejection

Chronic active antibody-mediated rejection

Category 3. Borderline changes: 'suspicious' for acute T-cell-mediated rejection

This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (t1) and at least i1. It is now defined more clearly that t2, t3 with i0 or i1 is also under the borderline category.

Category 4. Acute/active cellular rejection is now replaced with T-cell-mediated rejection and includes two subcategories:

Acute T-cell-mediated rejection

Chronic active T-cell-mediated rejection

Category 5. Chronic/sclerosing allograft nephropathy 'CAN' is now replaced with:

Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology

Category 6. Other, changes not considered to be due to rejection-acute and/or chronic. The specific diagnoses responsible for chronic allograft injury, given in Table 1, are represented under category 6.

macrophages/monocytes, B cells, NK cells and plasma cells could also present in the graft and might contribute to the alloimmune response. However, we think that the more definitive term 'T-cell mediated' should be regarded to be similar to the antibody-mediated category as indicating the immunological component that is specifically recognizing the alloantigens. It should also be emphasized that both rejection types have cellular participation (macrophages/monocytes, etc.). Thus the term of 'cellular rejection' is now replaced with 'T-cell mediated rejection' as category #4 with subcategories of 'acute T-cell mediated rejection' and 'chronic active T-cell mediated rejection'. Major changes from the previous Banff schema are summarized in Table 3.

The Pathology of Antibody-Mediated Rejection

Complement deposition as a mediator and/or marker for AMR was discussed in the context of kidney, liver and heart allografts. Method standardization and guidelines for interpretation of complement staining were provided by Drs. Collins and Colvin, summarized elsewhere (38). In the kidney, PTC staining appears quite specific for alloantibody using either monoclonal antibody with immunofluorescence detection on frozen tissue, or polyclonal antibody with immunoperoxidase (IP) detection on paraffin sections; the former, however, is more sensitive (39). Glomerular capillary staining may be a marker for alloantibody effects using polyclonal antibody and IP staining in paraffin embedded tissue (19), but it can also be caused by immune complex deposition in glomeruli. At this time the clinical significance of C4d deposition in a graft with normal histology is unknown. In contrast to patients with anti-HLA antibody, diffuse PTC staining for C4d is commonly detected in well-functioning allografts in patients with anti-A or -B blood group antibodies, without histological evidence of injury (40). The complexity of control of the complement cascade, and resistance to injury with possible arrest of the cascade as a marker for 'accommodation' were emphasized (41). However, recipients with positive cross-match (HLA-incompatible) were recently shown to have increased risk for TG one year after transplantation in comparison to ABO-incompatible and conventional allografts (22% vs. 13% vs. 8%, respectively), and prior AMR appeared as an independent determinant for development of TG (42).

Capillary margination of inflammatory cells is an important histological marker of AMR in kidney and heart allografts, and acute capillaritis in lung allografts may be an equivalent process. Marginating neutrophils are more specific for AMR (43), but both neutrophils and mononuclear cells/monocytes have been associated with PTC C4d staining (16,44). Aggregation of mononuclear cells in PTC is shown in Figure 3. Given the importance of PTC

Table 4: The proposal of quantitative criteria for peritubular capillary margination of inflammatory cells ('ptc') score[1]

ptc0—no significant cortical peritubular inflammatory changes
ptc1—cortical peritubular capillary with 3–4 luminal inflammatory cells
ptc2—cortical peritubular capillary with 5–10 luminal inflammatory cells
ptc3—cortical peritubular capillary with >10 luminal inflammatory cells

[1]Use asterisk (*) to indicate only mononuclear cells and absence of neutrophils.

margination of inflammatory cells as a histological feature of AMR, Ian Gibson proposed a scoring method for quantitation ('ptc' score) at the Banff 2003 conference and reviewed this at the 2005 conference. The proposal focuses on the most severely involved PTCs, in analogy to other inflammatory rejection features such as tubulitis (Table 4). The number of luminal inflammatory cells includes all types (neutrophil, monocyte/macrophage and lymphocyte), with an asterisk (*) used to indicate only mononuclear cells and absence of neutrophils. The extent of the PTC inflammation in the biopsy should be documented, either as focal (<50% of cortical area) or diffuse (>50% of cortical area). The presence of associated PTC dilatation may also be noted. Areas affected by acute pyelonephritis or necrosis, and subcapsular cortex with non-specific inflammation should not be scored. Inflammatory cells within PTC must be distinguished from interstitial inflammation by careful examination of basement membrane stains (PAS, silver). Inflammatory cells within veins and medullary vasa recta should not be scored.

Several groups represented at the Banff 2005 conference indicated that they are using this scoring system. It is particularly applicable to comparison of sequential biopsies from the same graft, for example, in assessing responses to rejection treatments, as well as for documenting biopsy features in clinical trials. Some provisional reports using the peritubular capillaritis scoring system have been published (45,46), confirming its applicability, and showing high 'ptc' scores associated with AMR, and that lower 'ptc' scores can be associated with progressive chronic graft injury (46). It must be emphasized that the 'ptc' score alone does not equate with any specific diagnosis, and ongoing reproducibility and diagnostic studies are required, but the 'ptc' score helps to direct the pathologist to careful examination of the PTC.

B Cells in the Renal Allograft

The role of B cells in allograft rejection and ischemic injury was also highlighted at the 2005 Banff conference. Memory B cells and long-lived plasma cells in bone marrow may persist for years. Initial B-cell activation leads to the formation of short-lived plasma cells that provide the first burst of antibody. Long-term antibody responses, however, are maintained by non-dividing, long-lived plasma cells that produce high-affinity antibody. It should be noted that the B cells or plasma cells reside in lymphoid compartments during AMR and antibodies enter the graft as the effector molecules of humoral immunity (47). 'Lymphoid neogenesis' has been described in renal allografts with prominent lymphoid aggregates (48), though not all lymphoid aggregates are associated with acute rejection (AR) (49). In other contexts (e.g. rheumatoid arthritis, SLE), such aggregates can locally secrete tissue-specific pathogenic antibodies. B cell tolerance may also be possible, as reviewed by Dr. Cascalho (50).

The presence of molecular markers associated with B cells has also been identified in a subset of clinical cases of AR; immunostaining of allograft biopsy tissue confirmed significant numbers of B cells in the inflammatory infiltrates (51). The presence of B cells/markers was associated with worse outcome in this series. However, the frequency of B cell infiltrates in allografts in either AR or non-specific injury has not been extensively studied, nor has the association of allograft B cell infiltrates and AMR/presence of DSA. Recent interest in B cells in allografts has been spurred by the availability of anti-B cell therapies such as rituximab. A few centers have begun to routinely perform immunohistochemistry for B cells in allograft biopsies that have inflammatory infiltrates, for quantitative assessment and pattern of localization. B cell-rich infiltrates should be denoted with an asterisk on the 'i' score in the Banff scoring system. In the short term, these observations could guide therapy for those cases of AR that are B cell-rich and resistant to standard immunosuppression. However, evidence is lacking at this point whether anti-B cell therapy can reverse a resistant episode of B cell-rich rejection. In the long term, detection of B cell markers will provide important data in regard to incidence of significant B cell infiltrates, effects of same on response to therapy, clinical correlates and effect on outcome.

Genomics Markers in Solid Organ Transplantation

Molecular approaches and techniques were the subject of a pre-meeting symposium as well as sessions during the 2005 Banff conference. Techniques discussed included gene expression profiling using high density and DNA microarrays, transcriptome (gene chips) or quantitative PCR, metabolomics and proteomics. The importance of a 'biological' approach was emphasized, correlating gene expression array data with RT-PCR and Western immunoblotting and other proteomic technologies that can validate the actual levels of differentially expressed proteins as well as their post-translational modifications, such as phosphorylation that determine activation and molecular network signaling. It was considered equally important to cross-validate the expression levels of both gene transcripts and proteins, and with the biopsy pathology and clinical data to derive the fullest possible picture. Potential applications of array-based data include definition of disease mechanisms, identification of targets for pharmacological intervention, calibration of indicator systems for drug development, revision of new end points for trials, and development of new diagnostic and monitoring systems that could be applied to blood, fluids (urine, bile) or tissue specimens. The importance of using these strategies to focus on 'real' clinical issues was emphasized, with cluster analysis to identify clinically relevant genetic information.

An ultimate aim is to develop a genomics supported 'Banff classification' for diagnosis and grading of rejection and

other processes in allografts. The potential pitfalls in using genomics markers exclusively for differential diagnosis of acute and chronic dysfunction in allografts were discussed, based on the burgeoning literature in this area (52). Studies are often based on small cohorts of patients and may not include individuals with allergic drug reactions, systemic or intragraft infections or other inflammatory processes. Therapeutic regimens may alter findings and correlations, as has been shown for steroid treatment (53). Many studies of molecular markers for AR have not addressed discrimination between subtypes of AR (tubulointerstitial vs. vascular, cell- vs. antibody-mediated). Sarwal reported that C4d-positive cases of AR fall in each of the 3 AR categories defined by gene profiling. In addition, no obvious differential gene expression could be defined in cases of allograft fibrosis due to different causes (51). At the molecular level, the predominant signatures for gene expression may reflect the predominant pathological mechanisms in the biopsied tissue, for example, fibrosis or inflammation. If this is the case, then a specific cause may be difficult to connect directly to a particular molecular profile without additional data including clinical and histopathological data.

Identification of a few relevant diagnostic markers may be more useful and reasonable for diagnostic application in the near future, particularly, since array data often need to be markedly pruned in order to provide discrimination between patient groups (54). Currently molecular screening of blood and urine represent a promising alternative to invasive biopsy procedures for surveillance to detect early AR, but do not provide enough discriminatory power. At the present time, the assays are not statistically robust enough for clinical guidance. At least for the foreseeable future, the biopsy remains the 'gold standard' for definitive allograft assessment, though exciting alternatives are on the horizon.

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OVERVIEW

ROLE OF DONOR KIDNEY BIOPSIES IN RENAL TRANSPLANTATION

PARMJEET RANDHAWA[1]

Division of Transplantation Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Recent years have seen an increasing use of marginal donors to expand the organ pool available for renal transplantation (*1–5*). Donors considered in the marginal category include those with age >55 years, hypertension, diabetes mellitus, acute tubular necrosis, disseminated intravascular coagulation, prolonged cold ischemia time, and non-heart-beating donors. Patient and graft outcome obtained with such suboptimal donors has been comparable to that obtained with ideal donors in some studies (*6*, *7*), but significantly worse in others (*2*, *8–10*). These variable results presumably reflect the use of organs with different degrees of functional reserve. It has been estimated that donor factors can account for 35–64% of the variation in recipient serum creatinine and creatinine clearance after transplantation (*11*, *12*). Hence, it stands to reason that demonstration of satisfactory donor kidney function before accepting an organ for transplantation would improve both short- and long-term graft function.

Donor assessment should begin with a review of clinical data, but in cases of traumatic death adequate prior medical records are not always available. Some centers have used an arbitrary age cut off to exclude donors with senile arterionephrosclerosis. However, this is not entirely a satisfactory approach, because of individual variability in the rate at which kidney tissue ages. Thus, the percentage of sclerotic glomeruli in human kidneys varies between 0.2–16.7% at age 55 years and 1.5–23.0% at age 75 years (*13*). Data from a study conducted at the University of Pittsburgh indicated that 17/30 (57%) donors aged 60–75 years had 0–10% glomeruli sclerotic: clearly a decision to reject these donors based on age alone would have been inappropriate (*3*). Conversely, mild histologic abnormalities can be present in younger individuals much more commonly than is generally appreciated. Arteriolar hyalinosis has been reported in 25- to 34-year-old subjects, and considered to be a marker for early onset atherosclerotic disease (*14*).

Laboratory evaluation of donor renal function is important, and should include urine examination as well as blood chemistry. It should be kept in mind that mild proteinuria can occur secondary to glomerular or tubular ischemia reflecting agonal changes occurring before death. Acceptable cut off values for proteinuria used by different centers range between 0.5–3.0 g/24 hr (*15*, *16*). Blood urea and serum creatinine are readily available parameters for the assessment of renal function, but can rise significantly due to conditions such as renal hypoperfusion and acute tubular necrosis, which do not per se contraindicate transplantation. Creatinine clearance has also been used for screening of donors, and is superior to serum creatinine in that it is not affected by donor age, muscle mass, or obesity, However, clear-cut guidelines on the use of creatinine clearance as a criterion for donor selection have not yet been developed. Some authors have suggested a donor creatinine clearance measurement >60–70 ml/min for accepting marginal organs for single kidney transplantation (*15*, *17*). In contrast, others investigators have recommended double kidney transplantation when the donor creatinine clearance is less than 90–100 ml/min (*18*). Allograft function cannot be simply predicted by evaluating the donor creatinine clearance, because of multiple post-transplant variables such as acute tubular necrosis, antibody or cell-mediated rejection, and calcineurin inhibitor nephrotoxicity. Another confounding factor is the occurrence of compensatory renal parenchymal hypertrophy, when the donor nephron mass is insufficient to meet the metabolic needs of the recipient. Compensatory changes have been shown to result in an approximately 20% rise in estimated creatinine clearance in the allograft kidney within 4–6 months of transplantation (*16*).

The remainder of this review will focus on the role that a pretransplant biopsy can play in helping to define the structural integrity and functional reserve of a donor kidney under consideration of transplantation. A biopsy should be considered mandatory when the donor in question is in the marginal category. At Pittsburgh, we have set an arbitrary cut-off age of 55 years, beyond which all donors are biopsied to evaluate the severity of senile arterionephrosclerosis. A strong case can be made to include a pretransplantation or postperfusion biopsy in the routine work up of all donors, irrespective of age and clinical setting. This would provide baseline anatomic data with which future biopsies can be compared. Preexisting lesions such as capillary thrombosis, arteriolosclerosis, glomerulosclerosis, and interstitial fibrosis can be recorded, so that the occurrence of the same lesions in posttransplantation biopsies is not misconstrued as evidence of calcineurin inhibitor nephrotoxicity or chronic allograft

[1] Address correspondence to: Parmjeet Randhawa, MD, C 903.1 Presbyterian University Hospital, Transplantation Pathology, 200 Lothrop St, Pittsburgh, PA 15213.

nephropathy. Lack of knowledge about the extent of preexisting changes in a donor kidney complicates the interpretation of posttransplant biopsies.

Biopsy techniques vary from institution to institution. I prefer a generous wedge biopsy about 1-cm long and 0.5-cm deep. This suggested size ensures that at least half the cortical depth is available for evaluation, and minimizes erroneous conclusions due to superficial subcapsular scarring secondary to senile arteriosclerosis. Some centers prefer that both a wedge and a needle biopsy be performed to provide assurance that the deep cortex has been adequately sampled. A needle biopsy alone may not permit reliable assessment of the extent of glomerulosclerosis due to limited sampling. One study has suggested that sample adequacy be defined by the presence of a minimum of 25 glomeruli (*19*). This contention was based on the observation that a statistically significant relationship between percent glomerulosclerosis and graft loss was observed only if biopsies with more than 25 glomeruli were analyzed. In another study, the relationship between percent glomerulosclerosis and graft function was found to hold irrespective of the number of glomeruli present at biopsy (*2*). However, in that study this relationship was lost on multivariate analysis if a correction was made for donor age. This led the authors to state that if the donor age is known, data on glomerulosclerosis do not add any additional vital information. However, as pointed out earlier, age-associated changes in the human kidney are extremely variable and can not be predicted without a biopsy. Additionally, a biopsy can detect the presence of previously undocumented chronic diseases such as hypertensive or diabetic nephropathy, and chronic tubulointerstitial nephritis.

Urgent histological processing of donor biopsies is needed, when the decision to use the donor kidney is contingent on the morphologic findings. Because prolonged cold ischemia can adversely affect long-term graft function, the biopsies need to be interpreted as soon as possible. Rapid processing protocols can allow permanent sections to be available for reading within 2 hr. Consistently providing this level of service, however, necessitates that both a histotechnologist and an anatomic pathologist to be on call round the clock. As an alternative, a frozen section service with only a pathologist being on continuous call can be offered. Frozen section morphology is adequate to recognize sclerotic glomeruli, advanced interstitial fibrosis, and arteriosclerosis. However, freezing artifacts can lead to interstitial widening, which can be confused with fibrosis, if one does not insist on demonstrating a definite collagenous matrix. Retraction of tubular epithelium from the basement membranes makes it difficult to recognize tubular atrophy. Frozen sections are also not reliable for assessment of mesangial cellularity, glomerular capillary wall thickening, and diabetic lesions such as small capsular drop lesions or early Kimmelstein-Wilson nodules. Gross thrombosis can be recognized at frozen section, but small fibrin thrombi in the capillaries are more difficult to evaluate.

Interpretation of a kidney biopsy from a donor with senile arterionephrosclerosis or other chronic renal disease calls for a semiquantitative evaluation of the degree of glomerulosclerosis, arteriosclerosis, and interstitial fibrosis present. The use of Banff criteria for grading chronic allograft nephropathy is suggested to ensure center to center uniformity in this assessment (*20*). If most of the glomeruli are patent, and there is only mild arteriosclerosis and interstitial fibrosis present, the donor kidney is suitable for use. However, the extent of acceptable chronic changes within the donor kidney has not yet been rigorously defined. A widely accepted empiric rule is that kidneys with more than 20% sclerotic glomeruli not be used (*8*). At Pittsburgh, surgeons are also hesitant to use any kidney with more than mild interstitial fibrosis (more than 25% of cortical area affected) or mild arteriosclerosis (more than 25% luminal occlusion). Glomerular, interstitial, and vascular lesions in any given biopsy are frequently proportional to each other, even though this interrelationship is somewhat imperfect (*21*). Hence, we have taken the approach that moderate or severe changes in any of the major anatomic compartments in a donor kidney should contraindicate transplantation. Recently, it has been shown that the maximal planar area of the nonsclerotic glomeruli is also a predictor of long-term graft function (*22*).

Several investigators have studied interobserver variability in grading morphologic changes in donor biopsies. Pokorna et al. reported moderate to good reproducibility with calculated weighted kappa scores of 0.66 for percent glomerulosclerosis, 0.78 for interstitial fibrosis, and 0.83 for arteriolar hyalinosis (*2*). Wang et al. addressed this issue by (1) comparing histological changes in paired baseline biopsies from the same donor, and (2) comparing baseline donor biopsies with sequential posttransplant biopsies from the same recipient (*19*). Using linear regression analysis, the precision of estimating percent glomerulosclerosis in paired biopsies was good only if analysis was restricted to biopsies with more than 14 glomeruli ($r=0.83$ for paired biopsies and $r=0.56$ for sequential biopsies). The κ statistic for arteriolar hyalinosis was 0.55 for paired biopsies and 0.38 for sequential biopsies. Discrepancies in grading arteriolar hyalinosis were found in 10% of paired biopsies and 20–30% of sequential biopsies. Sund et al. reported poor reproducibility in the grading of arteriosclerosis and arteriolar hyalinosis in sequential biopsies, based on calculated kappa scores of 0.046 and 0.122, respectively (*22*). These disappointing results presumably reflect variation in the distribution and severity of vascular lesions in this patient population. The lesions were more pronounced in the pretransplant biopsy compared to the posttransplant biopsy. It was suggested that this difference resulted from a propensity of vascular lesions to affect deeper vessels, which are more likely to be sampled when a biopsy gun is pointed directly at the surface of a donor kidney. In support of their contention, the authors pointed out that the cortico-medullary junction was indeed more often sampled in biopsies obtained before transplantation.

Several studies have validated the clinical utility of donor biopsies by formal statistical analysis. Seron et al. examined postperfusion biopsies, and showed a correlation between interstitial fibrosis and serum creatinine measured 12 months posttransplant (*9*). Leunissen et al. showed a correlation between a histological chronicity score obtained at postperfusion biopsy and creatinine clearance measurement performed 3 months later (*23*). Lehtonen et al. found the chronic allograft damage index in a donor biopsy to predict long-term graft function (*24*). Gaber et al. showed that postperfusion biopsies with >20% glomerulosclerosis ($n=8$) were associated with an 88% incidence of delayed graft function (7/8 grafts), 38% graft loss (3/8 grafts), and a mean serum creatinine of 2.6 ± 0.1 mg/dl at 6 months (*8*). Based on this

data, it was suggested that kidneys with >20% glomerulosclerosis not be used for transplantation. However, this conclusion was derived from a study group of only eight patients with an unusually high percent glomerulosclerosis (mean 39±6%). The control group of patients used for comparison had significantly lower glomerulosclerosis (8±1%). Pokorna et al. described a 3-year graft survival of 74.7% in 67 patients with 20.0–47.6% glomerulosclerosis, but 11% of these recipients had primary non-graft function, and a mean 1-year glomerular filtration rate of 41.4 ml/min (*2*). Several studies have demonstrated a relationship between donor arteriosclerosis and posttransplant function (*25–27*). Hyaline changes in the smaller arteriolar sized vessels also correlate with 1 year serum creatinine (*28*) and rate of graft failure (*19*).

The reason why interstitial fibrosis, glomerulosclerosis, arteriolar hyalinosis, or arteriosclerosis have variably been identified as the critical parameter in different studies is probably the result of patient selection and methodological considerations. For example, in one study where donor glomerulosclerosis, but not interstitial fibrosis, was found to predict graft function, cases with >55% and <55% interstitial fibrosis were compared with regards to the incidence of satisfactory graft function defined simply as a patient being alive without maintenance dialysis (*19*). In a second study, interstitial fibrosis was found to be predictive, if biopsies showing no interstitial fibrosis were compared with those showing any level of interstitial fibrosis, and graft function was assessed by calculated creatinine clearance (*3*). Failure to detect the effect of interstitial fibrosis in some studies may also partly reflect the patchy nature of this lesion, which in turn, may be due to the patchy nature of arteriosclerosis and arteriolar hyalinosis in the kidney.

In contrast to the literature discussed above, one can also find studies that fail to find any correlation between donor biopsy findings and posttransplantation graft function (*7*, *22*, *29–32*). This is surprising given the intuitively expected relationship between anatomic architecture and physiological function in the kidney. Closer analysis of many of these studies reveals methodological problems such as (1) small numbers of patients, (2) insufficient histological detail for critical evaluation, (3) studies limited to biopsies with only mild histological changes, (4) lack of correction for variables such as prolonged cold ischemia or acute rejection, and (5) use of only crude patient or graft survival rates in evaluating outcome (*1*, *7*). In some clinical settings, the expected effect of donor histology can probably be overshadowed by other confounding clinical variables. Thus, many surgeons prefer to give kidneys from older donors to older recipients, who have a weaker immune system. This may result in lower rejection and reasonable graft survival, despite changes of senile arterionephrosclerosis in the donor organ. One study has suggested that improvements in medical care have now reduced the importance of donor age as a critical factor in renal transplantation (*33*).

The preceding discussion has focused primarily on donor biopsies performed for old age, hypertension, or donor diabetes mellitus. Clinical concern about pretransplant ischemic injury is another relatively common reason for requesting a donor biopsy. Predisposing factors for such injury include a history of donor hypotension, use of pressors during donor medical management, prolonged cold/warm ischemia time, a non-heart-beating donor, and chronic parenchymal or vascular disease in the donor. Acute tubular necrosis, the histological counterpart of ischemic injury, is difficult to evaluate on frozen section, except in cases with frank coagulative necrosis or infarction. Even with ideal permanent section morphology, correlations between clinical renal dysfunction and histological acute tubular necrosis are imperfect. Solez et al. could not demonstrate any correlation between histological severity of acute tubular necrosis and duration of oliguric acute renal failure in the native (nontransplanted) kidney (*34*, *35*). Lehtonen et al. found that chronic changes in the donor biopsy did not correlate with immediate posttransplant graft function (*24*). A similar lack of correlation has been observed with donor vascular disease (*27*). However, others have reported that histological scoring for acute tubular necrosis (*2*, *26*, *36*) or apoptosis (*37*) predicts delayed graft function. There is evidence that prolonged cold ischemia and delayed graft function predisposes to vascular rejection in kidneys derived from older donors (*35*). Whether delayed graft function adversely affects long-term graft survival independently of rejection is controversial (*36*).

Donor biopsies performed in the setting of disseminated intravascular coagulation need to be evaluated for the extent of microvascular injury. Organs with diffuse and extensive glomerular thrombosis should be discarded. However, the presence of scattered capillary thrombi present in a minority of glomeruli does not necessarily contraindicate transplantation. When the donor serum creatinine is normal or marginally elevated, successful transplantation has been reported. Isolated fibrin thrombi can apparently be dissolved by an intact fibrinolytic system (*31*, *37*), although this may result in a transient microangiopathic hemolytic anemia in a few instances (*38*). Mate kidneys recipients from the same coagulopathic donor can have different graft outcomes due to variations in pre- and posttransplant factors (*39*).

Occasionally, pretransplant or postperfusion biopsies show changes consistent with glomerulonephritis, and allow the glomerular disease in the allograft kidney to be traced back to the organ donor. The risk of this scenario is probably the highest for IgA nephropathy, a disease with high prevalence in some geographic regions. Based on isolated case reports in the literature, it would appear that mild glomerular changes in a donor biopsy can probably be ignored. Thus, it has been documented that modest donor-derived IgA deposits do not cause significant graft dysfunction, and can spontaneously resolve with time (*40*, *41*). Similar observations have been made regarding donor-derived postinfectious glomerulonephritis, membranoproliferative glomerulonephritis type I and lupus nephritis (*42–44*). Focal segmental sclerosis attributable to donor disease has been shown not to progress in the posttransplantation period (*31*).

The final indication for a donor kidney biopsy is the presence of a grossly visible nodule noticed during harvesting of the organ. When histological examination shows a benign cyst, leiomyoma or angiomyolipoma, it is safe to proceed with transplantation. However, finding a small epithelial neoplasm can generate dilemmas that may be difficult to resolve, particularly when a high grade carcinoma is not demonstrated. The distinction between a so-called renal adenoma and a small low grade renal cell carcinoma is arbitrary, and traditionally based on the size of the lesion, although it is now increasingly recognized that lesions of any size can metastasize. If the donor lesion is small (less than 0.5 cm) and

completely excised, the risk of residual or recurrent carcinoma in the recipient is probably extremely small. Dr. Israel Penn has reported six cases, where wide excision of the donor nodule led to an uneventful course documented by up to 186 months of posttransplantation follow up (*45-48*). The rare occurrence of posttransplant renal allograft carcinoma, despite the estimated 7–25% incidence (based on routine autopsy data) of small renal cell neoplasms in donor kidneys, also suggests that the use of such kidneys might be reasonable, at least in the context of informed recipient consent. Nonetheless, this is a controversial issue, and some transplant centers may not accept organs with small epithelial neoplasms.

In summary, a kidney biopsy is essential in the clinical work-up of marginal donors who are being evaluated for renal transplantation. In fact, it should be the standard of care to obtain a baseline biopsy from all kidneys before implantation, irrespective of the donor's medical history. Such a practice can consistently document premature arterionephrosclerosis and other clinically unsuspected renal disease in the donor. Lack of knowledge about the extent of preexisting changes in a donor kidney complicates the diagnosis of chronic allograft nephropathy and drug induced hyalinosis in posttransplant biopsies. While examining donor biopsies, an effort should be made to grade the severity of glomerulosclerosis, interstitial fibrosis, arteriosclerosis, and arteriolar hyalinosis present. Review of available evidence suggests that donor organs with <20% glomerulosclerosis and mild interstitial fibrosis or arteriosclerosis give clinically acceptable results. If the biopsy changes are more pronounced, the prospect of implanting a suboptimal organ with reduced graft life has to be weighed against the alternate option of continuing to support the patient by dialysis. Double kidney transplantation can also be considered in the latter situation (*16*).

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Minireview

BK Virus Infection in Transplant Recipients: An Overview and Update

P. Randhawa[a,*] and D. C. Brennan[b]

[a] *Department of Pathology, Division of Transplantation Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA*
[b] *Department of Medicine, Division of Nephrology, Washington University, Saint Louis, Missouri, USA*
** Corresponding author: Parmjeet Randhawa, randhawapa@msx.UPMC.edu*

BK virus infection after kidney transplantation has been a subject of great interest in the past decade. This article traces the discovery of BK virus and the subsequent development of our knowledge about this emerging pathogen. The pathobiology of the virus is summarized with particular reference to epidemiology, interactions with host cell receptors, cell entry, cytoplasmic trafficking and targeting of the viral genome to the nucleus. This is followed by a discussion of clinical features, laboratory monitoring and therapeutic strategies. Finally, we present potential cellular mechanisms that explain the basis of virus-mediated damage to the human kidney.

Key words: BK virus, epidemiology, JC virus, kidney, pathobiology, pathogenesis, polyomavirus, renal, SV40 virus, transplantation

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Virology

Polyomavirus BK virus (BKV) is a double-stranded DNA virus with a 5-kb genome. It has been classified in the Polyomaviridae family, which includes JC virus (JCV), a well known cause of progressive multifocal leukoencephalopathy, and the simian virus SV40 (1). The BKV genome comprises the non-coding control region (NCCR), the early-coding region coding for the small and large T antigens, and the late-coding region coding for the viral capsid proteins (VP1, VP2 and VP3) and agnoprotein. The NCCR contains (a) the origin of replication (ori) and (b) the regulatory regions containing enhancer elements that can alter viral transcription. T antigen binds to tumor suppressor proteins Rb and p53 and initiates the cell cycle in host cells. VP1, VP2 and VP3 are structural proteins that make up the viral capsid. The VP1 gene displays considerable genetic heterogeneity, and this genetic variation has led to recognition of viral genotypes I, II, III and IV. Agnoprotein plays a role in several cellular processes, including cell cycle progression, DNA repair, viral capsid assembly and virion release from cell.

Historical Aspects

BKV was first isolated in 1970 from a Sudanese kidney transplantation recipient with a ureteric stricture. Epidemiological studies showed that up to 90% of some human populations become exposed to BKV by adulthood (1). After kidney transplantation, 10–60% of patients were noted to excrete virus in the urine. However, viruria was typically asymptomatic or associated with only transient graft dysfunction, though occasionally, virus-induced tissue damage was noted at allograft nephrectomy or at autopsy. A new era in the study of BKV began when BKV nephropathy (BKVN) was diagnosed by a needle biopsy in a renal transplant recipient suspected of having acute rejection. This case was diagnosed in 1993 at Pittsburgh and published in 1996. In the following years, additional cases were reported from kidney transplant centers worldwide (2–5). It is commonly believed that this epidemic of BKVN in the 1990s is the result of potent immunosuppressive drugs such as tacrolimus, mycophenolate mofetil and sirolimus.

Mode of Transmission

Given that polyomavirus is latent in the kidney, it is not surprising that the donor kidney itself appears to be an important source of infection in transplant recipients. Donor seropositivity has been implicated in development of BK viruria, viremia or BKVN in pediatric and adult transplant recipients (6–9). The mode of viral transmission in the general population is incompletely understood, but multiple routes of infection are likely involved (1). Thus, BKV DNA has been amplified from 0–40% of urine samples, and 1% of nasopharyngeal aspirates obtained from infants with respiratory infections. The possibility of feco-oral transmission has been recently raised by the demonstration of viral DNA in urban sewage. Blood, semen, genital tissues and normal skin biopsies have also been shown to contain BKV. Transplacental transmission of polyomaviruses from mother to fetus has been recorded.

Cell Entry and Intracellular Trafficking

BKV interactions with host cellular receptors have been the subject of only limited investigations. The primary receptor binding determinant on BKV is the VP1 protein. The host cell receptor for BKV appears to be an N-linked glycoprotein, in which GT1b and GD1b have been identified as component gangliosides (10). Both these gangliosides have an α-(2-8) linked di-sialic acid-motif as a common feature. An α-(2-3) sialic acid linkage has also been shown to be important (10,11). Despite considerable homology at the genetic level, BKV differs from other polyomaviruses with regard to the chemical nature of its receptor. Thus, the JCV receptor is an N-linked glycoprotein containing terminal α-(2-3)- and α-(2-6)-linked sialic acids. The mouse polyomavirus binds to receptors containing α-(2-3)-linked sialic acid N-glycoproteins as well as α4β1 integrins. SV40 VP1 interacts with major histocompatibility class I proteins and O-linked glycan molecules.

The mode of BKV entry into the cell and routes of intracellular trafficking are currently being clarified. Electron microscopic observations on human biopsy material show that BKV entry into host cells is similar to SV40, and mediated by non-clathrin coated vesicles resembling caveolae. In contrast, JCV enters the cell by clathrin-dependent endocytosis. The mechanisms of endocytosis and intra-cellular trafficking utilized by BKV have not been investigated in detail. However, it has been established that the route from cell membrane to the nucleus includes the endoplasmic reticulum and microtubules (12,13). There may also be participation of the Golgi apparatus, and other cytoskeletal elements such as actin, and microfilaments, as has been shown for other members of the polyomavirus family. The mechanism by which polyomavirus traverses the nuclear envelope to enter the nucleus is only partially understood. VP2 and VP3 contain a nuclear transport signal that may facilitate nuclear targeting of the viral mini-chromosome. Nucleoporin, a protein associated with the nuclear pore complex, has also been implicated. The uncoating process of polyomaviruses has been stated to occur after the virions have entered the cell nuclei, but it has been shown for SV40 virus that some disassembly can occur in the endoplasmic reticulum.

Risk Factors for Infection

Conflicting information has been reported on risk factors for BK infection in transplant recipients (9,14–16). Risk factors may be donor, recipient, transplant or virus related. Reported donor-related factors include deceased-donor versus living-donor transplant, the presence of active BKV or cytomegalovirus (CMV) infection, donor seropositivity and the absence of HLA-C7. Reported recipient-related risk factors include older age, male gender, Caucasian race, diabetes mellitus, CMV infection, prior renal tubule injury, recipient seronegativity and the absence of HLA-C7. Risk factors associated with transplantation include procurement injury, cold-ischemia time, delayed graft function, immunosuppression, especially with maintenance tacrolimus, mycophenolate mofetil or sirolimus, or treatment of acute rejection with lymphocyte depleting agents or steroids, drug-toxicity, and increased number of HLA mismatches. Viral-related factors include variants in VP1 and sequence alterations in the NCCR. Most of these risk factors are unavoidable, unable to be modified or their risk contribution has not been consistently shown from study to study, perhaps because the type and intensity of immunosuppression may override any individual or combination of risk factors. Thus, the type and degree of immunosuppression is the most modifiable factor. A randomized prospective trial of 200 kidney transplant recipients showed that the incidence of BK viruria or viremia was not increased with thymoglobulin induction as compared to no induction, use of tacrolimus as compared to cyclosporine or the use of mycophenolate mofetil as compared to azathioprine (15). However, using detection of BK viruria or viremia as a surrogate for the intensity of immunosuppression, the combination of tacrolimus and mycophenolate or cyclosporine and azathioprine were the most potent, and the combination of cyclosporine and mycophenolate the least potent for the development of BK viruria or viremia. An interventional strategy with discontinuation of the antimetabolite upon detection of viremia was used and no BKVN was observed. Thus, it appears that it may not be the type but rather the intensity of immunosuppression that is the greatest risk factor for BK infection and thus BKVN.

Systematic clinical observations of human subjects undergoing polyomavirus seroconversion have not been reported, and the role of BKV antibodies remains unclear. An increasing anti-BK antibody titer has been seen to develop with a decrease of immunosuppression and treatment of BKVN (17). However, high-titer anti-BK antibody in the donor has been associated with an increased likelihood of development of BK viruria and viremia in the recipient (18).

Clinical Features

The most frequent symptom associated with BKV infection is an upper respiratory infection. Sporadic reports of acute cystitis, with or without hematuria, are also reported. After primary infection has resolved, the virus enters a latent phase. It appears that viral latency can be maintained in a number of different sites, particularly the urogenital tract (kidneys, urinary bladder, prostate, cervix and vulva, as well as testes, prostate and seminiferous tubules, as detected in semen) and hematolymphoid tissues (peripheral blood mononuclear cells, tonsils). Reactivation of latent virus has been reported in old age, pregnancy and diabetes mellitus, and immunosuppression associated with congenital immunodeficiency, organ transplantation or HIV infection. The most striking feature of BK infection in kidney

transplant recipients is the lack of fever, malaise, myalgias, leukopenia, anemia, thrombocytopenia or other symptoms or signs typical of viral infection, despite viral loads exceeding a billion copies/mL in the urine or 100 000 copies/mL in the blood (15,16). BKVN presents with renal dysfunction without other clinical signs or symptoms.

Laboratory Diagnosis and Monitoring

Laboratory monitoring strategies for BKV are still evolving. Quantitative nucleic acid-based viral load assay of urine or blood are becoming widely used for BKV screening (15,19–22). Detectable virus in the blood is more predictive of BKVN than viruria alone. Some medical centers prefer urine cytology as the primary screening technique (23,24). While urinary 'decoy cells' have excellent sensitivity for the detection of overt BKVN, polymerase chain reaction (PCR) is four times more sensitive than urine cytology for monitoring asymptomatic viruria (25). Additionally, PCR provides a more objective estimate of true viral load, and can distinguish BK viruria from JC viruria. JCV excretion in the urine is usually insignificant, although very rare cases of JCV-associated interstitial nephritis are on record. Decoy cells are not stable, whereas DNA is, and PCR may be used for monitoring of patients at a distance from the transplant center. The relative costs of PCR versus cytology are a center-dependent variable. Laboratory screening for BKV should certainly be done for any unexplained rise in serum creatinine. In addition, it is very desirable to monitor patients periodically, and one potential monitoring strategy is shown in Figure 1. The cost-effectiveness of screening has been formally evaluated in only one study to date (26). In this investigation, it was determined that routine BKV screening becomes cost-effective only if the incidence of BKVN in a transplant program exceeds 2.1%. The cost of screening was found to be substantially offset by the savings related to reductions in immunosuppression following diagnosis of BKVN. No anti-viral agents were administered. The assumptions made in this study regarding the incidence of BKVN, cost of testing, management strategy and risk of graft loss may not be applicable to all medical centers in the United States.

Definitive diagnosis of BKVN requires a biopsy and demonstration of BKV inclusions in tubular epithelial or Bowman's capsular epithelial cells. Viral infection is accompanied by varying degrees of inflammatory cell infiltrates, tubular atrophy and fibrosis. The cytopathic effect seen by light microscopy is typical, but not pathognomonic for BKVN. Confirmatory immunohistochemistry or *in situ* hybridization studies are usually performed using antibodies against specific for BKV proteins or probes complementary to viral DNA (Figure 2). Electron microscopy can be used to demonstrate unenveloped, viral particles, approximately 40 nm in diameter. Since BKVN can be focal in distribution, ideally two biopsy cores should be examined. The availability of medullary parenchyma increases the diagnostic sensitivity. Negative biopsy results cannot rule out BKVN with certainty, and a diagnosis of 'presumptive BKVN' can be made if there is renal allograft dysfunction associated with BK viremia.

Biopsy findings have been shown to have prognostic significance and three histological patterns of BKVN have been proposed: (a) BKVN A: mild viral cytopathic changes, with little or no inflammatory infiltrates or fibrosis, (b) BKVN B: mild to moderate viral cytopathic changes with significant

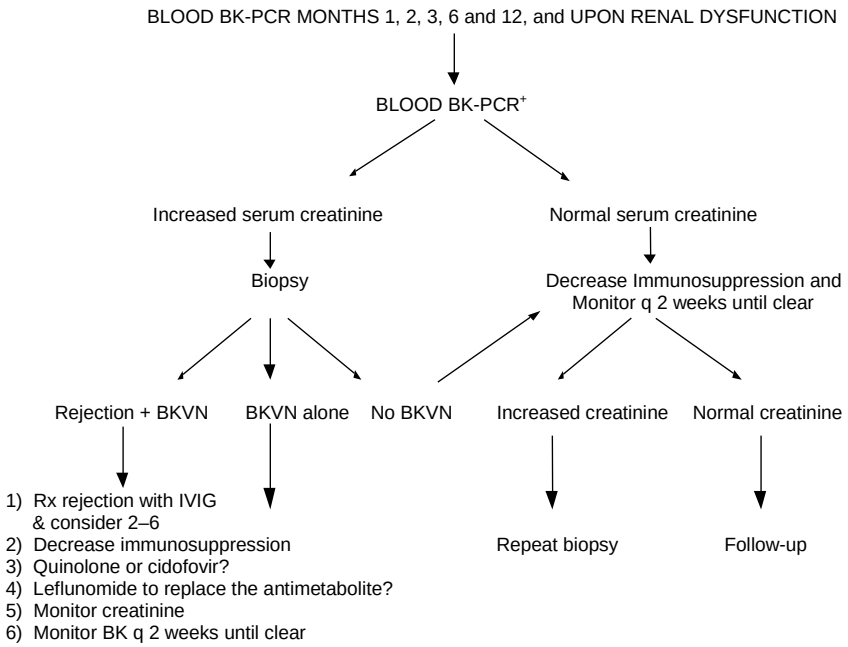

Figure 1: BK monitoring algorithm.

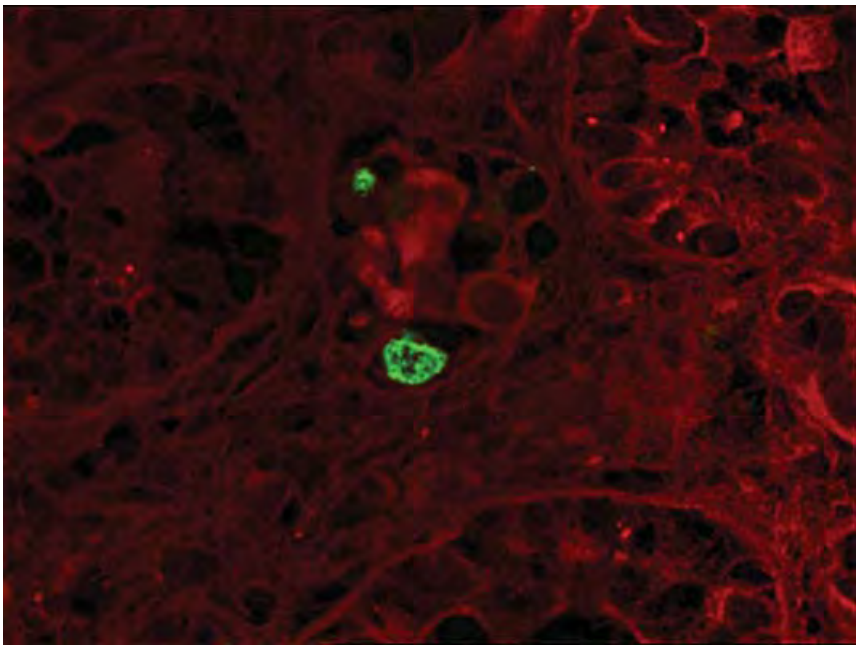

Figure 2: BK virus replication in the nucleus of a renal tubular epithelial cell, as demonstrated by a fluorescein-labeled antibody directed against VP1, a viral capsid protein that is synthesized late in the lytic life cycle.

inflammatory infiltrates, but limited fibrosis (≤ ci1+) and (c) BKVN C: prominent tubular atrophy and interstitial fibrosis, with usually sparse cytopathic changes, and variable inflammatory infiltrates. BKVN A carries the best prognosis, while BKVN C is associated with the worst long-term outcome (27).

Inflammatory cell infiltrates and tubulitis in biopsies with BKVN may represent an immune response to the infection or concurrent allograft rejection. A definitive diagnosis of rejection concurrent with viral nephropathy should only be made if there is endarteritis, fibrinoid arterial necrosis, glomerulitis or accumulation of the complement degradation product C4d along peritubular capillaries.

Pathogenesis

The pathogenesis of tissue damage in polyomavirus infected tissues is a subject of considerable interest. Transition from latent to lytic infection in the human kidney is likely initiated by ischemic, calcineurin inhibitor, or rejection-associated injury. This would explain, in part why most cases of BKVN occur in the allograft kidney, although disease in the native organ has been recorded. Using DNA microarray analysis of allograft kidney biopsies, it has been shown that BKVN is associated with up-regulation of several major groups of mRNAs, including CD8, Interferon-γ, CXCR3 and perforin. It is notable that these molecules are also up-regulated in acute cellular rejection, and this illustrates why the differential diagnosis between viral nephropathy and acute cellular rejection is problematic (28). Additionally, there is up-regulation of molecules associated with graft fibrosis, including matrix collagens, TGF-β, MMP2, MMP9 and markers of epithelial-mesenchymal transformation. The latter finding attests to the role of viral infection in promoting chronic allograft nephropathy.

Treatment

The treatment of BKVN is unsatisfactory, since no uniformly effective anti-viral drugs are currently available. Prevention of BKVN may be a better strategy than treatment of established disease. One large study of patients with prospective monitoring of urine and blood, and preemptive withdrawal of the anti-metabolite upon development of viremia, showed that this strategy resulted in clearance of viremia and viruria, and appeared to prevent progression to BKVN without increasing the risk of acute rejection (15). Another smaller prospective study showed that viremia and viruria could resolve or decrease over time with standard reductions in immunosuppression, without preemptive withdrawal of any component of the immunosuppressive regimen (16). Reducing the intensity of maintenance, immunosuppression currently represents the primary treatment of well established BK nephropathy. However, in patients with progressive graft dysfunction not responding to this maneuver, anti-viral treatment should be considered. Protocols and success rates are heterogeneous, with graft loss ranging from <10% to >80% (14). Anti-viral agents used with anecdotal success include cidofovir, leflunomide, quinolone antibiotics and intravenous immunoglobulin (14,29–32). The efficacy of these strategies is unclear, because reduction of immunosuppression has been used along with all of the strategies. Additionally, an *in vitro* study has shown that the 50%

effective concentration (EC50) for either leflunomide (39.7 μg/mL) or cidofovir (36.3 μg/mL) is higher than what may be achieved clinically with conventional dosing (33). Blood leflunomide levels above 40 μg/mL in the context of other reductions of immunosuppression have been associated with viral clearance or decrease in BK-viral load, but the pharmacokinetics of leflunomide are unpredictable, and even patients on 60 mg/day may fail to achieve 40 μg/mL (30,34). For cidofovir, the EC50 is much higher than peak plasma concentrations that are typically achieved with the low-dose (0.25–1.0 mg/kg) treatment regimens described in most publications related to BKVN (33). Esterification of cidofovir with hexadecyloxypropyl, octadecyloxyethyl or oleyloxyethyl groups results in up to 3-log lowering of EC_{50} and markedly increased selectivity index *in vitro*. Oral bioavailability and reduced nephrotoxicity are additional potential advantages of these derivatives over unmodified cidofovir (35). A cautiously conducted controlled clinical trial of these compounds in the management of BKVN appears to be warranted. Re-transplantation is safe and usually not complicated by recurrent BKV infection in the recipient. If allograft nephrectomy is performed, preemptive re-transplantation may be performed even during the phase of active viremia (36).

In conclusion, there is increasing recognition of BKV infections after kidney transplantation. Improved techniques of clinical monitoring and preemptive adjustment of immunosuppression have led to a reduction in the incidence of overt viral nephropathy. However, in patients who do develop BKV-induced allograft injury, we do not have reliable anti-viral drugs available at this time. The impact of long-term low-grade viruria or viremia on the development of chronic allograft nephropathy requires further study.

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Minireview

Recurrent Glomerulonephritis After Kidney Transplantation

B. Y. Choy*, T. M. Chan and K. N. Lai

Department of Medicine, Queen Mary Hospital, University of Hong Kong, Pokfulam, Hong Kong
** Corresponding author: Bo Ying Choy, choybyc@hku.hk*

Thirty to fifty percent of kidney transplant recipients have glomerular diseases as the underlying causes of end-stage renal failure. While recurrence of glomerulonephritis is an important cause of late renal allograft failure, the risk factors for recurrence are largely unknown or imprecise and prediction remains difficult. Recurrent disease usually presents with similar manifestations as the native disease. With regard to treatment of recurrent glomerular disease in the renal allograft, plasma exchange may be effective in reducing proteinuria in patients with early recurrence of focal and segmental glomerulosclerosis, but immunosuppressive therapy is generally ineffective in the prevention or treatment of recurrent disease. General supportive measures including strict blood pressure control and inhibition or blockade of the renninangiotensin pathway are helpful in retarding the rate of deterioration in renal allograft function. Despite the risk of recurrence, kidney transplantation following primary glomerulonephritides enjoys graft and patient survival rates comparable to other causes of end-stage renal failure. With a few exceptions, living related renal transplantation is not contraindicated in view of the favorable outcome and the donor shortage. This review discusses commonly encountered recurrent glomerulonephritides, with special emphasis on the influence of post-transplant prophylactic immunosuppression and emerging treatments.

Key words: Glomerulonephritis, recurrence, renal transplant, treatment

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Introduction

Glomerulonephritis is the underlying cause of end-stage renal failure in 30–50% of kidney transplant recipients (1). These patients are at risk of the recurrence of their original diseases. Recurrent glomerulonephritis was previously considered to be a minor contributor to graft loss. Introduction of newer immunosuppressive agents have reduced graft loss directly by decreasing the incidence of acute rejection and indirectly through the consequent reduction of chronic allograft nephropathy (1,2). With the prolongation of graft survival, the effect of recurrent disease on graft outcome assumes increasing importance. Studies on recurrent disease are difficult since not all patients have undergone native kidney biopsy and most centers perform graft biopsies only when there are abnormal clinical or laboratory features. The reported incidence of recurrent disease is thus influenced by prevailing clinical practice and could over- or underestimate the true occurrence. In this regard, it may be impossible to differentiate between *de novo* and recurrent disease. Accurate dissection of the contribution by recurrent disease toward graft dysfunction is also difficult in view of the often concomitant histological features of chronic allograft nephropathy or chronic nephrotoxicity due to calcineurin inhibitors. Many a time, full evaluation of biopsy specimen with combination of light microscopy, immunofluorescence and immunohistochemical studies and electron microscopy is needed to delineate different pathologies that coexist in the same patient. Despite these difficulties, there is accumulating evidence that recurrent glomerulonephritis is an important cause of graft loss in the long-term follow-up of renal allograft recipients (1,3,4).

The latest registry study, reported by Briganti et al. on 1505 patients with both native and graft biopsies, showed that graft loss due to recurrent glomerulonephritis was the third most frequent cause for graft loss 10 years after kidney transplantation. The risk of graft loss from recurrence increased with the years of follow-up, from 0.6% at first postoperative year to 8.4% at the 10th year (1). The recurrence rate, clinical course and impact on graft survival vary between different types of glomerulonephritis. This review aims to provide updated knowledge on recurrent renal diseases after kidney transplantation, focusing on recent findings with new post-transplant immunosuppressive regimens and treatment.

Immunoglobulin A Nephropathy

Immunoglobulin A Nephropathy (IgAN) is the most common type of glomerulonephritis worldwide and is the primary cause of renal failure in 20% of kidney transplant recipients. The pathogenetic mechanisms are complex and incompletely understood. It is likely to be related to the aberrant synthesis of abnormally *O*-glycosylated IgA1 in

Table 1: Recurrence rate of IgA nephropathy and risk of recurrence and graft loss from recurrent IgAN in relation to the donor type

				Recurrence rate[1]		Graft loss due to recurrence	
	Follow-up duration (mean) (months)	No. of allografts Total	(R/NR)	No. (%) Total	(R/NR)	No. (%) Total	(R/NR)
Berger et al. 1984 (6)	>24	32	(13/19)	17(53.1%)[2]	(9/8)	0	
Bachman et al. 1986 (9)	20 ± 13	13	(6/7)	6(46.2%)	(5/1)	1 (7.6%)	(1/0)
Odum et al. 1994 (7)	3–183	51	–	17(33.3%)	–	5 (9.8%)	–
Hartung et al. 1995 (10)	45.9 ± 10	128	–	47(36.7%)	–	9 (7.0%)	–
Kesser et al. 1996[3] (11)	68.1 ± 37.2	84	(3/25)[5]	13(15.5%)	(2/11)[5]	4 (4.8%)	–
Frohnert et al. 1997 (12)	78 (3–156)[6]	53	(41/12)	10(19%)	(8/2)	3 (5.7%)	(2/1)
Ohmacht et al. 1997[4] (13)	54 (7–127)	61	–	20(29.9%)	–	10 (16.4%)	–
Bumgardner et al. 1998 (14)	61 ± 37	61	(18/43)	18(29.5%)	(6/12)	7 (11.5%)	(4/3)
Freese et al. 1999 (15)	67 (11–159)[6]	104	(47/57)	13(12.5%)	(11/2)	6 (5.8%)	–
Kim et al. 2001 (16)	2–164	90	(60/30)	19(21.1%)	(13/6)	2 (2.2%)	–
Wang et al. 2001 (17)	52 (18–155)[6]	48	(17/31)	14(29.2%)	(6/8)	4 (8.3%)	(3/1)
Ponticelli et al. 2001 (18)	70.4 ± 50.5	106	(21/85)	37(35%)	(9/25)	4 (3.8%)	–
Andresdottir et al. 2001 (19)	67.2 ± 54	79	–	17(21.5%)	–	1 (1.3%)	–
Briganti et al. 2002 (1)	12–120	532	–	–	–	15 (2.8%)	–
Choy et al. 2003 (20)	100.0 ± 5.8	75	(32/43)	14(18.7%)	(9/5)	3 (4.0%)	(1/2)
Moriyama 2005 (21)	67.8 ± 19.9	49	(44/5)	13(26.5%)	(12/1)	5 (10%)	(5/0)

RD = related donor, NRD = nonrelated donor.
Recurrent rate for RD = 29.8%; NRD = 22.7%. Breslow–Day test of Homogeneity of odds ratio: chi-square = 10.29, df = 10, p = 0.416. Mantel–Haenszel estimate of Common odds ratio: 2.14 (95% CI = 1.42, 3.23; p < 0.001).
Percentage of graft loss from RD = 34.8%; NRD = 24.1%. Breslow–Day test of Homogeneity of odds ratio: chi-square = 7.37, df = 5, p = 0.194. Mantel–Haenszel estimate of Common odds ratio: 1.95 (95% CI = 0.64, 5.97; p = 0.243).
(%) = Percentage was calculated from number of graft loss due to recurrent IgAN/total number of patients with primary IgAN.
[1]Recurrence rate in patients with clinical symptoms of proteinuria/hematuria/renal impairment.
[2]Recurrence rate in patients with histological changes but clinically asymptomatic.
[3]Included 13 patients who suffered from underlying Henoch-Schonlein purpura.
[4]Included 4 patients who suffered from underlying Henoch-Schonlein purpura.
[5]Only 28 allografts had information with respect to the donor type.
[6]Median.

patients with IgAN. Mesangial deposition of polymeric IgA1 with abnormal *O*-glycosylation initiates glomerular inflammation and injury with progressive loss of renal function (5).

Recurrent IgAN is common after transplantation. Great variation in the incidence of recurrence has been reported because of difference in duration of follow-up and biopsy policy of different transplant centers (Table 1). Most centers performed renal biopsy only when patients presented with clinical symptoms of proteinuria, hematuria or decline in renal function. This would potentially underestimate the rate of recurrence as patients who were clinically asymptomatic but with histological changes in the graft kidneys would remain undiagnosed. For centers where routine protocol biopsies were being carried out in all transplant recipients, histological recurrence with mesengial IgA deposits and mesangial hypercellularity had been reported in 50–60% of patients (6,7). Recurrence rate reported for patients with renal biopsies for clinical symptoms ranged from 13–50% (9–21) (Table 1). Clinical manifestations are similar to primary IgAN and include microscopic hematuria, proteinuria and slow decline in renal function. Clinical course of recurrent IgAN had been reported to be benign initially (6,8). However, with increasing long-term data, it is apparent that recurrent disease is not as benign as had been reported previously (7,9–18,20,21). Graft loss from recurrence with histological features of diffuse mesangial proliferative expansion and glomerular sclerosis were reported between 1.3% and 16% (1,7,9–21) (Table 1). The estimated 10-year incidence of graft loss due to recurrence was 9.7% (CI = 4.7–19.5%) from the latest registry report containing the largest number of IgAN patients (1).

It is interesting to note that renal allograft survival for the first 5 years post-transplant is better in patients with primary IgAN compared to other primary diseases (8,11,19,20). The proposed mechanism included increased occurrence of allo-reactive IgA anti-HLA antibodies which may block the deleterious effect of IgG and IgM antibodies on the graft, and the immunological dysfunction of patients with IgAN (8). Despite the better graft survival of IgAN patients for the early post-transplant period, graft survival becomes comparable and might be worse than patients with other underlying renal diseases when data with follow-up beyond 10 years becomes available (16,18,20), suggesting other factors including recurrent disease contributing to graft loss becomes more apparent with long-term follow-up. No single parameter including age, gender, race, HLA

typing, pre-transplant course or biochemical characteristic of serum IgA can predict recurrence.

The relationship between the risk of recurrence and the donor type remains controversial. Some studies had reported a higher risk of disease recurrence in related donors (6,9,11,15,17), while others reported no added risk (12,14,16). Pooling all available data from literature that contained information on graft recurrence (6,9,11,12,14–18,20,21) and graft loss (9,12,14,17,20,21) in relation to donor type and estimate the risk by Mantel–Haenszel estimate of common odds ratio showed a higher risk of disease recurrence among transplant recipients with related donors (common odds ratio 2.14, $p < 0.001$), but the risk of graft loss was not increased (common odds ratio 1.95, $p = 0.24$) (Table 1). Whether this apparent paradox could be due to insufficient follow-up remains to be investigated. Given the fact that the graft survival of patients with primary IgAN is excellent for the first decade post-transplant, it is inappropriate to refrain from living related donor transplantation even though there may be a slight risk of recurrence. In contrast, familial IgAN should be rigorously excluded in potential living related donors since familial IgAN is associated with high risk of development of renal failure in affected members (22). Moriyama et al. reported higher risks of recurrence and graft loss in patients with latent IgA deposition from donor kidneys (majority were living related donors) (21). Whether such latent IgA deposition or the load of immune deposits might be detrimental to graft survival remain speculative.

The situation is quite different for patients with prior graft loss due to recurrent IgAN because the risk of recurrence in the second transplant (20–100%) is much increased (13–15,18). Ohmacht et al. reported a graft loss rate of 60% in their patients with a follow-up duration of 21–51 months (13) while two other series reported good graft function despite of recurrence in their patients up to 92 months of follow-up (14,18). In this regard, living donor transplant should be discouraged if recurrence and graft failure occur within few years after first transplant. However, such a transplantation is not a problem if their first graft functions beyond 10 years post-transplantation.

There is no effective therapy for the prevention or treatment of recurrent IgAN. Calcineurin inhibitors, in the presence or absence of induction therapy, do not influence the recurrent risk. Despite initial enthusiasm, newer immunosuppressive drugs are ineffective in preventing recurrence. Anecdotal reports that mycophenolate mofetil might have averted progression to allograft failure in recurrent IgAN are not substantiated by recent studies (18,23). Data on sirolimus are limited. Development of IgAN with nephrotic range of proteinuria had been reported in two transplant recipients after conversion from a calcineurin inhibitor-based immunosuppression to sirolimus (24). Steroid free or rapid steroid withdrawal regimen does not seem to affect the recurrent risk (25). The effect of fish oil in recurrent IgAN has not been systematically examined. Angiotensin converting enzyme inhibitor and angiotensin receptor blocker are commonly used for reduction of proteinuria and preservation of renal function in patients with recurrence as in IgAN of native kidneys (26,27).

Henoch-Schönlein purpura (HSP) has been regarded by many as the systemic variant of IgAN. Renal manifestation of HSP is indistinguishable from IgAN. Currently available data suggest that the recurrence rate after transplantation in patients with HSP is similar to that of IgAN (13,19,28).

Focal and Segmental Glomerulosclerosis

Focal and segmental glomerulosclerosis (FSGS) is a histological diagnosis that encompasses not only the idiopathic form (primary FSGS) but also a variety of secondary causes including glomerular hyperfiltration, toxic injury or viral infection leading to similar sclerotic lesions, recurrence risk of which depends on the underlying disorder. Primary FSGS has a recurrence rate of 20–50% after kidney transplantation leading to graft failure in 13–20% of patient in 10 years after kidney transplantation (1,4). Clinical manifestations of recurrent FSGS include early onset of massive proteinuria, usually within first year post-transplant, hypertension and graft dysfunction.

The pathogenesis of recurrent FSGS is unclear. A circulating permeability factor which increases the glomerular permeability to albumin and is removable by plasmapheresis or immunoadsorption therapy has long been suspected to play an important role. Savin et al. developed an *in vitro* bioassay for the permeability factor (29), and had shown that patients with high permeability factor activity in pretransplant sera were more likely to develop recurrence (29,30). However, recent data suggest that the absence or loss of an inhibitor of a normally present factor in plasma rather than the addition of a circulating factor could be the underlying cause for the glomerular permeability alteration (31,32). Further complicating the picture is the recognition of the pivotal role of the podocyte in the pathogenesis of proteinuria in various glomerulopathies. Acquired or inherited defect in the slit—diaphragm proteins (podocin [NPHS2], nephrin [NPHS1], α-actinin 4 and CD2AP) on the glomerular basement membrane have been reported in 15% of patients with primary FSGS (33,34). Recurrence which would not be expected in the genotypically normal donor kidneys have been reported in recipients with mutations of podocin, more so for the heterozygous than the homozygous mutations (34). This suggested that etiology of recurrent FSGS is likely multifactorial involving interaction between genetic and extra-renal mechanisms (putative permeability factor).

Risk factors for recurrence include younger age, rapid progression of original disease with development of end-stage renal failure within 3 years, mesangial hypercellularity of

native kidney, Caucasian race and a history of previous graft failure due to recurrence (4,35,36). Earlier reports have suggested a higher risk of graft loss with related donors (36) but recent reports showed that the risk of graft loss was similar between living donors and deceased donors (1,37). Patients who have recurrence of FSGS in the first year after transplantation with rapid loss of their graft are at a very high risk (>80%) of having recurrence and graft dysfunction in subsequent grafts (38). In this regard, living donor transplant should be avoided in patients who have lost their first graft in a rapid fashion.

Early institution of plasmapheresis is important as the effectiveness of treatment decreases with the increased number of sclerosed glomeruli. Relapse after cessation of plasmapheresis can be prevented or reversed by chronic plasmapheresis or concurrent treatment with cyclosporine or cyclophosphamide (29,30,35). Improved long-term renal outcome is observed in patients who achieve remission following treatment for early recurrence (35). Preemptive perioperative plasmapheresis for 2–8 sessions starting 1 week before operation for living donor transplant or immediately post-transplant for deceased donor transplant had been reported to reduce recurrence in children (39) and high-risk patients (40). The role of preemptive plasmapheresis in prevention of recurrence in high-risk group still awaits confirmation by larger clinical trial. There is a recent case report of complete resolution of proteinuria with rituximab (which was used to treat his post-transplant lymphoproliferative disease) in a patient who developed severe recurrent FSGS 2 weeks post-transplant with persistent proteinuria despite of prolong courses of plasmapheresis (41). The response of resistant proteinuria in this patient to the anti-CD20 antibody might shed some light in management of patients who failed to respond to conventional treatment although efficacy and long-term safety need further evaluation with prospective trial.

Newer immunosuppressive agents such as sirolimus have increasingly been used to replace calcineurin inhibitors to avoid calcineurin inhibitors associated nephrotoxicity and to treat chronic allograft nephropathy. However, a number of case reports have reported the development of *de novo* or recurrent FSGS when cyclosporine was replaced with sirolimus, with subsequent improvement after switching back to cyclosporine (24,42,43). The beneficial effect in this regard seemed specific to cyclosporine (43). Paradoxically, sirolimus had been reported in a recent study that 12 out of 21 patients with steroid resistant FSGS achieved complete or partial remission of their proteinuria after 6 months of therapy (44). In view of the accentuation of glomerular damage due to the proinflammatory effects of sirolimus and its derivatives in animal models (45), caution still need to be exercised when sirolimus is used in patients with underlying FSGS. Early steroid withdrawal did not lead to an increase in recurrent FSGS or graft loss from recurrent disease, although long-term data are still awaited (25,46). Data from small series have implicated increased recurrent FSGS with antilymphocytic antibodies (47) and anti-IL2 receptor antibodies (48).

Membranoproliferative (Mesangiocapillary) Glomerulonephritis

Secondary causes of membranoproliferative (mesangiocapillary) glomerulonephritis (MPGN) (type I) include infections such as viral hepatitis B or C and systemic diseases. Treatment of these underlying causes may thus reduce the risk of recurrence. Recurrent disease should also be differentiated from *de novo* MPGN which occurs as part of the histological changes in patients with chronic transplant nephropathy.

Both type I (with mesangial and subendothelial deposits) and type II (dense deposit disease) primary MPGN have high rates of recurrence after transplantation. Type I MPGN recurs in 20–50% of patients. Clinical manifestations include proteinuria and deterioration of renal function. Risk factors for recurrence include HLA-B8DR3, living related donors and previous graft loss from recurrence (49). The overall incidence of allograft loss at 10 years due to recurrence is around 15% (1). The risk of graft loss from recurrence in a second graft in patients who have experienced a recurrence in the first graft is as high as 80% (49).

Recurrent disease is much more frequent in type II disease, and up to 80–100% of patients are affected. These patients usually present with nonnephrotic range proteinuria within the first year posttransplant and slowly declining renal function. There is no correlation between complement level and recurrence risk. Graft loss due to recurrence occur in 15–30% of patients after 5 years (50). Type III MPGN (with both subepithelial and subendotherial deposits) has been considered as a variant of type I disease, and there are few data regarding its recurrence after kidney transplantation.

A recent report has suggested that the severity of histological abnormalities in the native kidney (interstitial fibrosis, crescent formation and mesangial proliferation) rather than the type of MPGN is related to recurrence risk. Nevertheless, type II MPGN usually has more aggressive glomerular changes and thus a higher risk of recurrence, and poorer prognosis (51). No effective therapy is available for prevention or treatment of recurrent MPGN.

Membranous Nephropathy

Secondary causes of membranous nephropathy (MN) including viral infections and malignancy should be screened. Treatment of these underlying causes may reduce the risk of recurrence in secondary MN. Idiopathic MN recurs in 10–30% of patients after kidney transplantation. Recurrent disease should also be differentiated from *de novo* MN,

which is the most common *de novo* glomerulopathy in renal allografts. The clinical presentation of recurrent disease is characterized by nephrotic range proteinuria. The mean onset time is approximately 10 months post-transplant as compared with the more insidious and later onset of symptoms in *de novo* MN, an entity thought to be related to chronic rejection (52,53). Recent demonstration of antibodies against 'neutral endopeptidase', a protein expressed on the human podocyte cell membrane, causing severe membranous glomerulonephritis in a fetus, suggested that 'neutral endopeptidase' probably plays a significant role in the pathogenesis of the membranous glomerulonephropathy (54). No risk factor for recurrence has been identified. The initial concerns with regard to the risk of recurrence with living related donors, presence of HLA-DR3 in the recipient, and the aggressiveness of native disease have not been substantiated (53). Graft failure from recurrence occurs in 10–15% of patients after 10 years (1). Cyclosporine and mycophenolate mofetil which have been used in treatment of primary MN do not prevent or change the course of recurrent disease (53). There is also no report to suggest therapeutic advantage of tacrolimus or cyclophosphamide over cyclosporine.

Antineutrophil Cytoplasmic Antibody-Associated Glomerulonephritis (Pauci-Immune Cresentic Glomerulonephritis)

Despite better recognition and improved treatment of antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis, a proportion of patients still progress to end-stage renal failure. Recurrence in patients with Wegener's granulomatosis (WG), microscopic polyangiitis (MPAN) and idiopathic necrotizing crescentic glomerulonephritis (CGN) have been reported. Nachman et al. pooled data from 127 patients and reported that 17% of patients had recurrence of vasculitis after 4–89 months of follow-up. Three-fifths of them had renal manifestation and two patients lost their grafts due to recurrence (55). A more recent study by Briganti reported a 10-year incidence of allograft loss of 7.7% in patients with pauci-immune cresentic glomerulonephritis (1).

Pre-transplantation disease course, cANCA or pANCA specificity, disease subtype (WG, MPAN or CGN), ANCA titer (in the absence of clinically active disease) at the time of transplantation, duration of follow-up or donor type do not predict recurrence (55). It is advisable to defer kidney transplantation until the disease is inactive (55). Patients with renal relapses generally showed good response to cyclophosphamide (55–57). For patients with cellular crescents on renal biopsies and high ANCA titer, favorable outcome with combination therapy comprising cyclophosphamide, plasmapheresis with or without intravenous immunoglobulin had been reported (57,58).

Systemic Lupus Erythematosis

Although the prognosis of lupus nephritis has improved over the past few decades, lupus nephritis remains an important cause of end-stage renal failure. Histological recurrence has been reported in up to 30% (59) of transplant recipients. Clinically significant recurrent disease occurs in 2–9% (2,60). With the higher morbidity and poorer general condition of patients during active disease, most centers would postpone renal transplantation until the disease become quiescent for at least 6–9 months (60,61). The duration of dialysis before transplantation and serological status in the absence of clinically active disease do not predict recurrence (59–61). There are anecdotal reports on the efficacy of mycophenolate mofetil in recurrent lupus nephritis (62,63). Graft loss due to recurrent lupus nephritis is uncommon, occurring in 2–4% (59–61). Long-term patient and graft survival are similar to kidney allograft recipients with other underlying diseases (59–61).

Antiglomerular Basement Membrane Disease

Histological recurrence had been reported in up to 50% of patients when kidney transplantation was performed while circulating antiglomerular basement membrane disease (anti-GBM) antibodies were still present (64). With the current practice of deferring transplantation until the disease become quiescent and circulating anti-GBM antibody levels become undetectable for at least 12 months, clinical recurrence is rare and consisted of isolated case reports only (1,3). Good treatment response had been reported in one patient who developed recurrence with positive anti-GBM antibody and cresentic glomerulonephritis treated with pulse steroid, plasmapheresis and cyclophosamide (65) while another patient responded to treatment with immunoadsorption and cyclophosamide (57).

Conclusions

With improving long-term renal allograft survival, recurrent disease has increased prominence as a significant contributor to late graft loss. Knowledge on the risk factors for recurrence, onset time and impact on graft function is prerequisite to informed decisions (Table 2). There are minimal data on the risk of recurrent disease with new immunosuppressive agents, although anecdotal observations caution cyclosporine and/or corticosteroid withdrawal in patients with a history of FSGS, and animal data suggest that it is pertinent to examine the impact of sirolimus on recurrent glomerular diseases. Apart from plasmaphresis for patients with recurrent FSGS, there is no consensus on strategies to prevent or treat recurrent glomerular disease in the kidney allograft. It is important to emphasize that the majority of patients with primary glomerulonephritis as the underlying cause of renal failure enjoy excellent graft and patient

Table 2: Risk of recurrence and graft loss and treatment strategies for different types of glomerulonephritis

	Clinically relevant[1] recurrent risk[2]	Risk of graft loss due to recurrence 5–10 years post-transplant[2]	Prevention/treatment strategies
IgAN	13–46%	2–16%	ACEI and/or ARB for patients with proteinuria ± renal impairment due to recurrent IgAN (26,27)
FSGS	20–50%	13–20%	Avoid living donors for patients with history of rapid graft loss from recurrence (38) Preemptive perioperative plasmapheresis (PP) for 2 weeks for patients with high risk of recurrence (39,40) Chronic PP with or without cyclophosphamide or cyclosporine for patients with relapse after initial course of PP (29,30,35) ? Avoid omission of calcineurin inhibitors in sirolimus based immunosuppressive regimen (24,42,43) ? Avoid induction therapy (47,48)
MPGN			
Type I	20–25%	~15%	No effective preventive or treatment measures
Type II	80–100%	15–30%	Exclude secondary causes
Membranous nephropathy	10–30%	10–15%	No effective preventive or treatment measures Exclude secondary causes
ANCA-associated glomerulonephritis	~17%	6–8%	Defer transplant till disease inactive (55) Cyclophosamide for recurrence (55,56) Combine therapy with PP, cyclophosphamide ± intravenous immunoglobulin for recurrence with high titer of ANCA and cellular crescents in renal biopsies (57,58)
SLE	2–9%	2–4%	Defer transplant till disease inactive (60,61) Consider mycophenolate mofetil for recurrence (62,63)
Anti-GBM	Rare	Rare	Defer transplant till disease inactive Combine therapy with PP/immunoabsorption and cyclophosphamide for recurrence with high anti-GBM titer and cellular crescents in renal biopsies (57,65)

[1] Clinical relevant refer to patients with clinical symptoms of proteinuria/hematuria/renal impairment.
[2] % of transplanted patients.

survival. Also, in spite of the controversy over the risk of recurrence with certain types of glomerulonephritis when the source of allografts is from living donors, the graft survival is largely comparable to patients with other causes of end-stage renal failure. Thus, living related kidney donation can still be encouraged in carefully selected patients and donors. Caution should be exercised in patients with previous rapid graft loss due to recurrent disease in view of the markedly increased risk with subsequent transplants. Research toward identification of biological or immunological markers for individual glomerulonephritis should provide tools to better identify and prevent recurrence.

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Grading of Acute Liver Allograft Rejection

Global assessment of rejection grade made on a review of the biopsy and after the diagnosis of rejection has been established.

Global Assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see reference below)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

* Verbal description of mild, moderate or severe acute rejection could also be labeled as Grade I,II and III, respectively.

Reference Anonymous. Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document. *Hepatology* 1997;25(3):658-63.

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

[FRAMES] [NO FRAMES]

REJECTION ACTIVITY INDEX (RAI)

Criteria which can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document.

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1

Venous Endothelial Inflammation	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3
Total RAI Score = _/9		
Reference Anonymous. Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document. *Hepatology* 1997;25(3):658-63.		

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

[FRAMES] [NO FRAMES]

Histologic Features of Early and Late Chronic Liver Allograft Rejection

Structure	Early CR	Late CR
Small bile ducts (<60 um)	Degenerative changes involving a majority of ducts (eosinophilic transformation of the cytoplasm; increased N:C ratio; nuclear hyperchromasia; uneven nuclear spacing; ducts only partially lined by biliary epithelial cells) Bile duct loss <50% of portal tracts	Degenerative changes in remaining bile ducts Loss in >=50% of portal tracts
Terminal hepatic venules and zone 3 hepatocytes	Intimal/lumenal inflammation Lytic zone 3 necrosis and inflammation Mild perivenular fibrosis	Focal obliteration Variable inflammation Severe (bridging) fibrosis
Portal tract hepatic arterioles	Occasional loss involving <25% of portal tracts	Loss involving >25% of portal tracts
Other	So-called "transition" hepatitis with spotty necrosis of hepatocytes	Sinusoidal foam cell accumulation; marked cholestasis
Large perihilar hepatic artery branches	Intimal inflammation, focal foam cell deposition without lumenal compromise	Lumenal narrowing by subintimal foam cells Fibrointimal proliferation
Large perihilar bile ducts	Inflammation damage and focal foam cell deposition	Mural fibrosis

Reference

- Demetris A, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. : Update of the International Banff Schema for Liver Allograft Rejection: Working Recommendations for the Histopathologic Staging and Reporting of Chronic Rejection *Hepatology 31(3):792-799, 2000*

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Modified HAI Grading: Necroinflammatory Scores

Periportal or Periseptal Interface Hepatitis (piecemeal necrosis) (A)	Score	Confluent Necrosis (B)	Score	Focal (spotty) Lytic Necrosis, Apoptosis, and Focal Inflammation* (C)	Score	Portal Inflammation (D)	Score
Absent	0	Absent	0	Absent	0	None	0
Mild (focal, few portal areas)	1	Focal confluent necrosis	1	One focus or less per 10x objective	1	Mild, some or all portal areas	1
Mild/moderate (focal, most portal areas)	2	Zone 3 necrosis in some areas	2	Two to four foci per 10x objective	2	Moderate, some or all portal areas	2
Moderate (continuous around <50% of tracts or septa)	3	Zone 3 necrosis in most areas	3	Five to ten foci per 10x objective	3	Moderate/marked, all portal areas	3
Severe (continuous around >50% of tracts or septa)	4	Zone 3 necrosis + occasional portal-central (P-C) bridging	4	More than ten foci per 10x objective	4	Marked, all portal areas	4
		Zone 3 necrosis + multiple P-C bridging	5				
		Panacinar or multiacinar necrosis	6				

References

1. Ishak K, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
2. Knodell RG, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1(5):431-5

Total Modified HAI = __/18

*****Does not include diffuse sinusoidal infiltration by inflammatory cells.

Additional features which should be noted but not scored: ❍ Bile-duct inflammation and damage ❍ Lymphoid follicles ❍ Steatosis, mild moderate or marked ❍ Hepatocellular dysplasia, large- or small-cell ❍ Adenomatous hyperplasia ❍ Iron or copper overload ❍ Intracellular inclusions (eg. PAS-positive globules, Mallory bodies)	*Immunohistochemical findings* ❍ Information on viral antigens, lymphocyte subsets or other features, when available, should be recorded and may be semi-quantitatively expressed

Modified Staging: architectural changes, fibrosis and cirrhosis*

Change	Score
No fibrosis	**0**
Fibrous expansion of some portal areas, with or without short fibrous septa	**1**
Fibrous expansion of most portal areas, with or without short fibrous septa	**2**
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	**3**
Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)]	**4**
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	**5**
Cirrhosis, probable or definite	**6**

References

1. Ishak K, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
2. Knodell RG, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1(5):431-5

*****_Additional features which should be noted but not scored:_ Intra-acinar fibrosis, perivenular ('chicken wire' fibrosis) and phlebosclerosis of terminal hepatic venules.

Click here to see guide to score interpretation.

Autoimmune Hepatitis: Revised Scoring System (1999)

(International Autoimmune Hepatitis Group, J. Hepatology 31: 929-938, 1999)

Feature	-5	-4	-3	-2	-1	0	+1	+2	+3
Sex						Male		Female	
Alk phos:ALT or Alk phos:AST (note 1)				>3		1.5-3.0		<1.5	
Serum globulins or IgG above normal						<1x normal	1-1.5x normal	1.5-2x normal	>2x normal
ANA, SMA, or LKM1 (note 2)						<1:40	1:40	1:80	>1:80
AMA		Positive				Negative			
Hepatitis viral markers (note 3)			Positive						Negative
Drug history (note 4)		Yes					No		
Average alcohol intake				> 60 gm/day				<25 gm/day	
Histology	Absence of all of the following: interface hepatitis, lympho-plasmacytic infiltrate, and liver cell rosettes		Biliary changes (note 5) or other defined changes (note 6) (-3 each)				Predominantly lympho-plasmacytic infiltrate, liver cell rosettes (1 each)		Interface hepatitis
Other autoimmune disease (note 7)						Absent		Present	

Seropositivity for other defined autoantibodies (note 8)								Present	
HLA DR3 or DR4 (note 9)						Absent	Present		
Response to therapy (note 10)								Complete	Relapse

Interpretation of scores: An aggregate score greater than 15 prior to therapy constitutes a definite diagnosis of AIH. A score of 10-15 is interpreted as probable AIH. A score greater than 17 following therapy is considered positive, and a score of 12-17 after therapy is considered probable, for the diagnosis of AIH.

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Note 1	The ratio refers to the degree of elevation above upper normal limits (UNL) of these enzymes, i.e., (IU/L alk phos/UNL alk phos)/(IU/L ALT/UNL ALT)	return
Note 2	As determined by indirect immunofluorescence on rodent tissues or, for ANA, on HEp-2 cells. Lower titers, esp. of LKM-1, are significant in children and should be scored at least +1	return
Note 3	SCore for markers of hepatitis A, B, and C viruses (i.e., positive or negative for IgM anti-HAV, HBsAg, IgM anti-HBc, anti-HCV and HCV-RNA). If a viral etiology is suspected despite seronegativity for these markers, tests for other potentially hepatotropic viruses such as CMV and EBV may be relevant.	return
Note 4	History of recent or current use of known or suspected hepatotoxic drugs.	return
Note 5	"Biliary changes" refers to bile duct changes typical of PBC or PSC, ie granulomatous cholangitis or severe concentric periductal fibrosis, with ductopenia, established in an adequate biopsy specimen, and/or a substantial periportal ductular reaction, so-called marginal bile duct proliferation with a cholangiolitis, with copper/copper-associated protein accumulation.	return
Note 6	Any other prominent feature or combination of features suggestive of a different etiology	return
Note 7	Score for history of any other autoimmune disorder(s) in patient or first-degree relatives.	return
Note 8	The additional points should be allocated only in patients seronegative for ANA, SMA, and LKM-1. Other "defined" autoantibodies include pANCA, anti-LC1, anti-SLA, anti-ASGPR, anti-LP, and anti-sulfatide.	return
Note 9	The additional points should be allocated only in patients seronegative for ANA, SMA, and LKM-1. HLA DR3 and DR4 are mainly of relevance to North European, Caucasoid, and Japanese populations. One point may be allocated for other Class II antigens for which there is published evidence of their association with AIH in other populations.	return

Note 10	Assessment of response to therapy is shown in the Table and may be made at any time. Points should be added to those accrued for features at initial presentation.	return

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Definitions of Response to Therapy (AIH Scoring System 1999)

(International Autoimmune Hepatitis Group, J. Hepatology 31: 929-938, 1999)

Response	Definition		
Complete	Either or both of the following: marked improvement of symptoms and return of serum ALT or AST, bilirubin and immunoglobulin values completely to normal within 1 year and sustained for at least a further 6 months on maintenance therapy, or a liver biopsy specimen at some time during this period showing at most minimal activity.	or	Either or both of the following: marked improvement of symptoms together with at least 50% improvement of all liver test results during the first month of treatment with AST or ALT levels continuing to fall to less than twice the upper normal limit within 6 months during any reductions toward maintenance therapy, or a liver biopsy within 1 year showing only minimal activity.
Relapse	Either or both of the following: an increase in serum AST or ALT levels of greater than twice the upper normal limit or a liver biopsy showing active disease, with or without reappearance of symptoms, after a "complete" response as defined above.	or	Reappearance of symptoms of sufficient severity to require increased (or reintroduction of) immunosuppression, accompanied by any increase in serum AST or ALT levels, after a "complete" response as defined above.

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Reference

- Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WGE, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston ALWF, Fainboim L, Heathcote J, Homberg J-C, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RNM, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde K-H, Mieli-Vergani G, Nakanuma Y, Nishioka M, Penner E, Porta G, Portmann BC, Reed WD, Rodes J, Schalm SW, Scheuer PJ, Schrumpf E, Seki T, Toda G, Tsuji T, Tygstrup N, Vergani D, Zeniya M. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatology 1999; 31:929-938.*

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

Histological Scoring System for Nonalcoholic Fatty Liver Disease

Components of NAFLD Activity Score (NAS) and Fibrosis Staging

Nonalcoholic Steatohepatitis Clinical Research Network

NAS Components (see scoring interpretation)			
Item	**Score**	**Extent**	**Definition and Comment**
Steatosis	**0**	**<5%**	**Refers to amount of surface area involved by steatosis as evaluated on low to medium power examination; minimal steatosis (<5%) receives a score of 0 to avoid giving excess weight to biopsies with very little fatty change**
	1	**5-33%**	
	2	**>33-66%**	
	3	**>66%**	
Lobular Inflammation	**0**	**No foci**	**Acidophil bodies are not included in this assessment, nor is portal inflammation**
	1	**<2 foci/200x**	
	2	**2-4 foci/200x**	
	3	**>4 foci/200x**	
Hepatocyte Ballooning	**0**	**None**	
	1	**Few balloon cells**	**The term "few" means rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline**
	2	**Many cells/prominent ballooning**	**Most cases with prominent ballooning also had Mallory's hyalin, but Mallory's hyaline is not scored separately for the NAS**
Fibrosis Stage (Evaluated separately from NAS)			
	0	**None**	
	1	**Perisinusoidal or periportal**	
	1A	**Mild, zone 3, perisinusoidal**	"delicate" fibrosis

Fibrosis	**1B**	**Moderate, zone 3, perisinusoidal**	"dense" fibrosis
	1C	**Portal/periportal**	This category is included to accommodate cases with portal and/or peri portal fibrosis without accompanying pericellular/perisinusoidal fibrosis
	2	**Perisinusoidal and portal/periportal**	
	3	**Bridging fibrosis**	
	4	**Cirrhosis**	

Total NAS score represents the sum of scores for steatosis, lobular inflammation, and ballooning, and ranges from 0-8. Diagnosis of NASH (or, alternatively, fatty liver not diagnostic of NASH) should be made first, then NAS is used to grade activity. In the reference study, NAS scores of 0-2 occurred in cases largely considered not diagnostic of NASH, scores of 3-4 were evenly divided among those considered not diagnostic, borderline, or positive for NASH. Scores of 5-8 occurred in cases that were largely considered diagnostic of NASH

Reference: Kleiner D.E., Brunt E.M., Van Natta M., Behlinh C., Contos M.J., Cummings O.W., Ferrell L.D., Liu Y.-C., Torbenson M.S., Unalp-Arida A., Yeh M., McCullough A.J., Sanyal A.J. for the Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313-1321, 2005.

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Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document

AN INTERNATIONAL PANEL COMPRISED OF ANTHONY J. DEMETRIS, KENNETH P. BATTS, AMAR P. DHILLON, LINDA FERRELL, JOHN FUNG, STEPHEN A. GELLER, JOHN HART, PEKKA HAYRY, WALTER J. HOFMANN, STEPHAN HUBSCHER, JOSEF KEMNITZ, GEORGE KOUKOULIS, RANDALL G. LEE, KLAUS J. LEWIN, JURGEN LUDWIG, ROD S. MARKIN, LIDIJA M. PETROVIC, M. JAMES PHILLIPS, BERNARD PORTMANN, JORGE RAKELA, PARMJEET RANDHAWA, FINN P. REINHOLT, MICHEL REYNÈS, MARIE ROBERT, HANS SCHLITT, KIM SOLEZ, DALE SNOVER, EERO TASKINEN, SWAN N. THUNG, G. WELDON TILLERY, RUSSELL H. WIESNER, D. G. DEREK WIGHT, JAMES W. WILLIAMS, and HIROHIKO YAMABE

A panel of recognized experts in liver transplantation pathology, hepatology, and surgery was convened for the purpose of developing a consensus document for the grading of acute liver allograft rejection that is scientifically correct, simple, and reproducible and clinically useful. Over a period of 6 months pertinent issues were discussed via electronic communication media and a consensus conference was held in Banff, Canada in the summer of 1995. Based on previously published data and the combined experience of the group, the panel agreed on a common nomenclature and a set of histopathological criteria for the grading of acute liver allograft rejection, and a preferred method of reporting. Adoption of this internationally accepted, common grading system by scientific journals will minimize the problems associated with the use of multiple different local systems. Modifications of this working document to incorporate chronic rejection are expected in the future. (HEPATOLOGY 1997;25:658-663.)

The success of hepatic transplantation has resulted in its widespread use for treatment of many patients with endstage liver disease; it is currently offered by more than 100 centers worldwide. One-year survival rates range from 70% to 90%; and long-term survival of 50% to 60% of patients is not uncommon.[1] Therefore, an increasing number of physicians, including pathologists, many of whom have no specific training in transplantation biology, will become involved in the care of organ allograft recipients.

Despite the good short-term and acceptable long-term survival after hepatic transplantation, the morbidity associated with long-term immunosuppression is significant and rejection remains a persistent, but usually manageable, problem. Clinical research to improve patient survival and lessen morbidity is, therefore, inherent to the clinical practice of hepatic transplantation. Because patient follow-up and successful application of developments could be simplified by a common scale of recognizing, naming, and grading the severity of acute liver allograft rejection, members of an international consensus panel recently agreed upon a common nomenclature and set of definitions.[2] The group next agreed to create an internationally acceptable grading system, which has already been developed for kidney,[3] heart,[4] and lung.[5] At the Third Banff Conference on Allograft Pathology, a group of specialists in liver transplantation from North America, Europe, and Asia met for this purpose.

DEFINITION OF ACUTE REJECTION

In general, organ allograft rejection can be defined as, "an immunological reaction to the presence of a foreign tissue or organ, which has the potential to result in graft dysfunction and failure."[2] This report is specifically concerned with acute rejection, recently defined by the international consensus document on terminology for hepatic allograft rejection[2] as, "inflammation of the allograft, elicited by a genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal veins and hepatic venules and occasionally the hepatic artery and its branches."[2] Early rejection, cellular rejection, nonductopenic rejection, rejection without duct loss, and reversible rejection are synonyms for acute rejection that appear in the literature, but their use is discouraged. The general clinical, laboratory, and histopathological abnormalities listed below were derived from the international consensus document.[2]

CLINICAL AND LABORATORY FINDINGS

Viewed from a biological perspective, any recipient's immune system will likely be perturbed after transplantation, resulting in immune activation.[2] However, viewed from a clinical perspective, because of baseline immunosuppressive therapy only some recipients manifest clinical symptoms of allograft recognition with, in the case of liver transplantation, liver biochemical abnormalities (most often), or frank hepatic dysfunction.[2] Therefore, it is important to distinguish between "biological" and "clinically relevant" rejection. The latter may require additional immunosuppressive treatment, although the distinction is not always achievable and treatment philosophies differ at various centers. This is particularly true for hepatic allografts, which are widely acknowledged to be unique. They are more resistant than others to humoral rejection, and are accepted without immunosuppressive therapy in some small and large experimental animal species. Of potential importance for human transplantation is the observation that in all animals in which a liver allograft is eventually accepted without drugs, the allograft undergoes a transient acute rejection crisis.[6-9] Thus, it should be understood that the histopathological diagnosis of acute rejection may not automatically signal that treatment is indicated, particularly if it is low grade. Adoption of a standardized histopathological grading system possibly could help determine if, and at what point, the histopathological severity of rejection can predict the need for, and success of antirejection

Abbreviations: RFH, Royal Free Hospital; RAI, rejection activity index.

From the Department of Pathology—Division of Transplantation, University of Pittsburgh Medical Center, Pittsburgh, PA.

Received February 9, 1996; accepted October 17, 1996.

Address reprint requests to A. J. Demetris, M.D., Director, Division of Transplant Pathology, University of Pittsburgh Medical Center, E1548 Biomedical Science Tower, Pittsburgh, PA 15261.

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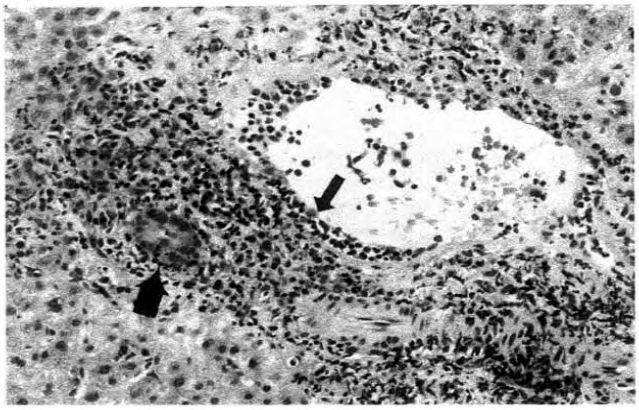

FIG. 1. Grading of acute rejection assumes the diagnosis has already been established: this portal tract shows all three of the typical histopathological features, two of which are required to make the diagnosis. There is: 1) a portal inflammatory infiltrate containing blastic lymphocytes and eosinophils; 2) subendothelial localization of the inflammatory cells in a portal vein branch (small arrow), and 3) inflammation and damage of small bile ducts (large arrow). If the subendothelial inflammation similar to this was present in most or all of the portal and/or hepatic venules, an RAI score of 2 for venous endothelial inflammation would be assigned.

therapy (see "Clinicopathological Correlation and Treatment of Acute Rejection").

When clinically apparent, acute rejection is usually first recognized between 5 and 30 days after transplantation. Earlier or later presentations can be seen in patients that receive less than therapeutic baseline immunosuppression. The clinical findings in early phases of mild acute rejection are often absent, although in late or severe cases, clinical findings include fever as well as swelling, cyanosis, and tenderness of the allograft. Bile often becomes pale in color and the flow is decreased. Occasionally, ascites develops because of liver swelling with increased intrahepatic pressure.[2]

Liver dysfunction, when present, usually manifests as concomitant nonselective elevations of the results of some or all of the standard liver injury tests, including total bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, and alkaline phosphatase.[2] Leukocytosis and eosinophilia are also frequently present. Unfortunately, all clinical and laboratory findings lack sensitivity or specificity. The diagnosis is considered on clinical grounds and confirmed by examination of a core needle biopsy specimen. Some centers find that fine-needle aspirates of the allograft are useful adjunct.

HISTOPATHOLOGIC FINDINGS

Most investigators have observed similar histopathological findings associated with acute rejection.[10-22] Core needle biopsy specimens may show the following: 1) mixed but predominantly mononuclear portal inflammation, containing blastic (activated) lymphocytes, neutrophils, and frequently eosinophils; 2) bile duct inflammation/damage; and 3) subendothelial inflammation of portal veins or terminal hepatic venules.[2] At least two of these three features are required for a histopathological diagnosis of acute rejection (Fig. 1). Biochemical evidence of liver damage manifests as increased results of tests for liver injury, usually elevation of serum γ-glutamyl transpeptidase and alkaline phosphatase activities, are also frequently present. The diagnosis is strengthened if > 50% of the ducts are damaged or if unequivocal endotheliitis of portal vein branches or terminal hepatic venules can be identified. Occasional cases show mild mononuclear inflammation of the perivenular regions with only focal portal tract changes. Additional findings such as ductopenia, spillover/piecemeal necrosis, eosinophilia, lobular inflammation, perivenular necrosis, arteritis, and inflammatory bridging, have been used in some systems for histopathological grading (see below).

Treatment of acute rejection with additional immunosuppression before a biopsy specimen is obtained may make the histopathological diagnosis more difficult, because of subsequent loss of the subendothelial infiltration of veins and of eosinophils, and a relative decrease in the number of mononuclear inflammatory cells.

GRADING OF ACUTE LIVER ALLOGRAFT REJECTION (CRITIQUE OF CURRENTLY POPULAR SYSTEMS)

The panel reviewed each system and agreed that the consensus scheme should fulfill the following criteria: scientific correctness, clinical relevance, simplicity, and reproducibility. They also recognized the need for flexibility and future modifications and therefore proposed a working formulation format for the current document.

The grading system used in Pittsburgh[23] is derived from those developed for kidney allograft.[24] It is based on the concept that serious injury from rejection is related to vascular compromise and ischemia, which can morphologically manifest as inflammatory or necrotizing arteritis and/or parenchymal necrosis and hemorrhage. The grading system developed in Minnesota by Snover et al.[19] is more specific to the liver and is based on a combination of an estimate of the severity of the inflammation and the presence and severity of damage or loss of key structures targeted for injury, such as the arterial vasculature or bile ducts. The above two systems have the advantage of simplicity[19, 23] and rely on pathophysiological concepts validated in renal transplantation. Prognostic significance has been shown at a single center.[19] Unfortunately, some of the features used in these schemes to define severe rejection are rarely found, poorly reproducible, or present so frequently in nonrejection complications that their usefulness in grading scheme is limited.[25] For example, while inflammatory or necrotizing arteritis[19, 23] represents a serious injury to the allograft, reproducibly identifying it in core needle biopsies is problematic.[25] In contrast, ballooning of perivenular hepatocytes[19] is frequently present in nonrejection graft syndromes and may not imply serious graft injury from an immunological insult. Bile duct loss, which has also been used to identify severe acute rejection more accurately reflects chronic rejection and possibly, a stage rather than a grade of rejection.

Kemnitz et al.[20,26] have devised a scheme similar to those mentioned above. However, increased emphasis is placed on precise numerical estimates of lobular injury, such as the percentage of necrosis, which may be difficult to reproduce and may not necessarily reflect rejection-related injury. Moreover, none of the systems was tested for reproducibility.

The European grading system for acute liver allograft rejection, developed by Hubscher and Dousset et al. at Birmingham[27-29], is based on a semiquantitative analysis of the diagnostic triad of Snover et al.[18] In this system, portal inflammation, bile duct damage, and venous endothelial inflammation are each graded semiquantitatively on a scale of 0 (absent) to 3 (severe). The individual scores are then added to produce an overall rejection score of 0 to 9, which is then converted to a rejection grade as follows: 0 to 2 = no rejection, 3 = borderline (consistent with), 4 to 5 = mild, 6 to 7 = moderate, and 8 to 9 = severe acute rejection. This system offers the attractive feature of quantifying the necro-inflammatory activity, as has recently become popular in the reporting and follow-up of patients with chronic hepatitis.[30-33] It also shows a good correlation between histological severity and clinical biochemical signs of graft dysfunction.[29] However, no obvious prognostic value has been shown.

The Royal Free Hospital, London (RFH) grading system[34] consists of a semiquantitative assessment of the diagnostic features of rejection, defined as immunosuppression responsive inflammation of rejection type, and identified by discriminant analysis. Mixed portal inflammation, eosinophils, endotheliitis, and bile duct damage were found to be independent, statistically significant contributors to the histological diagnosis of acute rejection. Each of the features are scored on a scale of 0 to 3, as in the European grading system, and a total score is derived by adding the individual scores together. Apart from the inclusion of eosinophils, which are of known diagnostic[35,36] and pathophysiological significance[37,38] as a separate variable in the RFH scheme, it is virtually identical to the European grading system. Like the European system, the RFH system offers a quantitative scale for the rejection-related activity, and is reproducible at the home institution.[34] However, neither the European system, nor the RFH system has been shown to have prognostic significance and the numerical cutoff points corresponding to the different degrees of rejection (and consequent therapeutic thresholds) need to be validated. In addition, there are no studies of inter-institutional scoring reproducibility.

The recently published scheme by the National Institute of Diabetes and Digestive Diseases and Kidney Diseases[39] had the advantages of being reproducible with prognostic significance documented at several centers. Unfortunately, the imprecise language used to explain the cutoffs for moderate and severe rejection makes the system difficult to follow, even for those experienced in the field.

INTERNATIONAL GRADING SYSTEM FOR ACUTE LIVER ALLOGRAFT REJECTION (RECOMMENDATIONS OF THE PANEL)

Grading of Rejection. The grading of rejection, as with hepatitis,[33] is a measure of the severity of the necro-inflammatory process. In addition, because rejection is more vasculocentric and vasculodestructive than hepatitis, some estimate of vascular or ischemic damage is needed to assess the full extent of the insult. This can be accomplished either by a global assessment of the biopsy using a "gestalt"[25] approach, or semiquantitatively with the assignment of numerical scores to different histopathological parameters. No data support one approach over the other, and in practice the two methods yield similar results (see below). Moreover, the semiquantitative approach could complement the global assessment by offering a greater degree of precision, by forcing the pathologist to critically evaluate important histopathological features. Conversely, the global approach can temper the semiquantitative analysis in cases with active inflammation and high scores, in which there is little architectural damage.

The panel agreed that existing grading systems for acute liver allograft rejection are conceptually similar, and that like chronic hepatitis, frequent monitoring and reporting of disease activity is an important function of biopsy analysis.[30-33] Therefore, in coming to a consensus, the panel drew upon the strengths, hopefully avoided the pitfalls, and corrected the weaknesses of the currently available grading systems. Portal inflammation, bile duct damage, subendothelial inflammation of portal veins, and terminal hepatic venules, strictly defined inflammatory or necrotizing arteritis and eosinophils (in the proper context) are features that the panel members regard as diagnostic of acute rejection. Portal inflammation, bile duct damage, strictly defined arteritis, and possibly confluent perivenular necrosis associated with perivenular inflammation are features that may also have prognostic significance, based on previous publication,[19,39] or personal experience. However, arteritis, as well as other findings such as bile duct loss, interstitial hemorrhage, and perivenular necrosis without inflammation are not included in the scheme, because they are poorly reproducible findings, considered to be part of chronic rejection, or also encountered frequently in nonrejection-related complications, respectively. If strictly defined arteritis can be shown to be a reproducible observation and present in more than a rare case, the current system can be modified to include it.

TABLE 1. Grading of Acute Liver Allograft Rejection

Global Assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see text)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

NOTE. Global assessment of rejection grade mode on a review of the biopsy and after the diagnosis of rejection has been established.

* Verbal description of mild, moderate, or severe acute rejection could also be labeled as Grade I, II, and III, respectively.

Being aware of the need for acceptability and thus simplicity, the panel agreed on a verbal grading of acute rejection based on the overall appearance of the biopsy according to the criteria listed in Table 1 (Fig. 2). It should be re-emphasized however, that any grading of acute rejection already presupposes that the diagnosis has been established. For example, use of the "indeterminate" category of acute rejection should be restricted to cases that have minor degrees of cellular infiltration that could possibly represent low grade or early acute rejection, but fail to meet the minimal diagnostic criteria. "Indeterminate" should not be used for cases in which one is unsure whether the inflammation is related to some other condition, such as chronic hepatitis C (see Complicating Conditions). After the global assessment, three specific features, portal inflammation, bile duct inflammation/damage, and venular inflammation, can be more critically evaluated and semiquantitatively scored on a 0 to 3 (mild, moderate, and severe) scale, according to the criteria listed in Table 2. The three are then added together to arrive at a final Rejection Activity Index (RAI) (Table 2), similar to the scoring developed for chronic hepatitis.[30-33] Modifications of the above system [19, 22, 29, 39] were made to arrive at a consensus scheme, so that features given the highest scores on the semi-quantitative analysis were the same as those shown to be of prognostic significance using the overall approach.

Potential problems using this method however, include: 1) the global assessment of rejection may under or overestimate the severity based on a semi-quantitative analysis and 2) the greater degree of "precision" achieved semiquantitatively may occur at the expense of reproducibility. We think that these pitfalls are unlikely to occur because both processes measure the same parameters or endpoints. Moreover, evaluation of a series of 50 posttransplantation liver allograft biopsy specimens using both methods by one of us (AJD) showed no significant differences between the systems. The reproducibility of the semiquantitative analysis will be the subject of future study by this group. The RAI, like other semiquantitative assessments of necro-inflammatory activity, is particularly attractive when evaluating new drugs or other treatment protocols and for comparison with previous biopsy specimens. Thus, it will be most valuable at academic centers involved with new developments in the field. Although strongly recommended for routine patient care, it is not required for day-to-day use if the pathologist chooses otherwise.

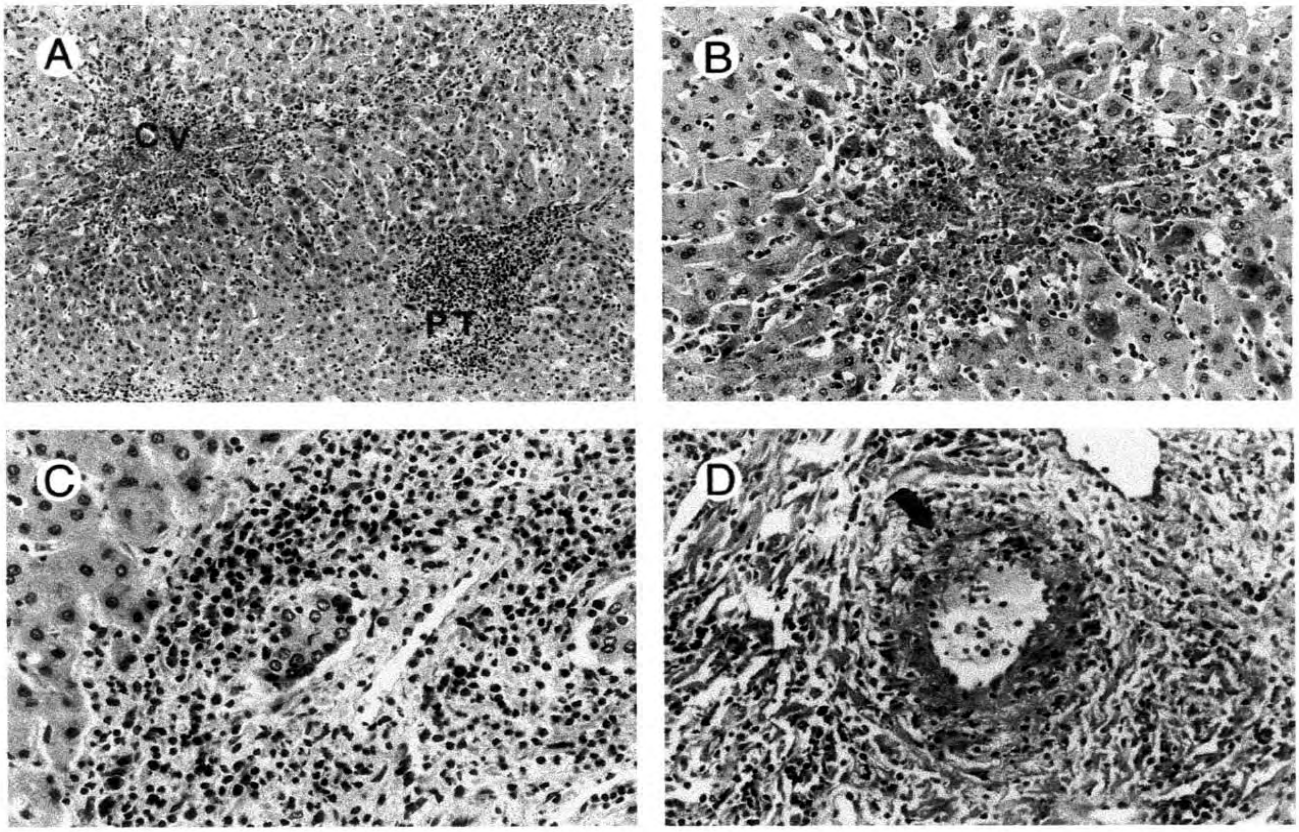

FIG. 2. (A) Low power photomicrograph of a failed liver allograft with severe acute rejection. Note the prominent portal tract (PT) and central vein (CV) inflammation, associated with confluent perivenular necrosis, which is shown at a higher magnification in (B). These findings would elicit a diagnosis of severe acute rejection. (C) In the same liver allograft, the bile duct inflammation and damage was widespread, and there was focal lumenal disruption, eliciting an RAI score of 3 for bile duct damage (Table 2). Both the portal and venous endothelial inflammation were also scored as severe, or "3," resulting in a total RAI score of 9/9. (D) Sections from the hilum of this failed allograft also revealed clear cut necrotizing arteritis (arrow), which is rarely detected with certainty in needle biopsies.

Staging of Rejection. Staging of a biological phenomenon is performed in an attempt to codify a process that is largely unidirectional and evolves in a predictable pattern over a relevant period of time. Acute liver allograft rejection is, for the most part, widely considered to be a completely reversible phenomenon. In the uncommon event of allograft failure from acute rejection, the evolution is relatively rapid. Therefore, acute rejection is not readily amenable to staging. Chronic rejection on the other hand, usually evolves more slowly and is often, but not always,[40,41] unidirectional or irreversible. At this time it is not clear whether acute and chronic rejection represent the ends of a spectrum of alloreactivity, or if they are completely different biological processes. Considerable data suggest the former, because both processes appear to be triggered by alloreactivity, and persistent or severe acute rejection can result in allograft failure from chronic rejection.

Clinicopathological Correlation and Treatment of Acute Rejection. As alluded to in the introductory sections, the histopathological diagnosis of acute rejection does not necessarily imply that the rejection is clinically significant or requires treatment with increased immunosuppression. In fact, Schlitt et al.[42] have shown that up to 40% of patients in whom a biopsy shows acute rejection, according to the criteria of Snover et al.[18,19] did not have clinically apparent graft malfunction or significant elevations of results of liver injury tests, and did not require additional immunosuppressive therapy. Similar conclusions were also reached in a study from Birmingham, in which 70% of histologically mild rejection episodes received no additional immunosuppression, without any adverse outcome.[28,29] A survey of the panel members showed no clear-cut consensus on the therapeutic approach to mild acute rejection (RAI $\leq$ 4) as defined in this report. In contrast, most centers report that patients with histopathological moderate or severe rejection (RAI $\geq$ 6) experience significant elevations of liver injury tests and the vast majority probably should, and usually are treated with additional immunosuppression. At present, no therapeutic recommendations can be inferred from the mild acute rejection grade, although some centers have exercised the option of routinely obtaining a follow-up biopsy after 1 to 2 weeks.

Complicating Conditions. Liver allografts are frequently affected by more than one condition. In the first few weeks after transplantation, preservation-related changes and mechanical problems with the vascular and/or biliary tree are the conditions that most commonly co-exist with acute rejection. Separation of the necro-inflammatory and ischemic damage of rejection from the same type of nonrejection insults is at times problematic, but achievable for the most part. For example, perivenular necrosis can occur in both preservation injury and severe rejection. However, the concomitant presence of mononuclear perivenular inflammation, portal changes of rejection, and absence of perivenular necrosis in a prior biopsy, are features that help to distinguish between the two. In contrast, more than several months after

TABLE 2. Rejection Activity Index

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
Venous Endothelial Inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

NOTE. Total Score = Sum of Components. Criteria that can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document.

transplantation, chronic viral hepatitis and recurrence of autoimmune chronic inflammatory disorders pose considerable difficulties in differential diagnosis and with grading or scoring of rejection related activity.

The problem of differentiating duct damage associated with complicating conditions such as viral hepatitis C from that seen in acute rejection can be minimized by applying strict diagnostic criteria: damage of more than an occasional bile duct, the presence of unequivocal endotheliitis, and absence of significant lobular disarray and necro-inflammatory activity favor a diagnosis of acute rejection. However, problematic cases will still be encountered, and implicit in any grading scheme for acute rejection (including this one), is the notion that grading can be reliably applied to biopsies only when rejection is thought to be the sole or predominant cause of graft damage. Therefore, in cases where other causes of cellular infiltration are suspected, neither the overall grade nor the scores can be reliably applied. In such cases, it is left to the judgment of the pathologist whether apportioning the necro-inflammatory activity to rejection or other concurrent conditions is appropriate.

CONCLUSIONS AND RECOMMENDATION

Although the adequacy of any particular biopsy is ultimately left to the judgment of the pathologist, the panel recommends that at least two hematoxylin and eosin stained sections from at least two different levels, of a core needle biopsy containing at least five triads be examined. The adequacy of the biopsy in the absence of any diagnostic findings when fewer than five portal tracts are identified, is again left to the pathologist's judgment.

The following format for the grading and reporting of acute liver allograft rejection is recommended, although all of this information is not needed in every case. The type of specimen and time after transplantation, if available, should be listed first. This is followed by the histopathological diagnosis(es). Although not necessary, some pathologists may prefer to list first the diagnosis perceived to be of greatest significance, followed by the second most important, and so forth. However, a comment on the presence or absence of acute rejection should be given for every biopsy, either in the diagnosis or comment section. This is followed by reporting of an RAI. The presence of chronic injury, such as bile duct loss or obliterative arteriopathy should also be listed. Lastly, a comparison with the most recent previous biopsy should be made if the pathologist feels that such a comparison is warranted. The following are several examples:

1. Liver allograft, needle biopsy (7 days posttransplantation)
   (a) Moderate preservation injury
   (b) No evidence of rejection (RAI = 0)
   (c) No previous biopsy for comparison
2. Liver allograft, needle biopsy (10 days posttransplantation)
   (a) Acute rejection, moderately active (RAI = 7)
   (b) Significantly worse than previous biopsy (S95-999 of 02/06/95{RAI = 2})
3. Liver allograft, needle biopsy (10 weeks posttransplantation)
   (a) Acute hepatitis, viral type C
   (b) No rejection (RAI = 0)
4. Liver allograft, needle biopsy (18 months posttransplantation)
   (a) Chronic hepatitis, viral type B, moderately active (HAI = 14)
   (b) Acute rejection, mildly active (RAI = 4)
   (c) Duct loss in 5/9 portal triads, suggestive of chronic rejection

We believe that this system will be easy to use and useful for physicians caring for allograft recipients. There already are data available to suggest that it will be both reproducible and have prognostic significance,[39] yet flexible enough to incorporate future development like the inclusion of chronic rejection or staging of rejection. We urge scientific journals to adopt this reporting system, classification, and grading of liver allograft rejection, to overcome the obstacles presented by the multiple schemes that currently exist and facilitate comparisons among different centers.

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SPECIAL ARTICLE

Liver Biopsy Interpretation for Causes of Late Liver Allograft Dysfunction

Banff Working Group[1]

Evaluation of needle biopsies and extensive clinicopathological correlation play an important role in the determination of liver allograft dysfunction occurring more than 1 year after transplantation. Interpretation of these biopsies can be quite difficult because of the high incidence of recurrent diseases that show histopathological, clinical, and serological features that overlap with each other and with rejection. Also, more than one insult can contribute to allograft injury. In an attempt to enable centers to compare and pool results, improve therapy, and better understand pathophysiological disease mechanisms, the Banff Working Group on Liver Allograft Pathology herein proposes a set of consensus criteria for the most common and problematic causes of late liver allograft dysfunction, including late-onset acute and chronic rejection, recurrent and new-onset viral and autoimmune hepatitis, biliary strictures, and recurrent primary biliary cirrhosis and primary sclerosing cholangitis. A discussion of differential diagnosis is also presented. (HEPATOLOGY 2006;44:489-501.)

Distinguishing among potential causes of late liver allograft dysfunction can be difficult because of overlapping clinical, serological, and histopathological features. Most problematic biopsies are obtained more than 1 year after transplantation. Currently, diagnoses are made using center-specific criteria, but a standardized set of criteria has not been generally agreed upon. Availability of standardized criteria[1,2] would enable centers to compare and pool results, improve therapy, and better understand pathophysiological disease mechanisms.

Native disease recurrence is a significant problem and can be categorized as follows: (1) infectious (viral hepatitis A, B, C, D.), (2) dysregulated immunity (autoimmune hepatitis [AIH], primary biliary cirrhosis [PBC], primary sclerosing cholangitis [PSC], and sarcoidosis),[3] (3) malignancies, (4) toxic (*e.g.*, alcohol, adverse drug reactions.), (5) metabolic disorders, including nonalcoholic steatohepatitis, and (6) other diseases, such as idiopathic granulomatous hepatitis,[4] postinfantile giant cell hepatitis,[5] and Budd-Chiari syndrome,[6] that are of uncertain etiology or multifactorial in origin. Recurrent infectious and dysregulated immunity diseases pose the most difficult diagnostic challenges and are addressed herein. Some diseases in the remaining categories can also recur, but because they do not usually present diagnostic challenges they are not discussed further.

Immunological Considerations

Immune recognition of differences in major histocompatibility complex antigens triggers a characteristically robust inflammatory response in the first few months after transplantation referred to as early acute rejection.[2] Like all other immune responses, acute and especially chronic rejection reactions[7,8] evolve over time and diversify via "epitope spreading."[9] Tissue damage during the initial phase releases cryptic antigens that activate endogenous danger signals. Recipient dendritic cell antigen uptake and self-reactive T and/or B lymphocyte priming[10] triggers "autoantibody" production and immunity directed against non–major histocompatibility complex determinants. Some non–major histocompatibility complex cytoplasmic, nuclear, and matrix protein antigens[11-14] (reviewed in Graft[15-18]) are shared by the donor and recipient, whereas others may be donor-specific.

Long-Term Protocol Biopsies

Most programs obtain biopsies when changes in liver tests represent a significant deviation from baseline values. Obtaining protocol allograft biopsies in asymptomatic

Abbreviations: AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; HCV, hepatitis C virus; LAR, late-onset acute rejection.

[1]*See end of article text for complete list of authors and affiliations.*

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Address reprint requests to: A. J. Demetris, M.D., E741 UPMC Montefiore, 200 Lothrop Street, Pittsburgh, PA 15213. E-mail: demetrisaj@upmc.edu; fax: 412-647-2084.

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long-term survivors with normal or near-normal liver tests is controversial. Considerations such as potential morbidity and mortality, cost, inconvenience, use of resources, and potential impact of unexplained histopathological findings should be weighed against potential individual and/or societal benefits.[4,19-24] These include (1) early detection of clinically inapparent disease,[19,24] (2) recognition of nonalcoholic steatohepatitis as a significant cause of cryptogenic cirrhosis in the United States[25] but not in England,[26] (3) identification of recipients that might be successfully weaned from immunosuppression,[27] (4) recognition of late-onset rapid hepatitis C virus (HCV) progression,[21] and (5) impact of alcohol use.[20]

Approximately 75% of biopsies from long-surviving recipients with abnormal liver tests or symptoms show significant histopathological abnormalities.[4,19-23] These abnormalities are usually attributable to recurrent disease or biliary tract strictures, some of which occur as a late complication of preservation injury.[4,19-23] The incidence and significance of histopathological abnormalities in long-surviving recipients without abnormal liver tests or symptoms is dependent on the original disease: up to 25% show significant abnormalities when obtained from recipients with original diseases that commonly recur (*e.g.*, HCV, PBC, AIH).[4,19-23]

Even in the absence of recurrent disease, minor histopathological abnormalities appear in approximately two thirds of biopsies obtained from long-surviving asymptomatic recipients with normal liver tests.[4,19-23] These include nodular regenerative hyperplasia changes and thickening/hyalinization of small hepatic artery branches[4,28] (probably side effects of immunosuppression) and "nonspecific" portal and lobular inflammation.[4,22-24] The pathogenesis, significance, and long-term consequences of nonspecific inflammation (*e.g.*, idiopathic posttransplantation hepatitis), portal venopathy, and nodular regenerative hyperplasia are in need of further study.

Recurrent HCV disease progression is significantly more rapid than HCV in native livers. Disease progression rates for recurrent hepatitis B virus, PBC, PSC, nonalcoholic steatohepatitis, and AIH are difficult to study because of the small number of long-term survivors with biopsies and chronic immunosuppression, as well as introduction of new medical therapies. Regardless, nearly all recurrent diseases can potentially cause allograft cirrhosis.

Practical Problems and Approach to Biopsy Interpretation

Most late causes of liver allograft injury are first detected because of abnormalities in routinely monitored liver tests; clinical signs and symptoms are much less common. When signs or symptoms do occur, they are similar to those seen in the general population with the same causes of liver injury. Examples include fever and upper right quadrant pain in ascending cholangitis; fatigue, nausea, vomiting, and jaundice in viral hepatitis; relapsing bacteria in hepatic infarcts, *etc.*

Many late posttransplantation biopsies show portal-based chronic inflammation with variable interface activity. Subtle histopathological differences relied upon to distinguish among several possible specific causes of dysfunction are not always present or reliable. Occasionally, rendering a definitive diagnosis may not be possible in the early stages of a disorder. A caveat of "features suggestive of early" emphasizes a tentative diagnosis.

Laboratory tests used to establish a diagnosis before transplantation may not have the same significance after transplantation. Antimitochondrial antibodies and antinuclear antibodies often persist after transplantation in patients with PBC or AIH, albeit at lower titers, even without histopathological evidence of recurrent disease. Patients without AIH before transplantation can develop autoantibodies either as a complication of otherwise typical rejection[11,12,29] or in association with new-onset AIH.[30-36] "Non–organ-specific" autoantibodies have been detected in up to 71% of patients after liver transplantation,[37] emphasizing the need for clinicopathological correlation.

More than 1 insult can contribute to late posttransplantation dysfunction. Biopsy analysis can help to determine the main component of injury, but careful clinicopathological correlation is needed. Levels of immunosuppression can influence biopsy findings and the severity of recurrent viral hepatitis, AIH, and rejection. For example, late-onset acute rejection (LAR) is often precipitated by inadequate immunosuppression and recipients with AIH and other autoimmune disorders usually require more immunosuppression to prevent rejection and disease recurrence. Too much immunosuppression can trigger cholestatic HCV hepatitis. Lymphoid depletion followed by rapid withdrawal of immunosuppression can precipitate aggressive HCV recurrence.[38]

Biopsy interpretation should include an assessment of adequacy, systematic examination, and thorough clinicopathological correlation. Adequacy is ultimately the subjective opinion of the pathologist, but in general, at least 6 small portal tracts should be sampled. The findings should then be correlated with the original disease, immunosuppression, liver tests, viral serology, and immunology and radiology findings.

Generalized Criteria

Criteria used to distinguish rejection from AIH can be melded into generalized criteria applicable to other causes

Table 1. Incidence, Risk Factors, and Clinical Observations

Diagnosis	Incidence at 5 Years of Recurrent Disease	Risk Factors for Disease Recurrence and/or Severe Recurrent Disease	Clinical/Immunological/Radiological Observations
Recurrent AIH	~30%	Suboptimal immunosuppression; type I > type II disease; severe inflammation in native liver before transplantation; longer duration of follow-up HLA DR3 or DR4 recipient status may reflect more severe disease	Usually need higher baseline immunosuprression (see text) HLA DR3 and/or DR4 genotype often present
De novo AIH	<5%	May be more common in children, but this assumption has been questioned recently	Same as above
Recurrent HBV	100% if HBV DNA is positive; less frequent if HBV DNA is negative	Anti-HBc–positive donor Inadequate anti-HBV treatment HBV mutants	Recurrent HBV disease not usually a significant problem because of treatment with effective antiviral drugs
Recurrent HCV	Nearly universal in those with HCV replication before transplantation	HCV RNA in blood helpful in differential diagnosis (>30,000,000 IU/L); increased risk of cholestatic hepatitis Significant acute or chronic rejection usually occurs only in association with relatively low HCV RNA levels (<5,000,000 IU/L)	Greater viral burden and more rapid progression of fibrosis than in general population Severity of hepatitis often worse with genotype 1 viruses Variable disease progression Subset of recipients with late-onset rapid progression
Recurrent PBC	20%-30%; increases with time	Tacrolimus as baseline immunosuppression; living-related donor; steroid and other immunosuppression withdrawal May recur as AIH	Initial diagnosis often made via biopsy in asymptomatic recipient with or without increased liver tests
Recurrent PSC	20%-30%; increases with time	Male sex; donor–recipient sex mismatch Intact colon at time of transplantation Patients at increased risk of rejection	Cholangiographically confirmed biliary strictures occurring >90 days after liver transplantation Mural irregularity, diverticulum-like outpouchings, and an overall appearance resembling PSC Patient and allograft survival not adversely affected up to 5 years; later outcome uncertain
Acute rejection	Variable; <30% of causes of late dysfunction	Inadequate immunosuppression Treatment with immune-activating drugs (*e.g.*, interferon) History of autoimmune liver disease	Much less common than early after transplantation May be more difficult to treat, perhaps related to delay in diagnosis.
Chronic rejection	~3 %	Inadequate immunosuppression Treatment with immune-activating drugs (*e.g.*, interferon) Refractory acute rejection Chronic rejection in a previous failed allograft	Important cause of late dysfunction Most cases occur within first year Does not appear to increase with time after transplantation, but more follow-up is needed.
Idiopathic posttransplantation hepatitis	5%-60%; wide variation		5%-15% of patients followed for a minimum of 10 years will develop progressive fibrosis resulting in established cirrhosis Incidence varies widely among centers

Abbreviations: AIH, autoimmune hepatitis; HBV, hepatitis C virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

of late liver allograft dysfunction,[39-56] including: (1) histopathological evidence of liver injury showing a pattern compatible with the diagnosis (liver tests are usually elevated in a pattern consistent with the diagnosis); (2) positive serological, molecular biological, immunological, or radiographic evidence of pathogen or possible cause of injury; and (3) other causes of similar histopathological changes and elevated liver tests, if present, have been reasonably excluded.

Table 1 shows approximate incidences, risk factors, and clinical, immunological, and radiological observations for common causes of late dysfunction. Specific diagnoses can be rendered when these observations are combined with histopathological findings (Table 2), tim-

Table 2. Histopathologic Features Most Commonly Detected With Various Causes of Late Liver Allograft Dysfunction

Histopathological Features	Autoimmune Hepatitis*	Acute Rejection	Chronic Rejection	Chronic Viral Hepatitis Types B and C	Primary Biliary Cirrhosis	PSC/BD Strictures
Distribution, severity, and composition of portal inflammation	Usually diffuse; predominantly mononuclear of varying intensity; often prominent plasma cell component	Usually diffuse; variable intensity; mixed "rejection-type" (see text) infiltrate	Patchy; usually minimal or mild lymphoplasmacytic	Patchy; variable intensity; predominantly mononuclear; nodular aggregates	Noticeably patchy and variable intensity; predominantly mononuclear; nodular aggregates and granulomas	Usually patchy to diffuse depending on stage; mild neutrophilic, eosinophilic, or occasionally mononuclear predominant
Presence and type of interface activity	Prominent and defining feature is usually necroinflammatory-type; often plasma cell–rich	Focally present and mild necroinflammatory type	Minimal to absent	Variable; usually not prominent: necroinflammatory- and ductular-type	Important feature later in disease development: ductular and necroinflammatory-type with copper deposition	Prominent and defining feature: ductular-type with portal and periportal edema
Bile duct inflammation and damage	Variable; if present, involves a minority of bile ducts	Present and usually involves a majority of bile ducts	Focal ongoing lymphocytic bile duct damage; inflammation wanes with duct loss	Variable; if present, involves a minority of bile ducts	Granulomatous or focally severe lymphocytic cholangitis is diagnostic in proper setting	Periductal lamellar edema; "fibrous cholangitis"; acute cholangitis; multiple intra-portal ductal profiles
Biliary epithelial senescence changes and small bile loss	Absent or involves only a minority of ducts/portal tracts, but may be focally severe	Absent or involves only a minority of ducts	Senescence/atrophy/atypia involve a majority of remaining ducts (see text)	Absent or involves only a minority of ducts	Small bile duct loss associated with ductular reaction	Small bile duct loss associated with ductular reaction
Perivenular mononuclear inflammation and/or hepatocyte dropout	Variable; can involve a majority of perivenular regions, similar to rejection (see text); may be plasma cell–rich.	Variable, if defining feature should involve a majority of perivenular regions; may also show subendothelial inflammation of vein (see text)	Usually present, but variable	Variable but generally mild; if present, involves a minority of perivenular regions	Variable but generally mild; if present, involves a minority of perivenular regions	Absent
Lobular findings and necroinflammatory activity	Variable severity; rosettes may be present and/or prominent	Variable; if present, concentrated in perivenular regions	Variable; if present, concentrated in perivenular regions	Disarray variable; variable severity; necroinflammatory activity	Mild disarray; parenchymal granulomas; periportal copper deposition and cholatestasis are late features	Disarray unusual; neutrophil clusters; ± cholestasis
Pattern of fibrosis during progression toward cirrhosis	Usually macronodular; posthepatitic pattern	Rare	Uncommon, if present usually a venocentric pattern; may evolve to biliary pattern over time	Usually macronodular, hepatitic pattern; may be micronodular (see text)	Biliary pattern	Biliary pattern

NOTE. The histopathological findings in this table should be combined with clinical, serological, radiographic, and important exclusionary criteria listed in Table 2 to arrive at a final diagnosis.
Abbreviation: PSC/BD, primary sclerosing cholangitis/bile duct.
*The same findings apply to recurrent and *de novo* autoimmune hepatitis.

ing and pattern of liver test elevations, and important exclusionary criteria (Table 3). A discussion of histological findings in late posttransplant biopsies and their differential diagnosis follows.

Late-Onset Acute Rejection. LAR, which occurs more than several months after transplantation, may show slightly different features than typical acute rejection seen early after transplantation (Fig. 1). Fewer blastic lymphocytes, slightly greater interface activity, less venous subendothelial inflammation, and slightly more lobular activity cause biopsies with LAR to resemble chronic hepatitis.[4,57] LAR can also present as isolated perivenular inflammation and hepatocyte dropout (so-called "central perivenulitis")[58-60] and evolve into typical chronic rejection with ductopenia.[61] Subendothelial inflammation of portal or central veins is not a required finding in such cases. LAR, however, is still most commonly characterized by: (1) predominantly mononuclear portal inflammation containing lymphocytes, neutrophils, and eosinophils; (2) venous subendothelial inflammation of portal or central veins or perivenular inflammation; and (3) inflammatory bile duct damage. Previously proposed criteria[2] should be used for grading unless LAR presents as isolated central perivenulitis. For these cases, the following descriptors are recommended:

- minimal/indeterminate: perivenular inflammation involving a minority of central veins with patchy perivenular hepatocyte loss without confluent perivenular necrosis

Table 3. Inclusionary and Exclusionary Criteria for the Diagnosis of Recurrent and New-Onset Chronic Necroinflammatory Diseases After Liver Transplantation and Timing of First Onset and Pattern of Liver Test Elevation

Diagnosis	Original Disease	Serology/Molecular Testing*	Timing and Liver Injury Test Profile†	Important Exclusionary Criteria
Recurrent AIH	AIH	Autoantibodies (ANA, ASMA, ALKM) usually in high titers (>1:160); elevated serum immunoglobulin G	>6 months hepatocellular	Acute and chronic rejection, HBV, HCV infection, as determined via third-generation ELISA and/or serum PCR
De novo AIH	Other than AIH	Same as above	>6 months hepatocellular	Same as above
Recurrent HBV or HCV	HBV- or HCV-induced cirrhosis	HBV or HCV infection using standard, third-generation serological criteria and/or positive molecular testing for HBV or HCV nucleic acids	Usually 6-8 weeks, but as early as 10 days Usually hepatocellular but may be cholestatic	Acute and chronic rejection AIH
Recurrent PBC	PBC	Positive AMA, but little additional benefit because AMA remains elevated in the majority of patients after transplantation	>1 yr Cholestatic	Biliary tract obstruction/strictures
Recurrent PSC	PSC	NA	Usually >1 yr Cholestatic	HA thrombosis/stenosis, chronic (ductopenic) rejection, abnormal surgical anatomy, anastomotic strictures alone, nonanastomotic strictures occurring <90 d after liver transplantation, and ABO incompatibility
Acute rejection	NA (see text for risk factors)	NA	Any time Usually hepatocellular; may be mixed if superimposed on chronic rejection	Inadequate immunosuppression usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Chronic rejection	NA (see text for risk factors)	NA	Any time, but usually <1 yr Cholestatic; rarely hepatocellular in veno-occlusive variant (see text)	Inadequate immunosuppression usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Idiopathic posttransplantation hepatitis	Nonviral and non-autoimmune hepatitis	Negative testing for HBV and HCV infection and autoantibodies	>1 yr Usually hepatocellular	Acute and chronic rejection, all other causes of chronic hepatitis, and biliary tract obstruction/strictures reasonably excluded; all attempts should be made to determine a cause

NOTE. See Table 1 for compatible histopathological findings.

Abbreviations: AIH, autoimmune hepatitis; ANA, antinuclear antibodies; ASMA, anti–smooth muscle antibodies; ALKM, anti-liver–kidney microsomal antibodies; HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

*Timing = usual timing of first onset.

†Sustained elevation for more than 1 month. Hepatocellular = alanine aminotransferase and/or aspartate aminotransferase > alkaline phosphatase and/or γ-glutamyltranspeptidase. Cholestatic = alkaline phosphatase and/or γ-glutamyltranspeptidase > aspartate aminotransferase and/or alanine aminotransferase.

- mild: as above, but involving a majority of central veins
- moderate: as above, with at least focal confluent perivenular hepatocyte dropout and mild moderate inflammation, but without bridging necrosis
- severe: as above, with confluent perivenular hepatocyte dropout and inflammation involving a majority of hepatic venules with central-to-central bridging necrosis.

"Minimal" and "mild" cases, as described above, may resolve spontaneously.[60] More severe perivenular changes probably warrant more aggressive treatment, but studies of long-term outcome according to therapy are needed to validate such an approach.

Chronic Rejection. Portal tracts and perivenular regions are primarily affected in chronic rejection, and changes are divided into "early" and "late" stages.[1] In a biopsy specimen, the minimum diagnostic criteria are: (1) biliary epithelial senescence changes affecting a majority of the bile ducts with or without bile duct loss; or (2) foam cell obliterative arteriopathy; or (3) bile duct loss affecting >50% of the portal tracts.[1]

Biliary epithelial senescence changes include cell and nuclear enlargement, multinucleation, uneven nuclear spacing, and cytoplasmic eosinophilia.[62] Some small bile ducts may be only partially lined by biliary epithelial cells. Perivenular hepatocyte dropout and central perivenulitis are typical of early chronic rejection.[63] Variable perivenular fibrosis occasionally progressing to veno-centric cir-

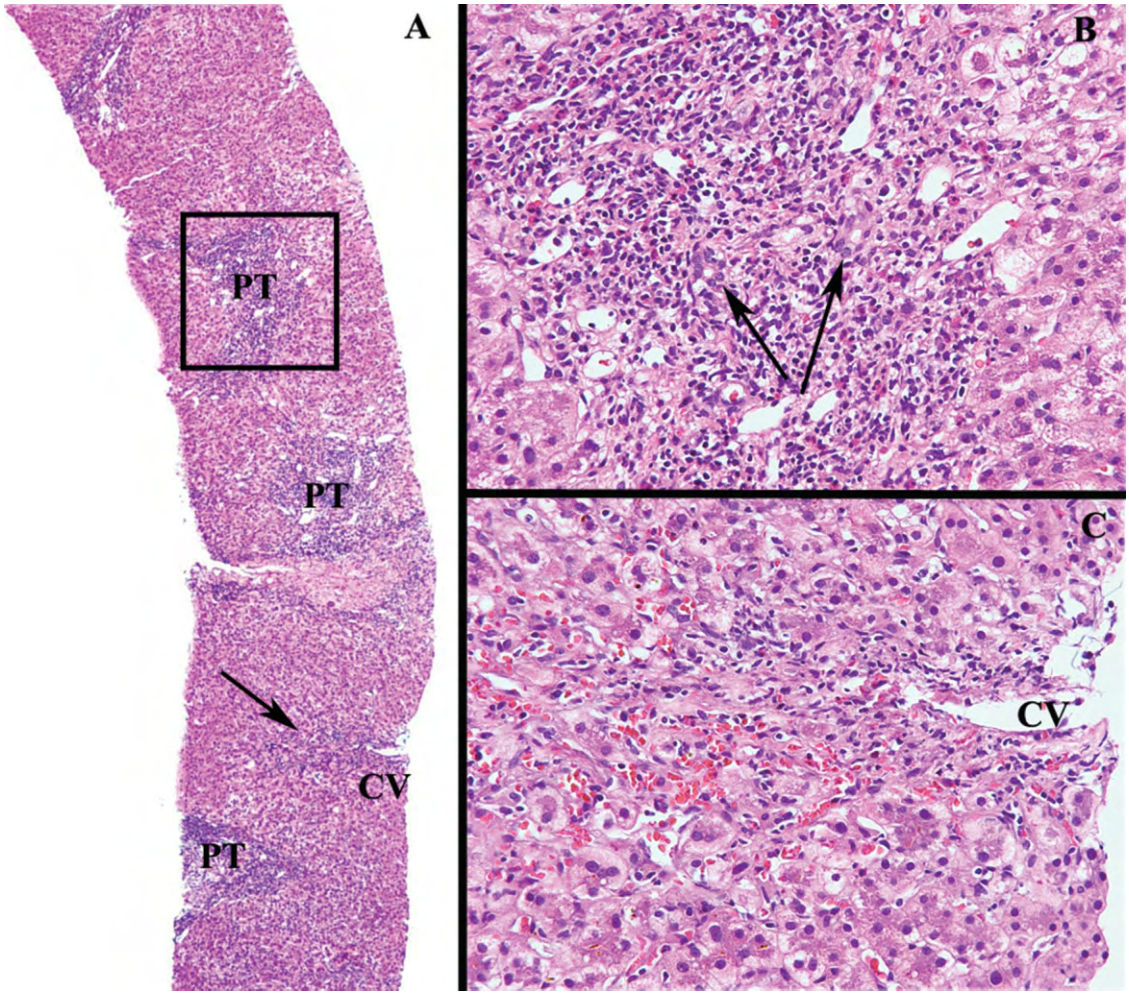

Fig. 1. Composite of late-onset acute rejection occurring more than 1 year after transplantation in a patient with low levels of baseline immunosuppression. (A) Low-magnification view (×20) shows prominent portal inflammation distributed evenly throughout the portal tracts, as well as perivenular mononuclear inflammation. Note also the irregular interface zone around the inflamed portal tracts. (B) Higher magnification (×200) of the portal tract outlined by the rectangle in panel A better illustrates the irregular interface zone and fewer blastic lymphocytes, which causes the biopsy to resemble chronic hepatitis. However, the prevalence and severity of lymphocytic cholangitis (**arrows**) are much worse than would be expected in chronic hepatitis and point toward acute rejection as the correct diagnosis. (C) Higher magnification (×200) of the central vein designated by the arrow in panel A better illustrates the perivenular mononuclear inflammation, or "central perivenulitis." Abbreviations: PT, portal tract; CV, central vein.

rhosis is typical of late chronic rejection.[64] Chronic rejection rarely results in a "posthepatitic" pattern of cirrhosis. If this pattern is present, other insults should be reasonably excluded.

The safest approach to a chronic rejection diagnosis in any setting is to review prior biopsies and correlate the histopathological findings closely with the clinical course. The typical scenario usually includes persistent/unresponsive acute rejection and/or inadequate immunosuppression.

Recurrent Diseases and New-Onset Diseases

Hepatitis C Virus. The predominant features of HCV include mononuclear portal inflammation, often arranged into nodular aggregates, necroinflammatory and ductular-type interface activity, and mild macrovesicular steatosis. Except for an association between steatosis and HCV genotype 3,[65] no histopathological features reliably distinguish among different viral genotypes. Lymphocytic cholangitis, if present, involves a minority of bile ducts without ductopenia. Lobular disarray and necroinflammatory activity are usually mild. Confluent or bridging necrosis with recurrent HCV alone is unusual. Central perivenulitis, if present, involves a minority of central veins.

There are two histopathological patterns of severe chronic HCV: (1) aggressive conventional hepatitis with prominent interface activity and (2) fibrosing cholestatic hepatitis. Features of fibrosing cholestatic hepatitis in-

clude centrilobular hepatocyte swelling and degeneration; cholestasis, hepatocyte apoptosis, and portal expansion because of a ductular reaction; fibrosis; and a mild mixed portal inflammation.[66] Fibrosing cholestatic hepatitis is associated with massive HCV replication (*e.g.*, >40-50 million IU/mL[67,68]).

Recurrent and New-Onset or* De Novo *Autoimmune Hepatitis. AIH is difficult to distinguish, histologically and conceptually, from rejection. Immune responses against self-antigens constitute an autoimmune response, whereas those against foreign antigens constitute rejection. Donor livers undoubtedly contain non–major histocompatibility complex antigens not expressed in the native liver, and theoretically all forms of AIH after transplantation could be classified as rejection.[34,42] Serological and histological findings used to distinguish AIH from rejection may reflect the nature, density, and location of antigenic targets. There are no conventional clinical tests that differentiate an autoimmune response from rejection, and distinctions based on clinical and histopathological findings may not reflect the true pathogenesis. Some new-onset AIH cases might be attributable to polymorphic expression of glutathione S-transferase T1[69]; transplantation of a mismatched graft into a nonexpressing recipient could trigger rejection that closely resembles AIH.

The International Autoimmune Hepatitis Group[70] scoring system and criteria for the diagnosis of AIH in native livers have not been tested in allografts; however, they do provide useful guidelines. AIH is established through a combination of serological, molecular biological, and histopathological findings. Non–organ-specific autoantibodies, a requisite for diagnosis, typically include anti–smooth muscle antibodies and antinuclear antibodies, as well as antibodies to liver kidney microsome type 1.[71] Their occurrence implies activation of immune mechanisms possibly involved primarily in disease pathogenesis or collateral responses to liver cell destruction and nonselective antigen release. Autoantibodies after liver transplantation do not establish the diagnosis of AIH, nor are they accurate parameters of inflammatory activity. Their principal value is to direct attention to the possibility of AIH.

The minimum diagnostic criteria for recurrent or *de novo* AIH in an allograft are: (1) interface hepatitis with portal lymphocytic infiltrates; (2) significant titers (≥1:160) of antinuclear antibodies, smooth muscle antibodies, or antibodies to liver kidney microsome type 1; (3) hyper-gammaglobulinemia; and (4) exclusion of virus-induced or drug-related hepatitis and late acute or chronic rejection. Titers ≥1:160 are unlikely to be nonspecific background reactivities and therefore compel a thorough evaluation for AIH.[70]

Initial manifestations include lobular hepatitis with hepatocyte rosetting[40] that usually evolves into the chronic phase characterized by lymphoplasmacytic portal inflammation with prominent interface activity. Plasmacytic infiltrates characterize AIH, but are not diagnostic requisites. Confluent and bridging necrosis are not uncommon, particularly in patients on suboptimal immunosuppression. Lymphocytic cholangitis, if present, involves a minority of ducts.

Central perivenulitis can occur in acute onset AIH in native livers[72-74] and in otherwise typical LAR. In native livers, perivenular hepatocyte injury associated with AIH usually wanes as interface hepatitis appears,[75] but the evolution of changes has not been studied in allografts. Panacinar hepatitis is also within the spectrum of histological findings in AIH,[70] but a cholestatic form is not recognized.

Idiopathic Posttransplantation Hepatitis. Idiopathic posttransplantation hepatitis is defined as chronic hepatitis that cannot be ascribed to a particular cause. By definition, bile duct damage and venous endothelial inflammation are not conspicuous. In adults, the prevalence is difficult to determine, because most native diseases have the potential to recur with features of chronic hepatitis. In some centers, up to 40% of adult patients subjected to biopsy more than 12 months after transplantation have unexplained chronic hepatitis.[76] A similar prevalence has been observed in the pediatric population, in which recurrent native disease is less of a problem; the frequency of "idiopathic" chronic hepatitis was 20% at 1 year of age, rising to 60% at 10 years of age.[77]

Cases presenting as central perivenulitis probably represent centrilobular-based acute rejection, or AIH if autoantibodies are also present,[57] because allograft dysfunction usually responds to increased immunosuppression.[59-61,78] Some idiopathic posttransplantation hepatitis cases may represent rejection with chronic hepatitic features.[79] However, a diagnosis of idiopathic posttransplantation hepatitis does not usually trigger treatment with increased immunosuppression. In some series, as many as 50% of such cases may develop bridging fibrosis or cirrhosis over a period of 10 years.[77] This observation supports the need for protocol biopsies and clarification of management policies in those with significant activity.[77]

Primary Biliary Cirrhosis. Recurrent PBC findings are nearly identical to those seen in native livers.[80,81] The pathognomonic lesion is noninfectious granulomatous cholangitis in the proper setting, which includes presence of antimitochondrial antibodies and absence of other causes such as infections and biliary strictures. Diagnostic

lesions are not always present. Patchy but easily recognizable and severe lymphocytic cholangitis accompanied by biliary epithelial cell eosinophilia, portal lymphoid nodules containing germinal centers, and development of a "biliary gestalt" can also be diagnostic of recurrent PBC in the proper setting. The biliary gestalt includes a ductular reaction at the interface zone combined with portal and periportal fibrosis, small bile duct loss, periportal edema (halo sign), and lysosomal pigment and copper/protein deposition in periportal hepatocytes. Plasma cell–rich periportal hepatitis may be an early marker predictive of later PBC recurrence.[82] Nonspecific lobular findings include mild spotty hepatocyte apoptosis, slight sinusoidal lymphocytosis, mild nodular regenerative hyperplasia, and Kupffer cell granulomas.

Primary Sclerosing Cholangitis. Findings are identical to those described for native livers with PSC and to other causes of biliary strictures. Subtle histopathological clues that suggest low-grade biliary strictures include mild portal edema; mild nonspecific acute and chronic "pericholangitis" often accompanied by a very mild type I ductular reaction; sinusoidal clusters of neutrophils; and centrilobular hepatocanalicular cholestasis. More significant strictures usually cause lamellar periductal edema, increased portal tract ductal profiles, and/or concentric periductal fibrosis.[83] Later-stage findings include the biliary gestalt. "Fibro-obliterative duct lesions" are not diagnostic of recurrent PSC, because they can also develop in patients with ischemic cholangitis and reflux cholangiopathy.

Differential Diagnosis

Rejection Versus Chronic Hepatitis. This commonly encountered and difficult problem has important therapeutic implications.[67] Unnecessary augmentation of immunosuppression can accelerate fibrogenesis in chronic HCV or trigger cholestatic hepatitis. Untreated acute rejection can progress to chronic rejection, particularly in interferon-treated recipients.

Mononuclear portal inflammation and lymphocytic cholangitis are features of chronic hepatitis and most cases of LAR. In LAR, however, the portal infiltrate tends to be more diffusely distributed throughout the portal tracts and throughout the biopsy rather than aggregated into nodules in occasional portal tracts, as in chronic hepatitis. In LAR and chronic rejection, lymphocytic cholangitis and/or biliary epithelial senescence changes, respectively, should involve a majority of bile ducts.[67] Central perivenulitis involving a majority of central veins also favors rejection. Damage limited to a minority of bile ducts favors acute or chronic hepatitis. Key features of acute and chronic hepatitis are lobular necroinflammatory activity and necroinflammatory and ductular-type interface zone activity, respectively, which are more prevalent and severe than in acute rejection.

Because acute and/or chronic rejection and chronic hepatitis can coexist, the predominant process should be identified. Key features of acute rejection in the context of recurrent HCV are prevalence and severity of mononuclear inflammatory bile duct damage and central perivenulitis. If either feature involves a majority of bile ducts or central veins, then acute rejection is present. However, coexistent acute rejection should be listed as the primary process only when rejection-related changes are obvious. Most such cases are graded as "moderate" according to the Banff schema.[67] Chronic rejection in the context of recurrent HCV is recognized by the same features as in allografts without recurrent HCV: small bile duct loss or biliary epithelial senescence or perivenular inflammation and fibrosis involving a majority of bile ducts or hepatic venules, respectively.

Chronic Rejection. Small bile duct damage and loss and perivenular fibrosis are relied upon for the diagnosis of chronic rejection because arteries with pathognomonic changes are rarely present in needle biopsy specimens.[1] Bile duct injury and ductopenia, however, can also be caused by biliary strictures, hepatic artery pathology, adverse drug reactions, and cytomegalovirus. Isolated ductopenia involving less than 50% of portal tracts can be seen occasionally without significant elevations of liver tests.[80] Whether these uncommon cases are an early phase or subclinical chronic rejection is uncertain. Angiography showing pruning of intrahepatic arteries with poor peripheral filling and segmental narrowing also supports a chronic rejection diagnosis.[84,85]

Perivenular fibrosis can also be caused by suboptimal hepatic venous drainage, adverse drug reactions,[86] and the various causes of veno-occlusive disease and Budd-Chiari syndrome in native livers.[87] In cases of chronic rejection identified by biliary epithelial senescence, bile duct loss, or perivenular fibrosis alone, non–rejection-related causes of ductal injury and loss or perivenular fibrosis should be reasonably excluded, particularly if the clinical scenario is not typical (Table 1).

Biliary Strictures Versus Acute and Chronic Rejection. Significant biliary strictures are usually recognized by the biliary gestalt and are reinforced by preferential elevation of γ-glutamyltranspeptidase and alkaline phosphatase. However, a thorough clinicopathological correlation is needed to distinguish among many underlying causes, such as recurrent PSC, ischemic cholangitis due to injury from prolonged preservation or non–heart-beating donors, imperfect biliary anastomoses, inadequate arterial

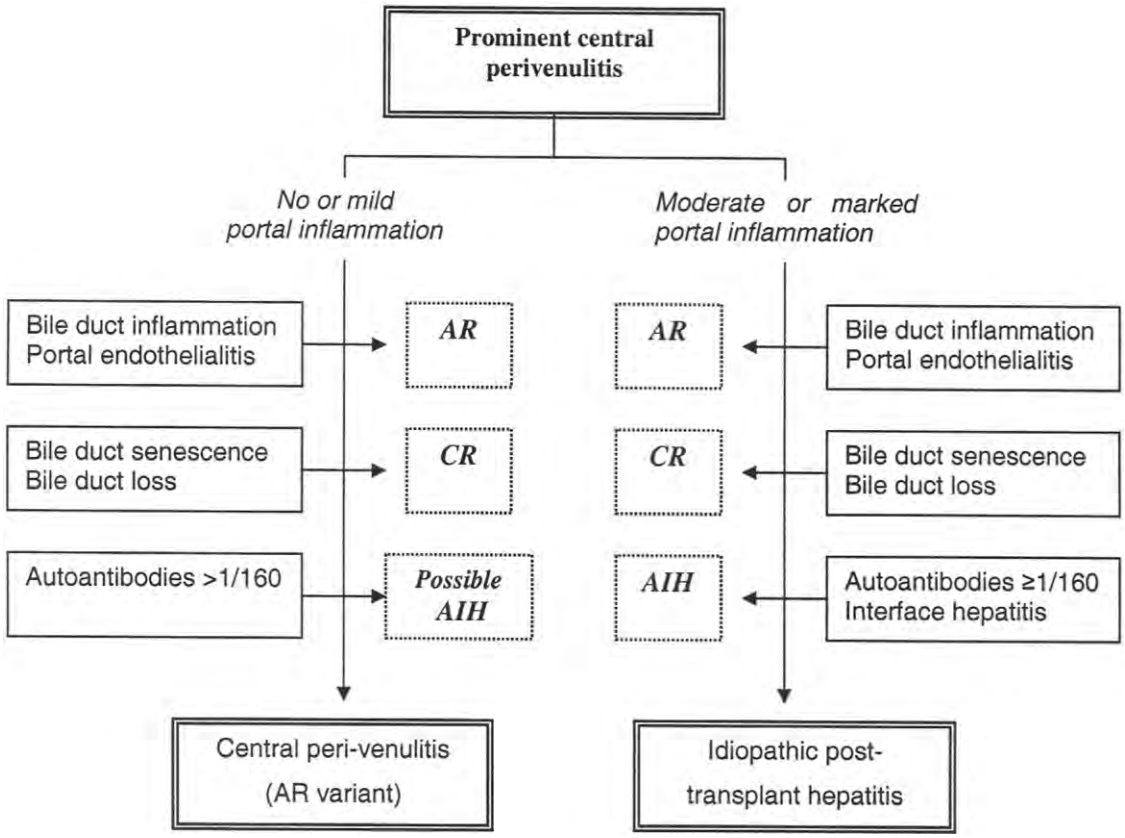

Fig. 2. Approach to biopsies showing posttransplantation central perivenulitis. In cases with no or minor portal inflammation, the differential diagnosis includes acute rejection, chronic rejection, and prediagnostic autoimmune hepatitis. If none of these changes is present, and vascular imaging is normal, the lesion is likely to represent a form of acute rejection. Cases with a more extensive portal inflammatory infiltrate have a similar differential diagnosis. It remains unclear whether idiopathic posttransplantation hepatitis is a form of rejection, and how it is related to pure central perivenulitis. Follow-up biopsies also frequently provide important diagnostic findings. Abbreviations: AR, acute rejection; CR, chronic rejection; AIH, autoimmune hepatitis.

flow, and antibody-mediated rejection.[4,88-92] Periportal hepatocyte copper deposition signals chronic bile flow impediments.

Mononuclear portal inflammation usually favors acute rejection, whereas neutrophilic or eosinophilic portal inflammation, late after transplantation, favors biliary strictures. However, chronic low-grade biliary strictures can occasionally cause predominantly mononuclear portal inflammation. Ductopenia in some portal tracts accompanied by a ductular reaction should raise the suspicion of biliary strictures. Cholangiography and/or angiography may be required to distinguish between chronic rejection and biliary strictures. Acute rejection occurring more than 6 months after transplantation is unusual in adequately immunosuppressed recipients. Therefore, checking baseline immunosuppressive drug levels and the liver test profile often point to the need for cholangiography before increased immunosuppression.

Acute and Chronic Rejection Versus Primary Biliary Cirrhosis. In acute rejection, portal inflammation and lymphocytic cholangitis are usually more diffusely distributed throughout the portal tracts and the biopsy and typically involve small bile ducts (<20 μm). Portal inflammation and lymphocytic cholangitis in recurrent PBC are typically patchy and involve medium-sized bile ducts (>40-50 μm). In the absence of a pathognomonic lesion, recurrent PBC is most commonly recognized by the biliary gestalt occurring in the absence of mechanical biliary strictures. This gestalt is unusual in rejection. Central perivenulitis is not a feature of PBC.

Central Perivenulitis. LAR can manifest primarily as central perivenulitis.[59-61,63,93-96] Because of its association with severe acute rejection[2] and transition to early chronic rejection,[63] central perivenulitis is sometimes portrayed as a poor prognosis lesion, but this is not necessarily correct.[59,60] As in native livers, central perivenulitis in allografts has several causes (Fig. 2), including various forms of rejection (pure perivenular rejection and early chronic rejection), early autoimmune hepatitis,[72,74,97] compromised afferent or efferent blood flow,[73,87,98] and adverse drug reactions. Perivenular rejection can be missed clinically and

present later as ascites because of a Budd-Chiari syndrome or veno-occlusive disease.[63,64,93,96,99]

An acute rejection diagnosis is obvious when central perivenulitis occurs in association with other portal-based changes typical of acute rejection; the severity is graded according to standard criteria.[2] Acute rejection is also the most likely diagnosis when central perivenulitis involves a majority of central veins with minimal or absent portal inflammation, except if the original disease was AIH. In this situation, isolated central perivenulitis may represent early recurrent AIH[34,42,74,75] or new-onset AIH. In native livers presenting with acute AIH central perivenulitis, chronic portal inflammation and interface activity usually develop over time.[72,74,97] Therefore, in allografts, re-examination of the native liver histopathology, serological studies for autoantibodies, and close follow-up for the development of changes more typical of chronic hepatitis[75] are warranted. Because increased immunosuppression effectively treats either rejection or AIH, any differences in assigned diagnoses may be semantic. Hepatic vein outflow obstruction and ischemia can also cause centrilobular necrosis, but any associated lymphocytic inflammation is usually minimal.

Mild focal central perivenulitis can coexist with other causes of late dysfunction. In such cases, central perivenulitis probably represents a focal alloreaction, because similar changes are rarely—if ever—seen with the same disorders in native livers. Therefore, we recommend mentioning its presence or suggesting a diagnosis of "indeterminate for rejection," unless a majority of central veins are involved.

Distinguishing Among the Various Causes of Chronic Hepatitis. Determining a specific cause of chronic hepatitis is not always possible, but subtle differences can suggest a specific etiology. Plasma cell and aggressive interface activity and confluent perivenular or bridging necrosis are suggestive of AIH, macrovesicular steatosis is suggestive of HCV, and viral inclusions are seen only in hepatitis B virus. Because potentially distinguishing features are inconsistently present and not entirely reliable, determining the underlying cause of acute and/or chronic hepatitis should be based on a complete clinicopathological evaluation (Tables 2 and 3). Steatohepatitis can coexist with other causes of injury.

Cholestatic or Biliary Disease Versus Chronic Hepatitis. A single granulomatous duct destructive lesion is diagnostic of PBC in the proper setting. Infectious causes of granulomatous cholangitis should be excluded, but they are uncommon. Portal granulomas without granulomatous cholangitis have been reported in native livers with HCV.[100] In the absence of pathognomonic lesions, recurrent PBC or PSC is most commonly distinguished from chronic hepatitis by a biliary gestalt.

Cholestatic viral hepatitis can be difficult to distinguish from biliary strictures with or without hepatic artery thrombosis. Portal edema and portal—rather than periportal—neutrophilia are common in biliary strictures. Cholangiolar proliferation and acute cholangiolitis without portal edema is more characteristic of cholestatic hepatitis. Lobular disarray and hepatocellular swelling and apoptosis are more usual for cholestatic viral hepatitis.

Banff Working Group

Anthony J. Demetris, University of Pittsburgh Medical Center, Pittsburgh, PA (Chairman)
Oyedele Adeyi, Princess Margaret Hospital, University of Toronto, Toronto, Canada
Chris O. C. Bellamy, University of Edinburgh, Edinburgh, Scotland, UK
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Ierachmiel Daskal, Albert Einstein Healthcare Network, Jefferson Health System, Philadelphia, PA
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Paulo Fontes, University of Pittsburgh Medical Center, Pittsburgh, PA
John Fung, Cleveland Clinic Foundation, Cleveland, OH
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Charles Lassman, David Geffen School of Medicine at the University of California–Los Angeles, Los Angeles, CA
Saverio Ligato, Hartford Hospital, Hartford, CT

John G. Lunz III, University of Pittsburgh Medical Center, Pittsburgh, PA
Amadeo Marcos, University of Pittsburgh Medical Center, Pittsburgh, PA
Marta Ida Minervini, ISMETT, Palermo, Italy
Johan Mölne, Sahlgrenska University Hospital, Göteberg, Sweden
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Aurelio Sonzogni, Ospedali Riuniti, Bergamo, Italy
Athanassios C. Tsamandas, University of Patras School of Medicine, Patras, Greece
Annika Wernerson, Karolinska University Hospital, Stockholm, Sweden
Tong Wu, University of Pittsburgh Medical Center, Pittsburgh, PA
Funda Yilmaz, Ege University School of Medicine, Bornova, Izmir, Turkey

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2004 Revision of Standardized Cardiac Biopsy Grading

go to 1990 Heart Grading System

Acute Cellular Rejection

Grade	Histopathological Findings	Comment
0R	No rejection	See example- Grade **0R**
1R	Focal OR diffuse interstitial AND/OR perivascular inflammation WITH up to one focus of myocyte damage	Incorporates old grades 1A, 1B and 2 into a single category. (See examples- **1A**, **1B**, **2).** Inflammation usually mononuclear with some eosinophils. Neutrophils not present except in severe forms, plasma cells not typically present. Myocyte damage may be manifest by clearing of cytoplasm and nucleus with nuclear enlargement, sometimes nucleolar prominence; also by irregular scalloped borders with associated inflammation

2R	Two or more foci of inflammation WITH associated myocyte damage (i.e., more than one focus of myocyte damage).	Equivalent to old grade 3A (See example- **3A**)
3R	Diffuse inflammation WITH multiple foci of myocyte damage WITH OR WITHOUT any combination of: edema, hemorrhage, vasculitis.	Incorporates old grade 3B and 4 into a single category (See **example**).
Acute Antibody-Mediated Rejection (AMR)		
AMR 0	No histologic or immunopathologic features of AMR	
AMR 1	Histologic features of AMR AND positive immunostain for AMR	Histologic features can include capillary endothelial swelling, denudation, intravascular macrophage accumulation, intravascular thrombi, interstitial edema, hemorrhage, myocyte necrosis without cellular infiltration. Immunostains include paraffin demonstration of diffuse capillary C4d and intracapillary CD68 positive macrophages, or frozen section immunofluorescence showing capillary immunoglobulin (IgG, IgM, and/or IgA) and complement (C3d, C4d and/or C1q) deposition

		NOTE: It was decided at Banff 2009 meeting to recommend screening ALL allograft heart biopsies for C4d.
Other (Non-Rejection) Biopsy Findings		
Early ischemia	Myocyte injury or necrosis (contraction band necrosis, coagulative necrosis) often with myocyte vacuolization, fat necrosis. Myocyte injury disproportionately high for small amount of inflammation, or no inflammation in early stages.	Related to perioperative ischemic injury, seen up to 6 weeks posttransplant. Over time, inflammation develops, contains neutrophils in addition to other cells. Acute rejection usually has more inflammation relative to myocyte injury; neutrophils uncommon in acute rejection unless severe. However, neutrophils may also occur in antibody mediated rejection. Trichrome stain may be helpful in detecting myocyte damage in some cases. See example: **Ischemia**
Late ischemia	Histologic features of ischemia similar to above	Related to graft coronary artery disease.
Quilty effect	Mononuclear cell infiltrate restricted to endocardium OR with infiltration into underlying myocardium.	Incorporates older terminology encompassing both infiltrating (Type A) and non-infiltrating (Type B) Quilty lesions. Tangential section may obscure relationship between infiltrating portion

		and endocardial portion. Additional sections may be helpful. Presence of B cells, plasma cells, background fibrosis and prominent vascularity favor Quilty effect over acute rejection.
IĂfection	Examine especially for CMV, toxoplasma	Both infections may have lymphocyte-predominant inflammation that may mimic acute rejection.
Lymphoproliferative disorder	Uncommon in heart, findings similar to spectrum seen elsewhere.	See example: **Lymphoproliferative disorder**

- **For evaluation need minimum of 3 samples, each at least 50% myocardium, and excluding prior biopsy site, scar, adipose tissue, blood clot. At least 3 levels H&E stained for microscopy.**

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Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection

Susan Stewart, FRCPath, Chair: Gayle L. Winters, MD, Michael C. Fishbein, MD, Henry D. Tazelaar, MD, Jon Kobashigawa, MD, Jacki Abrams, MD, Claus B. Andersen, MD, DMSc, Annalisa Angelini, MD, Gerald J. Berry, MD, Margaret M. Burke, FRCPath, Anthony J. Demetris, MD, Elizabeth Hammond, MD, Silviu Itescu, MD, Charles C. Marboe, MD, Bruce McManus, MD, PhD, Elaine F. Reed, PhD, Nancy L. Reinsmoen, PhD, E. Rene Rodriguez, MD, Alan G. Rose, MD, FRCPath, Marlene Rose, PhD, MRCPath, Nicole Suciu-Focia, PhD, Adriana Zeevi, PhD, and Margaret E. Billingham, FRCPath

In 1990, an international grading system for cardiac allograft biopsies was adopted by the International Society for Heart Transplantation. This system has served the heart transplant community well, facilitating communication between transplant centers, especially with regard to patient management and research. In 2004, under the direction of the International Society for Heart and Lung Transplantation (ISHLT), a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and to address recent advances in the knowledge of antibody-mediated rejection. This article summarizes the revised consensus classification for cardiac allograft rejection. In brief, the revised (R) categories of cellular rejection are as follows: Grade 0 R—no rejection (no change from 1990); Grade 1 R—mild rejection (1990 Grades 1A, 1B and 2); Grade 2 R—moderate rejection (1990 Grade 3A); and Grade 3 R—severe rejection (1990 Grades 3B and 4). Because the histologic sub-types of Quilty A and Quilty B have never been shown to have clinical significance, the "A" and "B" designations have been eliminated. Recommendations are also made for the histologic recognition and immunohistologic investigation of acute antibody-mediated rejection (AMR) with the expectation that greater standardization of the assessment of this controversial entity will clarify its clinical significance. Technical considerations in biopsy processing are also addressed. This consensus revision of the Working Formulation was approved by the ISHLT Board of Directors in December 2004. J Heart Lung Transplant 2005;24:1710–20.

Change is one thing, progress is another.
Change is scientific, progress is ethical.
Change is indubitable, progress is a matter of controversy.
Bertrand Russell
British philosopher
(1872–1970)

At the request of the International Society for Heart and Lung Transplantation (ISHLT), a standardized grading system for the pathologic diagnosis of rejection in cardiac biopsies was developed in 1990 to address the proliferation of diverse grading systems that occurred during the 1980s. The goal was to develop a uniform description and grading scheme for acute cardiac rejection, to improve communication between clinicians and investigators, to enable comparison of treatment regimens and outcomes between transplant centers, to facilitate multicenter clinical trials, and to promote further studies to determine the clinical significance of the various histologic patterns.[1] It was also intended to have a grading system that was easily learned, readily usable, reproducible, had defined clinical end-points, and could be modified as new information became available. The 1990 ISHLT grading system for cardiac biopsies was widely adopted and served the heart transplant community well for over a decade. However, several issues have arisen during this period requiring re-evaluation of the grading scheme.

First, it has become apparent that there were widespread inconsistencies in the use of the grading system as highlighted by multicenter therapeutic trials in which central pathology panel reviewers have been used for confirmation of endomyocardial biopsy diagnoses.[2,3] Major areas of diagnostic difficulty have included: Grade 1A vs Grade 2; Grade 1B vs Grade 3B; Grade 2 vs Grades 3A or 3B; Quilty B vs Grade 2 or 3A; and ischemic injury vs Grades 2 or 3A. Less common and less problematic areas of difficulty have included biopsy site(s) vs Grade 2 or 3A, Quilty B vs post-

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Reprint requests: Susan Stewart, FRCPath, Department of Pathology, Papworth Hospital, Papworth Everard, Cambridge CB3 8RE, UK. Telephone: 44-1480-364-308. Fax: 44-1480-364-777. E-mail: susan.stewart@papworth.nhs.uk.

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Table 1. ISHLT Standardized Cardiac Biopsy Grading: Acute Cellular Rejection[b]

2004		1990	
Grade 0 R[a]	No rejection	Grade 0	No rejection
Grade 1 R, mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage	Grade 1, mild A—Focal	Focal perivascular and/or interstitial infiltrate without myocyte damage
		B—Diffuse	Diffuse infiltrate without myocyte damage
		Grade 2 moderate (focal)	One focus of infiltrate with associated myocyte damage
Grade 2 R, moderate	Two or more foci of infiltrate with associated myocyte damage	Grade 3, moderate A—Focal	Multifocal infiltrate with myocyte damage
Grade 3 R, severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis	B—Diffuse	Diffuse infiltrate with myocyte damage
		Grade 4, severe	Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ± hemorrhage + vasculitis

[a]Where "R" denotes revised grade to avoid confusion with 1990 scheme.
[b]The presence or absence of acute antibody-mediated rejection (AMR) may be recorded as AMR 0 or AMR 1, as required (see Table 3).

transplant lymphoproliferative disorder (PTLD) and infection or PTLD vs rejection.

When the 1990 grading system was proposed, the clinical importance of Grade 2 (focal moderate rejection) histology was unknown and, therefore, a separate rejection grade was assigned to allow studies to determine the clinical significance of this histologic pattern. The proposal was made at that time to meet again and review the data regarding the clinical correlations of the grades and to modify the system as necessary. It should also be noted that the 1990 grading system was defined in biopsies from patients generally receiving triple-drug therapy (steroids, cyclosporine, azathioprine) for immunosuppression. Since that time, immunosuppressive regimens have changed, the incidence of rejection has changed, and it is possible that the histology of rejection may also have changed.

The advances in the understanding of transplant rejection and new therapeutic options to prevent and/or treat rejection have warranted re-examination of the grading system. An attempt was made in 1994–1995 to fine-tune the 1990 grading system and clarify those areas that had caused difficulty in interpretation, including Grade 2 acute rejection.[4] This revision drew mixed responses and was never officially adopted or published. The grading system was again discussed at the Sixth Banff Conference on Allograft Pathology in 2001, where a working group exchanged ideas and experience in using the 1990 grading system and recommended a review and update of the grading system, including the need to establish clear criteria for the pathologic diagnosis of humoral rejection.[5] In 2004, again under the direction of the ISHLT, a multidisciplinary review of the cardiac biopsy grading system was undertaken with task forces examining the areas of histopathology/cellular rejection, humoral (antibody-mediated) rejection, clinical issues and future research. In addition, comments solicited from the ISHLT membership at large were taken into account, which mainly concerned

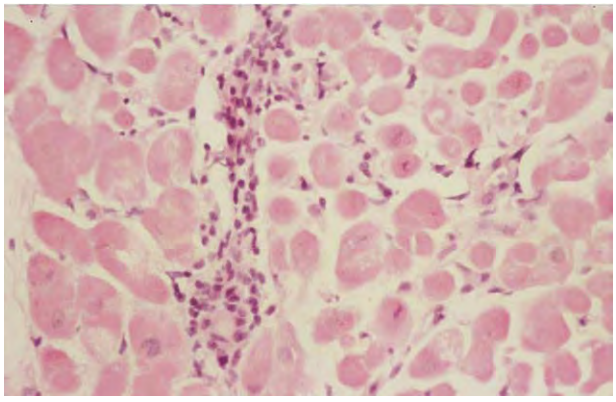

Figure 1. Myocardial biopsy showing acute cellular rejection with an inflammatory infiltrate composed of mainly lymphocytes in a perivascular distribution and not extending into interstitium or damaging myocytes. Hematoxylin and Eosin. (H&E)

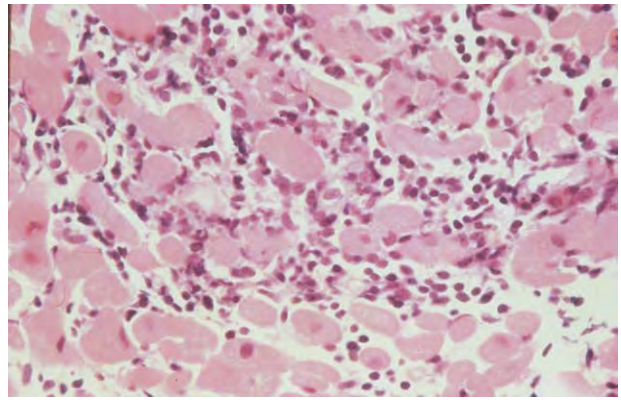

Figure 2. Myocyte damage characterized by encroachment of mononuclear cells at the perimeter of myocytes resulting in irregular, scalloped borders and distorting the cellular architecture. Several myocytes are surrounded by infiltrating cells. (H&E).

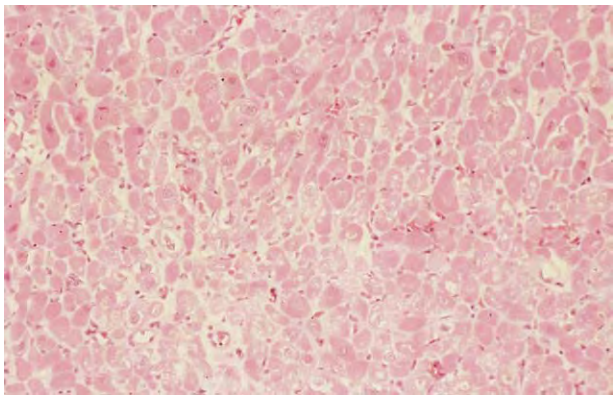

Figure 3. Grade 0 R: Normal endomyocardial biopsy showing no evidence of cellular infiltration. (H&E).

Grade 2 cellular rejection and humoral rejection. Consensus was reached and presented at the 24th Annual ISHLT scientific meeting. This study reports the consensus findings as a revision of the previous Working Formulation, which was approved by the ISHLT board in December 2004.

HISTOLOGIC DIAGNOSIS AND GRADING OF ACUTE CARDIAC ALLOGRAFT REJECTION

Biopsy-proven acute rejection on surveillance endomyocardial biopsies appears to be decreasing, due at least in part to improved immunosuppressive therapy.[6] In addition, there has been a shift in clinical response to some grades of rejection. In the middle to late 1980s, most (but not all) transplant centers treated any biopsy with myocyte injury (1990 ISHLT Grade 2 and higher) with some form of augmented immunosuppression, regardless of the clinical presentation. Several studies in the early to mid-1990s showed that lower grades of rejection resolve without treatment in a majority of cases.[7–14] Biopsies with 1990 ISHLT Grade 1, Grade 2 and even some sub-sets of Grade 3 rejection progress to high-grade rejection on the next biopsy in only 15% to 20% of cases. At the other end of the spectrum, Grades 3B and 4 are uniformly treated aggressively. Therefore, the consensus was to modify the 1990 ISHLT grading system as shown in Table 1. In brief:

- 1990 ISHLT Grades 1A, 1B and 2 would be combined into a new, revised 2004 ISHLT Grade 1 R.
- 1990 ISHLT Grade 3A would become 2004 ISHLT Grade 2 R; and
- 1990 ISHLT Grades 3B and 4 would become 2004 ISHLT Grade 3 R.

In addition, the Histopathology Task Force recommended that further characterization of the nature of the inflammatory infiltrate and definition of myocyte damage would be helpful in reducing inconsistencies in the application of the grading system (*vide infra*).

Inflammatory Infiltrate

Acute cellular rejection is characterized by an inflammatory infiltrate predominantly comprised of lympho-

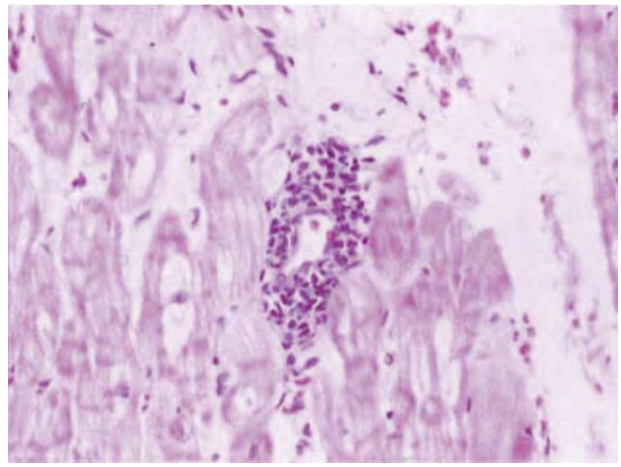

Figure 5. Grade 1 R: Higher power view of focal, perivascular mononuclear cell infiltrate without myocyte encroachment or damage. Previously Grade 1A. (H&E).

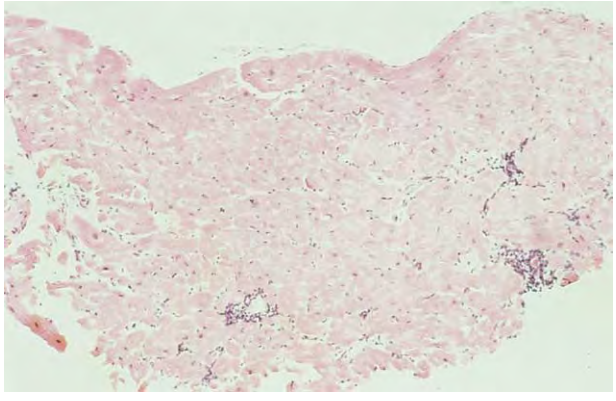

Figure 4. Grade 1 R: Low power view of endomyocardial biopsy showing three focal, perivascular infiltrates without myocyte damage. Previously Grade 1A (H&E).

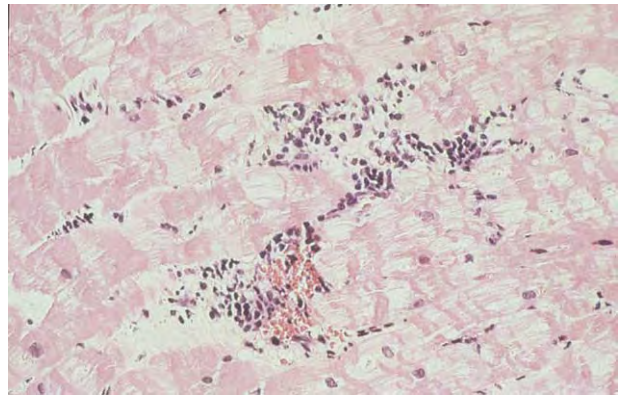

Figure 6. Grade 1 R: Both perivascular and interstitial infiltrates are present but without definite evidence of myocyte damage. Previously Grade 1A (H&E).

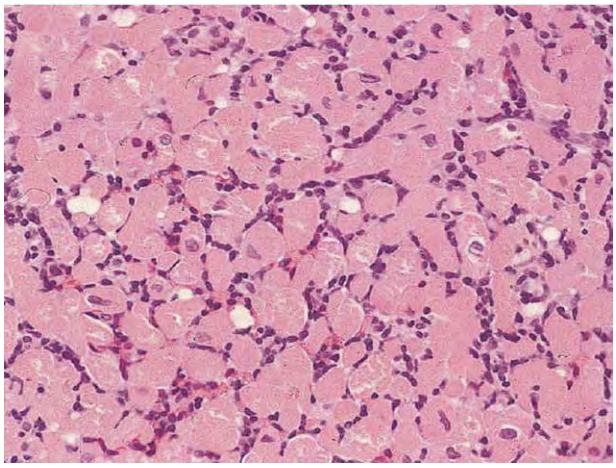

Figure 7. Grade 1 R: Diffuse mononuclear cell infiltrate with an interstitial pattern of lymphocytes between and around myocytes without associated myocyte damage. Previously Grade 1B. (H&E).

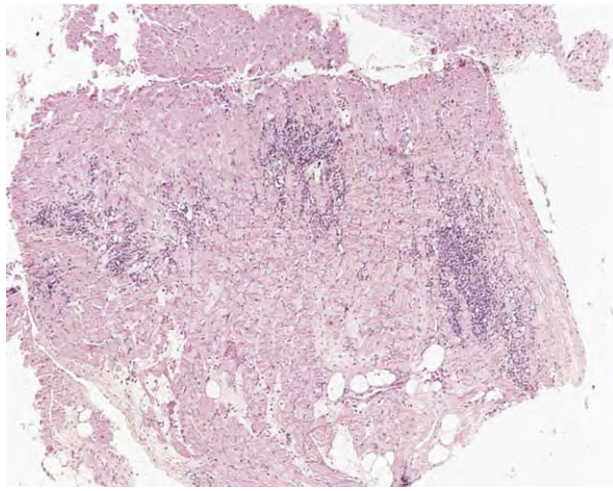

Figure 9. Grade 2 R: Low power view showing three foci of damaging mononuclear cell infiltrate with normal myocardium intervening. Previously Grade 3A. (H&E).

cytes, as well as macrophages and occasional eosinophils (Figure 1). The presence of neutrophils (except in the most severe form of rejection) should raise the question of an alternative process, such as healing ischemic injury, antibody-mediated (humoral) rejection or infection. Plasma cells are also not typically present in acute cellular rejection and suggest a Quilty lesion, healing ischemic injury (often in response to allograft coronary disease) or a lymphoproliferative disorder (plasmacytoid lymphocytes).

Myocyte Damage

Damage or injury to the myocardium, originally termed "myocyte necrosis," is an important but sometimes difficult feature to identify. Although readily distinguishable, cell death may be a feature of the most severe forms of rejection; myocyte damage in milder rejection is often characterized by myocytolysis and no contraction band or coagulation necrosis. Features of myocytolysis include clearing of the sarcoplasm and nuclei, with nuclear enlargement and occasionally prominent nucleoli. The presence of myocyte injury is frequently accompanied by encroachment of inflammatory cells at the perimeter of myocytes, resulting in irregular or scalloped myocyte borders, their partial or whole replacement, or distortion of the normal myocardial architecture (Figure 2). These features are often better appreciated by the examination of multiple levels of sectioning. It should also be noted that myocytolysis can be seen in both early and late ischemic injury.

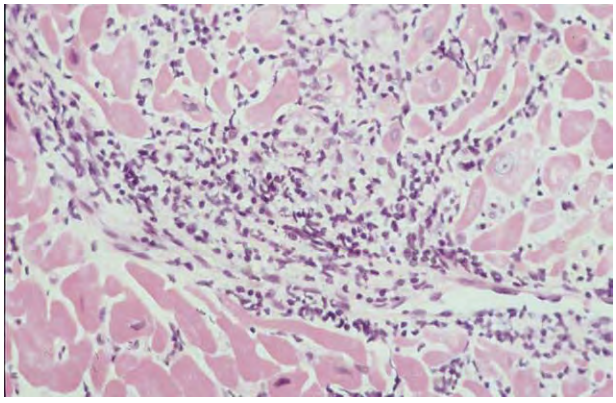

Figure 8. Grade 1 R: High power view of a mononuclear infiltrate extending from a perivascular position into adjacent myocardium with damage to myocytes and distortion of architecture. This is a single focus in the biopsy series and therefore is included in the revised mild grade of acute rejection, previously described as Grade 2. (H&E).

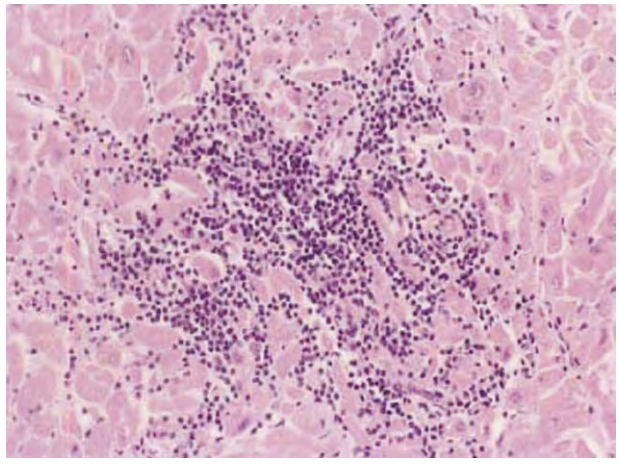

Figure 10. Grade 2 R: Higher power view of one focus of figure 9 damaging infiltrate with myocyte damage and architectural distortion (a "space occupying lesion"). (H&E).

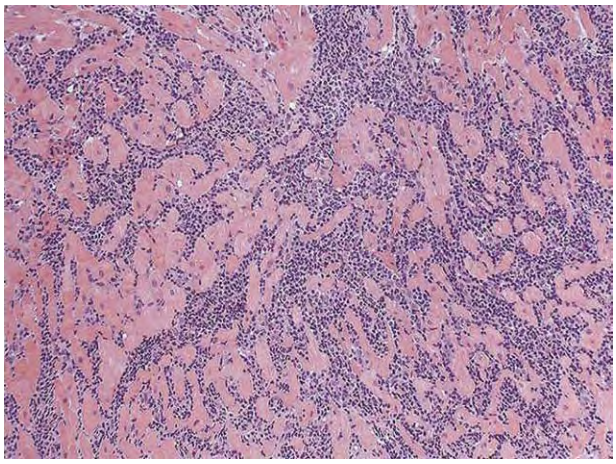

Figure 11. Grade 3 R: Diffuse damaging infiltrates with encroachment of myocytes and disruption of normal architecture. This contrasts with the non-damaging infiltrates of figure 7. Prevously Grade 3B. (H&E).

Grade 0 R (no acute cellular rejection)

In Grade 0 R there is no evidence of mononuclear (lymphocytes/macrophages) inflammation or myocyte damage (Figure 3).

Grade 1 R (mild, low-grade, acute cellular rejection)

Mild or low-grade rejection may manifest in one of two ways: (1) Perivascular and/or interstitial mononuclear cells (lymphocytes/histiocytes) are present. In general, these cells respect myocyte borders, do not encroach on adjacent myocytes, and do not distort the normal architecture (Figures 4, 5, 6 and 7). (2) One focus of mononuclear cells with associated myocyte damage may be present (Figures 2 and 8).

Table 2. Nonrejection Biopsy Findings

2004	1990
Ischemic injury	Ischemic injury
Early—up to 6 weeks post-transplant	A = up to 3 weeks post-transplant
Late—related to allograft coronary disease	B = late ischemia
Quilty effect	Quilty effect
	A = no myocyte encroachment
	B = with myocyte encroachment
Infection	Infection
Lymphoproliferative disorder	Lymphoproliferative disorder

Grade 2 R (moderate, intermediate-grade, acute cellular rejection)

In Grade 2 R two or more foci of mononuclear cells (lymphocytes/macrophages) with associated myocyte damage are present. Eosinophils may be present. The foci may be distributed in one or more than one biopsy fragment. Intervening areas of uninvolved myocardium are present between the foci of rejection (Figures 9 and 10). Low-grade (Grade 1R) rejection can be present in other biopsy pieces.

Grade 3 R (severe, high-grade, acute cellular rejection)

A diffuse inflammatory process, either predominantly lymphocytes and macrophages or a polymorphous infiltrate, is present, involving multiple biopsy fragments (Figures 11 and 12). In most cases, the majority of biopsy fragments are involved, although the intensity of the infiltrate may vary between pieces. Multiple areas of associated myocyte damage are present. In the most severe forms of cellular (and humoral) rejection,

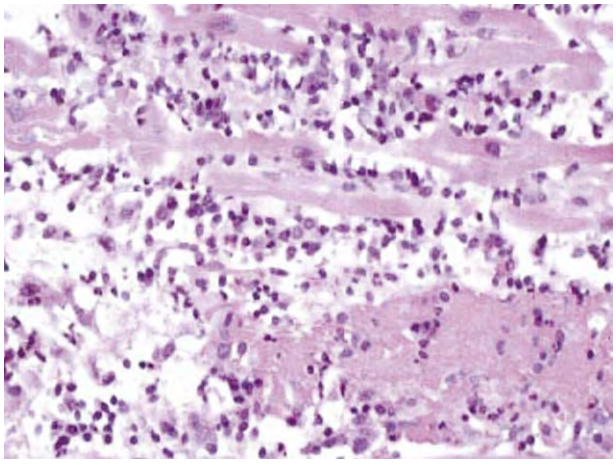

Figure 12. Grade 3 R: Severe acute rejection with widespread myocyte damage and some necrosis. The diffuse infiltrate includes polymorphs as well as lymphocytes, macrophages and plasma cells. Previously Grade 4. (H&E).

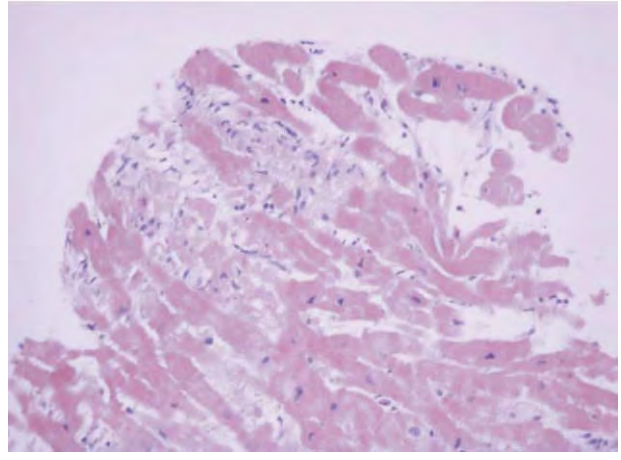

Figure 13. Peritransplant injury showing a focus of ischemic injury with myocytolysis and vacuolization. Note the relative lack of infiltrating inflammatory cells compared with acute cellular rejection. Macrophages are present. (H&E).

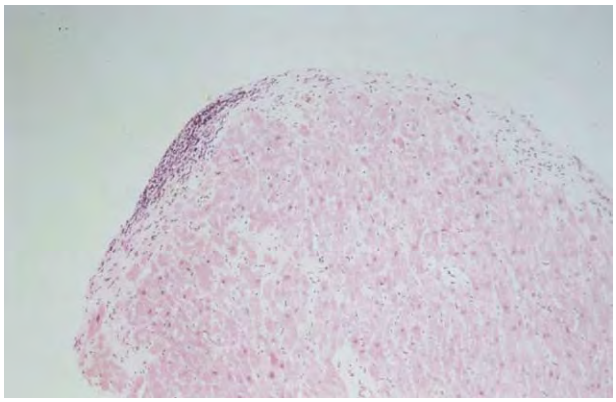

Figure 14. Low power view of non-encroaching endocardial infiltrate or Quilty lesion with normal underlying myocardium. (H&E).

edema, interstitial hemorrhage and vasculitis may be present.

NON-REJECTION BIOPSY FINDINGS

Peri-operative Ischemic Injury

Early (peri-operative) ischemic injury arises in the peri-operative period during the obligatory ischemic time that accompanies procurement and implantation of a donor heart (Table 2).[15] Such injury may be exacerbated by prolonged hypotension due to poor graft function, hemorrhage during the peri-operative period, and the effects of prolonged high-dose inotrope therapy. Ischemic injury is characterized initially by contraction band necrosis or coagulative myocyte necrosis, often with myocyte vacuolization and fat necrosis, and frequently extends to the endocardial surface. As healing ensues, biopsies may contain mixed inflammatory infiltrates, including

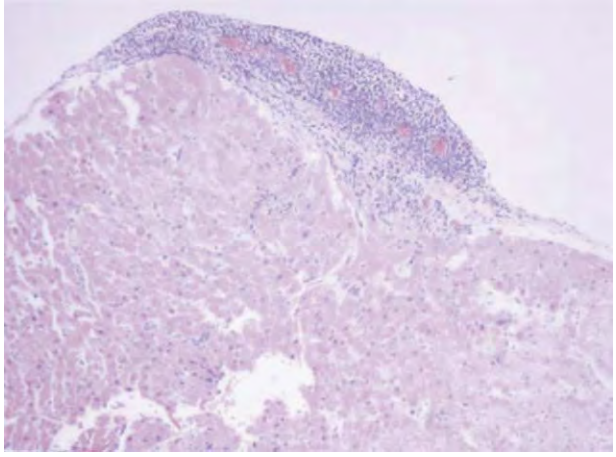

Figure 15. Higher power view of another area of the same biopsy as figure 14, showing some superficial encroachment of the endocardial lesion into underlying myocardium. Note the prominent vascularity of this endocardial infiltrate which can be a very useful feature for distinguishing tangentially cut infiltrates from foci of acute cellular rejection. (H&E).

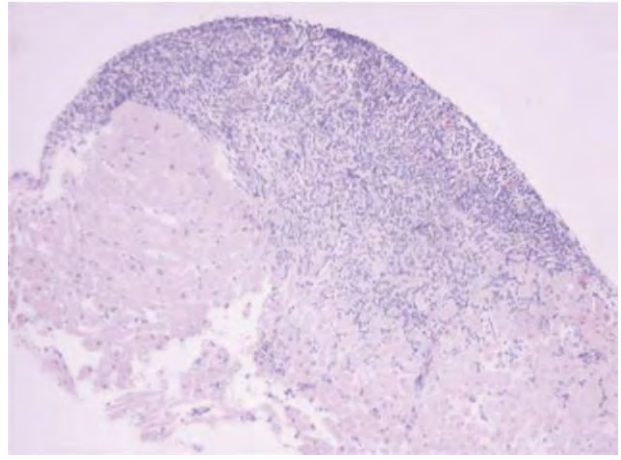

Figure 16. A deeper section of the biopsy in figure 15 showing much greater encroachment into myocardium and less vascularity. (H&E).

neutrophils as well as lymphocytes, macrophages and eosinophils, and it is at this point that confusion with acute rejection may occur. Ischemic injury, especially in its healing phase, is a common biopsy finding in the early post-transplant period (up to 6 weeks) and must be differentiated from acute rejection. In acute rejection, the inflammatory infiltrate frequently is proportionally greater than the degree of myocyte damage, whereas, in ischemic foci, it is usually the reverse (Figure 13). Peri-transplant injury with neutrophils may show overlapping features with antibody-mediated (humoral) rejection (*vide infra*).

Late Ischemic Injury (related to allograft coronary disease)

Assessing the arterial changes of allograft coronary disease in endomyocardial biopsy specimens is usu-

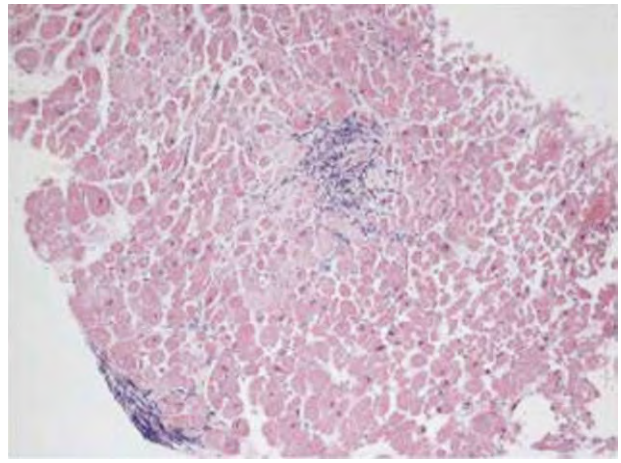

Figure 17. Endomyocardial biopsy showing a small endocardial infiltrate and focus of deeper intramyocardial cellular infiltration which raises the possibility of acute cellular rejection until deeper sections are examined. (H&E).

ally precluded by the lack of vessels large enough to permit such an evaluation. However, the ability to detect secondary myocardial changes, such as myocyte vacuolization and microinfarcts, may be helpful in determining the etiology of late cardiac failure.[16] In addition, the diagnosis of late ischemic injury may be helpful in determining the etiology of cardiac failure in transplant recipients. It may be especially helpful in ruling out other potentially treatable etiologies that are part of the differential diagnosis, such as acute rejection or PTLD.

Quilty Effect

Nodular endocardial infiltrates, or Quilty effect, occur in approximately 10% to 20% of post-transplant endomyocardial biopsies.[17,18] The infiltrates may be confined to the endocardium (1990 ISHLT Quilty A) or may extend into the underlying myocardium where associated myocyte damage may be present (1990 ISHLT Quilty B) (Figures 14, 15 and 16). The histologic sub-typing of Quilty A and Quilty B has never been shown to have any clinical significance and there is agreement that separating Quilty A from B has no clinical value.[19] The designations "A" and "B" have therefore been eliminated and the lesion is referred to simply as the Quilty effect.

The relationship of Quilty effect to acute rejection, if any, remains unknown. Traditionally, this lesion has been considered distinct from acute rejection, requiring no treatment with intensified immunosuppression. Differentiation of Quilty effect from acute rejection is not usually a problem when the former is confined to the endocardium. However, when it extends into the underlying myocardium, a tangential cut through the biopsy may not show a connection between the myocardial lesion and the endocardial lesion, making differentiation from acute rejection more difficult.[20] Cutting additional deeper sections may resolve this dilemma in some cases by demonstrating extension to the endocardium (Figures 17 and 18). In the absence of an endocardial extension, the density of the infiltrate, presence of B lymphocytes and plasma cells, background fibrosis and prominent vascularity favor a diagnosis of Quilty effect.

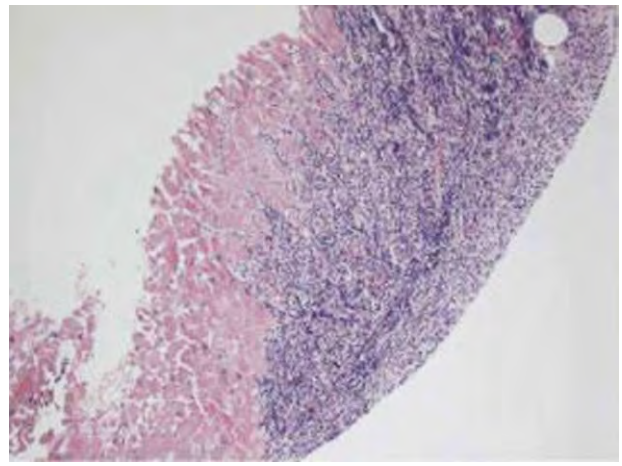

Figure 18. Deeper section of figure 17 clearly shows extension of the surface endocardial infiltrate into myocardium confirming the correct diagnosis of Quilty lesion rather than acute cellular rejection. (H&E).

Immunohistochemical staining of the infiltrate for B and T cells may be helpful in this regard.

Infection and PTLDs

Infection and PTLDs remain important causes of post-transplant morbidity and mortality, but are relatively rare in post-transplant cardiac biopsies. Notable among these are cytomegalovirus (CMV) and toxoplasmosis, both of which may be accompanied by lymphocyte-predominant infiltrates, which may be misinterpreted as acute cellular rejection, leading to inappropriate augmentation of immunosuppression. More specifically, targeted immunosuppression and improved prophylaxis protocols, especially for CMV, have decreased the incidence of some infections. Recognition of the relationship between immunosuppression and post-transplant neoplasms, especially PTLD, has favored less aggressive immunosuppression protocols. Although infection and PTLD are less controversial than other post-transplant biopsy interpretations, they require continued awareness and vigilance.

ACUTE ANTIBODY-MEDIATED (HUMORAL) REJECTION

Acute humoral rejection is recognized as a clinical entity in the grafted heart (Table 3). It remains controversial, however, with a highly varied incidence be-

Table 3. ISHLT Recommendations for Acute Antibody-Mediated Rejection (AMR)

	2004	1990
AMR 0	Negative for acute antibody-mediated rejection No histologic or immunopathologic features of AMR	Humoral rejection (positive immunoflourescence, vasculitis or severe edema in absence of cellular infiltrate) recorded as additional required information
AMR 1	Positive for AMR Histologic features of AMR Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)	

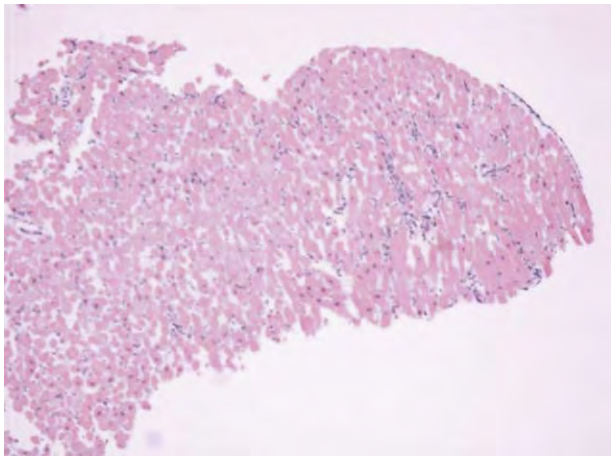

Figure 19. Antibody mediated rejection (AMR 1). Low power view of endomyocardial biopsy with scattered cellular infiltrates and intervening normal tissue. (H&E).

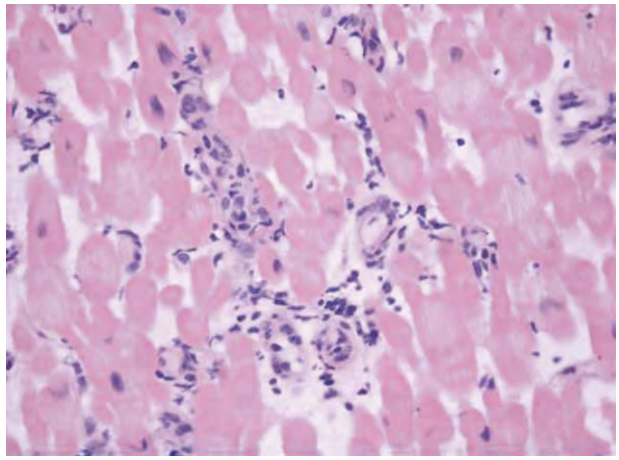

Figure 20. AMR 1. Higher power view shows that the cellular infiltrates are within vessels and include polymorphs. Endothelial cell swelling is present. The increased cellularity seen at low power is due to the presence of these intravascular cells and not perivascular inflammation. Compare with figures 1 and 5. (H&E).

tween different centers and no consensus has yet been reached on its recognition and diagnosis either histopathologically or immunologically.[21–25] The 2004 ISHLT meeting reviewed evidence from the immunopathology and clinical task forces and felt able to suggest diagnostic criteria in specific circumstances so that further assessment of this entity could be encouraged. In the 1990 Working Formulation, there was an option to record immunofluorescence studies for those centers that sought to pursue these on biopsy specimens in the first 6 weeks after transplantation.[1] Similarly, in utilizing the 2004 classification, pathologists can follow the guidance if they intend to investigate the possibility of antibody-mediated rejection as a cause of cardiac dysfunction. A separate companion study from the Immunopathology Task Force is available with a detailed discussion of antibody-mediated rejection. A summary of recommendations is provided here to allow incorporation, as required, into the revised Working Formulation.

Acute antibody-mediated rejection is associated with worse graft survival and is observed in allosensitized patients, including those with previous transplantation, transfusion or pregnancy and previous ventricular assist device use. The incidence may be up to 15% in the first year post-transplantation and the clinical presentation has no pathognomonic features. Pathologically, it can be recognized by myocardial capillary injury with endothelial-cell swelling and intravascular macrophage accumulation (Figures 19, 20 and 21). Interstitial edema and hemorrhage can be present together with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis without cellular infiltration can also be identified.[21,22] When these features are seen in the presence of unexplained cardiac dysfunction, typically early onset of hemodynamic compromise and myocardial dysfunction, it is proposed that immunostaining can be performed by immunofluorescence or immunohistochemistry as follows:

- Immunoglobulin (IgG, IgM and/or IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections (Figures 22 and 23); and/or

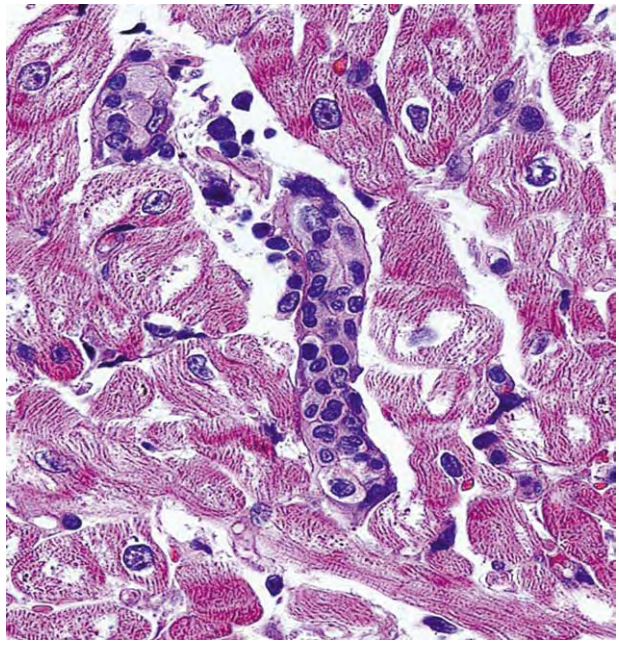

Figure 21. AMR 1. High power view confirms the intravascular location of the cells which have the appearance of macrophages and illustrates the endothelial cell swelling. (H&E).

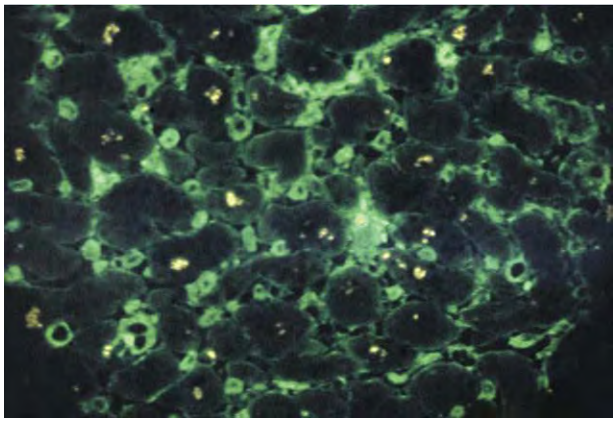

Figure 22. AMR 1. Immunofluorescence positivity for IgG clearly shown in and around capillaries.

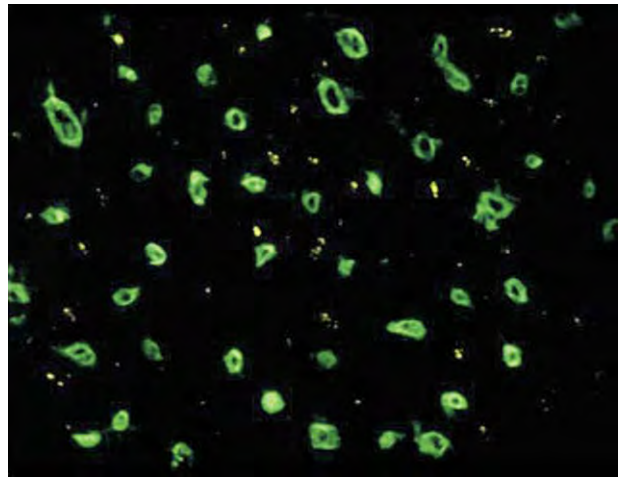

Figure 23. AMR 1. Immunofluorescence positivity for C4d in capillaries with characteristic "doughnut" appearance.

- CD68 staining of macrophages within capillaries (CD31- or CD34-positive) by immunohistochemistry (Figure 24); and
- C4 d staining of capillaries by paraffin immunohistochemistry (Figure 25).

It is recommended that patients with hemodynamic compromise undergo assessment for circulating antibodies.

The consensus meeting recommended that screening should not be advocated at this time, but every endomyocardial biopsy should undergo critical histologic evaluation for features suggestive of antibody-mediated rejection. If such features (as just detailed) are not seen, the biopsy should be designated negative for antibody-mediated rejection, or AMR 0. If features suggestive of antibody-mediated rejection are seen, the diagnosis of acute antibody-mediated rejection should be confirmed by immunohistochemistry, either immunofluorescence or immunoperoxidase, using antibodies directed against CD68, CD31 and C4d, and a serum should be drawn and tested for donor-specific antibody.[26,27] If these markers are positive, a positive diagnosis for AMR should be made (AMR 1). Patients who have several episodes of documented acute antibody-mediated rejection should be followed on future biopsies with at least one of these immunohistochemical methods and monitored for the production of donor-specific antibodies. It is also recognized that acute cellular and antibody-mediated rejection can co-exist, but further studies will be required to delineate these.

This recommended approach to the diagnosis of acute antibody-mediated rejection—if there is either a clinical indication or a research need—should encourage clinicians, histopathologists and immunologists to work together and clarify its existence, frequency and clinical significance.[28]

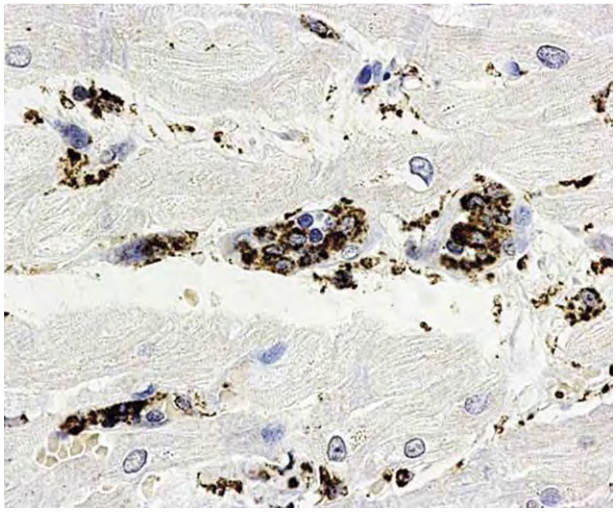

Figure 24. AMR 1. Immunoperoxidase staining is strongly positive for CD68, confirming the intravascular cells to be macrophages.

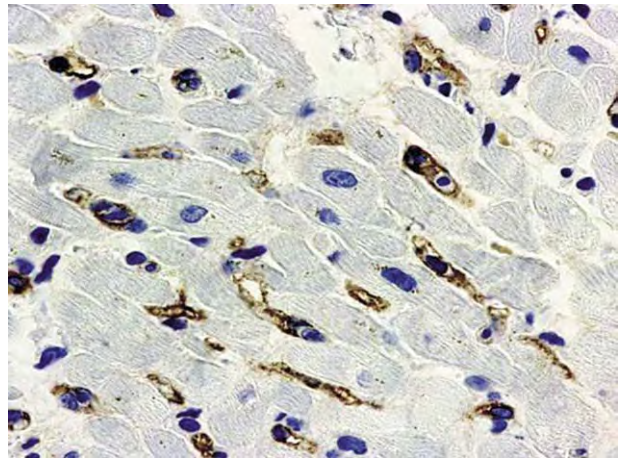

Figure 25. AMR 1. Immunoperoxidase staining is strongly positive for C4d in capillaries allowing a diagnosis of AMR to be made in the appropriate context. (see text).

Table 4. Technical Considerations

Minimum number of biopsy samples = 3
Number of hematoxylin and eosin slides = 3
Number of levels = 3
Routine special stains required = None

TECHNICAL CONSIDERATIONS

Due to the potential for sampling error in diagnosing acute rejection, multiple myocardial biopsy samples should be obtained from different right ventricle sites (Table 4). Samples should not be divided once procured in order to obtain the required number of pieces because this practice results in less representative sampling. Although the original ISHLT grading system required 4 samples of myocardium, the trend has been to accept 3 evaluable samples as the absolute minimum for interpretation. Therefore, a minimum of 3, and preferably 4 (or more), evaluable pieces of myocardium are now recommended for grading acute cellular rejection. An evaluable piece of myocardium contains at least 50% myocardium, excluding previous biopsy site, scar, adipose tissue or blood clot, which may comprise the remainder of the piece. Hematoxylin and eosin staining of at least 3 levels through the tissue samples are recommended. Additional spare slides may be saved unstained if additional studies are needed. Special stains are not routinely required and have been eliminated by many centers as the incidence of rejection has decreased. A trichrome stain may be helpful in selected cases for assessing myocyte damage and fibrosis, such as in the early post-operative period.

CONCLUSIONS

It is the intention of this consensus group that this revision of the grading system addresses and clarifies concerns that have developed in the 15 years since the adoption of the 1990 grading system. The plan is to supplement this revision with an educational program for pathologists and clinicians. As was the case for the 1990 grading system, the 2004 grading system will now be required for all ISHLT-sponsored meetings and publications.

There has been tremendous advancement in technology since the 1990 grading system was instituted, including many molecular techniques. Many of these advances have been used successfully in the research setting to further our knowledge of pathologic processes. The challenge will be to decide the appropriate time and choice of technique(s) to incorporate into routine clinical practice. For the ISHLT grading system to remain the standard worldwide, it must remain the lowest common denominator so that every transplant center has the technical ability and financial resources to incorporate any proposed changes. We must make sure, going forward, that we retain the universality of the grading system because this has always been a major component of its success. The consensus meeting task forces strongly urge the ISHLT to periodically review the grading system as immunosuppressive regimens evolve and as additional clinical and molecular monitors of cardiac function, coronary vasculopathy and immune responsiveness are developed and used in the management of heart transplant recipients.

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APPENDIX: PARTICIPANTS BY TASK FORCE

Chair of Consensus Meeting: Susan Stewart, FRCPath.

Histopathology

Chair: Gayle L. Winters, MD. *Participants:* Jacki Abrams, MD; Claus B. Andersen, MD, DMSc; Annalisa Angelini, MD; Gerald J. Berry, MD; Margaret M. Burke, FRCPath; Anthony J. Demetris, MD; Michael C. Fishbein, MD; Charles C. Marboe, MD; Bruce M. McManus, MD, PhD; Alan G. Rose, MD, FRCPath; Henry D. Tazelaar, MD.

Immunopathology

Chair: Michael C. Fishbein, MD. *Participants:* Elizabeth Hammond, MD; Silviu Itescu, MD; Elaine F Reed, PhD; Nancy L. Reinsmoen PhD; E. Rene Rodriguez, MD; Marlene Rose, PhD, MRCPath; Nicole Suciu-Foca, PhD; Adriana Zeevi, PhD.

Clinical Heart Transplantation

Chair: Jon Kobashigawa, MD. *Participants:* Manfred Hummel, MD, PhD; Sharon Hunt, MD; Anne Keogh, MD; James K. Kirklin, MD; Mandeep Mehra, MD; Leslie W. Miller, MD; Paul Josef Mohasci, MD; Jayan Parameshwar, MRCP; Branislav Radovancevic, MD; Heather J. Ross, MD; Randall Starling, MD.

Research

Chair: Anthony J. Demetris, MD. *Participants:* Bruce M. McManus, MD, PhD; E. Rene Rodriguez, MD; Gayle L. Winters, MD.

Update on Cardiac Transplantation Pathology

Carmela D. Tan, MD; William M. Baldwin III, MD, PhD; E. Rene Rodriguez, MD

● ***Context.*—The endomyocardial biopsy is the mainstay for monitoring acute allograft rejection in heart transplantation. Objective and accurate assessment of cellular and humoral types of rejection is important to optimize immunosuppressive therapy, avoid therapeutic complications, and improve patient outcome. The grading system for evaluation of heart transplant biopsies published in 1990 was revised in 2004 after more than a decade of implementation.**

***Objective.*—In this review, we focus on a practical approach to the evaluation of human heart transplant biopsies as diagnostic surgical pathologic specimens. We discuss the revised International Society of Heart and Lung Transplantation working formulation.**

***Data Sources.*—We reviewed pertinent literature, incorporating ideas and vast experience of participants in various work groups that led to the revision of the 1990 grading system.**

***Conclusions.*—The grading system for cellular rejection is presented with detailed light microscopic morphology and comparison of the 1990 and 2004 International Society of Heart and Lung Transplantation working formulations. We show how the pathologic recognition of cellular rejection and antibody-mediated rejection has evolved. We emphasize the interpretation of immunostains for complement components C4d and C3d in the diagnosis of antibody-mediated rejection. Evidence of regulation of complement activation in human heart transplant biopsies is presented in this context. We also discuss the pitfalls, caveats, and artifacts in the interpretation of allograft endomyocardial biopsies. Lastly, we discuss the pathology of human cardiac allograft vasculopathy in practical detail.**

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Heart transplantation remains the most effective therapy for end-stage heart disease of coronary and noncoronary etiology, with continued improvement in survival during the years. The most common indications for cardiac transplantation in the adult have not changed in the last 3 decades; 85% of cases are roughly equally divided between coronary heart disease and nonischemic cardiomyopathies.[1] In the pediatric age group, congenital heart disease is the leading diagnosis for recipients younger than 1 year old. Cardiomyopathy and congenital heart disease are the two most common indications for transplantation in children.[2]

The 10-year survival rate after cardiac transplantation currently approaches 50% and more in high-volume centers.[1,3] The success of heart transplantation, for the most part, has been achieved through better understanding of the immunology of transplant rejection and the application of strategies for the recognition, treatment, and prevention of rejection. In the early years of cardiac transplantation, failure resulted from a high incidence of acute cellular rejection that limited graft survival. The signs and symptoms of acute cellular rejection are often vague and there are no serologic markers of cardiac allograft rejection. The treatment of rejection, in turn, was often complicated by infection, malignancy, and drug toxicities that result from the difficulty in titrating immunosuppression to the desired end point according to the severity of rejection. The introduction of percutaneous transvenous endomyocardial biopsy by Caves et al[4] in 1973 provided an objective means of diagnosing rejection and allowed for careful monitoring and prompt treatment of cardiac allograft rejection.

ENDOMYOCARDIAL BIOPSY

Endomyocardial biopsy (EMB) remains the gold standard for rejection surveillance in the heart transplant patient.[5] It has a high sensitivity and specificity for the diagnosis of acute cellular rejection.[6,7] There are currently no cardiac imaging modalities or serum markers that can replace the performance of surveillance biopsies in the posttransplantation care and management of these patients.[8]

Ideally, an initial biopsy of the donor heart should be obtained in the operating room at the time of transplantation. This biopsy can be valuable because it provides a means to assess the status of the donor myocardium for hypertrophy, ischemia, or the presence of any pathologic process such as myocarditis. The frequency of posttransplant surveillance biopsies varies highly between different institutions. Typically, surveillance biopsies are performed once weekly for the first month, every 2 weeks for the second month, and every 6 to 8 weeks between the third and 12th months. After the first year, the frequency can be decreased to quarterly, biannually, or annually. In some centers, protocol biopsies are not done after 2 or more years unless there is a clinical suspicion of rejection. If rejection is diagnosed, the patient is treated and undergoes repeat biopsy after 1 to 2 weeks.

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From the Department of Anatomic Pathology, The Cleveland Clinic, Cleveland, Ohio (Drs Tan and Rodriguez); and the Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Md (Dr Baldwin).

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Reprints: E. Rene Rodriguez, MD, Cardiovascular Pathology, Department of Anatomic Pathology, L25, The Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195 (e-mail: rodrigr2@ccf.org).

The procurement of the tissue is made with a bioptome introduced from either the jugular or femoral vein to sample the right ventricular septal wall. Bioptomes are available in different sizes; therefore, the size of the pieces of tissue retrieved will differ slightly. The common sizes used are 7 F (French) and 9 F in adults and 3 F, 5 F, and 7 F in pediatric-age patients (Figure 1, A through D).[9]

Handling the Biopsy Specimen

To prevent introducing artifacts in EMB, the tissue should not be allowed to sit on filter paper, gauze, or any other surface that is impregnated with saline or other solutions that are not iso-osmotic, for a prolonged period of time. The tissue should be fixed immediately in the desired fixative, the most commonly used being 10% phosphate-buffered formalin that has been allowed to reach room temperature (25°C). Cold fixative enhances contraction band artifact. To avoid crushing artifacts, the tissue should not be handled with forceps or divided with a scalpel. The cardiac catheterization suite personnel should not triage the tissue based on gross appearance. All the pieces obtained should be submitted because they may have valuable information when examined histologically. Pieces that look white, suggesting that they are made up of thick endocardium, or pieces that look like blood clot may harbor a piece of myocardium in their core. Tissue is not routinely fixed in glutaraldehyde for electron microscopy of allograft biopsies.

Adequacy of the Biopsy Specimen

In the 1990 International Society for Heart and Lung Transplantation Working Formulation of Cardiac Allograft Pathology (ISHLT-WF1990), 4 to 6 pieces of tissue, depending on the size of the bioptome used, were required for light microscopic evaluation.[10] Because acute cellular rejection is not uniformly distributed in the heart, it is important to take multiple samples during the biopsy procedure. It has been shown that if 3 biopsy pieces taken show no rejection, there is a 5% and 0% chance of missing a mild and moderate-to-severe rejection, respectively. However, if 4 pieces are examined, the false-negative rate of mild rejection is further reduced to 2%.[11] Other investigators have suggested that the extent of infiltration is also important. Where mild rejection is the most severe grade observed in 3 or 4 fragments, the probability of missing moderate or severe rejection is 25.4% and 28.2%, respectively.[12] The 2004 revised working formulation (ISHLT-WF2004), however, currently recommends an absolute minimum of 3 biopsy pieces for evaluation, each of which must contain at least 50% myocardium and exclude a previous biopsy site or scar.[13] Studies of sensitivity to detect rejection with only 3 biopsy pieces using the current grading system have yet to be performed. Specimens that do not meet these criteria should be diagnosed as ''inadequate biopsy.'' If rejection is noted in a biopsy of fewer than 3 evaluable pieces, the rejection grade may be indicated in a diagnosis comment with the emphasis that a higher grade of rejection cannot be ruled out.

Gross Pathologic Evaluation

In addition to the demographic data of the patient, the gross description should include the number of tissue pieces, an aggregate measurement with the average size, and color. Careful gross examination provides, in most instances, important information regarding the presence of myocardium, thickened endocardium, adipose tissue, blood clot, or chordae tendineae (Figure 1, E and F).[14] It is good practice to state the number of pieces submitted in the requisition form to be verified on gross examination and always correlated with the number of pieces present in the paraffin block and in the hematoxylin-eosin–stained slides.

Histopathologic Evaluation

The current working formulation suggests a minimum of 3 step levels for microscopic examination.[13] No special stains are routinely required for evaluation. Unstained slides can be cut and saved for immunohistochemical staining if needed.

Frozen Section Evaluation

One or more pieces of tissue can be snap-frozen for immunofluorescence or other additional study (such as in situ nucleic acid hybridization, in situ polymerase chain reaction, and gene expression profiling) depending on the needs of a given patient and any research protocol used by the institution.

Following a careful freezing protocol is important in order to achieve the best preservation of morphology possible. The ISHLT-WF1990 suggests freezing 1 biopsy piece in OCT freezing compound (Miles Inc, Diagnostics Division, Elkhart, Ind). There is no specific recommendation in the ISHLT-WF2004 regarding either the manner of freezing or the number of biopsy pieces to be frozen. In our institution, 4 biopsy pieces are routinely obtained and all pieces are frozen. The tissue is quickly and gently blotted to remove any excess moisture before embedding them on a chuck containing partially frozen OCT. After proper orientation, the specimen is fully covered with OCT and submerged in liquid nitrogen until frozen. Three step levels are cut for hematoxylin-eosin staining. This technique yields excellent frozen sections that are comparable to those obtained from paraffin sections. Additional slides can be obtained for the application of immunoperoxidase and immunofluorescence studies. The tissue is then kept frozen and stored at −80°C for future study.

CARDIAC ALLOGRAFT REJECTION: MORPHOLOGIC ASPECTS

As in any other solid organ, cardiac rejection can result from humoral and cellular rejection. These are, in turn, subclassified into hyperacute, acute, and chronic rejection on the basis of mechanism and duration of the process.

HYPERACUTE REJECTION

Hyperacute rejection is graft injury triggered by preformed antibodies and occurs rapidly after implantation of the graft, usually within minutes to hours. In the older literature, hyperacute rejection has also been referred to as *humoral rejection, vascular rejection*, and *antibody-mediated rejection.* This type of rejection is extremely rare in the current practice of allograft cardiac transplantation. The morphologic findings are well described in experimental discordant xenografts[15] with similar findings in autopsy cases of cardiac allograft recipients.[16] Predisposing factors that may play a role are preformed antibodies to epitopes of the ABO and HLA systems and vascular endothelial cells,[17] previous pregnancies, multiple surgeries with the use of blood products and, especially, previous cardiac or

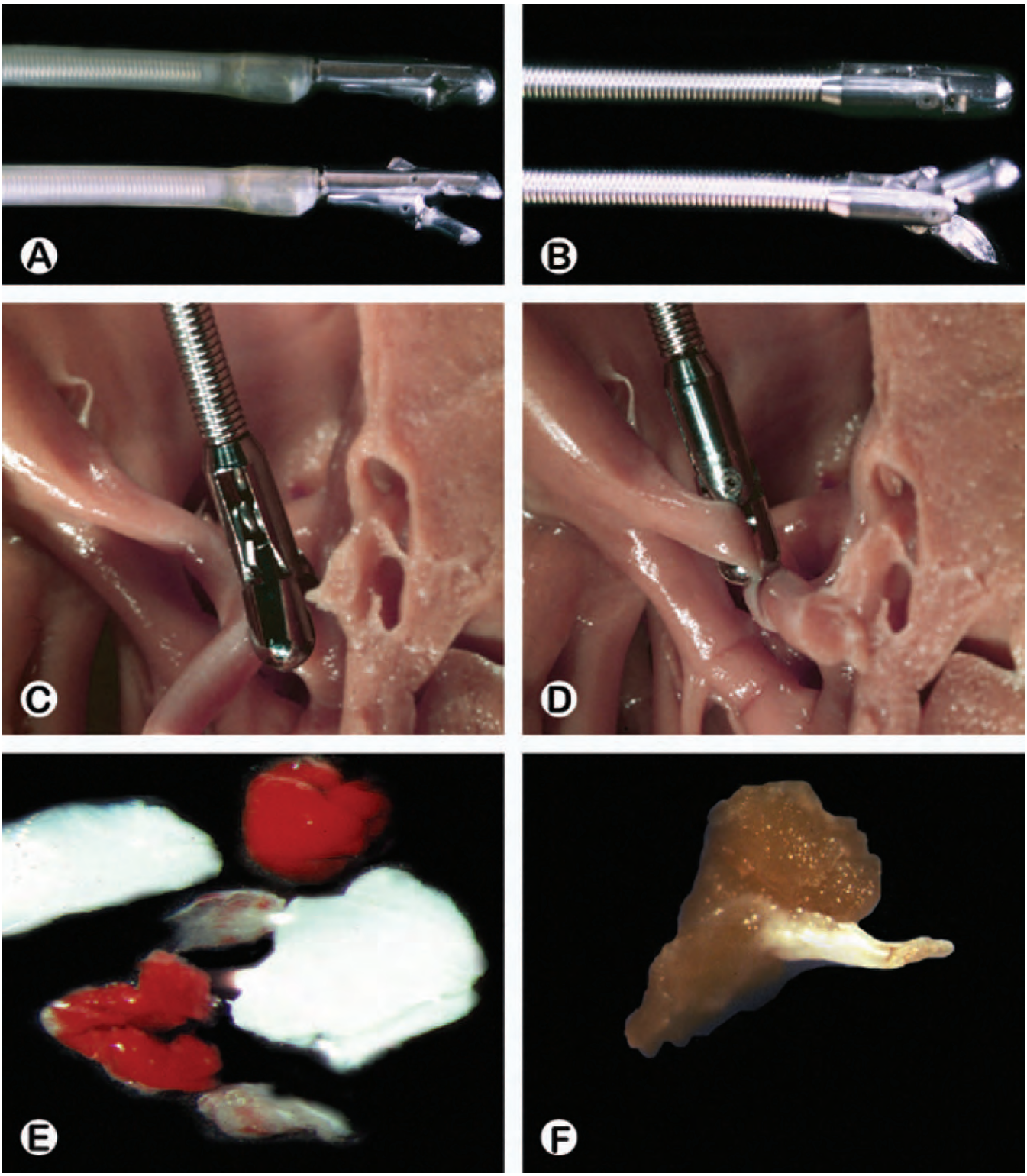

Figure 1. *The endomyocardial biopsy specimen. Bioptomes used for procurement of endomyocardial biopsy specimens are available in different sizes. A, Caves bioptome has a cutting mechanism that is composed of 1 rigid and 1 mobile jaw. B, The Cordis bioptome has 2 flexible jaws. C and D, The bioptome is seen in an open and closed position against the right side of the interventricular septum where trabeculations are usually abundant. E, Pieces of white thickened endocardium and blood clots can be seen in an endomyocardial biopsy specimen. They can be recognized grossly and should also be submitted for histologic examination as they may contain myocardium beneath. F, A fragment of papillary muscle with attached short segment of chorda is shown. Chordae tendineae are sometimes inadvertently sampled during the procedure. Their presence should be mentioned in the report.*

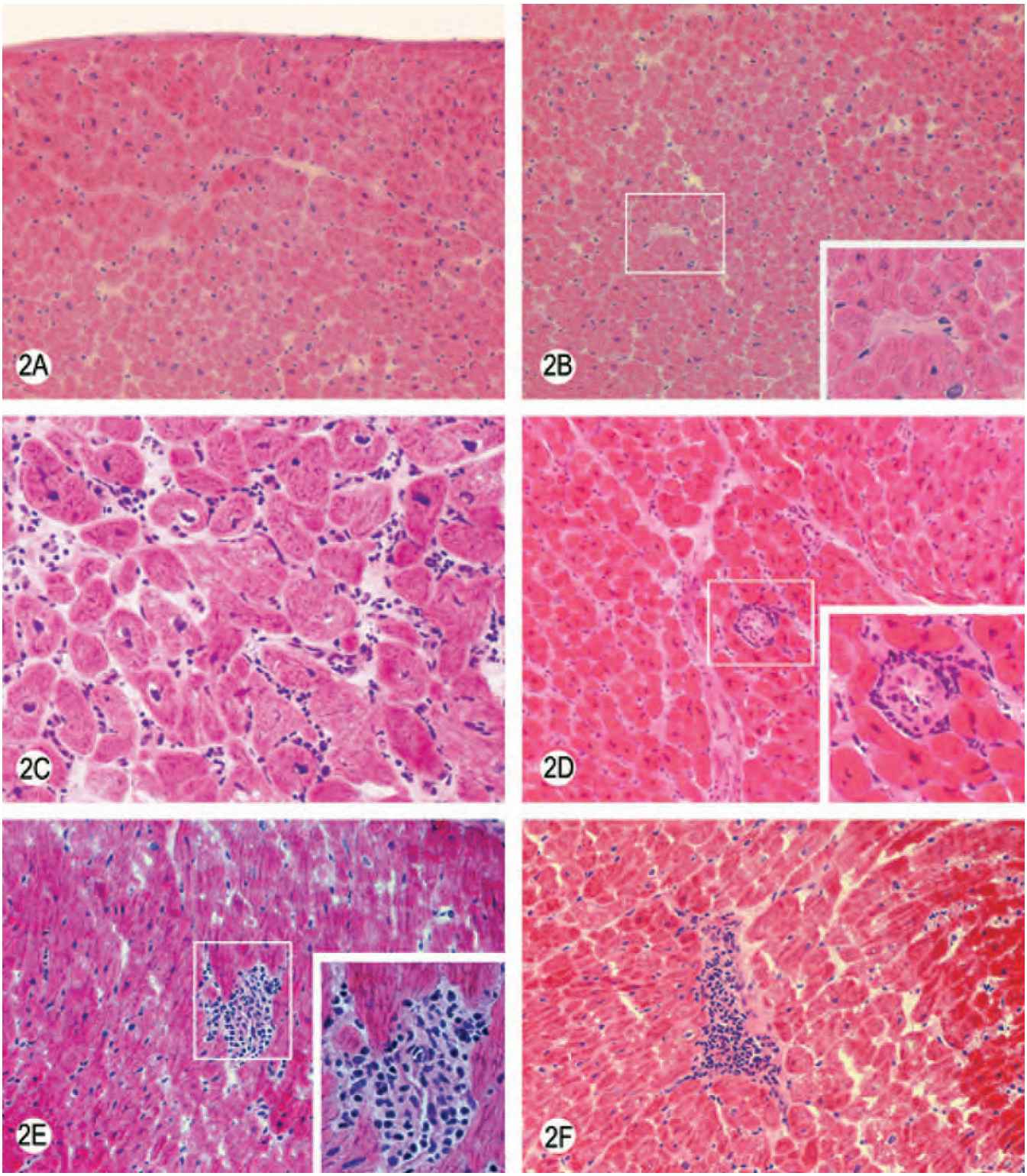

Figure 2 (Part 1). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004). A, Grade 0R. A well-oriented fragment of normal myocardium with no evidence of inflammatory infiltrates is illustrated. The endocardium is normal (frozen section, hematoxylin-eosin, original magnification ×100). B, Grade 0R. The inset shows a venule with flattened endothelial lining and no infiltrates in the interstitium or perivascular space (frozen section, hematoxylin-eosin, original magnifications ×100 and ×400 [inset]). C through F, Examples of Grade 1R. Mild rejection is seen as sparse interstitial infiltrates in between myocytes. Note the absence of interstitial expansion by the infiltrates (C, frozen section, hematoxylin-eosin, original magnification ×400). Scant inflammation is demonstrated around an arteriole (D) and a venule (E). Isolated mildly expansile perivascular infiltrate cut in a longitudinal orientation is shown (F). In the absence of significant myocyte encroachment or clear myocyte damage, it is graded as mild cellular rejection (D through F, frozen section, hematoxylin-eosin, original magnifications ×100 and ×400 [insets]).*

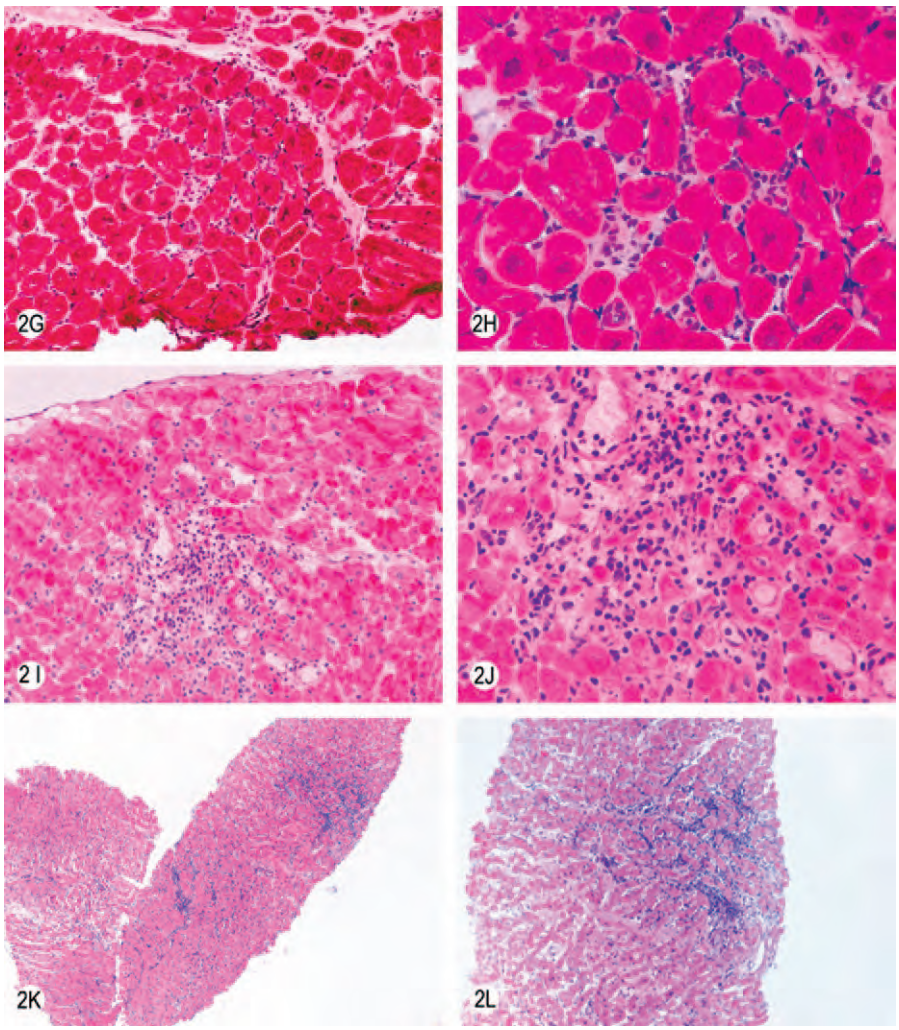

Figure 2 (Part 2). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004 [ISHLT-WF2004]). G and H, Grade 1R. A small focus of diffuse, predominantly interstitial mononuclear infiltrate is demonstrated in low and higher magnification (frozen section, hematoxylin-eosin, original magnifications ×200 [G] and ×400 [H]). This interstitial ''chicken wire'' pattern was previously referred to as 1B in the ISHLT-WF1990. I and J, Grade 1R with a focus of myocyte damage, formerly grade 2. A single focus of inflammation is located close to a normal endocardium in this biopsy fragment. At higher magnification (J), this area shows myocyte replacement or dropout, implying myocyte damage. Note the presence of fragmented and attenuated myocyte sarcoplasm (arrowheads) in the midst of the inflammatory cells as well as the loose stroma in the background consistent with an acute process (frozen section, hematoxylin-eosin, original magnifications ×100 [I] and ×200 [J]). K and L, Grade 2R with multifocal myocyte damage. Scanning magnification shows one fragment of tissue containing two distinct foci of more abundant inflammatory infiltrates with an intervening area of myocardium without inflammation (hematoxylin-eosin, original magnifications ×20 [K] and ×40 [L]).*

other organ transplants. The pathogenesis of hyperacute rejection is believed to be an antibody-mediated activation of the complement cascade, producing severe damage to the endothelial cells, as well as platelet activation followed by the clotting cascade and thrombosis. Although the widely accepted concept is injury to the capillary network of the graft, some investigators have suggested that endothelial damage occurs primarily in cardiac venules, resulting in venular thrombosis.[18] On gross examination, the heart is swollen and it is dusky on external inspection. The ventricles are dilated with scattered hemorrhages, mostly in the subendocardium. Histopathologic changes include swelling of the endothelial cells, vascular thrombosis, extravasation of red blood cells, prominent interstitial edema, and subsequent polymorphonuclear inflammatory infiltrates followed by tissue necrosis. These changes initially occur focally but rapidly spread through the organ. Immunohistochemical studies may show deposits of immunoglobulin (Ig) M, IgG, and complement in the vessel walls as well as fibrin deposits.

ACUTE CELLULAR REJECTION

Morphologically, acute cellular rejection consists of a mononuclear inflammatory infiltrate that is predominantly a T-cell–mediated response directed against the cardiac allograft. In severe cases, there is also participation of granulocytes in the rejection process. Characterization of the phenotype of lymphocytes in cardiac biopsy tissue has shown no good correlation between the extent and composition (CD4:CD8 ratio) of T lymphocytes infiltrating the graft and the histologic grading of rejection.[19,20] However, other studies report a good correlation between the mean number of $CD8^+$ T cells and the severity of rejection grade.[21] The discrepancy in these studies may be related to the fact that the immune response to the allograft is a continuous process in flux that is usually dissected in small ''time-lapsed'' views for pathologic study. Some support to this notion is provided by the observation that if subsets of T lymphocytes are further classified on the basis of the presence of naive cells (CD45RA) and memory or activated cells (CD45RO), naive cells of the CD4 phenotype are more abundant in biopsy tissue during mild rejection. A shift toward activated CD8 phenotype is seen in moderate rejection.[22] An increase in the number of antigen presenting cells (ie, macrophages and dendritic cells) is also observed as a function of the severity of rejection.[23–26] B-cell infiltrates are rarely present in mild rejection. However, a substantial increase in activated B lymphocytes and natural killer cells are seen in moderate rejection, suggesting their important role as promoters and effectors of cellular rejection.[26]

Grading of Acute Cellular Rejection

Historically, several methods to assess the histologic grade of rejection have been used by different transplant

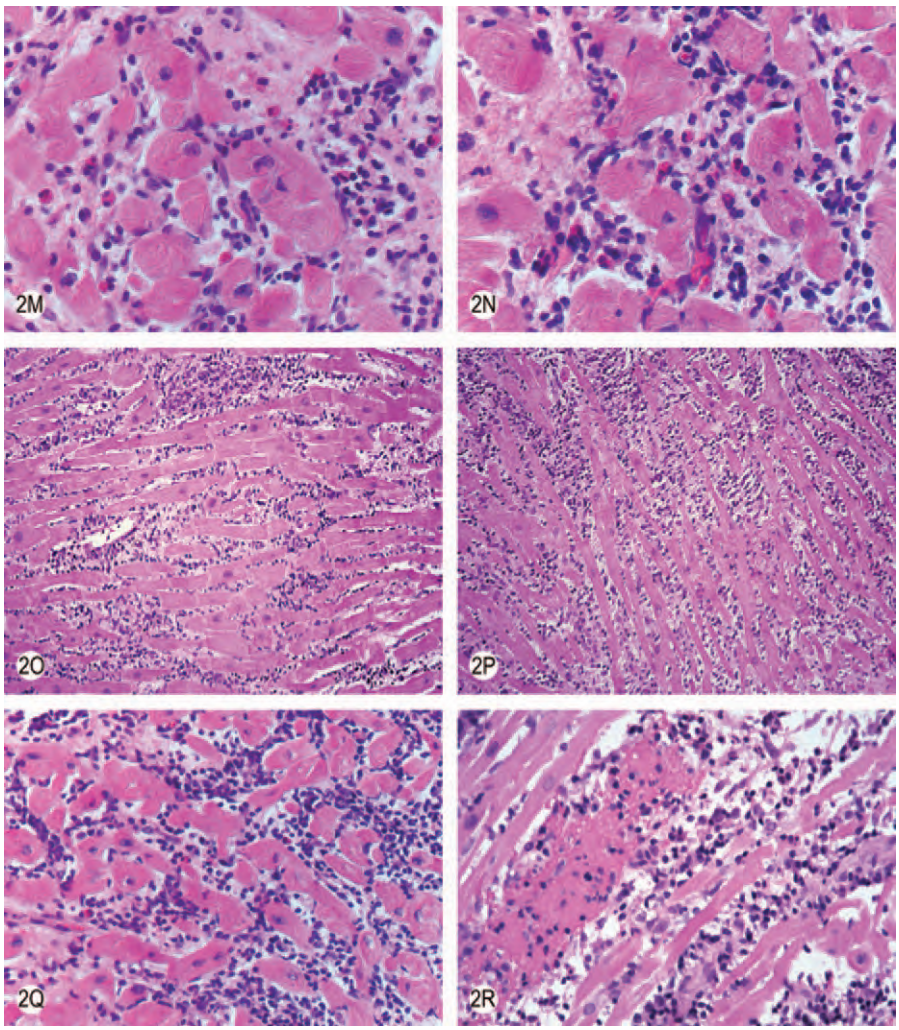

Figure 2 (Part 3). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004 [ISHLT-WF2004]). M and N, Grade 2R. Higher magnifications of 2 different areas of moderate rejection in a biopsy demonstrate widening of the interstitium. The lymphocytes appear to be in close contact with the myocyte borders. Numerous eosinophils are present in these images. These lesions would be 3A in the ISHLT-WF1990 (hematoxylin-eosin, original magnification ×400). O through Q, Grade 3R with diffuse inflammation. These myocardial pieces are diffusely infiltrated by dense mononuclear inflammatory infiltrates. These represent a grade 3B in the ISHLT-WF1990 (hematoxylin-eosin, original magnifications ×100 [O and P] and ×200 [Q]). R, Grade 3R with edema and hemorrhage. There is a mixed inflammatory infiltrate including neutrophils in severe rejection. The blood vessel shown here is necrotic. There is interstitial edema that separates damaged myocytes (hematoxylin-eosin, original magnification ×200).*

centers and will not be reviewed here. In 1990, the ISHLT published a standardized international grading system for the purpose of effectively communicating outcomes in multicenter drug trials and among institutions using different treatment regimens. The grades proposed in the ISHLT-WF1990 were mainly based on the amount of inflammatory infiltrate and the presence of myocyte damage.[10] The absence of cellular rejection was called grade 0 (Figure 2, A and B). Because rejection is a patchy process, the severity of inflammation may differ from one fragment to the next. Rejection is generally graded on the worst area of involvement. The pattern of inflammatory infiltration was reflected in the subdivisions A and B in grades 1 and 3. In mild rejection, it played a minor role and does not imply that a diffuse pattern (1B) is worse than a focal infiltrate (1A) (Figure 2, C through H).[27] It must also be noted that the grading of rejection was designed to assess rejection in endomyocardial biopsies and not the whole grafts.

As this grading scheme was widely adopted after its publication, variability in the interpretation of histologic grading among pathologists became evident and resulted in a lack of consensus with regard to the treatment of specific grades of cellular rejection. In 2001, the Banff Allograft Pathology Group invited pathologists, cardiologists, and cardiac surgeons to discuss their experiences after more than 10 years of using the ISHLT-WF1990. These discussions pointed out some of the more difficult

Comparison of the 1990 and 2004 Grading System of the International Society of Heart and Lung Transplantation for Acute Cellular Rejection

1990	2004
Grade 0 (no acute rejection)	Grade 0R (no acute cellular rejection)
Grade 1A (focal, mild acute rejection)	Grade 1R (mild, low-grade, acute cellular rejection): interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
Grade 1B (diffuse, mild acute rejection)	
Grade 2 (focal, moderate acute rejection)	Grade 2R (moderate, intermediate-grade, acute cellular rejection): 2 or more foci of infiltrate with associated myocyte damage
Grade 3A (multifocal moderate rejection)	
Grade 3B (diffuse, borderline severe acute rejection)	Grade 3R (severe, high-grade, acute cellular rejection): diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis
Grade 4 (severe acute rejection)	

issues for clinical practice and for use of the pathology information as end points in clinical trials.[5] In 2004, under the direction of the ISHLT, a working group composed of an international, multidisciplinary team of subspecialists in cardiac transplantation met to review the ISHLT-WF1990 definitions of cellular and antibody-mediated rejection, identify areas of difficulty in interpreting transplant biopsies, and revise the grading system. There was strong consensus that any changes in the formulation should reflect current pathologic practice and should not affect the grading of historic samples. The issue then was not one of changing the 1990 ISHLT grading scales, but one of more clearly defining how pathologists and cardiologists should interpret the grading system.

A major controversy in the ISHLT-WF1990 is the diagnosis and clinical significance of grade 2 rejection (Figure 2, I and J).[28,29] It is a grade that has been used in many transplant centers as a discrete defining point in therapeutic decisions. The misdiagnoses of grade 2 lesions by pathologists and the clinical data indicating that grade 2 rejections resolve without treatment in the majority of cases prompted the working group to now include grade 2 rejection with the revised mild rejection category. The old grade 3A (Figure 2, K through N) has been reclassified as grade 2R in the new working formulation (Table). Disagreement in the diagnosis between grade 3B (diffuse, borderline, severe acute rejection) and grade 4 (severe acute rejection) (Figure 2, O through R) also occurred previously as both of these can show the same severity of diffuse destructive infiltrates. The difference rests mainly on finding additional neutrophilic infiltrates and demonstrating edema and hemorrhage in the biopsy. It seemed more logical then that grades 3B and 4 were placed together in the severe category of the revised grading system because these minor discrepancies do not affect clinical therapeutic decisions.

The different histologic grades in the revised ISHLT-WF2004 classification are indicated by a suffix, ''R'' (Table). Absence of inflammation is reported as no rejection. A perivascular or interstitial infiltrate of mononuclear cells without architectural distortion is considered mild rejection. A focus of inflammation with myocyte damage, previously termed *grade 2* in the ISHLT-WF1990 classification, has been incorporated in the mild rejection category. Moderate, intermediate-grade rejection consists of 2 or more foci of mononuclear cell infiltrates associated with myocyte damage. Eosinophils may be present in moderate rejection. Severe, high-grade rejection is a diffuse process with multiple areas of myocyte damage and often a polymorphous inflammatory infiltrate that may be accompanied by edema and hemorrhages. A comparison of the 1990 working formulation and the revised grading system is presented in the Table.

Pitfalls and Caveats in Evaluating Endomyocardial Biopsies for Cellular Rejection

Although an enormous effort has been put forth to create a standard method for grading rejection that is easily reproducible, there were some controversial points that have been identified by both pathologists and clinicians in using the ISHLT-WF1990 and these warranted further clarification in the revised grading scheme.[5] Some of these controversies are discussed in the following sections.

Definition of Myocyte Damage.—A major source of discordance in histologic grading is the criteria used for the interpretation of ''myocyte damage'' in light microscopy, which is a required feature in higher grades of rejection.[5] The morphologic spectrum of myocyte damage is wide and has subtle changes that can be difficult to ascertain. Various forms of myocyte injury described by experienced cardiac pathologists include vacuolization, perinuclear halo, ruffling of the cytoplasmic membrane, irregular myocyte border, splitting or branching of myocytes, and myocyte encroachment with partial disruption of the myocytes.[6,30] Hypereosinophilia and nuclear pyknosis would indicate myocyte necrosis. Ultrastructural studies have shown that actual myocyte necrosis is rare, and reversible myocyte injury and myocyte regeneration occur even in moderate-to-severe acute cellular rejection.[31–33] In the revised working formulation, myocyte damage is described as ''clearing of the sarcoplasm and nuclei with nuclear enlargement and occasionally prominent nucleoli.''[13] Architectural distortion, myocyte encroachment with irregular myocyte borders, and myocyte dropout also frequently indicate myocyte damage in cellular rejection.

Does Grade 2 Lesion Exist?—One of the criticisms in relying on EMB to monitor rejection is the low interobserver agreement in the diagnosis of grade 2 rejection.[34,35] A corollary to this is the controversy of whether or not grade 2 rejection exists. Recognition of a grade 2 lesion is indeed problematic because of the obvious implications for therapy. Earlier on, most centers treated moderate rejection (grade 2 or higher) with adjustment in the immunosuppressive regimen. It is believed that a major source of confusion in grade 2 rejection is the difficulty in distinguishing the histologic features of this grade from Quilty lesions. Quilty lesions, named after the first patient in whom they were observed at Stanford University, are also known as *endocardial lymphocytic infiltrates*, which we think is a better term (Figure 3, A through D).[36,37] These are collections of predominantly T lymphocytes with admixed B cells, occasional macrophages, and plasma cells seen in the endocardium of transplanted hearts that vary in size from 0.007 to 1.89 mm^2.[38] (The detailed pathology of endocardial lymphocytic infiltrates is discussed under ''Redefinition of the Quilty Effect.'') Small capillaries, sometimes with prominent endothelial cells, and dense endocardial collagen (Figure 3, B) are seen within the infiltrate and are diagnostically useful clues. Quilty infiltrates can extend deep into the subjacent myocardium and the lesion is designated type B in the ISHLT-WF1990 (Figure 3, C and D). Quilty B lesions can be big and may be associated with architectural distortion that does not represent acute rejection. One may imagine how a tangential section through the deeper (myocardial) end of a Quilty B lesion may show inflammatory infiltrates with myocyte encroachment that can easily be mistaken for moderate rejection if only a few levels of section are examined. However, if additional sections are made, one can usually ascertain the continuity of such a lesion from the myocardium to the endocardium. This type of artifact has prompted some observers to question whether or not grade 2 cellular rejection even exists.[39] Our personal experience shows that sectioning through the entire tissue block and examining alternatively stained slides almost always resolves the question (Figure 4).

Another solution offered to this problem is to stain the biopsy section with antibodies to RANTES (regulated on activation, normal T cell expressed and secreted). This is helpful in differentiating a focus of cellular rejection from

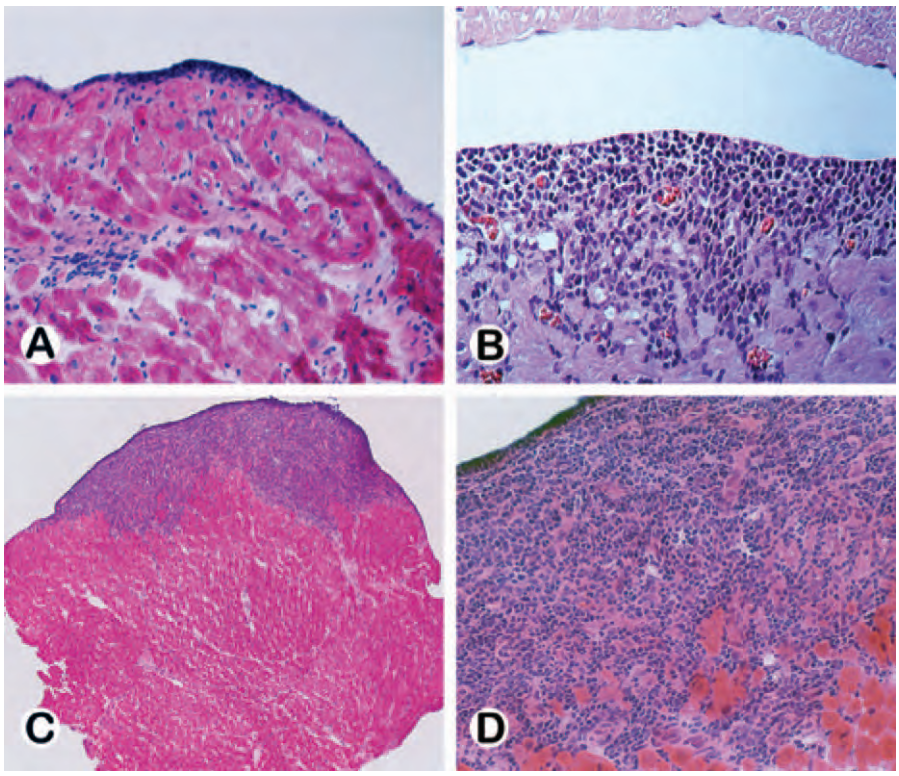

Figure 3. *Endocardial lymphocytic infiltrates (Quilty effect). A, Endocardial lymphocytic infiltrates are confined to the endocardium in this Quilty lesion (frozen section, hematoxylin-eosin, original magnification ×200). B, Another example of a Quilty lesion is shown that extends into the myocardium (invasive Quilty lesion). There are numerous capillaries present within the dense infiltrate. Cytoplasmic vacuoles can be seen in the adjacent myocytes. If this focus is not oriented properly in the biopsy, a tangential cut or a section through the deeper portion of the lesion can easily be misinterpreted as rejection with myocyte damage (hematoxylin-eosin, original magnification ×200). C and D, Large Quilty lesions are frequently seen in biopsies. Proliferation of small blood vessels and fibrous stromal background are typical of these lesions. In contrast, cellular rejection lesions show no fibrosis or small vessel formation during the acute process. In these images, isolated myocytes and small groups of myocytes appear to be entrapped within the lesion. This infiltrative type of Quilty lesion was formerly called Quilty type B in the 1990 International Society of Heart and Lung Transplantation Working Formulation (frozen section, hematoxylin-eosin, original magnifications ×40 [C] and ×200 [D]).*

Quilty B lesions because the RANTES-positive cells are more abundant in acute rejection.[40]

Characterization of the Inflammatory Infiltrate.—In the ISHLT-WF1990, the inflammatory infiltrates are called ''aggressive'' but are not further defined. Pathologists have difficulty in determining what is meant by ''large aggressive lymphocytes.'' This descriptive term is therefore deleted in the current grading system. Immunostains for phenotyping inflammatory cells are not routinely performed for diagnostic or prognostic purposes.

Additional Information to be Included in the Biopsy Report

The following sections show morphologic findings that may be confusing for the novice pathologist in the differential diagnosis of rejection. Some of these features do not represent rejection but need to be clearly recognized. Furthermore, the ISHLT-WF2004 requires that these features be recorded in the report.

Ischemic Injury.—The presence or absence of ischemic damage should always be documented. The ISHLT-WF1990 makes a distinction during allograft monitoring between ischemia commonly seen in the biopsy up to 3 weeks posttransplant representing perioperative injury (ischemia A) and late ischemia that occurs after 3 or more months (ischemia B). In the revised grading system, ischemia is divided into early (up to 6 weeks) and late ischemic injury (Figure 5). Late ischemic injury may explain cardiac allograft dysfunction secondary to severe allograft atherosclerosis.

Perioperative ischemia is seen in a majority of transplanted hearts and is strongly associated with prolonged total ischemic time.[41] Other causes of ischemic injury include events that affect the donor such as catecholamine discharge, pressor therapy given during acute care, severe donor trauma, reimplantation damage, or early postoperative damage. In the early stage, it consists of subendocardial foci of myocytes showing coagulation necrosis (with or without contraction bands) and macrophages with variable amounts of polymorphonuclear leukocytes. These areas are usually sharply demarcated with necrotic myocytes occurring in small groups and highlighted by staining with Masson's trichrome. Some lesions can lack an acute inflammatory reaction (Figure 5, A and B). Ischemic foci may persist for several weeks because of a depressed inflammatory response in these immunosuppressed patients. In the healing phase, these ischemic foci usually show pigment-laden macrophages with a few lymphocytes, a somewhat loose connective tissue stroma, and scant granulation tissue (Figure 5, C). Once they mature, ischemic lesions are indistinguishable from scars produced by previous endomyocardial biopsies (Figure 5, D and E).

Ischemic injury should be differentiated from cellular rejection. The extent of myocyte necrosis is usually out of proportion to the inflammatory infiltrate in ischemic injury, with the infiltrates consisting mostly of neutrophils and macrophages. In cellular rejection, the infiltrates are predominantly lymphocytic. A more difficult distinction to make is between the healing phase of ischemic injury and the resolving phase of moderate rejection in the early posttransplant period. This is usually resolved with clinical correlation and proper communication with the cardiologists.

Most early ischemic injury is clinically silent, but if the injury is extensive, myocyte necrosis can compromise the function of the graft postoperatively. Another possible implication in hearts that had damage during the peritransplant period is the subsequent development of interstitial fibrosis.[42]

Redefinition of the Quilty Effect.—The ISHLT-WF1990

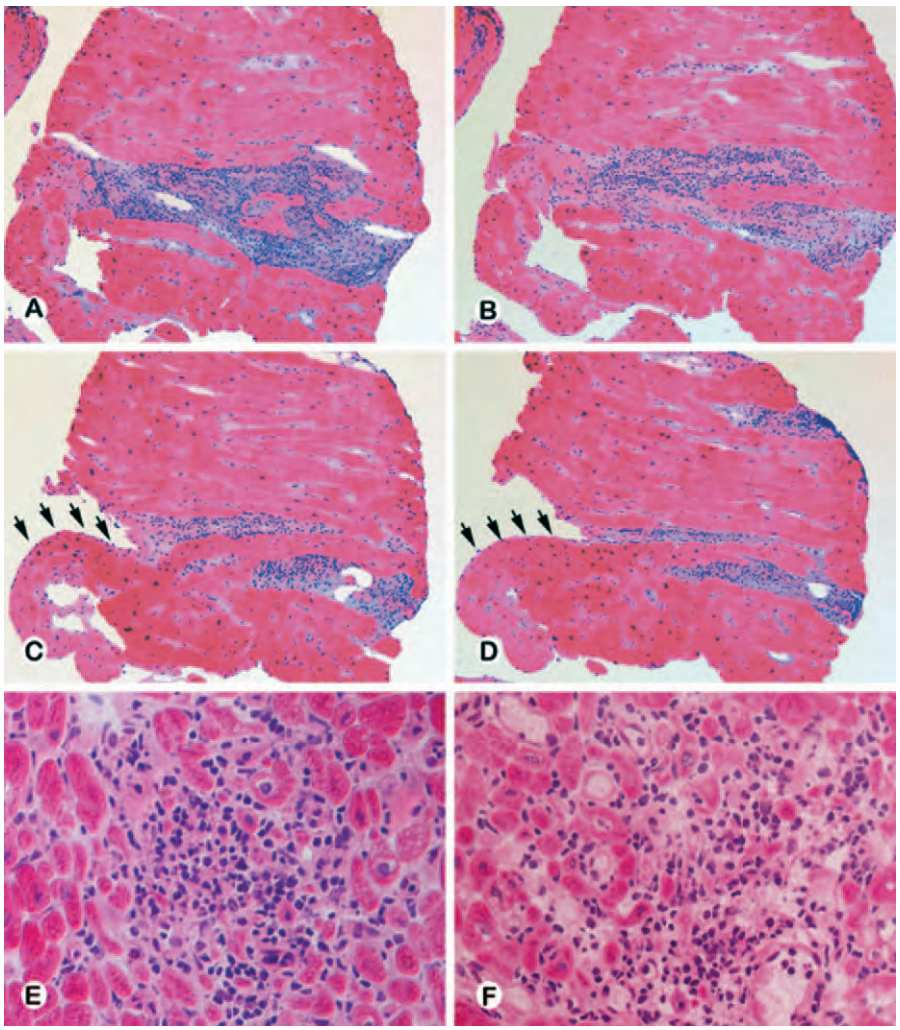

Figure 4. *Endocardial lymphocytic infiltrates versus cellular rejection. A through D, Sequential and deeper sections are helpful in differentiating a focus of rejection with dense inflammatory infiltrates and apparent disruption of the myocytes from a tangential cut of an invasive Quilty lesion. In the first section, a few myocytes are noted on the left side and no endocardial surface is clearly identified around the fragment. Deeper sections show a decrease in the amount of infiltrates. The infiltrate connects to the endocardial surface, which becomes identifiable on the left side in C and D (arrows) (frozen section, hematoxylin-eosin, original magnification ×40). E and F, Differentiation between a Quilty lesion (E) and a focus of rejection with myocyte damage (F) based on a few sections is difficult. Step levels have to be examined. A very useful feature in our observation is the difference in the character of the stroma between the two entities. The stroma in Quilty lesions is fibrotic (frozen section, hematoxylin-eosin, original magnification ×200).*

recommends that the presence or absence of the Quilty effect should be recorded. In the revised grading system, distinction between infiltrates exclusively confined to the endocardium (Quilty A) (Figure 3, A) and those that extend into the underlying myocardium (Quilty B or invasive Quilty) (Figure 3, B through D) is no longer indicated. There appears to be no clinical significance in subtyping Quilty lesions into A and B.[36] Both these lesions are now referred to as the *Quilty effect*. Several hypotheses have been proposed to explain the pathogenesis of these infiltrates and include the use of cyclosporine-based immunosuppression,[43] idiosyncratic responses to cyclosporin A,[37] reduced endocardial levels of cyclosporine A,[44] and concomitant infection with Epstein-Barr virus.[45] None of these have been proven conclusively. One striking observation is that the Quilty effect was not found in the hearts of patients who were also treated with cyclosporin A for other solid-organ transplantation including the liver and kidney.[46] The Quilty lesion seems to be a phenomenon that occurs only in the endocardium of cardiac allografts. Clear and consistent associations of Quilty lesions with grade of cellular rejection, viral infection, subsequent development of vasculopathy, or survival have not been established. As alluded in the section ''Does Grade 2 Lesion Exist?,'' Quilty effect lesions are sometimes misinterpreted by inexperienced pathologists and the diagnosis of rejection is rendered. Serial sections are very useful to differentiate these two lesions, as shown in Figure 4, A through D. Furthermore, the histologic detail of these two lesions is rather distinct. The Quilty lesions usually have extracellular matrix (collagen) between the lymphocytes as these cells are infiltrating the endocardium (Figure 4, E). These lesions frequently show capillaries in the middle of the infiltrate. On the other hand, the rejection lesions that were previously called grade 2 are indeed foci of rejection in which the lymphocytes are attacking the graft and not infiltrating connective tissue. Thus, one does not find collagen bundles surrounding the lymphocytes (Figure 4, F).

Previous Biopsy Site.—A previous biopsy site is a common finding in transplant surveillance biopsies and can be seen in up to 69% of biopsies.[47] This high frequency occurs because, for a given patient, the anatomy of the inflow tract to the right ventricle is constant. During the biopsy procedure using the transjugular approach, the ridges of the atrial or caval anastomotic sites, the right ventricular trabeculations, and the moderator band all contribute to guide the tip of the bioptome toward the same site in the interventricular septum. Figure 6, A through G, illustrates different stages of lesions related to previous biopsy site. Gross examination at autopsy may show a patch of thickened endocardium measuring 1 to 2

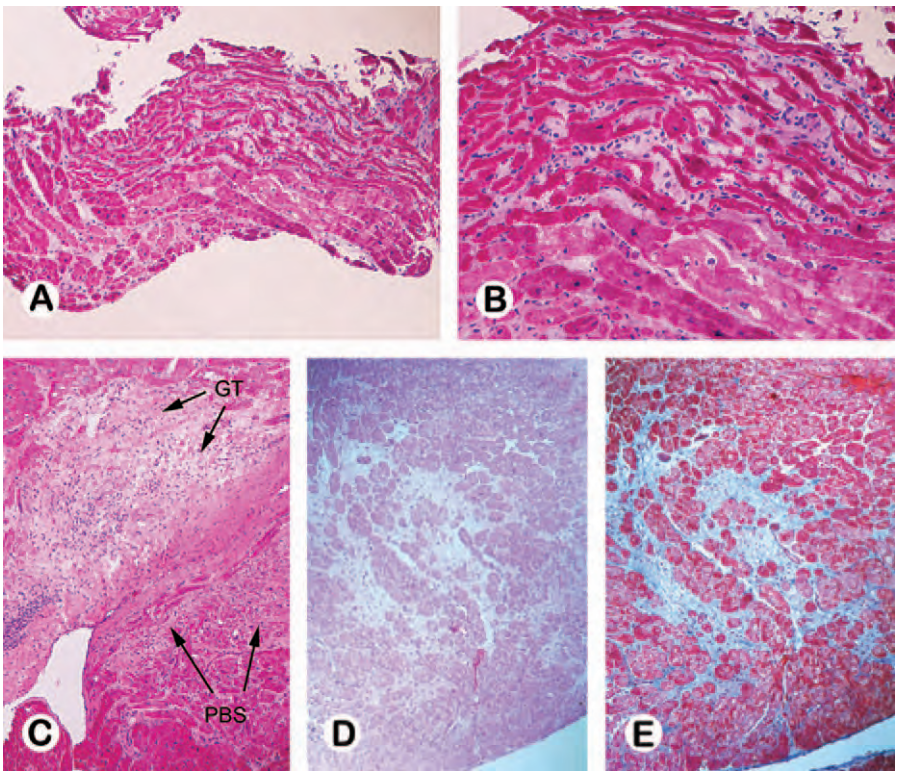

Figure 5. *Acute and healed ischemic injury. A and B, This specimen is the first biopsy taken after transplantation and shows a focus of ischemic myocytes with thin, stretched, and wavy cytoplasm in the upper half of the myocardium. Ischemic myocytes are often found in small groups, subendocardial in location, with absent or pyknotic nuclei and typically hypereosinophilic cytoplasm (frozen section, hematoxylin-eosin, original magnifications ×40 [A] and ×200 [B]). C, Healing ischemic focus with loose granulation tissue (GT) and mild lymphocytic infiltrates in the left upper corner. A previous biopsy site (PBS, also see Figure 7) is also present in the right lower corner (frozen section, hematoxylin-eosin, original magnification ×40). D and E, Interstitial fibrosis and small replacement scars in a transplant biopsy should always raise the suspicion for the presence of allograft vasculopathy (hematoxylin-eosin [D] and Masson trichrome [E], original magnification ×40).*

cm in diameter in the mid third of the right ventricular septum in patients who survived several months to years after the transplant. On light microscopy, the findings of this repetitive sampling of a small area of the septum will include several stages of healing. Recent biopsy sites will show thrombus and granulation tissue (Figure 6, A). Later, there is fibrosis with entrapped myocytes that often exhibit disarray and a variable amount of mononuclear cell infiltrate (Figure 6, E). Old biopsy sites present as endocardial scars (Figure 6, F and G).

Lymphoid Neoplasia.—Posttransplant lymphoproliferative disease has been reported to occur in 1.2% to 9% of cardiac transplant patients, more commonly within the first year of transplantation.[48–50] Recent studies are lacking, and this diagnosis is indeed rare in large-volume centers, perhaps as a result of modern immunosuppression regimens. Identified risk factors for the development of lymphoid neoplasms in these patients are infection with Epstein-Barr virus and type of immunosuppressive regimen received, particularly OKT3.[50–53] Histology of lymphoid neoplasia can range from polymorphic lymphoid hyperplasia to monomorphic malignant lymphomas.[54] Posttransplant lymphoproliferative disease can be diagnosed in the transplant biopsy and should be distinguished from that of acute rejection because early diagnosis and reduction of immunosuppression may lead to regression.[55] Molecular studies can be performed using allograft biopsy material to confirm the diagnosis, including DNA analysis for immunoglobulin gene rearrangement and detection of Epstein-Barr virus genome by in situ hybridization or polymerase chain reaction.[48,56–59] The majority of posttransplant lymphoproliferative diseases seen today are malignant lymphomas of B-cell origin. Their clinical presentation, in decreasing order of frequency, involves lymph nodes, lung, gastrointestinal tract, liver, central nervous system, spleen, and the heart itself.[60] T-cell lymphomas also occur and usually present in extranodal sites.[61–63] Development of multiple myeloma after cardiac transplantation is rare.[64,65]

Opportunistic Infections.—Chronic immunosuppressive therapy to control rejection predisposes transplant patients to a large number of opportunistic infections. Bacterial infection is the most common type of infection, accounting for 47% of the cases. Viral infections are second in frequency (41%), with fungal and protozoal pathogens being responsible for the remaining 12%.[66,67] Identification of infectious pathogens in cardiac biopsy is rare. The two most commonly reported opportunistic infections seen in EMB specimens are *Toxoplasma* and cytomegalovirus (Figure 7, A and B, respectively).[68–70] When examining a biopsy, unusual inflammatory infiltrates such as the presence of granulocytes, plasma cells, and/or macrophages in a focus of inflammation without overt myocyte necrosis or dropout should alert the pathologist to consider a possible infectious process. One should also look for viral inclusions in the nuclei of endothelial cells, smooth muscle cells, or miscellaneous perivascular cells. Cytomegalic inclusions within cardiac myocytes are extremely rare. Both infections can also be associated with a paucity of inflammatory infiltrates and can therefore be easily overlooked. Figure 7, C and D, show examples of fungal infections.

Fibrosis.—Development of interstitial fibrosis in the transplanted heart has been associated with cyclosporine therapy, total ischemic time, rejection episodes, and donor cause of death.[42,71–75] Other investigators, however, did not find a significant association between increase in myocardial collagen and prolonged ischemic time or cyclosporine immunosuppression.[76] The perception of the amount of fibrosis in endomyocardial biopsies may be influenced by the size of the bioptome used; larger pieces of biopsy fragments appear to have lesser quantitated area of fibrosis.[77] Perimyocytic fibrosis is seen most often in areas adjacent

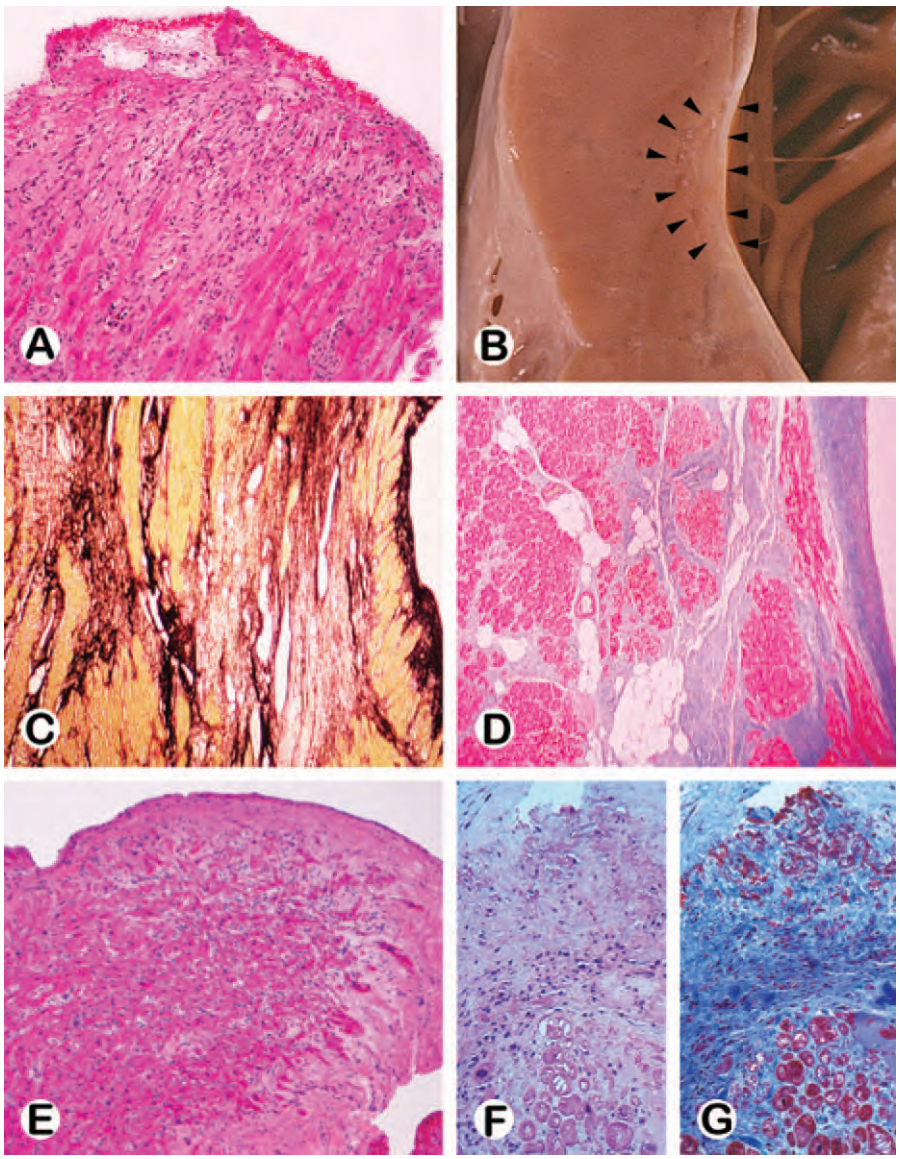

Figure 6. *Previous biopsy sites versus rejection or ischemic injury. A, A recent biopsy site that is about 1 week old and composed of granulation tissue with chronic inflammation is shown. There is a microscopic fibrin clot occurring on the superficial aspect of the biopsy site. Inflammation in biopsy sites or scar is not considered in the evaluation of rejection grade (hematoxylin-eosin, original magnification ×200). B, A slightly depressed, crescent-shaped, scarred myocardium along the septal wall (arrowheads) indicates the site of previous biopsies in the heart of this transplant patient. C and D, Connective tissue stains reveal endocardial fibroelastosis and interstitial fibrosis in the myocardium adjacent to the biopsy site (C, elastic stain, original magnification ×20; D, Masson trichrome, original magnification ×20). E, A healed previous biopsy site shows endocardial thickening with subendocardial fibrosis. Entrapped myocytes show disarray. Variable amount of inflammatory cells can be present (frozen section, hematoxylin-eosin, original magnification ×40). F and G, An old biopsy site with thick fibrotic endocardium is illustrated. Some of the subendocardial myocytes also show colliquative myocytolysis. Previous biopsy sites are common findings in endomyocardial biopsy specimens as the same areas are repeatedly sampled (hematoxylin-eosin [F] and Masson trichrome [G], original magnification ×200).*

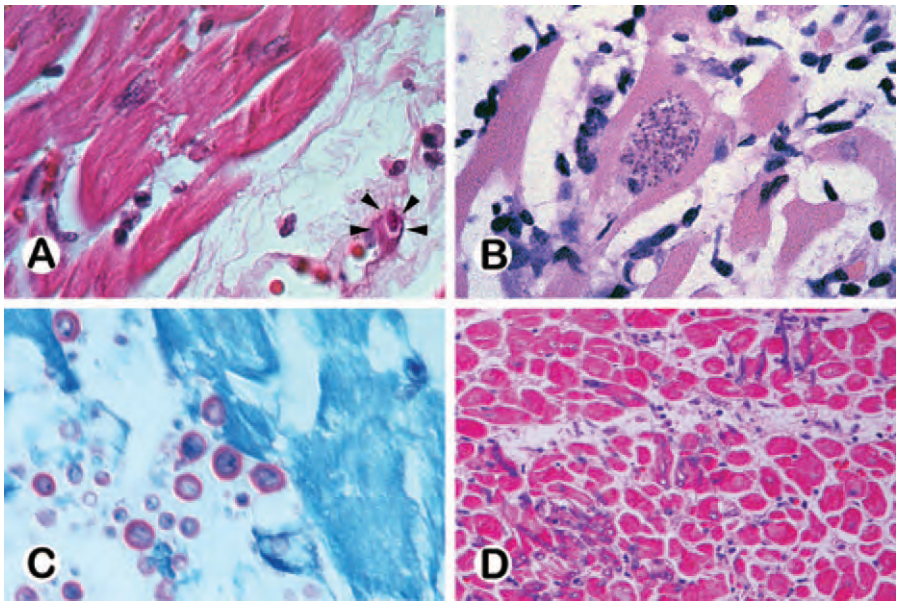

Figure 7. *Opportunistic infections. A, Nuclear inclusion with cytomegaly is noted in an endothelial cell (arrowheads) in a case of cytomegalovirus infection (hematoxylin-eosin, original magnification ×1000). B,* Toxoplasma *bradyzoites are evident as small basophilic structures within the sarcoplasm of a myocyte. Scant lymphocytic infiltrates are present in the interstitium in this image. However, polymorphonuclear leukocytes can also be present (hematoxylin-eosin, original magnification ×1000). C, Variably sized round yeast forms of* Cryptococcus *are present without inflammatory reaction in the myocardium of a posttransplant patient who died of overwhelming infection (mucicarmine, original magnification ×1000). D, Septated fungal hyphal elements invading the myocardium are demonstrated in an autopsy case of invasive* Aspergillosis *(hematoxylin-eosin, original magnification ×200).*

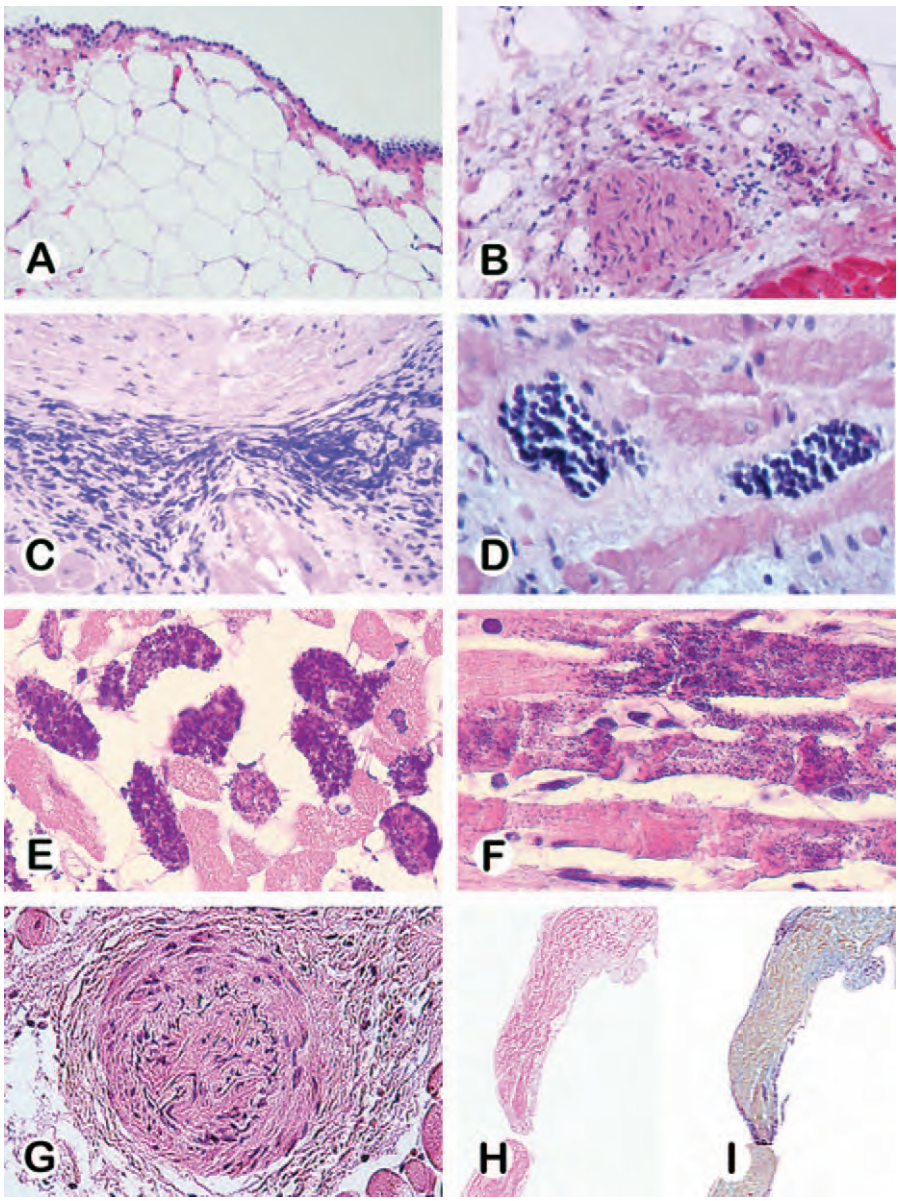

Figure 8. *Interpretation of other findings and artifacts in heart biopsies. A, The presence of mesothelial lining overlying adipose tissue in endomyocardial biopsies is indicative of perforation of the ventricular wall. Mesothelial lining is present in the visceral layer (also called* epicardium*) and parietal layers of the pericardium (hematoxylin-eosin [H&E], original magnification ×100). B, One fragment of epicardial fat with a small nerve bundle and scant inflammation but absent mesothelial lining is noted in a transplant surveillance biopsy. Inflammation in the epicardial fat is commonly seen early in the postoperative period, but in the absence of mesothelial cells, one cannot conclude that this represents a perforation of the ventricular wall (frozen section, H&E, original magnification ×100). C, Crush artifact may be so extensive as to render a piece of myocardium difficult to interpret (H&E, original magnification ×200). D, Occasional lymphatic vessels are seen in biopsies that are distended with lymphocytes. Note that the endothelial cells are not swollen or prominent. This is an infrequent finding. The International Society for Heart and Lung Transplantation Working Formulation does not provide guidelines to interpret this finding (H&E, original magnification ×400). E and F, Mitochondrial calcification appears as basophilic granules in these necrotic myocytes cut in cross and longitudinal sections. Eventually, these myocytes become completely calcified (H&E, original magnification ×400). G, Telescoping (intussusception) within the lumen of this small artery can be confused with luminal occlusion. Note the presence of elastic lamina within the smooth muscle cells that fill up the lumen of the artery (H&E, original magnification ×400). H and I, Chordae tendineae can occasionally be seen in specimens (Figure 1, F) and are characterized by parallel arrays of dense collagen fibers covered by thin endocardium in all their surfaces (H&E [H] and Movat pentachrome [I], original magnification ×100).*

to previous biopsy sites. A causal relationship between interstitial fibrosis and diastolic dysfunction in the cardiac allograft is still uncertain.[78] Furthermore, fibrosis may in fact be a feature already present in a donor heart. Low availability of hearts has led cardiologists and surgeons to the practice of accepting hearts from older donors, which, in some instances, show interstitial and/or replacement fibrosis despite having ''no history'' of coronary artery disease.

Adipose Tissue, Perforation Versus Infiltration.—Adipocytes are normal cellular components of the heart, mostly present in the epicardium. In addition, microscopic foci of adipose tissue are usually present in the subendocardium and, less frequently, within the myocardium.

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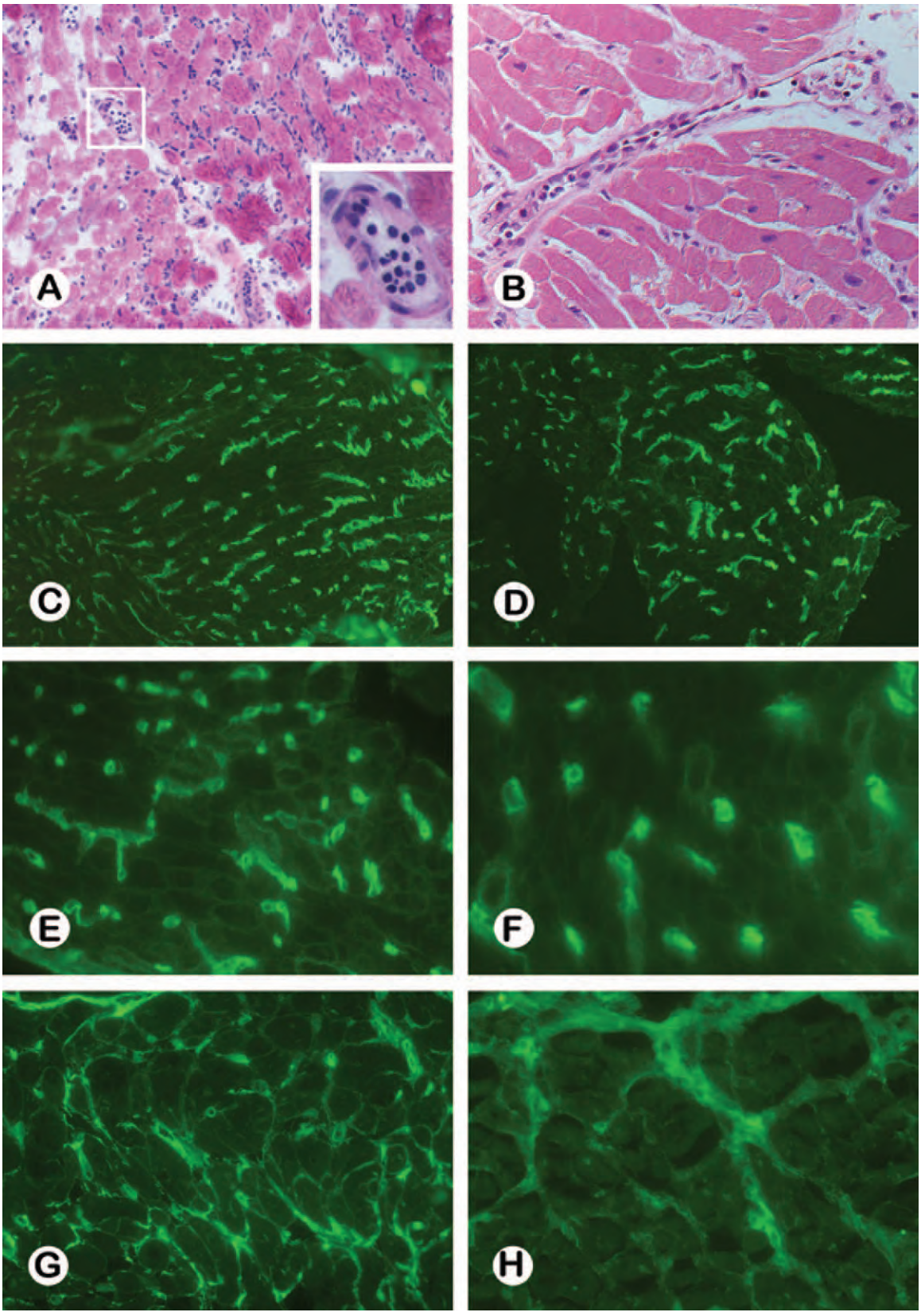
Figure 9. *Antibody-mediated rejection (AMR). A and B, On visible light microscopy, this sample shows a striking low magnification feature, which is the presence of conspicuous endothelial cell nuclei in the interstitial compartment. The small arterioles also appear to be filled with cells. The inset shows mononuclear inflammatory cells within the lumen of an arteriole (A, frozen section, hematoxylin-eosin, original magnifications ×200 and ×400 [inset]; B, hematoxylin-eosin, original magnification ×400). C and E, C4d staining shows intense linear deposits in capillaries that are mostly oriented longitudinally in this frozen section (C, fluorescein isothiocyanate [FITC] anti-C4d, original magnification ×100). Higher magnification demonstrates cross sections of the capillaries (E, FITC anti-C4d, original magnification ×400). D and F, The same biopsy also shows an identical pattern of intense deposits in capillary endothelium with anti-C3d (FITC anti-C3d, original magnifications ×100 [D] and ×400 [F]). G, Repeat biopsy of the same patient after 1 week shows persistent but weaker staining in capillaries. In addition, the biopsy now shows linear staining around myocytes, indicating that the complement split products (C4d and/or C3d) are redistributed to the interstitium. This type of pattern is commonly seen in resolving AMR and after therapy with plasmapheresis (FITC anti-C4d, original magnification ×200). H, After resolution of AMR, a biopsy of the same patient shows artifactual staining in the perimysial collagen. There is no staining in vascular endothelium. The biopsy is now negative for AMR. This staining pattern can persist for several weeks (FITC anti-C4d, original magnification ×200).*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These foci can be seen in all chambers but are more commonly found in the right ventricular wall. In obese patients, older patients, and patients taking steroid hormones, fat infiltration is more common and can be grossly visible. Thus, the presence of adipose tissue per se is not pathologic. The goal of the right ventricular biopsy procedure is to obtain samples from the right side of the interventricular septum; however, on rare occasions, the bioptome may actually sample the right ventricular free wall. Therefore, when a focus of adipose tissue is found in an EMB, the pathologist should make an effort to determine if this is subendocardial or subepicardial adipose tissue. This can sometimes be easily determined by looking for the presence of mesothelial cell lining, indicating the epicardial surface (Figure 8, A). Because of the fibrinous and eventually fibrous pericarditis that usually develops after the transplant, it may be difficult to find mesothelial cells; in the latter case, the presence of nerves and ganglion cells or inflammation in the fat is suggestive of epicardial location (Figure 8, B). In time, the organized pericarditis usually forms a dense, fibrous, protective layer around the myocardium that prevents the development of tamponade if there is perforation. In one study, the presence of adipose tissue was reported to occur in 4.62% of transplant biopsies.[79] There is also some tendency to see fat deposits in areas of previous biopsy site or foci of healing ischemic damage. Whether the use of steroids for the treatment of rejection increases the amount of adipose tissue in the subendocardium is not known.

Nonrejection Lymphocytic Infiltrates.—Lymphocytes from Quilty lesions can be trapped in previous biopsy sites and then are crushed during subsequent biopsies (Figure 8, C). In other instances, lymphocyte clusters can be seen in postcapillary venules that become engorged with lymphocytes as these prepare to migrate into the interstitial space of the graft (Figure 8, D).

Dystrophic Calcification.—There have been reports of various forms of calcification in the heart after transplantation. In some patients, evidence of calcification has been shown histologically in biopsy tissue and radiographically in the native atria.[80,81] In our experience, it is also uncommon to see dystrophic calcification of the ventricular myocardium in biopsies. Calcium deposition within mitochondria is known to occur during ischemia and catecholamine-induced myocardial injury. In the posttransplant patients, a relationship between calcification and cyclosporine therapy has been suggested.[82] In some cases, several episodes of rejection requiring therapy, temporary uremia, and septicemia appear to be associated with the development of dystrophic calcification.[80] On light microscopy, the dystrophic calcification of the mitochondria is easily recognized as dark blue granular material in the cytoplasm of myocytes, ranging from 1 to 2.5 μm in diameter (Figure 8, E and F). The granules may be seen in perinuclear location and in between the myofibers. When they are abundant, they follow the contour of the whole myocyte. Dystrophic calcification is usually found in the subendocardium, affecting single myocytes or small groups of myocytes.

"Telescoping" or Intussusception of Small Arteries.—When a small muscular artery is sampled by the bioptome, telescoping or intussusception occurs. Just before the jaws of the bioptome completely cut through the tissue, the small artery is stretched and then recoils into its own lumen as soon as it is severed. This can give the appearance of an occluded vessel or a small artery with vasculopathy. The birefringent internal elastic lamina within the lumen can be recognized easily on closer examination of small arteries (Figure 8, G).

Chordae tendineae and valvular tissue.—Fragments of chordae tendineae are occasionally seen in the biopsy specimen and should be described in the report when present (Figure 8, H and I). Chordae to the tricuspid valve can arise from the septum and thus can be entrapped, torn, or biopsied during the procurement of tissue. Chordal rupture may or may not result in clinically significant tricuspid regurgitation.[14,83–85]

Procedural Artifacts

Procedural artifacts are common and should be recognized in the interpretation of the endomyocardial biopsy.[86] *Contraction bands* are a very common artifact seen in transplant and nontransplant heart biopsies. Several factors may influence the presence of contraction bands in the biopsy. It may be the result of trauma to the myocardium induced when the bioptome cuts the tissue. It may also be induced by poor osmolarity of the medium in which the biopsy is placed before and during fixation, as well as the cool temperature of the medium. We rarely observe contraction bands in frozen sections. Because of the high likelihood of finding contraction bands, they should never be the only criterion used to make a diagnosis of myocyte necrosis or ischemic damage in heart transplant biopsies. *Pinching or forceps artifact* represents mechanical distortion of the tissue induced by the bioptome itself during extraction. It can also be induced during processing of the tissue in the pathology laboratory. An effort should be made to handle biopsy tissue with care because this artifactual deformation may render the specimen uninterpretable. *Foreign bodies* introduced at the time of the transplant, such as gelatin foam, occasionally can be seen. At other times, actual sampling of fragments of indwelling catheters or the soft plastic cover of pacemaker leads may occur. *Pseudohemorrhage* occurs when red blood cells are embedded into the tissue by the pressure of the bioptome on the myocardium being sampled. This produces artifactual pools that mimic hemorrhage. They are usually not accompanied by inflammatory cells or pathologic changes in the myocytes, thus making the distinction between artifact and rejection fairly easy.

ANTIBODY-MEDIATED REJECTION

Transplants are capable of eliciting strong cellular and humoral immune responses. Antibody-mediated rejection (AMR) is an immunopathologic process in which injury to the graft is, in part, the result of activation of the complement system. This was first recognized in kidney transplantation as a distinct clinicopathologic entity characterized by acute allograft rejection associated with the production of antidonor reactive antibodies and poor prognosis.[87] It is poorly responsive to conventional immunosuppression, which targets the cellular arm of the immune response. Old terminology such as *vascular rejection, microvascular rejection,* and *humoral rejection* should be avoided as it has only led to confusion in the literature. The preferred terminology in the ISHLT-WF2004 is *AMR.*

Risk factors for developing AMR include pregnancy, previous transplantation, blood transfusions, sensitization by OKT3 induction therapy, use of ventricular assist devices, presence of positive B-cell flow cytometry cross-

match, and elevated panel-reactive antibodies.[13,88,89] The long-term outcome of AMR is not yet fully established in heart transplantation but it has been associated with the development of cardiac allograft vasculopathy (CAV) and with decreased survival.[90,91]

A detailed pathologic classification of ''humoral rejection'' in biopsies was not well defined in the ISHLT-WF1990. Consequently, the true incidence of AMR is unknown and recognition of AMR as a real entity was not widely accepted for several reasons. There was no uniform set of diagnostic criteria provided to guide different transplant programs in the detection of this entity. The antibodies used in evaluation of immunofluorescence changed over time. Positive immunofluorescence with the markers suggested then (IgG, IgM, C3, C1q, and fibrinogen) did not always correlate with hemodynamic compromise or incidence of CAV, which resulted in decreased usefulness of this test.[92] Lastly, it was believed that most AMR occurs early and the ISHLT-WF1990 recommends AMR monitoring by immunofluorescence on all biopsies up to 6 weeks posttransplant only. This is clearly incorrect, as it is now known that AMR can and most commonly does occur months and even years after transplantation.

Diagnostic Criteria

The histologic features that allow for the identification of this type of rejection on endomyocardial biopsies as defined in the ISHLT-WF2004 and its companion article on AMR include: ''capillary endothelial changes (swelling or denudation with congestion), macrophages in capillaries [Figure 9, A and B], neutrophils in capillaries, interstitial edema and/or hemorrhage and fibrin in vessels.'' [93] If these features are observed in the biopsy and there is unexplained cardiac dysfunction, the revised working formulation proposed that immunofluorescence or immunohistochemistry, in the absence of frozen tissue, be performed. Immunopathologic evidence of AMR include[13]

''—Immunoglobulin (IgG, IgM and/or IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections; and/or
—CD68 staining of macrophages within capillaries (CD31- or CD34-positive) by immunohistochemistry; and
—C4d staining of capillaries by paraffin immunohistochemistry.''

Examples of the capillary pattern of complement deposition are shown in Figure 9, C through F.

It is also recommended that these patients undergo assessment for circulating antibodies to HLA class I or II as well as non-HLA donor antigens. An EMB with no histologic or immunopathologic evidence of AMR is graded 0 (AMR 0). If the immunofluorescence or immunohistochemical staining supports the histologic features of AMR, the biopsy is considered positive (AMR 1).

Mixed Acute Cellular and AMR

Although most AMRs are associated with absent, or at most mild, acute cellular rejection, mixed rejections have also been reported that carry a significant risk of mortality.[94,95] Mixed rejections usually occur early in the course of transplantation and are also associated with allograft dysfunction.

Practical Issues in the Diagnosis of AMR

Histologic Features of AMR.—The ISHLT-WF2004 recommends that if histologic features suggestive of AMR are not seen, no further testing (immunofluorescence or immunohistochemical) needs to be pursued. However, a recent report[96] describes that the sensitivity of histologic criteria (ie, light microscopic features such as endothelial cell swelling, intravascular macrophages, edema, and hemorrhage) is too low to serve as screening parameters for AMR. The authors thus recommend the addition of immunostaining to screen for the presence of AMR.

Diagnostic Considerations of Complement Split Products.—Immunofluorescence methods for detection of AMR in tissues have evolved in the last decade. Some complement components, specifically C3d and C4d, are found to be more readily detected than antibodies and serve as very sensitive markers of rejection in endomyocardial biopsies for several reasons.[97] Antibodies bind to antigens with different avidity and either dissociate at varying rates or are eliminated by shedding or internalization. In contrast, the process of complement activation yields split products of C4 and C3 that bind to the tissue where complement was activated. This increases the sensitivity of complement detection by prolonging their half-lives. Among the components of the complement system, C3 is present in the highest concentration, followed by C4; therefore, their split products are also deposited in tissues in the largest quantities (Figure 10).[98] Furthermore, the amplification steps in the complement cascade results in the generation of more C3 split products.[99]

Although complement is activated through antibody in the classic pathway, one must remember that complement can also be activated during procedures such as extracorporeal circulation during surgery,[100,101] by ischemia/reperfusion injury,[102] and by induction therapy before transplant with antithymocyte globulin.[103] Thus, the mere presence of C4d and/or C3d in capillaries should not be equated with AMR.

In our experience, the use of C4d immunostaining alone is not a reliable tool. Instead, evaluation of endomyocardial biopsies for AMR should include staining for both C4d and C3d (Figure 9). A recent prospective study of heart transplant patients evaluated the usefulness of IgG, IgM, IgA, C1q, C4d, and C3d as markers for the diagnosis of AMR.[104] In this study, the authors performed routine staining of all biopsies for these five markers. These authors' institution reported 3% incidence of AMR in 165 nonsensitized patients. Immunoglobulin G, IgM, IgA, and C1q did not prove to be useful in discriminating patients with AMR. Conversely, the usefulness of C4d and C3d was confirmed. Immunostaining for C4d alone can be misleading because about 10% of the patients showed either C4d or C3d deposits alone in capillaries without clinical evidence of dysfunction of the allograft. Within the study period of 3 years, some patients demonstrated persistent activation of complement with C4d deposition over time without the development of allograft dysfunction. Another important observation made was that AMR occurred many months to years after transplantation in most patients. This study showed that the diagnosis of AMR must be a correlative diagnosis in which pathologic and clinical criteria play a role.

Discrepancy Between Pathology and Clinical Presentation.—Activation of the complement cascade detected

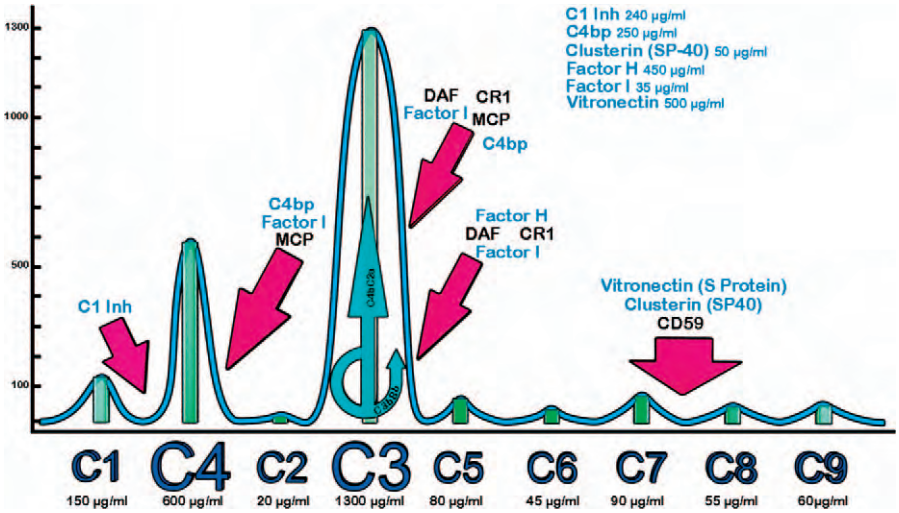

Figure 10. *Serum concentration of complement components and regulators of complement activation. This figure shows the serum concentration of the different complement factors in micrometers per milliliter. The activation of C3 is critical as it augments both cellular and humoral immune response. C3 is enzymatically cleaved and activated by C4b2a of the classic pathway and C3bBb through an amplification loop of the alternative pathway. Its activation is an important amplification step because C3 is present in a larger molar amount and, once activated, it can further increase the activation of the rest of the cascade. Regulators of complement activation (RCA) are composed of both plasma (blue letters) and membrane (black letters) proteins that inhibit the proteolytic subunits of classical and alternative pathways, thereby preventing the progression of the complement pathway to the membrane attack complex (MAC) formation. MCP indicates membrane cofactor protein; DAF, decay accelerating factor; CR1, complement receptor 1; C1 Inh, C1 inhibitor; and C4bp, C4 binding protein.*

by immunostains for C4d and/or C3d is not always accompanied by dysfunction of the graft. Some authors have referred to this apparent lack of graft injury despite evidence of complement activation as ''accommodation'' in animal models[105] and in ABO-incompatible renal transplants.[106] One possible explanation is that complement activation is interrupted by a protective mechanism in the host. This suggests that unless the complement cascade proceeds to the formation of the membrane attack complex, there is no expected injury to the allograft. This complex is needed to form a ''pore'' that leads to loss of integrity of the cell membrane. In humans, it is well known that there are regulators of complement activation that can prevent the completion of the complement cascade at different stages of activation.

Regulators of complement activation exert their effects at different points in the complement activation cascade, whether the activation occurs through the classic, alternate, or mannose binding lectin pathways. All these pathways converge at the point of generation of the enzymatic complexes known as the *C3 convertases*, which, in turn, proceed to activate the remaining complement components required for the formation of the membrane attack complex. There are two main types of proteins that can regulate the activation of complement. These can be divided into the membrane-bound and soluble types. In humans, the membrane-bound regulators are CD35 or complement receptor 1, CD46 or membrane cofactor protein, CD55 or decay-accelerating factor, CD59 or protectin, and C8-binding protein or homologous restriction factor.[107,108] The soluble factors include the C1 inhibitor, C4 binding protein (C4bp), factor I, factor H, clusterin, and S protein (vitronectin). Their points of action are shown in Figure 10.

There is little information about the expression of these regulators of complement activation molecules in human heart transplantation. A recent abstracted study shows that decay-accelerating factor or CD55 is expressed locally in the myocardium in heart transplant patients. In this study, a group of patients with complement deposition in endomyocardial biopsies was examined. The biopsies were stained by immunofluorescence for C4d, C3d, and decay-accelerating factor (Figure 11, A through F). There were 2 subgroups identified on the basis of present or absent allograft dysfunction. All patients had biopsy-proven C4d (Figure 11, A and C) and C3d (Figure 11, B and D) deposits. Patients with good response to therapy and resolution of the AMR episode showed intense tissue expression of CD55 in the endothelium of the allograft (Figure 11, E and F). Patients with poor outcome had low or absent tissue expression of CD55. Thus, the local expression of decay-accelerating factor correlates with absence of allograft dysfunction in spite of C4d and C3d deposition in capillaries.[109] In the same study, there was no evidence of detectable CD35, CD46, or CD59 in the biopsy tissue of this cohort of patients. At this juncture, there are no studies published that address the role of the soluble regulators of complement activation in human heart transplantation.

Complement Staining Artifacts.—Common artifactual staining seen in immunofluorescence microscopy of transplant biopsy includes autofluorescent lipofuscin deposits (Figure 12, A), nonspecific binding to collagen in the interstitium (Figure 12, B), and to the internal elastic lamina of arteries (Figure 12, C). Necrotic myocytes likewise bind complement (Figure 12, D).

CARDIAC ALLOGRAFT VASCULOPATHY

Currently, the most challenging problem in attaining a long-term successful outcome in cardiac transplantation is the development of CAV (Figure 13, A through E), also known as graft coronary artery disease, graft coronary vascular disease, transplant coronary artery disease, accelerated graft arteriosclerosis, and chronic rejection. This problem is not unique to the heart; it occurs in other solid organ grafts in a somewhat similar manner.[110,111] Cardiac allograft vasculopathy develops in a majority of transplanted hearts at a variable rate, sometimes as early as 3 months after transplantation.[112] According to the most recent ISHLT registries, only 47% of adults are free of CAV as detected by angiography at 9.5 years; in children, the incidence is much lower compared with adults, with 75%

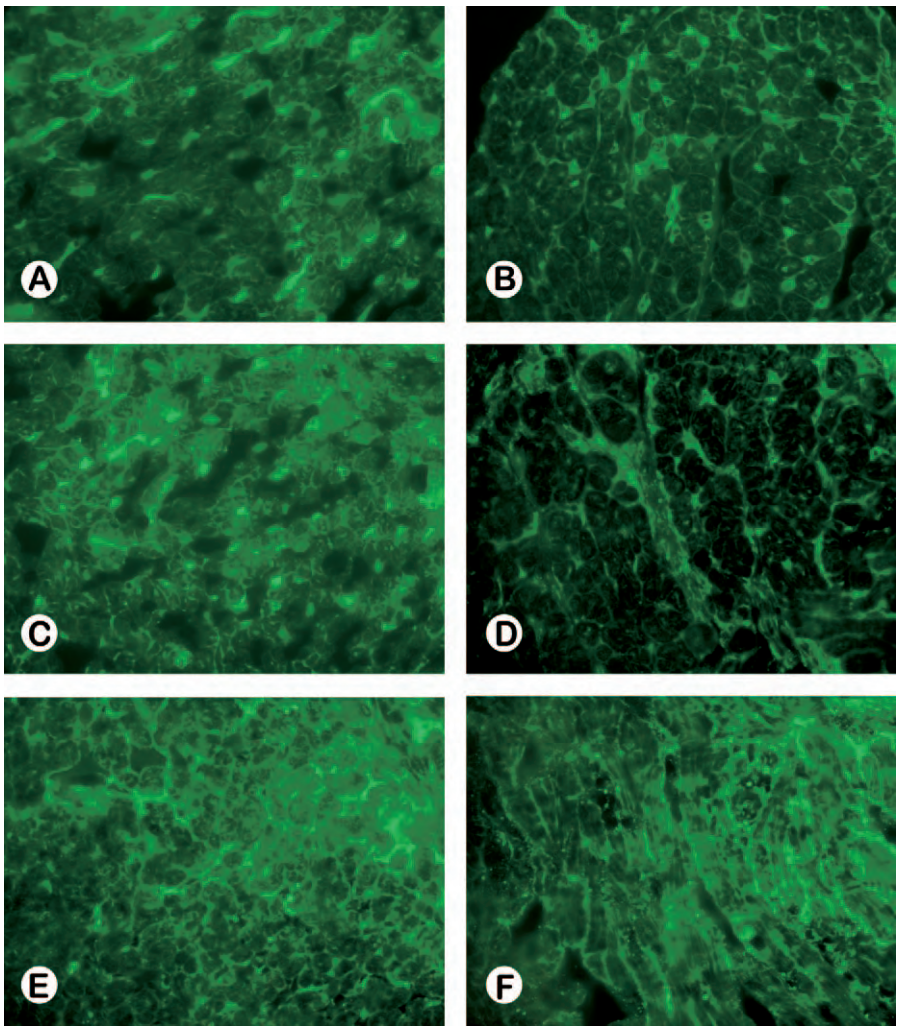

Figure 11. *Immunofluorescence detection of regulators of complement activation (RCA). Injury to the graft can be limited by regulating complement activation in the tissue. This panel illustrates a female patient who presented with hemodynamic compromise at 154 weeks posttransplant with evidence of capillary staining with C4d (A) and C3d (C). Two weeks after therapy (B and D), there was rapid recovery with normal ejection fraction on echocardiography. C4d remained positive with linear perimyocytic staining (B) while C3d shows rare positive capillaries and nonspecific interstitial staining (D). Staining for the membrane-bound RCA CD55, also known as decay accelerating factor (DAF), shows focal granular staining with DAF along capillaries at 154 weeks (E). Clinical improvement is accompanied by increased number of capillaries expressing DAF at 156 weeks (F) (fluorescein isothiocyanate, original magnification ×400).*

of patients free of CAV at 7 years posttransplant.[113,114] Most patients cannot experience typical angina associated with myocardial infarction or ischemia because of denervation of the donor heart. Therefore, CAV commonly presents clinically as congestive heart failure, ventricular arrhythmias, and sudden death. Risk factors for developing early CAV (occurring within 3 years posttransplant) include donor hypertension, infection within 2 weeks posttransplant requiring intravenous antibiotics, and rejection during the first year.[1,2,115] Risk factors associated with late CAV (occurring within 7 years posttransplant) include donor history of diabetes and intracranial hemorrhage as donor cause of death. Independent continuous risk factors for both early and late CAV are donor age, recipient age (inverse relationship), center volume, and recipient pretransplant body mass index.[1,116] Female donors are associated with a lower risk.[1]

The events leading to this type of vasculopathy are complex and interdependent. The mechanisms can be divided into immunologic and nonimmunologic. Endothelial cells express major histocompatibility complex class I and class II antigens, and thus appear to be primary targets of cell-mediated and humoral immune response.[117–119] Activated T lymphocytes secrete cytokines (interleukins, interferons, and tumor necrosis factors), which promote proliferation of alloreactive T cells, activate monocytes and macrophages, and stimulate expression of adhesion molecules by endothelial cells.[120] Macrophages are then recruited to the intima where they elaborate cytokines and growth factors, leading to smooth muscle cell proliferation and synthesis of extracellular matrix.[121] The role of humoral immune response in CAV relates to the antibody production against HLA and endothelial cell antigens.[122–125] The relationship between acute cellular rejection, histocompatibility mismatch, and development of CAV remains controversial.[126–132] Endothelial cell dysfunction resulting from sustained inflammatory injury also predisposes to thrombosis, vasoconstriction, and vascular smooth muscle proliferation.[133–135]

Some of the nonimmune factors that have been associated with the development and progression of CAV include myocardial ischemia,[136,137] donor-transmitted coronary atherosclerosis,[138,139] cytomegalovirus status,[140–143] lipid profile,[144,145] arteritis,[146] deficient fibrinolysis,[147,148] hormonal milieu,[149] and immunosuppressive therapy.[150–152] Excellent reviews of the pathobiology of vasculopathy have been written.[110,153–155]

Allograft vasculopathy involves both epicardial and intramural coronary arteries. The whole length of the coronary vessels is usually affected. Formation of collateral vessels is lacking. In some patients, the pathology predominantly involves only the small intramyocardial branches.[156] In these cases, early diagnosis is limited by

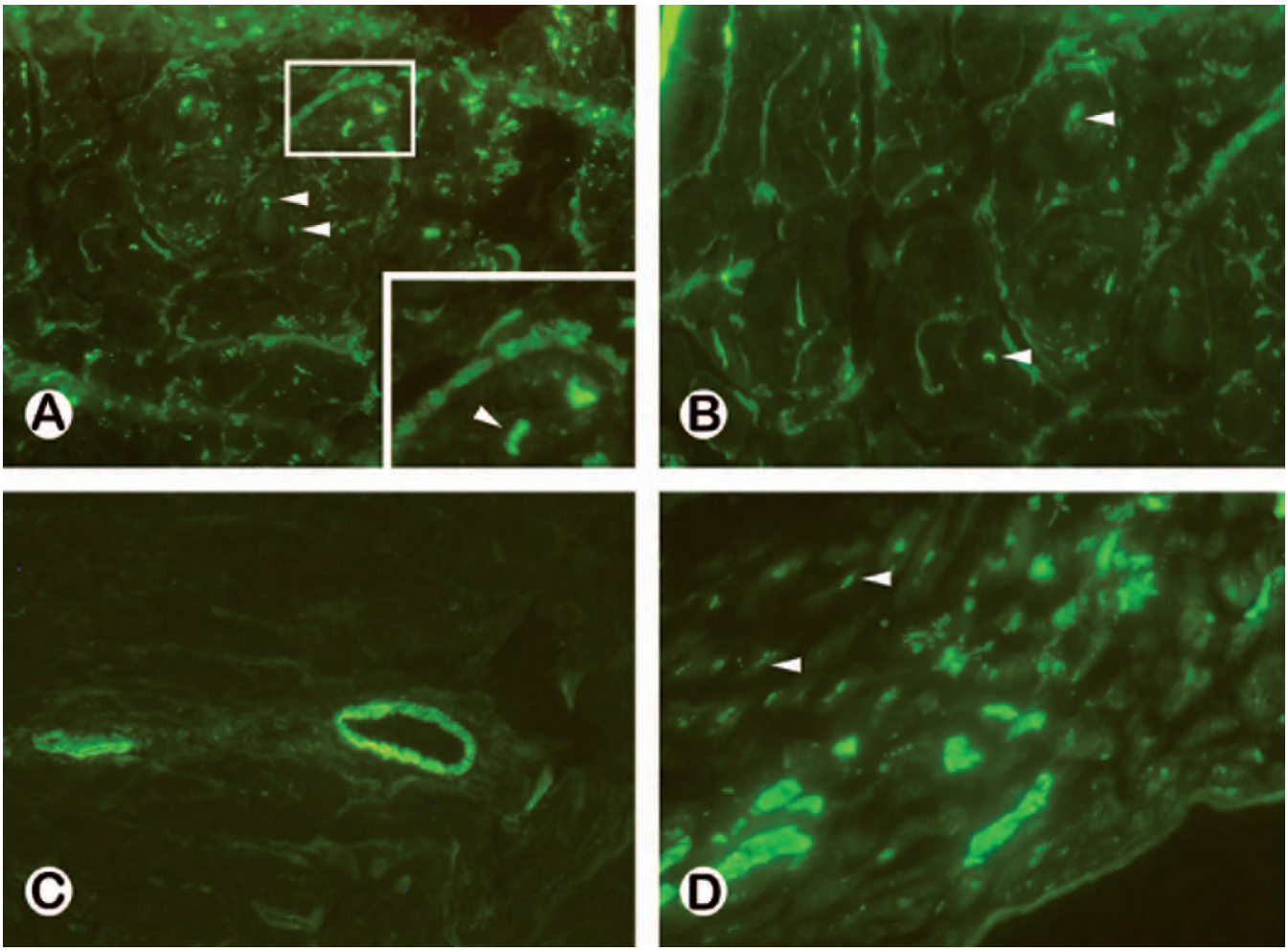

Figure 12. *Artifacts in immunofluorescence microscopy. A, Lipofuscin pigments (arrowheads) appear as irregularly shaped autofluorescent coarse granules that are distributed within the myocytes (fluorescein isothiocyanate [FITC] anti-C4d, original magnifications ×200 and ×400 [inset]). Lipofuscin is also evident in two other parts in this figure (B and D). B, Interstitial collagen can be artifactually stained with complement in some patients and appear as interrupted linear to squiggly strands around myocytes with uneven staining intensity (FITC anti-C4d, original magnification ×400). C, The internal elastic lamina of small arteries stains positive with complement (FITC anti-C4d, original magnification ×200). D, Necrotic myocytes are easily identified in immunofluorescence microscopy. They are artifactually stained intensely with complement and to a lesser extent with immunoglobulin G because of nonspecific absorption (FITC anti-C4d, original magnification ×200).*

inaccessibility of distal lesions to evaluation by coronary angiography or intravascular ultrasonography.

The endomyocardial biopsy has limited sensitivity in the recognition of vasculopathy because it samples only the smallest of intramyocardial arteries and arterioles, which often do not show histologic features of CAV.[157] Proliferative intimal lesions are usually not prominent in the coronary microvasculature (vessels less than 100 μm in diameter) within the first few years posttransplantation when most of the surveillance biopsies are being performed on a regular basis.[158] Moreover, investigators have suggested discordant progression of CAV because of differences in the structural and functional abnormalities between small intramyocardial and large epicardial arteries.[158–160] Reported histologic changes seen in the small arteries in endomyocardial biopsies include concentric intimal thickening with or without foamy macrophages, subendothelial accumulation of lymphocytes (called by some, *endothelialitis*), and perivascular fibrosis.[156,157] Evidence of myocardial ischemia, such as colliquative myocytolysis, frank coagulation necrosis, and healing ischemic lesions as well as interstitial, perivascular, and replacement fibrosis, can be seen in endomyocardial biopsies.[161] Identification of myocardial injury should raise the suspicion of CAV as the cause of graft dysfunction. Absence of these findings, however, does not necessarily rule out the presence of CAV. One study of the predictive value of endomyocardial biopsies in a series of patients with CAV confirmed on autopsy reveals a sensitivity of only 21% for the detection of myocardial ischemic changes.[162]

The classic feature of CAV is that of diffuse concentric narrowing with luminal stenosis (Figure 13, A and B). If atherosclerotic plaques were present in the donor heart prior to the transplantation, the morphology of the lesion is one of eccentric atheromatous plaques with superimposed intimal proliferation of transplant-related vasculopathy. Sometimes, long-term lesions of epicardial coronary arteries may eventually look like conventional atherosclerosis and be indistinguishable from CAV. Careful examination of the cut surfaces of ventricles often reveals intramural arteries (with a range in diameter from 0.2 to 0.5 mm) that are thickened with abundant perivascular

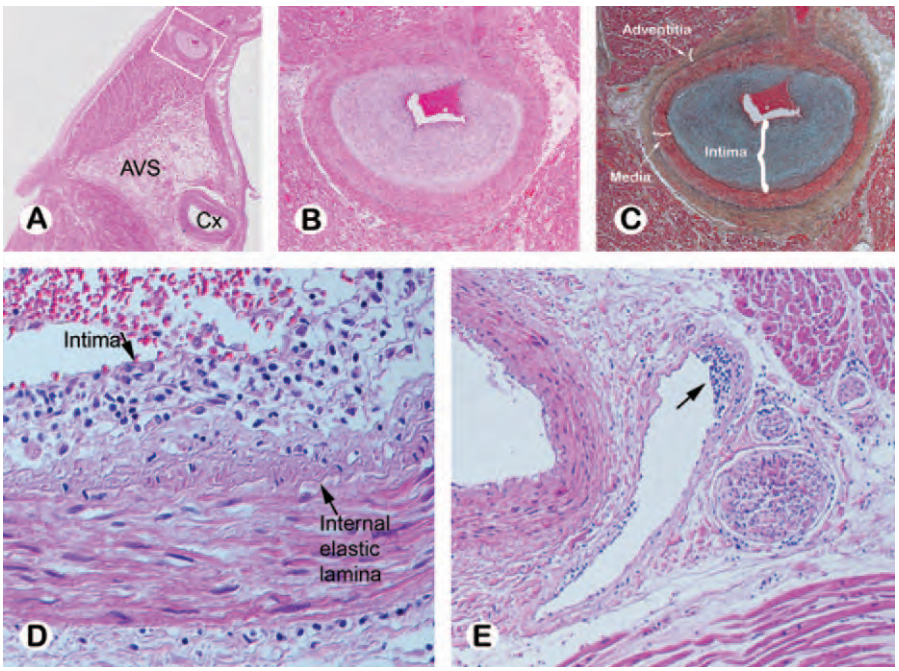

Figure 13. *Cardiac allograft vasculopathy (CAV). A, Scanning photomicrograph of left atrium and ventricle with the left circumflex (Cx) artery noted at the atrioventricular sulcus (AVS) from a patient who died of CAV 3 years posttransplant. The epicardial coronary arteries did not show occlusive lesions (hematoxylin-eosin, original magnification ×10). B and C, Intramyocardial branch of circumflex artery (from inset in A) supplying the atrium shows marked intimal proliferation with preservation of the elastic lamina and normal medial layer (hematoxylin-eosin [B] and Movat pentachrome [C], original magnification ×20). D and E, Subendothelial lymphocytic infiltration is seen in a small epicardial artery (D) and in an intramyocardial vein (arrow, E). This lesion has been called* endothelialitis *and is part of a spectrum of pathologic changes seen in CAV. Its clinical significance, however, is uncertain (hematoxylin-eosin, original magnifications ×200 [D] and ×40 [E]).*

fibrosis. In addition, focal areas of myocardial scarring may be evident.

The histology of allograft vasculopathy is slightly different in epicardial arteries compared with medium-sized or small arteries.[146,163,164] Microscopically, allograft vasculopathy in large epicardial vessels shows concentric intimal proliferation composed of smooth muscle cells and less-differentiated spindled cells (myofibroblasts or ''myointimal'' cells) (Figure 13, B and C). There is accompanying abundant deposition of proteoglycans with different immunohistochemical staining pattern and distribution, compared with conventional atherosclerosis, and more similar to angioplasty-related restenotic lesions (Figure 13, C).[165] Calcification and large pools of extracellular lipid are rare unless associated with atheromatous plaques that may develop in long-term survivors. Early lesions tend to be more cellular than those in the late stages, where the smooth muscle cells decrease in number and the intima becomes fibrotic. Mononuclear inflammatory cells are usually present in variable number, consisting mostly of T lymphocytes admixed with macrophages and foam cells. The internal elastic lamina is intact or only focally disrupted. The media is of normal thickness and shows little to no lipoprotein deposition. Medial fibrosis in the outer half is associated with lymphocyte-mediated injury of the vasa vasorum. An adventitial cuff of fibrous tissue with or without mononuclear inflammatory infiltrates is commonly observed (Figure 13, B and C). Atheromatous plaques, if present, are found in the proximal to middle segments of large epicardial arteries, produce an eccentric type of luminal stenosis, and histologically are indistinguishable from those of conventional atherosclerosis.

In the small epicardial and intramyocardial branches, allograft vasculopathy is also concentric but foam cells are not prominent. Endothelialitis is frequently observed in autopsy material (Figure 13, D and E). Occasionally, vasculitis with transmural inflammation by lymphocytes and plasma cells and disruption of internal elastic membrane is present in distal coronary arteries, usually associated with acute cellular rejection in the myocardium.[164,166] A second pattern of vasculitis that is characterized by severe inflammation in the adventitia that extends to the medial layer with destruction of external elastic membrane and is correlated with myocardial rejection has also been recognized by some investigators.[146] Fibrinoid necrosis of the media and thrombosis in small epicardial and intramural arteries can sometimes be seen.[146,163,164] Recanalized vessels may represent healed vasculitis with thrombosis. It is not clear whether this necrotizing vasculitis is due to cellular or humoral rejection or a combination of both.

The myocardium oftentimes show bilateral, patchy microscopic acute and healing ischemic injury[161] because it is believed that intramyocardial vessels are totally occluded first before the large epicardial arteries become critically stenosed. Chronic ischemic changes including myocytolysis and interstitial fibrosis are also frequent. Large myocardial infarcts are uncommon in the absence of thrombosis in the major epicardial vessels.[164] The pathology of CAV in children is practically identical.[166]

POSTTRANSPLANT MORBIDITY AND MORTALITY

Complications of chronic immunosuppression include drug toxicities, development of malignancy, and increased risk of infection. In time, most patients also develop hypertension, hyperlipidemia, diabetes mellitus, and renal insufficiency. Other notable adverse effects of therapy include bone marrow suppression and gastrointestinal symptoms.

Despite the use of newer and less toxic immunosuppressive drugs that decrease the incidence of acute cellular rejection, immunosuppression is still a significant cause of morbidity and mortality in the first year posttransplant. The majority of patients will have at least one episode of rejection. The rates for freedom from rejection at 1 year ranged from 10% to 23% covering the era before and after the introduction of cyclosporine in the Stanford experience.[167] Other centers have shown a significant decrease in the incidence of moderate and severe cellular rejection at-

tributed to improved immunosuppressive therapy in the 1990s.[168] Other major causes of death within the first year are early graft failure, multiorgan failure, and infection other than cytomegalovirus.[1,169] Early graft failure in the absence of cellular rejection can be the result of right ventricular failure from pulmonary hypertension, acute humoral rejection, and ischemia related to donor atherosclerosis, prolonged ischemic time, or poor donor preservation.[116]

Infections after cardiac transplantation often occur in the first few months after transplantation, with the highest risk of death at approximately 2 months after transplantation. Risk factors identified for fatal infections are very old and very young recipients, ventilator support at time of transplant, older (more than 50 years) donor heart, and prolonged donor ischemic time.[67] The most common site of infection reported was the lung.[66] Late infection is usually associated with recurrent high-grade rejection that requires augmentation of the immunosuppressive regimen.

Between 1 and 4 years, rejection, malignancy, and CAV account for most number of deaths. After 5 years, malignancy and CAV remain as the leading causes of death. A study involving 7290 patients who received transplants in multiple institutions between 1990 and 1999 reported malignancy (29%) as the leading cause of death, followed by CAV (23%) after the fourth year following transplantation.[169] The ISHLT registry with an 8-year cumulative data shows a 26% incidence of malignancy.[114] Transplant recipients do not appear to have increased risk of developing common cancers, including carcinomas of the lung, breast, prostate, and colon. However, an increased incidence of lymphoproliferative disorders, squamous cell carcinomas of the skin, sarcoma including Kaposi sarcoma, renal cell carcinoma, carcinomas of the vulva and perineum, and hepatobiliary tumors are observed.[170] The etiopathogenesis of posttransplant malignancies can be multifactorial. Association between the development of lymphoproliferative diseases and cytolytic induction therapy has been reported in renal and cardiac transplant recipients.[53,171] In another study, no increase in the incidence of malignant neoplasms was found between patients who received Thymoglobulin induction therapy and those who did not. However, it has been reported that patients who are treated with rabbit anti-thymocyte immunoglobulin develop malignant neoplasms earlier than those without induction therapy and have worse prognosis of their malignancies.[172] Long-term use of azathioprine has also been implicated in the development of myelodysplastic syndrome and acute myelogenous leukemia.[173]

Oncogenic viral infection may also play an important role in the development of malignancy in the immunocompromised hosts, including Epstein-Barr viruses, human papillomavirus, and hepatitis B and C virus. Recurrence of prior malignancy in a transplant patient may be because of defective immune surveillance. Transmission of cancer from donor to recipient is also possible. Transmitted malignancies in recipients of cardiac allografts include high-grade primary brain tumors, choriocarcinoma, lung adenocarcinoma, and melanoma.[174]

Autopsy studies of sudden unexpected cardiac deaths in transplant recipients reveal arrhythmias, CAV with diffuse involvement of distal coronary arteries, and cellular or humoral rejection as the most frequent causes of death.[175,176]

THE FUTURE

Endomyocardial biopsy is an invasive procedure that carries a low but finite risk of complications. There is associated patient discomfort with frequent biopsies. It is also a costly procedure and is resource-intensive. In addition, criticisms for EMB are the lack of concordance among pathologists in grading acute cardiac allograft rejection and failure to recognize AMR because of poorly defined histologic diagnostic criteria. However, no viable alternative exists to date.

Noninvasive monitoring of allograft rejection is an area of active research.[177] The need for proteomic and genomic markers to predict cardiac transplant rejection, correlation with outcomes, and risk of graft failure has been well recognized.[5] A recent study correlated gene expression profiling in peripheral blood mononuclear cells to the presence of acute cellular rejection in endomyocardial biopsies.[178] Their results indicating a high negative predictive value for the test show a promising diagnostic role for molecular testing. Reproducibility of these results in large-scale clinical settings has to be further demonstrated.[179]

In summary, the ultimate goal of any heart transplant team is a successful long-term outcome for the patients. This can be achieved with the pathologist working closely with the cardiac transplant team before and after the transplant. The recent revision of the ISHLT grading scheme should improve the interobserver reproducibility for cellular rejection and allow objective recognition of AMR. This, in turn, will result in more accurate diagnosis and better assessment of the effectiveness of therapy. Targeting proper therapy for host cellular or humoral response to the allograft may reduce the development and progression of CAV and other causes of graft dysfunction.

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2007 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection	
Acute Rejection	
Grade	**Histopathological Findings**
A0 (None)	**No mononuclear inflammation**, hemorrhage or necrosis
A1 (Minimal)	**Scattered infrequent perivascular mononuclear infiltrates in alveolated parenchyma**. Blood vessels, particularly venules, are cuffed by small round, plasmacytoid, and transformed lymphocytes forming a ring of 2 to 3 cells thick in the perivascular adventitia. Cuffing is loose or compact but generally circumferential. No eosinophils or endothelialitis present.
A2 (Mild)	**More frequent perivascular mononuclear infiltrates** surrounding venules and arterioles readily recognizable at low magnification, Usually a mix of activated lymphocytes, small round lymphocytes, plasmacytoid lymphocytes, macrophages, and **eosinophils**. Frequent subendothelial infiltration by the mononuclear cells with hyperplastic or regenerative changes in the endothelium (**endothelialitis**); although there is expansion of the perivascular interstitium by inflammatory cells, there is no obvious infiltration by mononuclear cells into the adjacent alveolar septae or air spaces. **Concurrent lymphocytic bronchiolitis** is not uncommon.
A3 (Moderate)	Readily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are usually associated with endothelialitis; eosinophils and occasional neutrophils are common. By definition, there is **extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septae and air spaces**. This may be single cell infiltration of alveolar walls or more prominent infiltration with septal expansion. Collections of alveolar macrophages and/or type 2 alveolar cell hyperplasia may be seen in the airspaces in the zones of septal infiltration.
A4 (Severe)	**Diffuse** perivascular, interstitial, and air space infiltrates of mononuclear cells and **prominent alveolar pneumocyte damage and endothelialiti**s. May have intra-alveolar necrotic cells, macrophages, hyaline membranes, hemorrhage, and neutrophils; there may be associated parenchymal necrosis, infarction, or necrotizing vasculitis. Perivascular inflammation may actually appear to be diminished due to extension of inflammation into alveolar septa and spaces. However, the obvious presence of numerous perivascular and interstitial

	mononuclear cells seen with grade A4 rejection permits distinction from peri-operative (reperfusion/ischemic) lung injury.
Airway Inflammation: Lymphocytic Bronchiolitis	
Grade	**Histopathological Findings**
B0 (None)	No small airway inflammation
B1R (Low-grade inflammation)	Scattered to band-like collections of mononuclear cells with or without eosinophils within the submucosa of the bronchioles. No epithelial damage or intraepithelial lymphocytic infiltration.
B2R (High-grade inflammation)	Mononuclear cells in small airways are larger and activated; more frequent eosinophils and plasmacytoid cells. Epithelial damage present (variable necrosis, metasplasia, marked intraepithelial infiltration). Note: disproportionately high number of neutrophils is suggestive of infection.
BX (Ungradeable inflammation)	Sampling problems, infection, tangential cuts, or other artifacts preclude accurate assessment of small airway inflammation
Chronic Airway Rejection (Bronchiolitis Obliterans)	
Classification	**Histopathological Findings**
C0 (No evidence of obliterative bronchiolitis)	No changes of obliterative bronchiolitis
C1 (obliterative bronchiolitis present)	Submucosal eosinophilic hyaline fibrosis in membranous and respiratory bronchioles with variable luminal occlusion, variable inflammation, variable secondary effects such as injury to smooth muscle and elastica, downstream mucostasis and/or foamy histiocyte collections
Chronic Vascular Rejection	
Also known as accelerated graft vascular sclerosis. Fibrointimal thickening of arteries and veins. Venous changes may be more common in older individuals. Generally evident only on open biopsy material.	
Acute Antibody-Mediated (Humoral) Rejection	
No histologic features for antibody-mediated rejection in the lung were agreed upon. Likely that capillary injury and small vessel intimitis suggest humoral rejection, but these can beĂnonspecific and a multidisciplinary approach is recommended to reach consensus before issuing specific criteria. If humoral rejection is suspected, stains for C4d, C3d, CD31, CD68 might be performed.	
Non-Rejection Biopsy Findings and Other Differential Diagnostic Considerations	
Condition	**Histopathologic Findings**
CMV pneumonitis	May have perivascular inflammation, more prominent alveolar septal inflammation relative to perivascular cuffing; perivascular edema; neutrophilic inflammation with or without microabscesses; pneumocyte atypia; intranuclear and intracytoplasmic inclusions

Pneumocystis pneumonia	May have perivascular and interstitial inflammation mimicking acute rejection; granulomatous inflammation; diffuse alveolar damage, focal necrosis
Granulomatous pneumonitis	May occur with mycobacterial, fungal or herpesvirus infection; can be seen with Pneumocystis (see above)
Aspiration pneumonitis	May show exogenous material with foreign body giant cell reaction; Lipid droplets, macrophages with large vacuoles; distal organizing pneumonia may occur
Organizing pneumonia	Intra-alveolar fibromyxoid tissue with variable interstitial inflammation (may occur in multiple settings, e.g., infection; reperfusion/ischemic injury; prior severe acute rejection; may also be idiopathic/cryptogenic).
Reperfusion/ischemic injury	Usually early posttransplant; usu. associated with neutrophils and acute lung injury; may have perivascular and interstitial infitrates in some cases, may lead to organizing pneumonia (see above)
Large airway inflammation	Most often seen in association with infection or aspiration; scarring of large airways is considered nonspecific (but should also prompt a search for similar changes in small airways).
Bronchus associated lymphoid tissue	Generally B lymphocyte nodules normally without true germinal centers, in submucosa of distal bronchi and bronchioles usu. at bifurcation points. May have prominent vascularity, well circumscribed with macrophages containing particulate matter. No epithelial injury, eosinophilia or neutrophilia.
Smokers' type respiratory bronchiolitis	Macrophages with brown or black pigment with or without iron accumulate around respiratory bronchioles. May have other features of chronicity such as goblet cell metaplasia, mucostasis, bronchiolar metaplasia, interstitial thickening with variable inflammation. May be donor-related.
Alveolar septal fibrosis	Potential association with late onset upper lobe fibrosis; currently considered nonspecific and difficult to interpret.

Reference Stewart S et al., Revision of the 1996 Working Formulation for the Standardization of NomenclatureĂin the Diagnosis of Lung Rejection. J Heart Lung Transplant 26:1229-42, 2007.

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Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection

Susan Stewart, FRCPath, Michael C. Fishbein, MD, Gregory I. Snell, MD, Gerald J. Berry, MD, Annette Boehler, MD, Margaret M. Burke, FRCPath, Alan Glanville, MD, F. Kate Gould, FRCPath, Cynthia Magro, MD, Charles C. Marboe, MD, Keith D. McNeil, FRACP, Elaine F. Reed, PhD, Nancy L. Reinsmoen, PhD, John P. Scott, MD, Sean M. Studer, MD, Henry D. Tazelaar, MD, John L. Wallwork, FRCS, Glen Westall, MD, Martin R. Zamora, MD, Adriana Zeevi, PhD, and Samuel A. Yousem, MD

In 1990, an international grading scheme for the grading of pulmonary allograft rejection was adopted by the International Society for Heart and Lung Transplantation (ISHLT) and was modified in 1995 by an expanded group of pathologists. The original and revised classifications have served the lung transplant community well, facilitating communication between transplant centers with regard to both patient management and research. In 2006, under the direction of the ISHLT, a multi-disciplinary review of the biopsy grading system was undertaken to update the scheme, address inconsistencies of use, and consider the current knowledge of antibody-mediated rejection in the lung. This article summarizes the revised consensus classification of lung allograft rejection. In brief, acute rejection is based on perivascular and interstitial mononuclear infiltrates, Grade A0 (none), Grade A1 (minimal), Grade A2 (mild), Grade A3 (moderate) and Grade A4 (severe), as previously. The revised (R) categories of small airways inflammation, lymphocytic bronchiolitis, are as follows: Grade B0 (none), Grade B1R (low grade, 1996, B1 and B2), Grade B2R (high grade, 1996, B3 and B4) and BX (ungradeable). Chronic rejection, obliterative bronchiolitis (Grade C), is described as present (C1) or absent (C0), without reference to presence of inflammatory activity. Chronic vascular rejection is unchanged as Grade D. Recommendations are made for the evaluation of antibody-mediated rejection, recognizing that this is a controversial entity in the lung, less well developed and understood than in other solid-organ grafts, and with no consensus reached on diagnostic features. Differential diagnoses of acute rejection, airway inflammation and chronic rejection are described and technical considerations revisited. This consensus revision of the working formulation was approved by the ISHLT board of directors in April 2007. J Heart Lung Transplant 2007;26:1229–42. Copyright © 2007 by the International Society for Heart and Lung Transplantation.

The original 1990 working formulation for the classification of pulmonary allograft rejection resulted from an International Society for Heart and Lung Transplantation (ISHLT) workshop to develop a standardized grading system for the pathologic diagnosis of rejection in transplant lung biopsies.[1] A core group of pathologists developed a grading scheme for pulmonary allograft rejection that allowed data to be compared between institutions as a result of uniformity of grading. The grading system was intended to be simple, easily taught, and readily reproducible, and was adopted at the majority of institutions performing lung transplantation at the time.

In 1995, an expanded group of international pathologists convened to revise the original 1990 proposal in response to developments in the field and their experience with using the working formulation.[2] On this occasion, the lung rejection study group critically assessed the merits of the initial working formulation and improved it on the basis of both published data and practical experience across many centers. The goal was again to maintain a uniform description and grading scheme for lung rejection, to improve communication between clinicians and investigators, to enable comparison of treatment regimes and outcomes between transplant centers, to facilitate multi-center clinical trials, and to promote further studies to determine the clinical significance of the various histologic patterns. The revised classification was based on histologic findings of acute and chronic lung rejection by primarily using transbronchial biopsies for allograft monitoring in both adults and children. It was emphasized that all biopsy data needed to be interpreted in an integrated clinical context to allow optimum patient management and clinical decisions. It was also noted that infection/rejection often occur together and can be confused histologically and that infection needs to be rigorously

From the Papworth Everard Pathology Department, Papworth Hospital, Cambridge, UK.

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Reprint requests: Susan Stewart, FRCPath, Papworth Everard Pathology Department, Papworth Hospital, Cambridge CB3 8RE, UK. Telephone: 44-1480-364-304. Fax: 44-1480-364-777. E-mail: susan.stewart@papworth.nhs.uk.

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excluded for the accurate and reproducible interpretation of pulmonary allograft biopsies.

The 1996 revision was itself widely adopted by the lung transplant community and has served it well for over a decade.[3,4] The revised working formulation represented a simplification of the original classification scheme, but it also highlighted some unresolved and complex issues such as the diagnosis and significance of airway inflammation. In 2004, again under the direction of the ISHLT, a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and also to address recent advances in the knowledge of antibody-mediated rejection. The revised consensus classification was accepted by the board of directors and published in 2005.[5] It was clear that the success of the multidisciplinary approach could be usefully adopted for a further revision of the diagnosis of lung rejection to take into account a decade of developments in the clinical, pathologic and immunologic fields. Toward this end, a multi-disciplinary consensus meeting was held at the ISHLT 2006 meeting in Madrid and its conclusions form the basis of this consensus report. The multidisciplinary task forces examined the histopathology of cellular rejection, humoral (antibody-mediated rejection) and clinical issues and future research.

Comments solicited from the ISHLT membership at large and from the transplant pathology community were also taken into account. Compared with the numerous responses from ISHLT members in 2004 regarding the cardiac grading system, only a small number of responses were received concerning lung grading. This was interpreted as most likely reflecting an overall higher level of satisfaction with the existing scheme compared with the 1990 cardiac working formulation. The present study reports on the consensus of revisions to the pathologic classification (Table 1) and is supplemented by the consensus of lung transplant physicians and surgeons focusing on the clinical viewpoint.[6]

Table 1. Revised Working Formulation for Classification and Grading of Pulmonary Allograft Rejection

A: Acute rejection
Grade 0—none
Grade 1—minimal
Grade 2—mild
Grade 3—moderate
Grade 4—Severe
B: Airway inflammation
Grade 0—none
Grade 1R—low grade
Grade 2R—high grade
Grade X—ungradeable
C: Chronic airway rejection—obliterative bronchiolitis
0—absent
1—present
D: Chronic vascular rejection—accelerated graft vascular sclerosis

"R" denotes revised grade to avoid confusion with 1996 scheme.

HISTOLOGIC GRADING OF PULMONARY ALLOGRAFT REJECTION

The histopathology task force again recognized that alloreactive injury to the donor can affect both the vasculature and the airways in acute and chronic rejection. Acute rejection is characterized by perivascular mononuclear cell infiltrates, which may be accompanied by sub-endothelial infiltration, so-called endothelialitis or intimitis, and also by lymphocytic bronchitis and bronchiolitis.[1,2,7] However, chronic rejection is manifest by fibrous scarring, which is often dense and eosinophilic, involving the bronchioles and sometimes associated with accelerated fibrointimal changes affecting pulmonary arteries and veins. As in the original and revised classifications, the histologic changes have been divided into grades based on the intensity of the cellular infiltrate and the presence or absence of fibrosis. The presence of presumed irreversible dense eosinophilic hyaline fibrosis in airways and vessels remains the key histologic discriminator between acute and chronic rejection of the lung.

A. ACUTE REJECTION

A diagnosis of acute rejection is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates. The intensity of the perivascular mononuclear cell cuffs and the distribution of the mononuclear cells, including extension beyond the vascular adventitia into adjacent alveolar septa, form the basis of the histologic grade. Acute rejection usually affects more than one vessel (particularly in adequate transbronchial biopsy samples) but is occasionally seen as a solitary perivascular infiltrate. This finding should be evaluated with the same criteria as those applied to multiple infiltrates as outlined in what follows. In the setting of multiple foci of rejection, the grade reflects the most advanced pattern of rejection rather than the predominant pattern. The infiltrates surrounding small vessels in the sub-mucosa of airways are again interpreted as part of the spectrum of airway inflammation rather than being diagnostic of acute rejection, Grade A.

Grade A0 (No Acute Rejection)

In Grade A0 acute rejection, normal pulmonary parenchyma is present without evidence of mononuclear cell infiltration, hemorrhage or necrosis.

Grade A1 (Minimal Acute Rejection)

In Grade A1 acute rejection, there are scattered, infrequent perivascular mononuclear infiltrates in alveolated lung parenchyma (Figures 1, 2 and 3). Blood vessels,

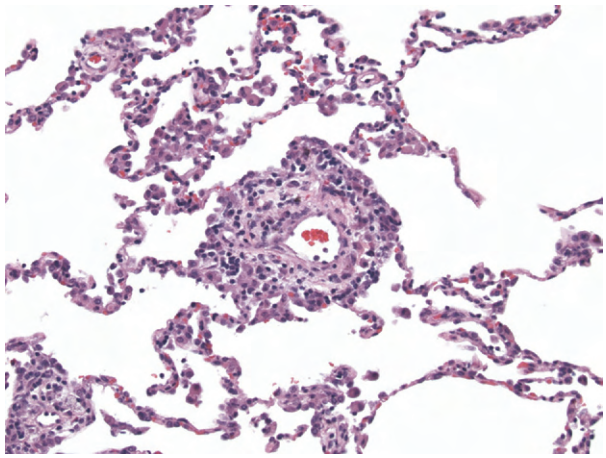

Figure 1. Minimal acute cellular rejection (A1). The characteristic feature of minimal acute cellular rejection is circumferential infiltration of the perivascular interstitium by mononuclear cell inflammatory infiltrate. This typically involves the small veins and consists of scattered mononuclear cells within loose perivascular connective tissue. No significant expansion of the perivascular interstitium or extension of mononuclear cells into adjacent alveolar septa is present. Haematoxylin and eosin (H&E).

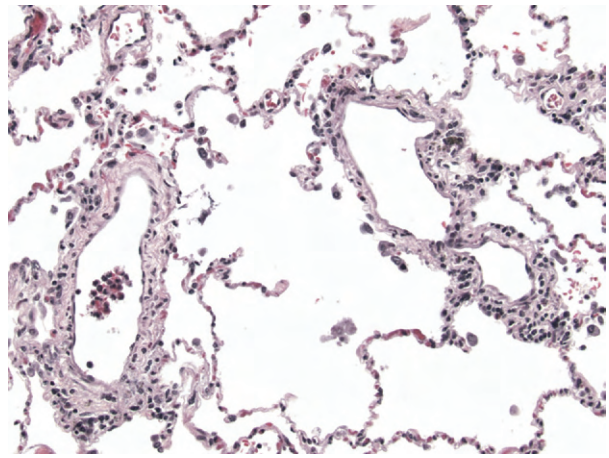

Figure 3. Minimal acute cellular rejection (A1). In this example of A1 rejection, the vessel at right displays a perivascular mononuclear infiltrate which in one segment of this vessel appears to be circumferential therefore warranting a diagnosis of minimal acute rejection. At left the vessel contains an ill-defined noncircumferential infiltrate of mononuclear cells of low intensity which would be regarded as a nonspecific morphologic finding. H&E.

particularly venules, are cuffed by small, round plasmacytoid and transformed lymphocytes forming a ring of two or three cells in thickness within the perivascular adventitia. This cuffing may be loose or compact and is generally circumferential. Eosinophils and endothelialitis are not present. The previous grading schemes suggest that these minimal infiltrates are not obvious at low magnification, but it was believed that this criterion can be misleading. Grade A1 infiltrates can be seen at scanning magnification if the specimen is adequately alveolated and free from artifact. The consensus was that evidence of infrequent perivascular infiltrates at low-power (scanning) magnification is not a reliable discriminator between Grade A1 and A2 acute rejection.

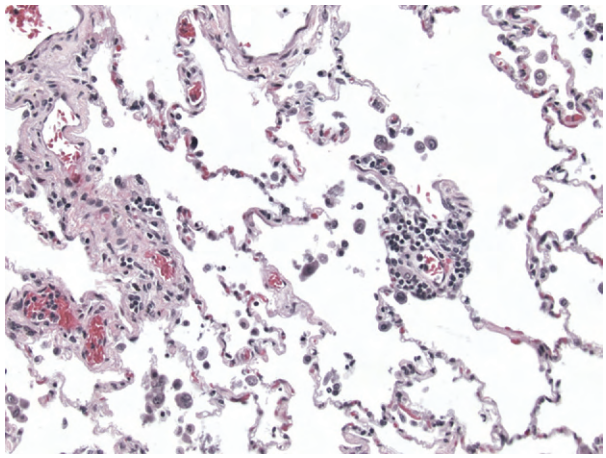

Figure 2. Minimal acute cellular rejection (A1). A sparse mononuclear cell infiltrate is present in the perivascular zones in this example of A1 minimal rejection at right. At left is a nonspecific mononuclear inflammatory infiltrate which fails to show the circumferential cuffing of vessels or the density of mononuclear cells that is sufficient for a diagnosis of minimal acute cellular rejection. H&E.

Grade A2 (Mild Acute Rejection)

In Grade A2 mild rejection, more frequent perivascular mononuclear infiltrates are seen surrounding venules and arterioles and are readily recognizable at low magnification (Figures 4, 5, 6 and 7). They may be densely compacted or loose. These infiltrates usually consist of a mixture of small, round lymphocytes, activated lymphocytes, plasmacytoid lymphocytes, macrophages and eosinophils. Eosinophils are not a feature of Grade A1 minimal rejection. In Grade A2 rejection there is frequently sub-endothelial infiltration by mononuclear cells, which may be associated with hyperplastic or regenerative changes in the endothelium, that is, endothelialitis. In making the distinction between Grade A2 and higher grade acute rejection it is important to note that the perivascular interstitium can be expanded by mononuclear cells in A2 rejection but there is no obvious infiltration by mononuclear cells into the adjacent alveolar septa or air spaces. Concurrent lymphocytic bronchiolitis (see later) may be seen in association with mild acute rejection (Grade A2), but is less common with minimal acute rejection (Grade A1).

Mild acute rejection is therefore distinguished from minimal acute rejection by the presence of unequivocal mononuclear infiltrates, which are more easily identified at scanning magnification. In addition, endotheliali-

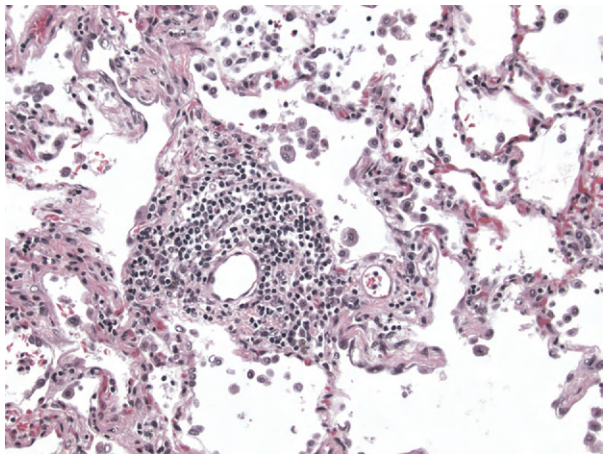

Figure 4. Mild acute cellular rejection (A2). In mild acute cellular rejection, the perivascular interstitium of small vessels, venules and arterioles, demonstrates significant circumferential expansion of the perivascular interstitium by mononuclear inflammatory infiltrate. The infiltrate consists largely of mononuclear cells with occasional activated lymphocytes and plasmacytoid lymphocytes. The mononuclear inflammatory infiltrate within the perivascular zones may be accompanied by alveolar macrophages. No infiltration of adjacent alveolar septa by the mononuclear infiltrate is present. H&E.

tis, the presence of eosinophils and co-existent airway inflammation favor mild Grade A2 over minimal A1 acute rejection.

Grade A3 (Moderate Acute Rejection)

Grade A3 acute rejection shows easily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are commonly associated with endothelialitis (Figures 8, 9, 10 and 11). Eosinophils and even occasional neutrophils are common. This grade is defined by the extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septa and airspaces, which may be associated with collections of intra-alveolar macrophages in the zones of septal infiltration and Type 2 alveolar cell hyperplasia. The interstitial infiltration can take the form of cells percolating singly into alveolar walls or more sheet-like infiltration with corresponding expansion of the septa. There is continuity with the

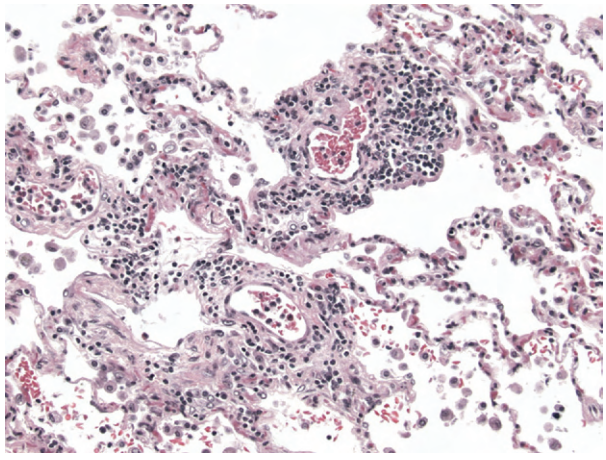

Figure 5. Mild acute cellular rejection (A2). In this example of mild acute cellular rejection, a tortuous small vessel in the lung parenchyma is cuffed by an inflammatory cell infiltrate which expands and tracks along the perivascular interstitium. The infiltrate remains associated with the perivascular interstitium without infiltration or expansion of adjacent alveolar septa by mononuclear cells. Subendothelial lymphocytic infiltration is also noted in upper right and such endothelialitis is a common finding in mild acute cellular rejection. H&E.

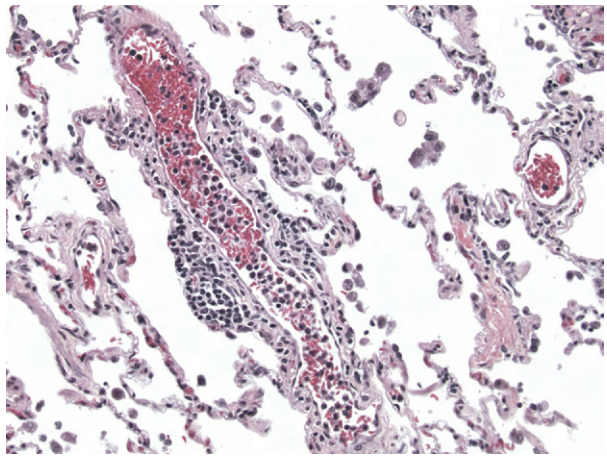

Figure 6. Borderline minimal-mild acute cellular rejection (A1-A2). In this example, a blood vessel is cut along its long axis. For the most part, the lymphoplasmacytic infiltrate is loosely distributed within the perivascular connective tissue. In only one focus is there expansion of the perivascular interstitium by the mononuclear infiltrate and therefore this case would be classified as mild acute cellular rejection even though the majority of this vessel shows minimal changes. H&E.

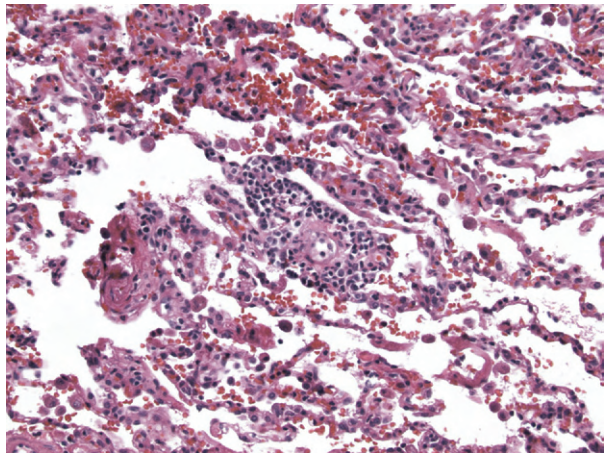

Figure 7. Borderline minimal-mild acute cellular rejection (A1-A2). This solitary perivascular infiltrate is associated with endothelial cell hyperplasia and expansion of the perivascular interstitium by mononuclear cells. The expansion, however, is rather slight and not pronounced and such a case would fall along the borderline of minimal-mild acute cellular rejection. H&E.

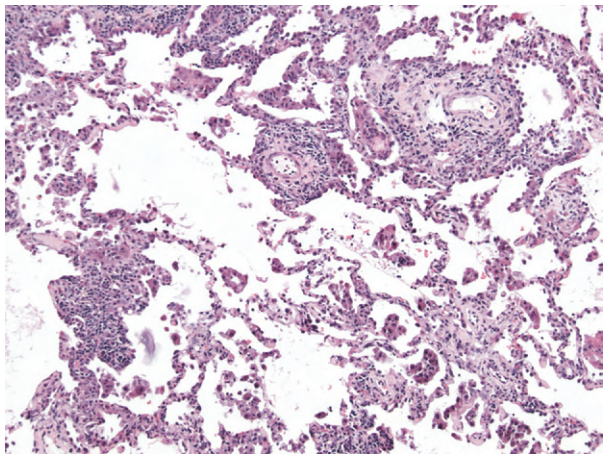

Figure 8. Moderate acute cellular rejection (A3). At scanning power, the perivascular mononuclear inflammatory infiltrate within the lung is easily identified. In addition, mononuclear cells are seen to percolate from the perivascular interstitium of small vessels into the alveolar septa where they are accompanied by alveolar pneumocyte hyperplasia. Mononuclear cells percolate into the perivascular airspaces where they are accompanied by a pronounced intraalveolar macrophage reaction. H&E.

perivascular infiltrates. True interstitial infiltration characterizing moderate acute rejection should be distinguished from the expansion of the potential space of the perivascular adventitia in mild acute rejection.

Grade A4 (Severe Acute Rejection)

In Grade A4 severe rejection there are diffuse perivascular, interstitial and air-space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endothelialitis (Figures 12, 13 and 14). These

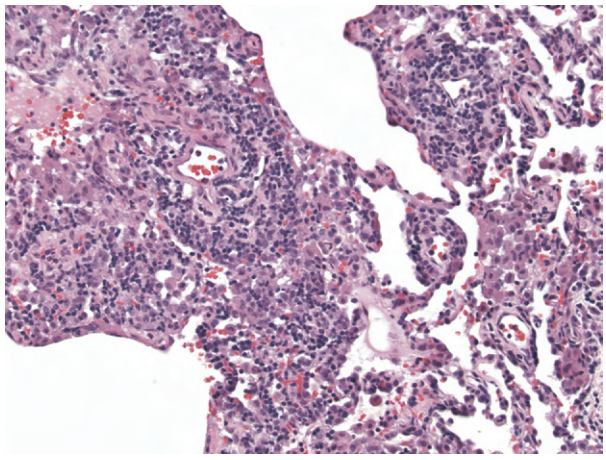

Figure 9. Moderate acute cellular rejection (A3). In this transbronchial biopsy, the lung parenchyma appears rather collapsed and atelectatic and yet, the perivascular mononuclear inflammatory infiltrate, with plasmacytoid lymphocytes and occasional eosinophils, expands the perivascular interstitium and extends into alveolar septa resulting in an "interstitial pneumonitis". Alveolar pneumocytes are prominent and endothelialitis is readily identified. H&E.

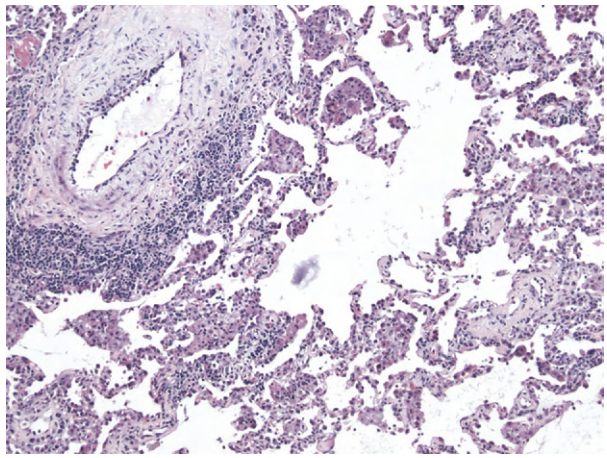

Figure 10. Moderate acute cellular rejection (A3). The characteristic feature of moderate acute cellular rejection is the expansion of the perivascular interstitium by mononuclear cells and the extension of the same cells into adjacent perivascular alveolar septa. Such cells may extend into the airspaces resulting in collections of macrophages and lymphocytes within alveoli. H&E.

may be associated with intra-alveolar necrotic epithelial cells, macrophages, hyaline membranes, hemorrhage and neutrophils. There may be associated parenchymal necrosis, infarction or necrotizing vasculitis, although these features are more evident on surgical rather than transbronchial lung biopsies. There may be a paradoxical diminution of perivascular infiltrates as cells extend into alveolar septa and spaces where they are admixed with macrophages.

Grade A4 acute rejection must be distinguished from post-transplantation acute lung injury by the presence of numerous perivascular and interstitial mononuclear cells, which are not a feature of reperfusion-related damage.

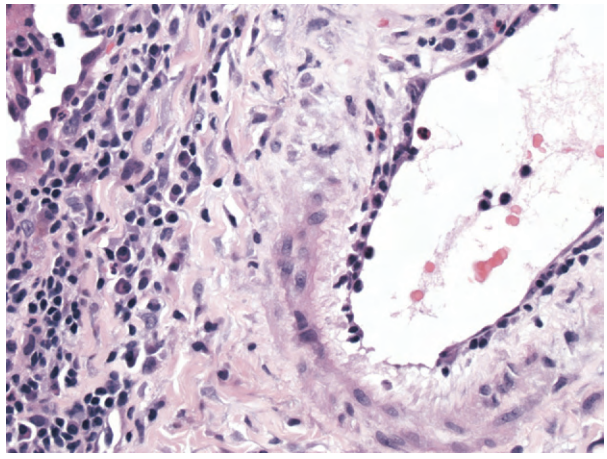

Figure 11. Moderate acute cellular rejection (A3). In almost all cases of moderate acute cellular rejection, subendothelial infiltrates of small round and plasmacytoid lymphocytes are characteristic, often accompanied by eosinophils i.e. endotheliitis or endothelialitis. H&E.

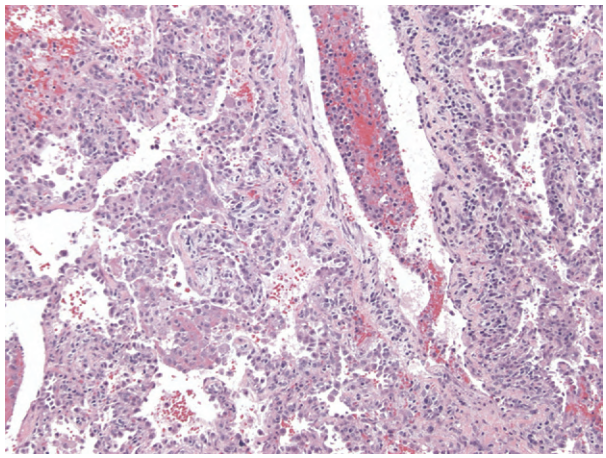

Figure 12. Severe acute cellular rejection (A4). At low magnification, the perivascular spaces and alveolar septa are expanded by a mononuclear inflammatory infiltrate, which paradoxically seems less intense than that seen in moderate acute cellular rejection. Percolation of mononuclear cells into the alveolar septa is readily identified and in severe rejection is accompanied by such pronounced alveolar pneumocyte injury that airspace fibrin and hyaline membranes form, with varying degrees of organization. This is accompanied by a nonspecific neutrophilic infiltrate. Such injury to the alveolar septa with fibrin exudation and neutrophil infiltration is characteristic of severe acute cellular rejection. Endothelialitis is almost uniformly seen in these cases. H&E.

In summary, the diagnosis of acute rejection is based on the presence of perivascular and interstitial mononuclear cell infiltrates. After much debate about the merits or otherwise of collapsing the A1 to A4 grades into fewer grades, the consensus was to retain the existing 5-point system while recognizing that, in most pathologists' experience, Grade A4 is uncommon. The nature of the tissue damage in Grade A4, however, was

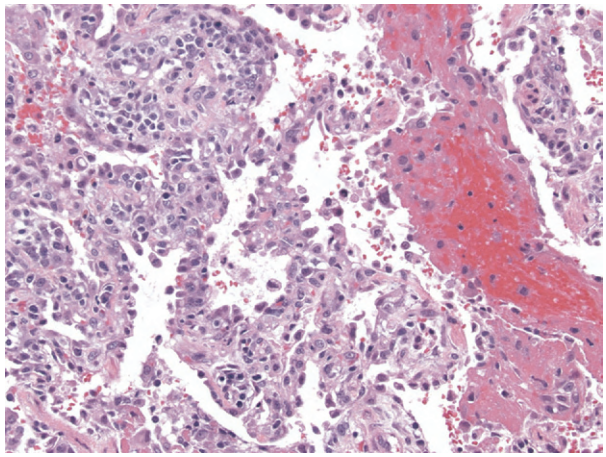

Figure 13. Severe acute cellular rejection (A4). In this example of severe acute rejection, perivascular mononuclear infiltrates are seen surrounding a small vessel at upper left and within alveolar septa. Injury to alveolar septa has resulted in hemorrhage and airspace fibrin undergoing varying degrees of organization. H&E.

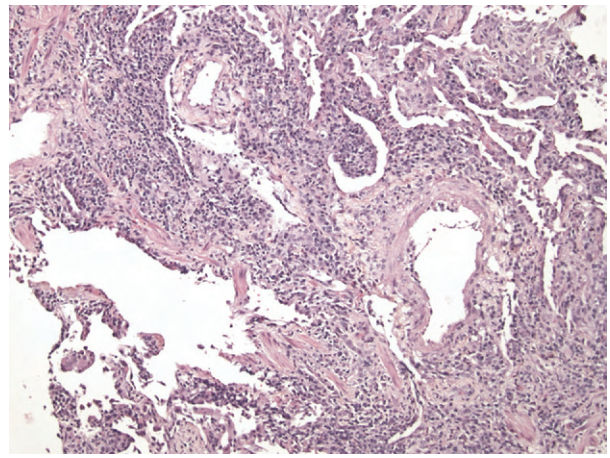

Figure 14. Severe acute cellular rejection (A4). In this example of severe acute cellular rejection two additional features are worthy of note. In addition to the perivascular and alveolar septal mononuclear infiltrate, there is significant injury to a small airway at lower left with an intense peribronchiolar mononuclear infiltrate. At upper right, airspace organization is noted with fibrin and hyaline membranes undergoing transition to granulation tissue. Such a finding can be a marker of the prior alveolar septal injury observed in moderate and severe acute rejection. H&E.

identified as having a potential relationship with an antibody-mediated form of acute rejection (see later) and therefore potentially useful in contributing to further understanding of lung rejection in the future, albeit in an infrequently diagnosed grade. The histopathology task force also recommended that perivascular infiltrates related to acute rejection should be truly circumferential and that incomplete vascular cuffing is unlikely to represent acute rejection. It is advised that further samples, deeper serials or levels into the tissue block should be obtained when the infiltrates are equivocal to discriminate both between rejection and non-rejection pathology and between the various grades of acute cellular rejection.

The participants also noted that the transbronchial biopsy diagnosis of acute rejection represents but one component of an integrated approach to the assessment of lung allograft recipients. The diagnosis of acute lung rejection therefore requires integration with clinical and particularly microbiologic data.[8] In relation to the treatment of acute rejection the task force noted that different clinical groups have different therapeutic algorithms and that, since the 1996 working formulation, the potential long-term significance of Grade A1 minimal acute rejection has emerged.[9–11] It was decided to retain this minimal grade for further evaluation in light of better guidance for its recognition.

B: AIRWAY INFLAMMATION: LYMPHOCYTIC BRONCHIOLITIS

The 1996 working formulation allowed airway inflammation to be graded from B0 (no inflammation) to B4

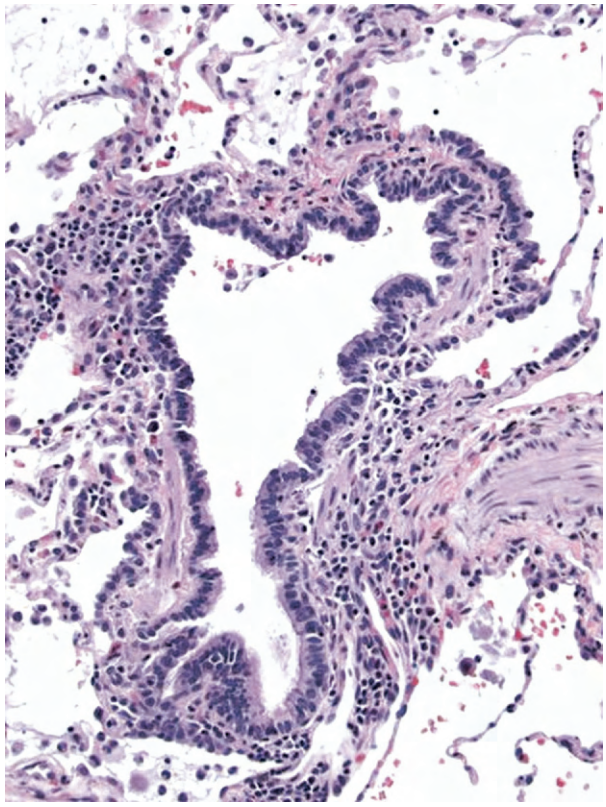

Figure 15. Low grade lymphocytic bronchiolitis (B1R). In this example the bronchiole shows a mild patchy peribronchiolar mononuclear cell infiltrate which spares the respiratory epithelium and is unassociated with epithelial injury. The infiltrate forms an incomplete circumferential band in places. There is no evidence of fibrosis in lymphocytic bronchiolitis in comparison with obliterative bronchiolitis. H&E.

(severe airway inflammation).[2] The earlier 1990 formulation recommended airway inflammation co-existent with Grade A acute rejection to be recorded as present or absent, but did not reflect the intensity of the inflammatory infiltrates.[1] The 1996 grading of airway inflammation was not accepted by all members of the lung rejection study group for several reasons, including the lack of convincing evidence that airway inflammation could be used solely to grade rejection because of its frequent co-existence with airway infection. Also, there are frequent problems with adequate sampling of small airways in transbronchial biopsies and with technical issues such as tangential cutting, etc. An ungradeable category was designated for those biopsies limited by sampling problems, infection, tangential cutting, etc. It was accepted that the scientific and clinical usefulness of airway inflammation grades would need revisiting over the course of time.[12] However, the format of Grades A and B in the 1996 classification emphasized the need to retain perivascular infiltrates as the primary focus in the histologic classification of acute lung rejection.

At the 2006 consensus meeting, the majority of pathologists felt that the criteria for separating four grades of airway inflammation were poorly defined and difficult to discriminate on transbronchial biopsy. Previous studies of reproducibility of the 1996 working formulation both in terms of inter- and intra-observer variability had shown significant problems with the airway inflammation B grades in comparison to the acute rejection A grades and it was recognized that new recommendations must improve reproducibility.[3,4,13] The revision of the B grades has collapsed the four previous grades into two and retained B0 (no airway inflammation) and BX (ungradeable for reasons just stated). The B grade designation applies only to small airways, that is, bronchioles, and the description of inflammation in cartilage-containing large airways is covered later. It is recognized that airway inflammation can be present in the absence of perivascular infiltrates and that rigorous exclusion of infection is necessary before ascribing the features to acute rejection of the airway.

Grade B0 (No Airway Inflammation)

In Grade B0 there is no evidence of bronchiolar inflammation.

Grade B1R (Low-grade Small Airway Inflammation)

In Grade B1R there are mononuclear cells within the sub-mucosa of the bronchioles, which can be infrequent and scattered or forming a circumferential band (Figures 15 and 16). Occasional eosinophils may be

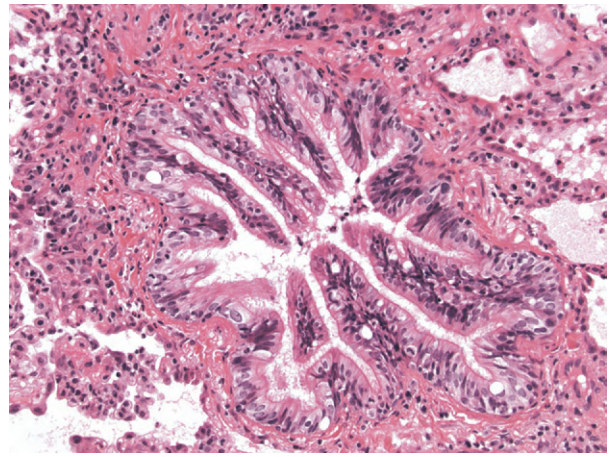

Figure 16. Low grade lymphocytic bronchiolitis (B1R). This terminal bronchiole shows epithelial hyperplasia and some epithelial undulation but is accompanied by a very sparse mononuclear inflammatory infiltrate which does not home to the basement membrane or injure the mucosal epithelium. H&E.

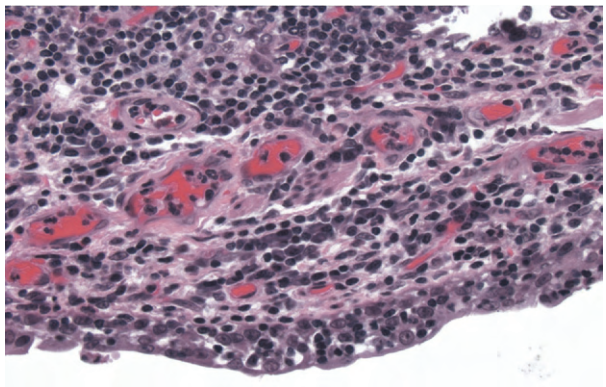

Figure 17. High grade lymphocytic bronchiolitis (B2R). In high grade lymphocytic bronchiolitis, in contrast to the low grade variant, mononuclear cells expand the submucosa and home to the epithelial basement membrane where they percolate through the basement membrane into the overlying respiratory epithelium. Epithelial cell necrosis and apoptosis is observed. H&E.

seen within the sub-mucosa. There is no evidence, however, of epithelial damage or intra-epithelial lymphocytic infiltration. This grade combines and replaces the previous B1 and B2 grades.

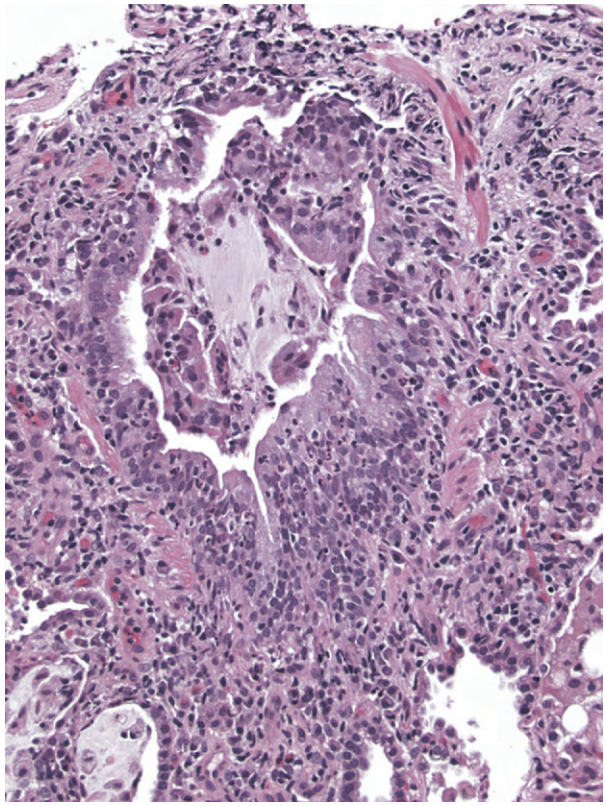

Figure 18. High grade lymphocytic bronchiolitis (B2R). This small bronchiole shows an intense mucosal and peribronchiolar mononuclear cell inflammatory infiltrate involving the epithelium with focal epithelial damage. Neutrophils are present in the epithelium and should not be confused with infectious bronchiolitis if correlation with microbiology is undertaken. H&E.

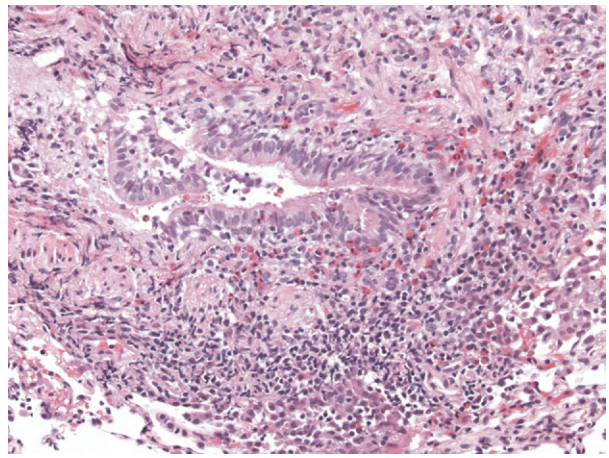

Figure 19. High grade lymphocytic bronchiolitis (B2R). In this example of a small bronchiole in a transbronchial biopsy, the mononuclear inflammatory cell infiltrate is accompanied by an intense eosinophilic infiltrate with eosinophils and lymphocytes traversing the epithelium accompanied by epithelial cell necrosis. Infection should be excluded as a cause of the eosinophilia. H&E.

Grade B2R (High-grade Small Airway Inflammation)

In Grade B2R the mononuclear cells in the sub-mucosa appear larger and activated, with greater numbers of eosinophils and plasmacytoid cells (Figures 17, 18 and 19). In addition, there is evidence of epithelial damage in the form of necrosis and metaplasia and marked intra-epithelial lymphocytic infiltration. In its most severe form, high-grade airway inflammation is associated with epithelial ulceration, fibrino-purulent exudate, cellular debris and neutrophils. The presence of a disproportionate number of neutrophils within the epithelium and sub-mucosa in relation to the numbers of sub-mucosal mononuclear cells is highly suggestive of infection rather than rejection. Any accompanying lavage or aspirate may also be purulent and/or show evidence of organisms.

Grade BX (Ungradeable Small Airways Inflammation)

In Grade BX the changes are ungradeable due to sampling problems, infection, tangential cutting, artifact, etc.

The consensus group recommended that the diagnosis of acute rejection with co-existent airway inflammation be in the same form as the 1996 formulation—that is, acute rejection grade with airway inflammation grade. For example, moderate acute cellular rejection in which there is intense small airways inflammation would be designated moderate acute rejection, Grade A3, with airways inflammation being Grade B2R. The category of lymphocytic bronchiolitis is graded as A0, B1R or A0, with B2R depending on the severity of the airway inflammation.

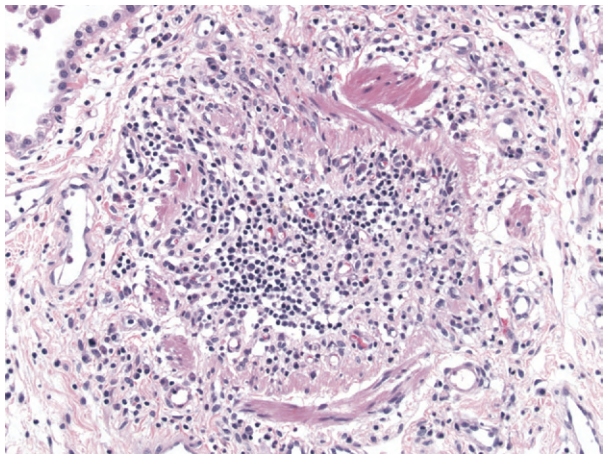

Figure 20. Obliterative bronchiolitis. In this example of obliterative bronchiolitis, the entire airway lumen has been obliterated by scar tissue and mononuclear cells, with the circumference of the small airways defined by an interrupted layer of smooth muscle bundles. H&E.

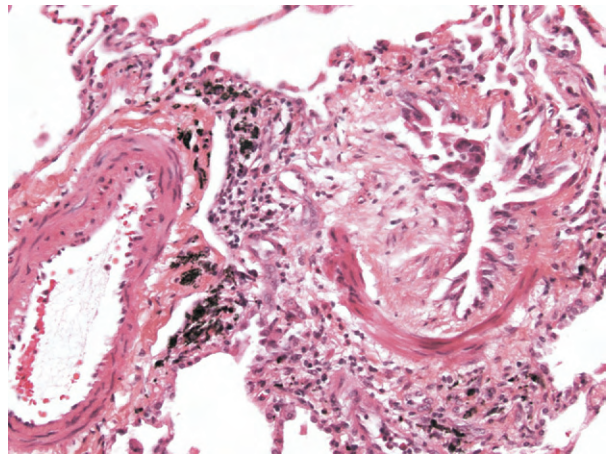

Figure 22. Obliterative bronchiolitis. In this transbronchial biopsy, an eccentric polypoid plaque of dense eosinophilic scar tissue is superimposed between attenuated respiratory epithelium and the smooth muscle wall of the airway. Such focal scarring of the airways is classified as obliterative bronchiolitis. H&E.

C: CHRONIC AIRWAYS REJECTION: OBLITERATIVE BRONCHIOLITIS

Obliterative bronchiolitis describes dense eosinophilic hyaline fibrosis in the sub-mucosa of membranous and respiratory bronchioles, resulting in partial or complete luminal occlusion (Figures 20, 21, 22, 23 and 24). This tissue can be concentric or eccentric and may be associated with fragmentation and destruction of the smooth muscle and elastica of the airway wall. It may extend into the peri-bronchiolar interstitium. Mucostasis and/or foamy histiocytes in the distal air spaces are commonly associated with obliterative bronchiolitis and may be observed in transbronchial biopsies in the absence of bronchiolar occlusion or any bronchiolar tissue.

The 1996 working formulation concluded that the 1990 distinction between sub-total and total forms of obliterative bronchiolitis was not useful, but retained the designation of active vs inactive, depending on the presence and degree of accompanying inflammation.[2] The consensus in 2006 was that the distinction between active and inactive obliterative bronchiolitis is no longer useful and the condition should be designated merely as C0, indicating a biopsy with no evidence of obliterative bronchiolitis, and C1, indicating that obliterative bronchiolitis is present in the biopsy. Transbronchial biopsy is an insensitive method for detecting obliterative bronchiolitis and the clinical use of bron-

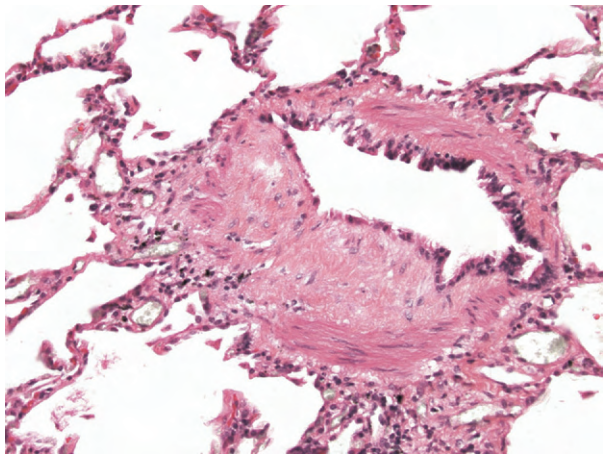

Figure 21. Obliterative bronchiolitis. This small bronchiole shows eccentric scarring of the submucosa of the small airway associated with an inconspicuous peribronchiolar mononuclear infiltrate. The overlying epithelium appears attenuated, while the lumen of the airway is distorted. Such partial occlusion of the small airways may be responsible for significant increases in airflow resistance. H&E.

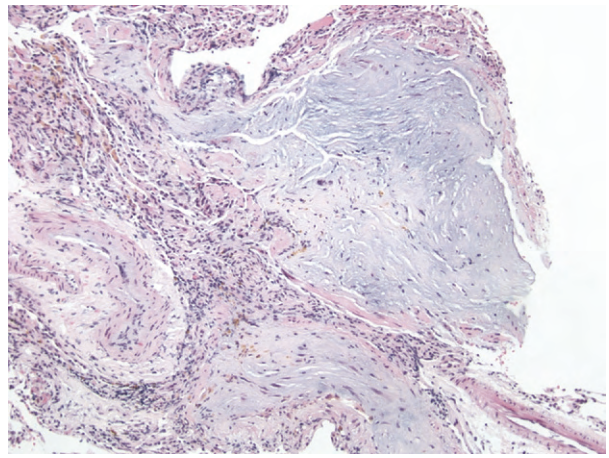

Figure 23. Obliterative bronchiolitis. In this distorted transbronchial biopsy, the scar tissue which is obliterating the airways has a loose myxoid quality but still shows dense lamellae of irreversible fibrous scar tissue in the airways. Once again the location of these scars adjacent to pulmonary arteries and the residual smooth muscle within the walls of these airways alert the pathologist to small airway disease. H&E.

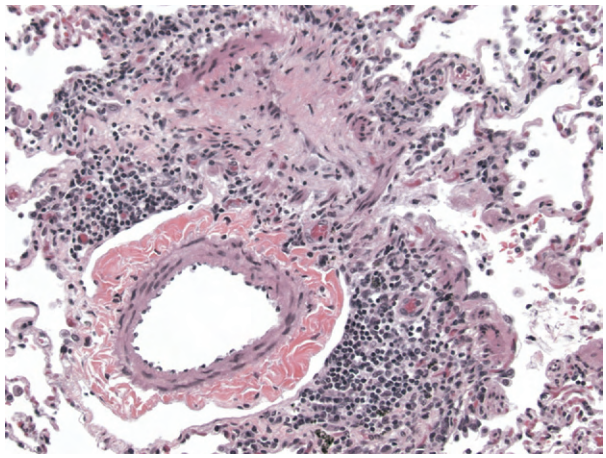

Figure 24. Obliterative bronchiolitis. The hint to underlying obliterative bronchiolitis in this case is the interrupted cords of smooth muscle forming a tubular structure associated with dense scar tissue in a position adjacent to a pulmonary artery. H&E.

chiolitis obliterans syndrome (BOS) with its functional grading is the preferred means of diagnosing and monitoring chronic airway rejection.[14]

D: CHRONIC VASCULAR REJECTION

In chronic vascular rejection/accelerated graft vascular sclerosis there is fibrointimal thickening of arteries and veins, which is similar to coronary artery disease in transplanted hearts (Figure 25). In the veins, the histologic appearance is usually of poorly cellular hyaline sclerosis and it is recognized that the use of older donors is associated with a higher incidence of this phlebosclerosis in biopsy material. Chronic vascular rejection is not applicable to transbronchial biopsies but may be noted on open biopsy material.

Acute Antibody-mediated (Humoral) Rejection

Acute humoral rejection is now recognized as a clinical entity in heart and renal transplants, although it remains controversial with a highly varied incidence between different centers.[15–17] There is no consensus on its recognition and diagnosis either histopathologically or immunologically, nor on its significance and treatment. The 2004 ISHLT cardiac rejection meeting reviewed evidence from histopathology, immunopathology and clinical task forces and was able to suggest diagnostic criteria in specific clinical circumstances so that further assessment of this entity could be encouraged.[5] Pathologists can follow the guidance in that consensus report if they intend to investigate the possibility of antibody-mediated rejection as a cause of cardiac dysfunction. Recommendations were published to allow incorporation, as required, into the revised working formulation for heart rejection. It was noted that acute antibody-mediated rejection is associated with worse graft survival and is observed in allosensitized patients, including those with previous transplantation, transfusion or pregnancy, and those with prior use of a ventricular assist device.[15]

The diagnosis and recognition of antibody-mediated rejection of the lung is more controversial and less well developed than for other solid-organ grafts.[16–18] However, the presence of serum anti-HLA antibodies and the deposition of complement in alveolar tissue after transplantation suggest a role for humoral immune responses in lung transplantation.[19] A significant portion of the lung consensus meeting was devoted to reviewing evidence for antibody-mediated acute lung rejection. Pulmonary transplant recipients with evidence of sensitization, as demonstrated by elevated titers of panel-reactive antibodies, have significantly more ventilator days post-operatively compared with non-sensitized patients.[20] Humoral immune responses are also implicated in the pathogenesis of obliterative bronchiolitis, possibly due to anti-HLA antibodies contributing to the development of scarring fibrosis via stimulation of epithelial cells within the airway.[21]

Historically, acute antibody-mediated rejection of the lung has been associated with "hyperacute rejection," which is clinically manifested by primary graft failure occurring very early after transplantation in the setting or pre-formed antibodies to donor HLA antigens or endothelial cells.[16] Morphologically, this is associated with fibrin thrombi in alveolar septa, fibrinoid necrosis of alveolar septal walls and hemorrhage. In 2006, no histologic features for antibody-mediated rejection in the lung were agreed upon. However, there was a consensus that, although pulmonary capillaritis has been described as possibly related to acute lung rejection, it is not recognized in transbronchial biopsies in

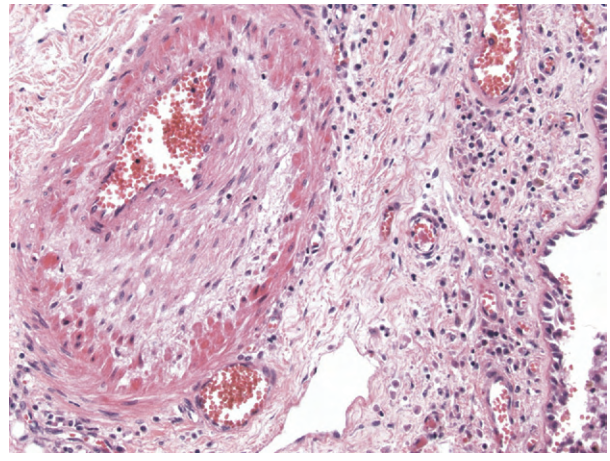

Figure 25. Graft atherosclerosis. In this example of accelerated vascular atherosclerosis due to alloreactive injury the pulmonary arteries adjacent to airways show fibro-intimal thickening of the subendothelial zones with atrophy of the media. H&E.

the majority of institutions performing pulmonary transplants and this term should not be used to indicate the histologic hallmark of antibody-mediated rejection.[22] Pulmonary capillaritis should also be distinguished from neutrophil margination and congestion. It was agreed that the term capillary injury is more useful as it can indicate a morphologic spectrum of capillary damage, although it can be a non-specific finding occurring in infection, diffuse alveolar damage and severe cellular rejection.[23]

Extrapolating from other solid-organ descriptions of antibody-mediated rejection, it was agreed that small vessel intimitis could raise the suspicion of humoral rejection. It was also agreed, on an empirical basis, that, should antibody-mediated rejection be suspected clinically, immunopathologically or with histologic evidence of capillary injury, immunohistochemistry could be performed on the transbronchial biopsies for C3d, C4d, CD31 and CD68. This extrapolates from experience in heart and kidney grafts. The use of broad immunofluorescence panels and electron microscopy was not recommended. It was emphasized that antibody-mediated rejection in the lung is not as well developed as an entity as in the heart and kidney and more work is required for its evaluation.

The use of agreed-upon immunohistochemical markers may prove helpful in understanding the diagnosis. The use of C4d staining in particular may allow the humoral response to a lung graft to be interpreted along the lines of the NIH recommendations from the 2003 national conference (Table 2). However, recent studies of C4d staining of pulmonary allograft biopsies have shown conflicting results with immunohistochemistry by indicating positive staining in a variable, focal, non-specific pattern without a consistent staining pattern within different diagnostic groups.[24] Specifically, C4d deposition has been variably demonstrated as present or absent in the microvasculature of lung biopsies in patients with acute and chronic rejection.[25,26] Specific immunohistochemical sub-endothelial C4d deposition has been suggested as a marker for the involvement of HLA antibodies in lung allograft rejection.[19] However, the patchy nature and low sensitivity and specificity of the C4d staining suggested limited clinical use in protocol biopsies, but raised the possibility of specific C4d deposition serving as a marker of co-existent antibody-mediated rejection in patients with refractory acute cellular rejection.

No recommendations could be made on the diagnosis of concomitant acute cellular rejection and antibody-mediated rejection at this time, although it is likely to occur by extrapolation from other solid-organ grafts. The true specificity and sensitivity of a diagnosis of antibody-mediated rejection (with and without concomitant acute cellular rejection, infection or even primary graft dysfunction) requires further careful study. Caution is urged in the diagnosis of acute antibody-mediated rejection in the lung until this evidence is forthcoming and a multidisciplinary approach is again recommended in view of the wide differential diagnosis and the potential toxicity of treatments.

Table 2. Putative Stages of Humoral Response to an Organ Graft (From Reference[16])

I: Latent humoral response
Circulating antibody[a] alone (but without biopsy findings or graft dysfunction)
II: Silent humoral reaction (accommodation vs pre-rejection state)
Circulating antibody[a] + C4d deposition (but without histologic changes or graft dysfunction)
III: Sub-clinical humoral rejection[b]
Circulating antibody[a] + C4d deposition + tissue pathology (but without graft dysfunction)
IV: Humoral rejection
Circulating antibody[a] + C4d deposition + tissue pathology + graft dysfunction

[a]Circulating antibody to HLA or other antigens expressed on donor endothelial cells.

[b]May differ among organs, as the ability to detect particularly mild degrees of graft dysfunction varies among organs.

GENERAL RECOMMENDATIONS

Adequacy of Specimens

Transbronchial biopsy has been the mainstay of lung allograft evaluation. It was again the uniform opinion of the consensus meeting that at least five pieces of well-expanded alveolated lung parenchyma are required for an assessment of acute rejection. The bronchoscopist may need to submit more than five biopsies to provide this minimum number of adequately alveolated pieces, and possibly further biopsies if small bronchioles are required to be present. A strip of bronchus may be attached to the alveolated parenchyma and this should be distinguished from bronchiolar tissue. Specimens can be gently agitated in formalin to inflate the fragments and require tender handling in the laboratory to avoid crush artifacts that can render interpretation difficult or nearly impossible.

Histologic Examination

Histologic examination should include a minimum of sections from three levels of the paraffin block for hematoxylin and eosin (H&E) staining with connective tissue stains to evaluate any sub-mucosal fibrosis, essential for the diagnosis of bronchiolitis obliterans and arteriosclerosis. Silver stains can be performed for fungi, including pneumocystis, but have not been absolutely mandated by the group in view of the numerous microbiologic, serologic and molecular techniques presently in use for the diagnosis of opportunistic infections in these patients. Beyond this minimum H&E

and connective tissue stain work-up, investigators may wish to augment their evaluation with histochemical, immunohistochemical and in situ hybridization studies. Bronchoalveolar lavage may be performed at the time of biopsy and is useful for the exclusion of infection and for research investigations, but it has no clinical role in the diagnosis of acute rejection.

Differential Diagnosis of Perivascular and Interstitial Infiltrates

Perivascular mononuclear infiltrates are not specific for acute rejection and many other conditions may simulate or mimic alloreactive lung injury.[27] Differential diagnostic considerations include cytomegalovirus pneumonitis, *Pneumocystis jiroveci* (previously *P carinii*) pneumonia and post-transplantation lymphoproliferative disease, which can itself range from pneumonitis to active lymphoproliferation with tumor nodules. These conditions have been described elsewhere. Cytomegalovirus (CMV) pneumonitis often shows disproportionate alveolar septal cellular infiltrates as compared with any perivascular cuffing, and may include perivascular edema. In addition to infected cells with intranuclear and intracytoplasmic viral inclusions, the presence of abundant neutrophils with the formation of microabscesses and marked atypia of alveolar pneumocytes may also contribute to the diagnosis.

Molecular and serologic methods for monitoring and diagnosing CMV disease are also extremely helpful in suggesting the diagnosis. Transbronchial biopsy, however, remains the only standard for assessing concomitant CMV infection/pneumonitis and acute rejection. Although pneumocystis can exactly mimic acute rejection with perivascular and interstitial infiltrates, it can also manifest atypical histologic reactions, including granulomatous inflammation, diffuse alveolar damage and foci of necrosis. Granulomatous inflammation is not a feature of acute rejection and should always raise the possibility of mycobacterial or fungal, including pneumocystis, infection. Punctate zones of necrosis should also raise the possibility of mycobacteria, fungi or herpesvirus infections rather than acute rejection. Further differential diagnoses of perivascular and interstitial infiltrates include recurrent primary disease such as sarcoidosis and, in the early post-transplant period, reperfusion injury, although the latter is more often associated with neutrophils and evidence of acute lung injury.

OTHER NON-REJECTION BIOPSY FINDINGS

Aspiration

The pulmonary allograft is not protected by a cough reflex and patients are highly predisposed to recurrent aspiration. Helpful features in making this diagnosis include the identification of exogenous material with associated foreign-body giant-cell reaction within the airways and parenchyma (Table 3). Large lipid droplets and/or macrophages with large vacuoles are helpful markers of aspiration. Distal organizing pneumonia can also be seen. Since the last revision of lung rejection grading, aspiration has emerged as a significant cause of chronic allograft dysfunction, which may be ameliorated by treatment.[28,29] It can occur early or late after transplantation and is therefore within the differential diagnosis throughout the post-operative period.

Table 3. Other Pathologic Features to Note in Transbronchial Biopsies

Infection
Aspiration
Organizing pneumonia
Post-transplant lymphoproliferative disorder
Large airway inflammation
Bronchus-associated lymphoid tissue
Smoker's-type respiratory bronchiolitis
Diffuse alveolar damage
Recurrent native disease
Hemosiderosis

Organizing Pneumonia

Organizing pneumonia with intra-alveolar fibromyxoid tissue associated with variable interstitial inflammation is another common finding in biopsies from lung allografts.[30] It can occur in a variety of clinical contexts and requires microbiologic correlation where infection is suspected. Organizing pneumonia can be seen as a sub-acute form of infectious lung damage. Patchy organizing pneumonia may also represent reperfusion/ischemic injury where there may have been evidence of primary graft failure. The histologic pattern of organizing pneumonia can also be seen in association with acute rejection of Grade A3 severity and greater where there is alveolar extension of the acute inflammatory response with subsequent organization. Idiopathic/cryptogenic organizing pneumonia can also manifest identical histologic features in biopsies from a lung transplant recipient, but many other causes must be excluded before the reaction is attributed to an idiopathic origin.

Large Airway Inflammation

The importance of distinguishing large and small airways inflammation was again the subject of much discussion and dissent.[7,31] No definite evidence was produced to support a separation of small and large airway inflammation as useful in the diagnosis of acute rejection. Large airway inflammation is most commonly associated with infection and aspiration (see earlier). Scarring can be seen in the large airway in addition to the bronchiolar scarring of bronchiolitis obliterans, but this feature is regarded as so non-specific as to not

warrant a separate comment. However, the presence of large airway scarring, like the presence of intra-alveolar, foamy macrophages, can alert the pathologist to the possibility of obliterative bronchiolitis and the need to examine further sections.

Bronchus-associated Lymphoid Tissue

Bronchus-associated lymphoid tissue consists of subepithelial mucosal lymphoid follicles that are distributed along the distal bronchi and bronchioles. It is scattered throughout the lung in adults, tending to be most prominent at the bifurcation points of airways. The lymphoid follicles contain mainly B lymphocytes and normally lack true germinal centers. These follicles are associated with specialized bronchial and bronchiolar epithelium, which is composed of modified cuboidal, non-ciliated, non-mucinous cells allowing for the trans-epithelial migration of antigens and cells.[32] Attention to these histologic features and recognition of the often prominent vascularity should enable distinction to be made between bronchus-associated lymphoid tissue (BALT) and rejection-related airway inflammation.[32,33] BALT is often well circumscribed and may contain macrophages with particulate matter. There should be no evidence of epithelial injury, neutrophils or eosinophils in a BALT collection. BALT aggregates can trail off into fibrovascular septa and should not be confused with perivascular or interstitial infiltrates.

Smokers'-type Respiratory Bronchiolitis

In respiratory (smokers') bronchiolitis, biopsies show an accumulation of tan-colored alveolar macrophages around respiratory bronchioles. Macrophages may contain flecks of brown or black material and show Prussian blue positivity. There may be associated interstitial thickening and variable accompanying chronic inflammation. There may be other features of chronic obstructive pulmonary disease with goblet-cell metaplasia, mucostasis and bronchiolar metaplasia. This appearance should be distinguished from rejection-related inflammation and BALT. The incidence of smokers'-type respiratory bronchiolitis in transbronchial biopsies from lung transplants has increased with the expansion of the donor pool to include smokers' organs. Occasionally, dust macules/nodules are seen of donor origin. The persistence of smokers' macrophages in the donor lung should not be confused with recipient smoking.

Alveolar Septal Fibrosis

Some members of the consensus group had observed fibrotic thickening of the alveolar septal walls in transbronchial biopsies from pulmonary allografts and noted the clinical entity of upper-lobe fibrosis, which has been described as a newly identified late-onset complication after lung transplantation.[34,35] However, due to the lack of specificity and the difficulty in interpretation of interstitial fibrosis in transbronchial biopsy specimens it was considered to be an unhelpful observation.

CONCLUSIONS

This multidisciplinary review of the classification of lung allograft rejection has taken place more than a decade since the previous revision.[2] There was continued support for retaining the previous acute rejection grades and for collapsing of the previous lymphocytic bronchiolitis (B) grades. The consensus group concluded that more detailed descriptions of the various grades and differential diagnoses, mainly in the form of additional photomicrographs, would enhance the usefulness of the 2006 revision and thereby improve reproducibility. The group also tackled the contentious issue of antibody-mediated rejection in the lung and reviewed the available literature. The consensus was that the available evidence supports the possibility of antibody-mediated rejection after lung transplantation but that more studies are required to determine which of the previously described pathologic lesions could be the histologic counterparts of this form of acute rejection.

Proposals for a standardized approach to investigating possible antibody-mediated rejection have been suggested to focus research endeavors in this difficult field. The consensus meeting again emphasized the importance of amalgamating the clinical, histologic, radiologic, immunologic and microbiologic data in a multidisciplinary setting to achieve the most accurate diagnosis for a particular patient episode. As always, the working formulation is regarded as a live document that will no doubt require further modification in the future with the advent of further molecular and other diagnostic refinements for the diagnosis and management of this complicated group of allograft recipients.

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APPENDIX: PARTICIPANTS BY TASK FORCE

Chair of consensus meeting: Susan Stewart, FRCPath.

Histopathology

Chair: Samuel A. Yousem, MD. *Participants:* Gerald J. Berry, MD, Margaret M. Burke, FRCPath, Michael C. Fishbein, MD, Charles C. Marboe, MD, Henry D. Tazelaar, MD.

We also acknowledge the invaluable contribution of the following international lung transplant pathologists who answered the questionnaire on the re-evaluation of the 1996 working formulation: Philip Cagle, MD, Belinda Clarke, FRCPA, Aliya Husain, MD, David Hwang, MD, Alberto Marchevsky, MD, N. Paul Ohori, MD, Jon Ritter, MD, Dani S. Zander, MD.

Immunopathology

Chair: Michael C. Fishbein, MD. *Participants:* Cynthia Magro, MD, Elaine F. Reed, PhD, Nancy L. Reismoen, PhD, Adriana Zeevi, PhD.

Clinical Lung Transplantation

Chair: Gregory I. Snell, MD. *Participants:* Annette Boehler, MD, Alan Glanville, MD, F. Kate Gould, FRCPath, Keith D. McNeil, FRACP, John P. Scott, MD, Sean M. Studer, MD, John Wallwork, FRCS, Glen Westall, MD, Martin R. Zamora, MD.

Lung Transplantation: Current Status and Challenges

Richard N. Pierson III

The lung is an anatomically complex vital organ whose normal physiology depends on actively regulated ventilation and perfusion, and maintenance of a delicate blood–air barrier over a huge surface area in direct contact with a potentially hostile environment. Despite significant progress over the past 25 years, both short- and long-term outcomes remain significantly inferior for lung recipients relative to other "solid" organs. This review summarizes the current status of lung transplantation so as to frame the principle challenges currently facing end-stage lung-failure patients and the practitioners who care for them.

Keywords: Lung transplant, Lung allocation, Reperfusion injury, Acute Lung injury, Chronic rejection, Bronchiolitis obliterans syndrome.

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Evolution of Current Practice

Successful clinical lung transplantation was first achieved in the 1980s (*1*, *2*) after nearly 40 instructive failures over almost 20 years (*3–5*). Reduced steroid doses, a dominant risk factor for both infection and airway anastomotic dehiscence (*3*, *6*, *7*), became possible with cyclosporine-based immunosuppression (*8*). Encouraged by preclinical results (*8–11*), Reitz and colleagues at Stanford achieved long-term survival after combined (en bloc) heart-lung transplantation, and demonstrated that rejection and various infections of the lung could be accurately distinguished and thus more safely and successfully treated (*1*, *12*). Subsequently, single and then double lung transplantation were reintroduced clinically at Toronto by Cooper and colleagues (*2*). Improved donor management, lung preservation, immunosuppression, and antimicrobial therapy contributed to 1-year survival approaching 70% by 1990, justifying broad acceptance in the pulmonary medicine community, and explosive growth of the waiting list through the 1990s. Worldwide activity increased from a few patients per year at one center in 1984 to over 1,500 at about 100 centers in the mid-1990s. Annual lung transplant activity in the United States (about 1,000) and the rest of the world (about 500) has remained constant over the past decade (*13*, *14*), while the U.S. waiting list has grown from about 1,500 to nearly 4,000. Consequently, only about 25% of wait-listed patients will receive a transplant in the next year, down from 60% in the mid-1990s; not counting candidates removed from the waiting list (whose reason for delisting and subsequent fate are not tracked), at least 15% will die before a suitable lung becomes available. These statistics document the practical consequences associated with the donor lung shortage but not its human toll.

Surgical practice in lung replacement has evolved considerably over two decades. Excellent clinical results have been reported with several different bronchial anastomotic techniques (*15–19*). Thus, at least in the context of improving graft preservation and immunosuppression and as long as a technically accurate anastomosis is performed without tension between well-vascularized tissues, intussusception, absorbable sutures, and interrupted technique are not critical. Neither omental nor intercostal pedicle flaps nor primary bronchial artery revascularization were found to decrease the incidence of bronchial anastomotic complications or the incidence or kinetics of chronic rejection (*20*, *21*). More recently, lung transplantation through a limited access anterior thoracotomy has been introduced to minimize surgical trauma (*22*).

Concomitant uncorrectable cardiac pathology in the setting of end-stage lung disease still warrants en bloc replacement of the heart and lungs. However, bilateral sequential ("double") lung transplantation has largely replaced heart-lung transplant as the preferred approach for those patients in whom chronic suppuration mandates replacement of both lungs, as in cystic fibrosis or "wet" bronchiectasis. This shift occurred based on evolving priorities for allocation of increasingly scarce hearts and lungs; simpler logistics and superior outcomes (although in expert hands results are similar (*23*, *24*)); and ethical issues associated with assessing the quality of "domino" heart donors and the added risk for a lung recipient of cardiac allograft rejection and vasculopathy. In the United States, more patients now die on the heart-lung waiting list each year than receive a transplant (*13*).

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Division of Cardiac Surgery, Department of Surgery, University of Maryland and Baltimore VAMC, Baltimore, MD.

Address correspondence to: Richard N. Pierson III, M.D., Cardiac Surgery, N4W94, 22 South Greene Street, Baltimore, MD, 21201.

E-mail: rpierson@smail.umaryland.edu

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The optimal procedure for the majority of end-stage lung failure patients remains controversial. For most candidates, either a single or bilateral sequential procedure provides symptomatic relief, independence from supplemental oxygen, and minimal activity restriction, and 1-year mortality and quality of life are similar (*13*, *14*). On the other hand, 5-year survival (38% vs. 47%), time to onset of chronic rejection, exercise tolerance, objective measures of lung function, and quality of life tend to favor double-lung recipients (*25*). However, this observation is based on retrospective nonrandomized data, and ignores prevalent programmatic practices whereby two lungs go preferentially to younger, healthier candidates who have a better prognosis independent of their transplant outcome (*26*). Even if results are superior, as seems biologically plausible, especially for younger candidates, the net impact on access and outcomes must be considered for the entire population of lung transplant candidates (*27*).

Living donor lobar lung transplantation, pioneered by Starnes and colleagues (*28*), has established a niche primarily for small candidates, because an adult lower lobe is sufficient in size to occupy the pleural space only for children or small adults. It is usually performed bilaterally for patients with cystic fibrosis or ventilator dependence of various etiologies who likely will not survive the wait for a suitable cadaveric donor and have two willing, healthy, biologically compatible donors. Short- and long-term survival are similar to cadaveric transplantation despite application in extraordinarily high-risk recipient populations (*28*, *29*). However, the procedure is ethically and logistically complicated (*30*), putting three lives at risk to save one, and thus is performed in significant numbers at only a few centers.

Short- and intermediate-term retransplantation outcomes similar to primary transplant have been reported for carefully selected patients (<2% of lung transplants performed) at a few programs, particularly when performed for chronic rejection (obliterative bronchiolitis [OB]) rather than primary graft failure (*14*, *31*, *32*). Because anecdotally the second allograft usually develops OB within a shorter interval than did the first, retransplantation seems sensible mainly for young patients with normal renal function and late-onset OB in the first graft.

Lung Donor Demographics

In the United States, about 15% of 6,500 cadaveric organ donors each year yield lungs that are transplanted (*13*, *14*). As a consequence of international initiatives to reduce ethanol-related road traffic accidents, the composition of the donor pool has changed adversely: the average age (now well over 30 years) and incidence of significant comorbidities (hypertension, diabetes) and other putative risk factors for initial lung dysfunction (smoking history, radiologic abnormalities, high A-a O_2 gradient, prolonged intubation) have increased among donors whose lungs are used. The proportion of organ donors whose lungs are transplanted varies dramatically by geographic region, from 5–10% in many US organ procurement areas to about 40% in Ontario and Australia. These striking disparities reflect differences in regional demographics of the donor and recipient pools, heterogeneous donor management strategies, and variably aggressive transplant program practices. Expansion of the lung donor pool will require the lung transplant community to identify and disseminate "best practices" to optimize lung function in organ donors (*33*); to systematically match high-risk donors with physiologically appropriate consenting recipients; and to optimally preserve, assess, and even "resuscitate" marginal or deceased donor lungs (*34*, *35*). Lung donation after cardiac death is feasible (*35–39*), but not yet widely disseminated.

Lung Donor Allocation

The U.S. rules for lung allocation have recently changed dramatically (*40*). Until 2005, lung allocation was driven almost exclusively by accumulated time since listing (*27*, *41*). Based on extensive data analysis, modeling, and iterative interactions among the lung transplant community, in April 2005 the United Network for Organ Sharing (UNOS) grouped wait-listed patients into five relatively homogeneous categories. Disease-specific relative risk criteria collected over the previous 6 months were used to estimate for each waitlist candidate the risk of dying before transplant, which was mathematically combined with the probability of survival for that patient after transplant to derive an integrated allocation priority score. Importantly, each patient-specific component of the score is an objective test result that can be obtained serially and updated as often as the transplant center chooses to do so, allowing rate of patient decline to be reflected in prioritization. The allocation model is intended to be revised to address inadvertent inequities or incorporate evolving priorities; it is expected to more fairly balance access for the sickest recipients with efficacy (net increase in years of life) over the entire population of waiting candidates.

Other countries utilize a wide variety of allocation algorithms, few of which incorporate objective recipient disease severity measures or probability of survival after transplant. Most allow transplant programs local to the donor to make allocation decisions before offering organs on a regional or national basis (*42*).

Primary Graft Dysfunction

Clinically evident ischemia/reperfusion (I/R) injury remains quite common after lung transplantation, with interstitial infiltrates and increased A-a O_2 gradient observed in a substantial minority of lung recipients (*43–46*). Although I/R injury delays withdrawal of ventilator support in less than 30% of recipients, when severe (in about 10% of recipients), "primary graft failure" significantly prolongs intensive care unit and hospital stay and is associated with 20–30% additional 90-day mortality and 30% of deaths within 30 days (*14*). How the mode of brain injury influences lung function in the donor and early and late outcomes is poorly understood (*48*); imperfect preservation of organ function during explant and storage, and reperfusion injury following the obligatory ischemic interval also contribute to the pathogenesis of this clinically important problem. It has persisted despite introduction of an extracellular preservation solution specifically tailored to the lung (Perfadex), and wide adoption of improved procurement techniques such as retrograde perfusion through the pulmonary veins (*49*, *50*). Controlled reperfusion with leukocyte-depleted autologous blood is a clinically available, technically straightforward approach that appears to be associated with significantly lower incidence of initial graft dysfunction, and deserves study on a broader scale (*51*).

Although never evaluated in a prospective, randomized study, over the past decade extracorporeal membrane oxygenation (ECMO) has increasingly been used to rescue patients with severe primary allograft failure and is usually associated with lung recovery (*52–55*), perhaps because of reduced ventilator-associated barotrauma.

Acute lung injury (ALI)—whether associated with I/R, systemic infection, transfusion, or hemorrhagic shock—is mediated by a variety of cytokines, chemokines, and adhesive ligand/receptor interactions (*56–62*). Complement activation, oxygen free radical and eicosanoid generation, and coagulation pathway interactions also contribute to inflammation, cell injury, and loss of endothelial barrier function. In the context of lung transplantation, various parenchymal cell populations as well as passenger donor macrophages or neutrophils sequestered in the lung may be primed by brain death and associated stressors. Targeting each of these cell types and pathways is logistically impractical. Rather, pivotal common mechanisms governing pathogenic pulmonary responses to inflammation represent attractive therapeutic targets. In experimental systems, S-1-P (*63*, *64*), PAR-1 (*64*, *65*), and adenosine-2 receptor agonists (*66*, *67*) can reverse established ALI, perhaps by modulating the balance between Rac- and Ras-mediated signaling pathways, and might thus reverse even established primary graft dysfunction.

Once established, ALI may resolve with minimal sequelae, or the lung may undergo fibroproliferative remodeling with loss of compliance and diffusing capacity via mechanisms that are poorly understood. Recently developed scoring systems for stratifying donor risk and recipient lung injury severity (*45–47*) and multicenter cooperative study groups should facilitate expeditious evaluation of preventive approaches, like soluble complement receptor type 1 (*68*), or candidate therapeutic agents. Expression of various protective proteins during the ischemic interval after lung harvest (*69*, *70*) awaits advances in efficient industrial-scale vector development (G.A. Patterson, personal communication, 2006).

Recipient Selection

The proportion of patients receiving lung transplant with emphysema and A1AT deficiency (50%), cystic fibrosis (15%), and idiopathic pulmonary fibrosis (15%) have remained fairly constant since 1990, with primary pulmonary hypertension, sarcoidosis, retransplant, and an assortment of other diagnoses accounting for the remainder (*13*). Recipient selection criteria (*71*) have been substantially relaxed at many programs (*72*). As one consequence, the average age of lung candidates and recipients is steadily increasing (*13*, *14*, *40*). Of note, some U.S. registry studies suggest that patients with emphysema derive no survival benefit from transplantation (*73–75*), a phenomenon perhaps related to U.S. organ allocation strategies because it is not suggested in a European analysis (*76*). Improving medical therapies for pulmonary hypertension have dramatically reduced the need for transplant for this diagnosis (*40*).

Recipient Management and Associated Outcomes

One-year survival has improved slightly over the past 10 years, from about 75% to 82% (*40*). Since use of extended criteria and older donors has expanded during this interval and in older recipients (increasing recipient age is an independent risk factor (*14*)), expected adverse consequences of these donor trends have apparently been mitigated by improving program practices in other areas (organ preparation, perioperative support, immunosuppression). However, 5-year survival remains stubbornly below 50% (*13*, *14*, *40*).

In the absence of one clearly superior approach, a wide variety of maintenance immunosuppression strategies are currently being used in lung recipients. Mycophenolate mofetil and FK506 may be associated with reduced rates of acute infection and/or improved survival relative to azathioprine and cyclosporin, respectively (*77–79*), but these observations are not universally replicated (*80–82*). Few immunosuppressive agents have been formally studied in this population, but from the ever-broadening array of agents approved by the U.S. Food and Drug Administration or European Standards Agencies for use in kidney recipients, a regimen can now be tailored to each lung recipient's risk factors, evolving clinical circumstances, and financial situation. Newer immunosuppressive agents are now being evaluated for lung transplant indications, in part because the room for improvement in 1-year survival remains substantial and favorable effects are thus easier to measure (*83*). Calcineurin inhibitor-associated renal insufficiency is very common among lung recipients within 5 years (creatinine >2.5 mg/dl in >30%; dialysis or renal transplant in 5–10%). Because their antiproliferative effects may also prevent or retard progression of OB, many centers are exploring conversion from calcineurin inhibition to a "renal-sparing" target-of-rapamycin inhibitor, but a high incidence of bronchial anastamotic dehiscences halted substitution of sirolimus at transplant (*84*, *85*). Another approach to minimize cumulative pharmacologic toxicities utilizes "induction." Although popular, interleukin (IL)-2R blockers have not yet consistently decreased acute rejection or infectious complications (*86*). High-dose ATG or Campath 1H (*87*) with low-dose conventional immunosuppression do not prevent chronic rejection despite profound long-term lymphocyte depletion, and at intermediate-term follow-up mortality and infectious complications appear similar to other regimens. Rapid steroid withdrawal or avoidance (*84*) is rarely attempted in lung allograft recipients because acute rejection is common and a strong risk factor for chronic rejection, whereas chronic rejection is prevalent despite relatively intense current immunosuppressive regimens.

Looking forward, aerosols deliver high concentrations of various drugs directly to the lung, thereby increasing their therapeutic index (*88*), an important opportunity unique to the lung. Because its phosphorylated form is an Edg-1 receptor agonist and should promote enhanced endothelial barrier function, preoperative loading with FTY720 may be particularly useful to prevent primary graft dysfunction in lung recipients, in addition to any effects it may have on cell trafficking and adaptive immunity (*89*). Induction therapy could provide a foundation for peripheral or central tolerance based on immunomodulatory costimulation (*90*, *91*) or chemokine pathway blockade (*92*, *93*).

Control of infection, particularly of herpes-family viruses, remains a particularly important issue for lung allograft recipients, perhaps because their immune suppression is relatively intense, and the lung is relatively vulnerable to local

and systemic insults that accompany acute or recrudescent infection with these organisms. Prolonged prophylaxis for cytomegalovirus with ganciclovir or an orally bioavailable variant usually prevents viremia and disease during therapy, but after prophylaxis is stopped viral activation is prevalent and, anecdotally, the organism is more often resistant to conventional pharmacotherapy. Based on the notion that recipient immunity is integral to long-term control, and taking advantage of increasingly reliable tests to diagnose presymptomatic infection, an expectant "bait-and-switch" approach was effective at preventing symptomatic cytomegalovirus disease and facilitating short- and long-term viral control, at significantly reduced fiscal and physiologic costs (*94*). Likewise, invasive aspergillus, either at the bronchial anastomosis or occasionally in native or graft parenchyma, is a highly morbid complication; prophylactic inhaled or systemic antifungal therapy is probably unnecessary unless airway ischemia at bronchocopy, sputum culture results, or environmental circumstances (e.g., construction or preoperative colonization) alter a particular patient's a priori risk. When less common viruses such as adenovirus and respiratory syncytial virus invade the lung, survival is unusual because, at present, these organisms are essentially untreatable except by supportive measures.

OB describes fibrotic occlusion of small airways, the pathologic hallmark of chronic lung allograft rejection (*95*). OB can usually only be proven by histologic demonstration of OB on large tissue samples obtained at open lung biopsy, retransplant, or autopsy. The typical physiologic correlate of OB, bronchiolitis obliterans syndrome (BOS)—defined as a decline in FEV1 of more than 20% not attributable to acute infection or rejection—has been generally accepted as a valid proxy for OB (*96*). However, about 50% of patients with BOS do *not* have OB when pathologic material is comprehensively audited (*25*, *97*). In these patients recent evidence shows that BOS may be caused by silent gastroesophageal reflux and chronic aspiration (*98*), and performing early antireflux surgery reduces the incidence of BOS (*99*). Adaptive immunity mediated by antibody and T-cell mechanisms clearly plays a central role in OB pathogenesis (*100–103*), as do immunity to lung autoantigens (*104*) and innate immune activation (*105–109*). Pharmacologic approaches to inhibit innate immune system activation, such as azithromycin (*110*), are moving into the clinic.

Malignancy accounts for about 10–15% of deaths during late follow-up, similar to other organ recipient populations. Given the terrible results with chemotherapy for posttransplant lymphoproliferative disease in lung recipients (*111*), the advent of relatively safe, effective treatment with anti-CD20 offers a welcome alternative when reduction in immunosuppression is not curative (*112*).

On balance, the evidence to date supports the logical notion that antibody reactive with donor antigens contributes to both acute and chronic lung injury. In a large series of Harefield's heart-lung recipients, a positive NIH lymphocytotoxic crossmatch was associated with 50% survival among 32 patients, compared to 61% survival with a negative result (*113*). Strikingly, in a subset of patients with a positive T cell–directed crossmatch, zero of four patients survived beyond 70 days, compared to 68% of 100 patients with a negative crossmatch, suggesting that antibody detected by this assay is highly injurious to the graft. Among 656 first-time lung recipients from the combined Toronto and Duke experience, 20 (3%) who had a panel-reactive antibody (PRA) titer >25% exhibited significantly decreased median (1.5 vs. 5.2 years) and 1-month survival (70% vs. 90%) (*114*). Thus, a high PRA (anti-HLA antibodies) reflects a propensity to humoral alloreactivity and is a risk factor for acute and chronic allograft injury (*102*). Recent reports suggest that antibody directed against non-HLA antigens can also trigger acute lung injury (*115*, *116*). Although two ABO-incompatible lung reported in the popular press were associated with early death (*117*, *118*), two published cases demonstrate that anti-ABO antibody can be effectively managed by available therapy (*119*, *120*).

In addition to crude survival statistics and costs, patients and health care payors are increasingly focused on quality of life and return to work as important measures of successful transplant outcome (*121*, *122*). Although over 80% of surviving lung recipients report no activity restrictions at 1 and 5 years, less than 40% return to work (*14*): in the United States, return to gainful employment is often impeded by astronomical ongoing medication costs and unaffordable insurance premiums, effectively trapping patients on disability. Although piecemeal remedies specific to transplantation are conceivable, resolution of this catch-22 will probably require fundamental restructuring of U.S. health care financing, a daunting challenge.

Future Advances

Xenotransplantation from genetically modified pigs offers the most likely near-term prospect for alleviating the lung donor shortage, but lung xenografting poses formidable problems (*123–125*). Whether triggered primarily by cellular adhesive interactions or coagulation pathway incompatibilities, to fully prevent acute lung injury in this context, substantial additional work appears necessary. Investment in primate heart and lung allograft tolerance models should yield new knowledge applicable to xenografts, and to address chronic allograft rejection issues that are specific to thoracic organs (*104*, *126*, *127*). In the longer term, durable fully implantable artificial lung technology will probably require evolution of ECMO to a self-renewing biological interface propagated on new biocompatible materials (*128*, *129*).

SUMMARY

Stable overall lung transplant activity for the past decade reflects the net effect of competing forces. Some restrict activity and patient access (low lung donation rates, adverse donor demographics, preferential double lung use), while countervailing influences include liberalization of recipient age and comorbidity criteria, relaxing donor acceptance standards, and initiatives to disseminate optimal donor management practices. Incremental improvement in 1-year lung transplant outcomes have been achieved despite use of older donors in older, sicker recipients, likely due to improved donor management and increasing availability of newer antibiotic and immunosuppressive regimens. However, 5-year survival remains disappointing at below 50%, with OB, infection, renal insufficiency, and malignancy all contributing to late attrition. These persistent problems un-

derscore the imperative to develop tolerance induction strategies for clinical lung transplantation, and to better understand the contribution of innate immune and nonimmune mechanisms to BOS and OB. Improved policies based on wait-list and posttransplant risk factors are being implemented to fairly allocate organs, and may aid patients and their physicians in deciding whether to accept marginal organs. In addition, socioeconomic barriers will need to be addressed for the full therapeutic potential of lung transplantation to be realized.

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2007 Banff Schema for Grading of Acute Pancreas Allograft Rejection

Link to: Acute cellular rejection; Chronic active cellular rejection; Antibody-mediated rejection; Chronic active antibody-mediated rejection;Chronic rejection

Acute Cell-Mediated Rejection(return to top)

Category	Histopathology	Comments
Normal	No inflammation OR inactive septal mononuclear inflammation not involving veins, arteries, ducts, or acini	1. Fibrous tissue limited to septa in appropriate amounts; no injury or atrophy of acinar regions
Indeterminate for Acute Rejection	"Active" septal inflammation without other criteria for rejection (see below)	1. Any venulitis or ductitis qualifies for at least mild acute rejection (or more depending on other features) 2. Active inflammation refers to blastic lymphoctyes with variable numbers of eosinophils
Grade I (Mild acute cell-mediated rejection)	"Active" septal inflammation with involvement of septal veins (venulitis) and/or ducts (ductitis) AND/OR focal (1-2 foci/lobule) acinar "active" inflammation with minimal/no	1. Any venulitis or ductitis is sufficient for diagnosis; nerve branches usually involved but rarely sampled; focal acinar "active" inflammation alone also adequate for diagnosis

	acinar cell injury	
Grade II (Moderate acute cell-mediated rejection)	Minimal intimal arteritis AND/OR multiple (3 or more foci/lobule) foci of acinar "active" inflammation with single cell injury/dropout	1. Minimal intimal arteritis refers to occasional subendothelial lymphocytes without obvious endothelial swelling/activation or injury
Grade III (Severe acute cell-mediated rejection)	Widespread acinar inflammation with confluent areas of acinar cell injury/necrosis AND/OR moderate to severe intimal arteritis AND/OR necrotizing arteritis	1. Any of these three findings is sufficient for the diagnosis 2. Acinar inflammation may contain variable lymphocytes, eosinophils, and neutrophils as well as edema and/or hemorrhage 3. Moderate/severe intimal arteritis consists of more frequent subendothelial lymphocytes with evidence of intimal injury, such as cell swelling, fibrin leakage, etc. 4. Necrotizing arteritis may also occur in antibody-mediated rejection and C4d stain should be performed.
Chronic Active Cell-Mediated Rejection (return to top)		
Category	Histopathology	Comments
Chronic active cell-mediated rejection	Arterial luminal narrowing due to intimal proliferation of fibroblasts, myofibroblasts.	1. May represent transition between intimal arteritis and chronic transplant arteriopathy related to suboptimal immunosuppression 2. Rarely seen in needle biopsies, more often seen in

	smooth muscle cells, with admixed T lymphocytes and macrophages ("active" transplant arteriopathy)	allograft resection related to chronic rejection
Antibody-Mediated Rejection (return to top)		
Category	Histopathology	Comments
Hyperacute antibody-mediated rejection	Widespread deposition of immunoglobulin (usu. IgG) and complement (e.g., C4d) with resultant arteritis and venous thrombosis, hemorrhagic necrosis and allograft failure usually within 1 hour after revascularization	1. In all cases, diagnosis is dependent upon demonstration of a) graft dysfunction, b) capillary complement deposition (i.e., C4d positivity), AND c) donor specific antibodies in serum. 2. If C4d and only 1 of the other 2 features is found, then the diagnosis"suspicious for" antibody-mediated rejection is more appropriate 3. In cases in which vascular thrombosis is the predominant finding, the differential diagnosis lies between antibody-mediated rejection and "technical failure".
Accelerated antibody-mediated rejection	Similar to hyperacute, but changes evolve over hours to days after revascularization.	
Acute antibody-mediated rejection	Allograft dysfunction in first posttransplant weeks; histology varies from normal to margination of neutrophils and	

	mononuclear cells to thrombosis and necrosis;	
Chronic Active Antibody-Mediated Rejection (return to top)		
Category	Histopathology	Comments
Chronic active antibody-mediated rejection	Features of chronic rejection/graft sclerosis together with C4d positivity in capillaries	1. May also have vascular fibrinoid necrosis/thrombosis indicating ongoing antibody-mediated rejection 2. C4d positivity, graft dysfunction, and donor-specific antibodies are all required for a diagnosis as in other forms of antibody-mediated rejection
Chronic Rejection (Graft Sclerosis) (return to top)		
Category	Histopathology	Comments
Chronic allograft rejection Stage I (mild graft sclerosis)	Fibrous septa expanded but comprise less than 30% of biopsy surface area. Acinar lobules have irregular contours due to erosion	
Chronic allograft rejection Stage II (moderate graft sclerosis)	Fibrous septa expanded to 30-60% of biopsy surface area. Most lobules have irregular contours and central areas are affected, with fibrous strands extending between	

	lobules	
Chronic allograft rejection Stage III (severe graft sclerosis)	Fibrous septa comprise over 60% of biopsy surface area with only a few areas of residual acini and/or islets	
Reference: Drachenberg CB et al. Banff schema for grading pancreas allograft rejection: Working proposal by a multi-disciplinary international consensus panel. Am J Transplant 8:1-13, 2008.		

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Banff Schema for Grading Pancreas Allograft Rejection: Working Proposal by a Multi-Disciplinary International Consensus Panel

C. B. Drachenberg[a,*], J. Odorico[b], A.J. Demetris[c], L. Arend[d], I. M. Bajema[e], J. A. Bruijn[e], D. Cantarovich[f], H. P. Cathro[g], J. Chapman[h], K. Dimosthenous[i], B. Fyfe-Kirschner[j], L. Gaber[k], O. Gaber[l], J. Goldberg[m], E. Honsová[n], S. S. Iskandar[o], D. K. Klassen[p], B. Nankivell[h], J. C. Papadimitriou[a], L. C. Racusen[q], P. Randhawa[c], F. P. Reinholt[r], K. Renaudin[s], P. P. Revelo[t], P. Ruiz[u], J. R. Torrealba[v], E. Vazquez-Martul[w], L. Voska[n], R. Stratta[x], S. T. Bartlett[y] and D. E. R. Sutherland[z]

[a] *Department of Pathology, University of Maryland School of Medicine, Baltimore, MD*
[b] *Department of Surgery, University of Wisconsin-Madison, Madison, WI*
[c] *Division of Transplantation Pathology, Department of Pathology, University of Pittsburgh, Pittsburgh, PA*
[d] *Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center, Cincinnati, OH*
[e] *Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands*
[f] *Department of Nephrology, CHU – Hôtel Dieu, Nantes, France*
[g] *Department of Pathology, University of Virginia, Charlottesville, VA*
[h] *Department of Renal Medicine, University of Sydney, Westmead Hospital, Sydney, Australia*
[i] *Department of Pathology, Evangelismos Hospital, Athens, Greece*
[j] *Department of Pathology, Robert Wood Johnson University, New Brunswick, NJ*
[k] *Department of Pathology, University of Tennessee Health Science Center, Memphis, TN*
[l] *Department of Surgery, The Methodist Hospital, Houston TX*
[m] *Instituto de Nefrologia, Buenos Aires, Argentina*
[n] *Department of Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*
[o] *Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC*
[p] *Department of Medicine, University of Maryland School of Medicine, Baltimore, MD*
[q] *Department of Pathology, John Hopkins University, Baltimore, MD*
[r] *Institute of Pathology, University of Oslo and Division of Pathology, Rikshospitalet University Hospital, Oslo, Norway*
[s] *Anatomie et Cytologie Pathologiques CHU – Hôtel Dieu, Nantes, France*
[t] *Department of Pathology and Laboratory Medicine, University of Utah, Salt Lake City, UT*
[u] *Departments of Pathology and Surgery, University of Miami, Miami, FL*
[v] *Department of Pathology and Laboratory Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI*
[w] *Departmento de Patologia, CHU Canalejo, A Coruňa, Spain*
[x] *Department of General Surgery, Wake Forest University School of Medicine, Winston-Salem, NC*
[y] *Department of Surgery, University of Maryland School of Medicine, Baltimore, MD*
[z] *Department of Surgery, Division of Transplantation and Diabetes Institute for Immunology and Transplantation, University of Minnesota, Minneapolis, MN*
[*] *Corresponding author: Cinthia B. Drachenberg, cdrac001@umaryland.edu*

Accurate diagnosis and grading of rejection and other pathological processes are of paramount importance to guide therapeutic interventions in patients with pancreas allograft dysfunction. A multi-disciplinary panel of pathologists, surgeons and nephrologists was convened for the purpose of developing a consensus document delineating the histopathological features for diagnosis and grading of rejection in pancreas transplant biopsies. Based on the available published data and the collective experience, criteria for the diagnosis of acute cell-mediated allograft rejection (ACMR) were established. Three severity grades (I/mild, II/moderate and III/severe) were defined based on lesions known to be more or less responsive to treatment and associated with better- or worse-graft outcomes, respectively. The features of chronic rejection/graft sclerosis were reassessed, and three histological stages were established. Tentative criteria for the diagnosis of antibody-mediated rejection were also characterized, in anticipation of future studies that ought to provide more information on this process. Criteria for needle core biopsy adequacy and guidelines for pathology reporting were also defined.

The availability of a simple, reproducible, clinically relevant and internationally accepted schema for grading rejection should improve the level of diagnostic accuracy and facilitate communication between all parties involved in the care of pancreas transplant recipients.

Key words: Acute allograft rejection, acute cellular rejection, allograft arteriopathy, allograft function, allograft loss, allograft monitoring, anti-HLA antibodies, antibody-mediated rejection, Banff schema, biopsy specimen, pancreas, pancreas allograft, pancreas and kidney, pancreatic islets, pathology

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Introduction

Pancreas transplantation is an effective treatment option for patients with either brittle or complicated diabetes mellitus (DM). A successful pancreas transplant results in disappearance of the acute complications of DM (i.e. hypoglycemia, severe hyperglycemia, and ketoacidosis), and may stabilize or even reverse some of the long-term complications of the disease (1–3).

The first pancreas transplant was performed in 1966, but routine application of this procedure did not occur until the 1980s. The slower progress for pancreas transplantation in comparison to other organ transplants was related both to technical and immunological challenges inherent to the graft itself (4). Maintenance of parenchymal architecture and preservation of endocrine function in whole pancreas transplantation requires concurrent surgical management of exocrine secretions. This is most commonly accomplished by pancreatico-duodenal transplantation with anastomosis of a portion of the donor duodenum either to the recipient small intestine or urinary bladder. Venous drainage of the pancreas (and consequent insulin delivery) can be performed either into the systemic (iliac vein or vena cava in cases of bladder or enteric exocrine drainage) or portal system (mesenteric vein in cases of enteric exocrine drainage) (5). Pancreas transplantation is performed in three different situations, depending on the patient's native kidney function: simultaneous pancreas-kidney (SPK) is used in diabetic patients with uremia/end-stage renal disease. This is the most common type of pancreas transplant performed, and currently accounts for 60–65% of new cases. Alternatively, the pancreas can be transplanted after a successful (previous) kidney transplant (sequential pancreas after kidney, (PAK)). Pancreas transplantation alone (PTA) is used to treat nonuremic diabetic patients (6).

Results of pancreas transplantation have continued to improve, with current 1-year graft survival (complete insulin independence) rates of 85% for SPK, 78% for PAK and 77% for PTA. One-year patient survival rates are excellent in all three categories, ranging from 95% to 97% (7). As of 2007, more than 20 000 pancreas transplants have been performed in the United States (8).

The mechanisms of acute rejection in the pancreas allograft are not different from those in other solid transplants, although distinctive rejection patterns are seen for the exocrine and endocrine components that likely reflect variations in major histocompatibility complex (MHC) expression, type and quality of the micro-vasculature, and sensitivity to ischemia (9–13). Experimental, as well as clinical, studies have shown that vessels, ducts and acini are the preferential targets of cell-mediated rejection, in contrast to the islets of Langerhans that are neither directly nor immediately affected (14–25) (Figures 1–4). On the other hand, the few documented cases of antibody-mediated rejection of the pancreas have presented with hyperglycemia, suggesting that the islets may be susceptible to micro-vascular injury associated with antibody deposition (26–28). MHC disparities in general, and specifically class II alloantibodies, have been associated with an increased risk of pancreas allograft loss (28). Antibody-mediated rejection has become increasingly recognized in pancreas transplantation (29) (Figure 5).

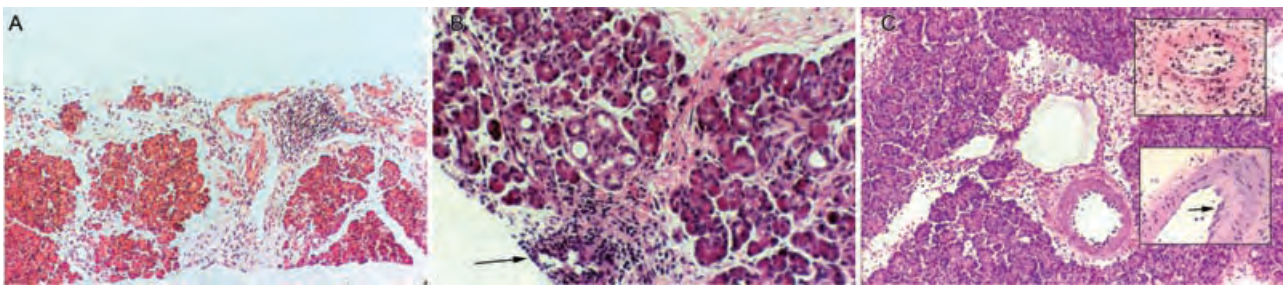

Figure 1: Inflammation in septa and septal structures. (A) Inactive appearing, mononuclear septal infiltrates that are not involving septal structures or acini. In the absence of more specific findings this case would be classified as 'indeterminate' for rejection. (B) Venulitis (arrow) representing mild/grade I ACMR in a biopsy done 5 days posttransplantation. There is minimal acinar inflammation (toward the left). The acini show patchy luminal dilatations (rounded empty spaces) consistent with ischemic injury. (C) Moderate/grade II ACMR defined by active septal inflammation in association with venulitis in two cross-sections of veins and intimal arteritis in the artery below. Top insert: from a different biopsy, necrotizing arteritis (the wall is replaced by amorphous eosinophilic material). There is also transmural inflammation and moderate-severe intimal arteritis. Note endothelial cell injury (swelling, sloughing). Bottom insert: minimal intimal arteritis consisting of a few of lymphocytes beneath the endothelium (arrow). There is no clear evidence of endothelial injury.

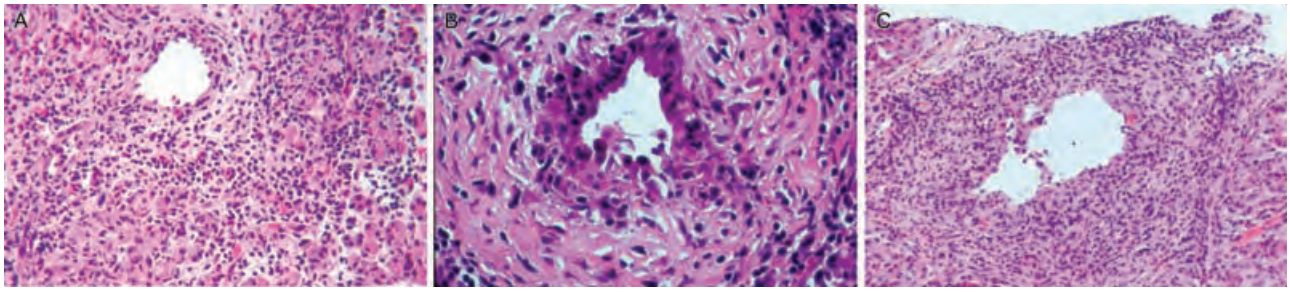

Figure 2: Venous and ductal inflammation in ACMR. (A) Active septal inflammation with numerous eosinophils and venulitis (upper middle field). (B) Ductal inflammation and associated reactive/regenerative epithelial changes. (C) Severe ductal inflammation. Dense infiltrates around a duct with extensive denudation of its epithelial lining. Few epithelial clusters on the left upper contour were positive for cytokeratin stain (not shown).

Significant and/or persistent acinar damage and chronic vascular injury (transplant arteriopathy) trigger a progressive fibrogenic reaction, which eventually impairs endocrine function (Figure 6). As with all other allografts, repeated episodes of acute rejection, and particularly late acute rejection, significantly increase the risk for graft loss due to chronic rejection (19,30–34).

Clinical diagnosis of acute rejection

Pancreas allograft rejection is usually asymptomatic so the clinical diagnosis relies heavily on laboratory markers of acinar cell injury (i.e. increase in serum amylase and lipase levels) or abnormalities in the exocrine or endocrine functions (decrease in urine amylase in bladder-drained grafts or hyperglycemia, respectively).

Increase in serum amylase and/or lipase is seen in a majority of rejection episodes, correlating with biopsy-proven rejection in approximately 80% of cases (35,37,38). Additionally, in patients with bladder exocrine drainage, decrease in amylasuria from baseline has been reported to correlate with histologically proven acute rejection in 53% of cases (35,36,39).

Hyperglycemia occurs only in the more severe, often irreversible, forms of acute rejection (16,17). The differential diagnosis in patients with hyperglycemia also includes early or late large vessel thrombosis, recurrence of autoimmune disease, islet cell drug toxicity and advanced stages of chronic rejection/graft sclerosis (30,31,40–42). Serial serum creatinine levels, with confirmation by renal allograft biopsy, are used as surrogate markers for pancreatic acute rejection in patients with simultaneous pancreas kidney transplants. Despite the general acceptance of this practice, the occurrence of asynchronous rejection has been well documented with isolated rejection of either the pancreas or the kidney allograft occurring in up to 30% of cases (43–45).

Rejection occurs earlier and is more common in PTA (9,46), which also has a higher rate of graft loss from irreversible rejection in comparison to SPK (9% and 30% in PTA vs. 2% and 7% in SPK transplants at 1 and 5 years, respectively) (8). Routine performance of percutaneous pancreas allograft biopsies has significantly improved outcomes in PTA because as mentioned above, timely diagnosis and treatment of acute rejection are essential to prevent irreversible graft sclerosis (6,43,46,47).

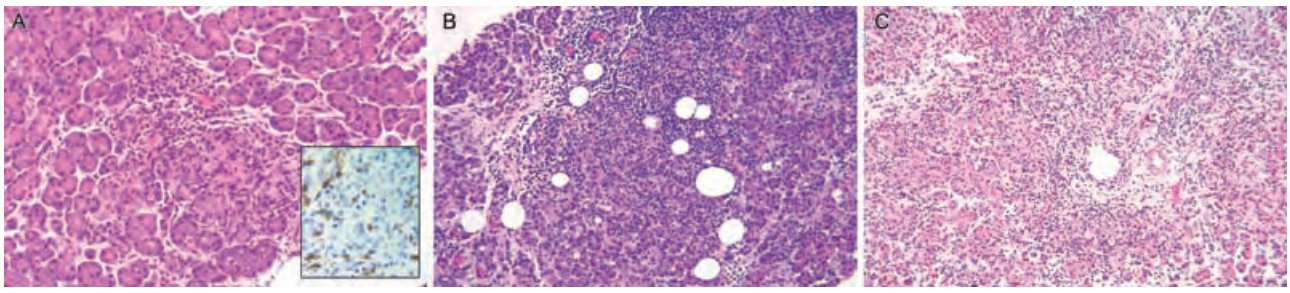

Figure 3: Acinar inflammation in ACMR. (A) Acinar inflammatory focus (center field), that is in continuity with a small inflamed septal area above. Insert: CD3 stain highlights T lymphocytes and their tight relationship to the acinar cells as is typically observed in ACMR. Also see Figure 6A. (B) Dense septal inflammation (upper left) that spills extensively in the neighboring acinar areas. There is also mild fatty infiltration. (C) Example of grade II/moderate acute cell-mediated rejection defined by the presence of multi-focal acinar inflammatory infiltrates in addition to the septal inflammation. All acinar areas are affected, but high magnification (not depicted) showed that acinar cell injury/necrosis was only seen in isolated cells (i.e. multi-cellular or confluent acinar cell injury/necrosis was not present).

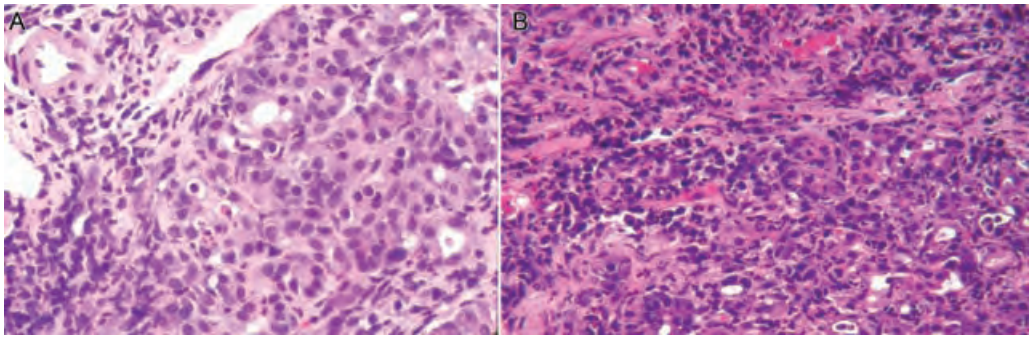

Figure 4: Focal versus multi-cellular/confluent acinar cell injury/necrosis. (A) Acinar cell injury/necrosis in occasional acinar cells. Note an apoptotic cell distinguished from the adjacent cells by a clear halo and few apoptotic bodies (lower left of apoptotic cell). Most acinar areas in this biopsy were free of inflammation. (B) Extensive septal and acinar inflammation with multi-cellular acinar cell injury/necrosis. The acinar architecture is distorted, there are empty spaces indicating acinar cell drop-out and patchy amorphous eosinophilic foci representing necrosis (top right and lower right). The depicted findings were seen throughout the acinar areas indicating severe/grade III ACMR.

Needle core biopsies

In contrast to kidney and liver transplantation, procurement of tissue samples from pancreas allografts was uncommon until the early 1990s when Allen et al. introduced a safe and reproducible technique of percutaneous needle biopsy (15). Due to the lack of specificity of serum and urine markers, needle core biopsies have become the standard for the diagnosis of acute pancreas allograft rejection (47–53).

Core biopsies are usually performed with 18 or 20 gauge needles, under ultrasound or computer tomographic guidance (53,54). Adequate tissue can be obtained in 88% to 90% of cases and complications are rare (2–3%, i.e. bleeding, mild pancreatitis) (38,46,52–55). Laparoscopic or open biopsies may be required if bowel loops are interposed between the abdominal wall and the graft (56). Cystoscopic transduodenal biopsies are now performed rarely in patients with bladder drainage, with adequate samples of pancreatic tissue obtained in 57–80% (57,58).

Biopsy adequacy

The adequacy of any particular biopsy sample is ultimately determined by the examining pathologist, but based on the current understanding of pancreas allograft rejection, it is recommended that at least three lobular areas and their associated inter-lobular septa are evaluated. Arterial branches follow a less-predictable course and are sampled with more difficulty. Due to the diagnostic importance ascribed to the arterial lesions, the absence of arterial branches in the biopsy core should be noted in the pathology report (17,59,60).

The presence of islets in pancreatic core biopsies is similarly unpredictable and is not necessary in the determination of allograft rejection (or biopsy adequacy) because

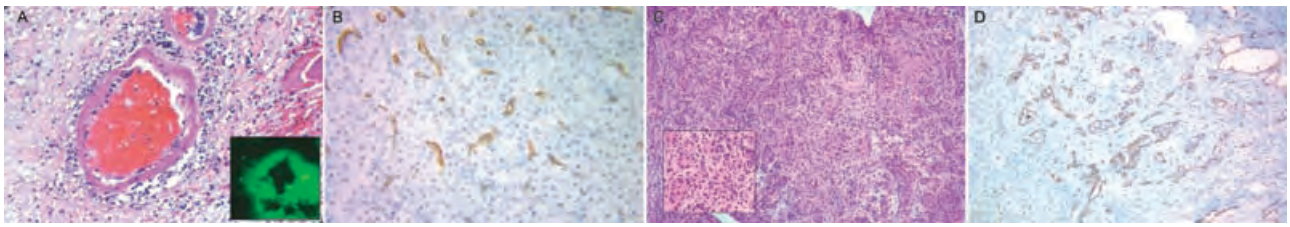

Figure 5: Antibody-mediated rejection (AMR). (A) Arterial fibrinoid necrosis due to accelerated AMR in a graft pancreatectomy performed 30 h posttransplantation. Insert: immuno-fluorescence stain is strongly positive for IgG. C4d stain (not represented) was also positive in all size vessels. (B) C4d stain in pancreatic capillaries in patient with acute AMR biopsied 10 days posttransplantation. (C) Same patient as part B, biopsied 18 days posttransplant, continues to have strong positivity for C4d (not represented) and extensive inter-acinar neutrophilic inflammation. Note foci of necrosis (upper right). (D) Same patient as parts B and C: strong C4d staining in pancreas lost due to persistent AMR, 3 months posttransplantation. Note extensive fibrosis with associated obliteration of the endocrine and exocrine components (chronic active AMR).

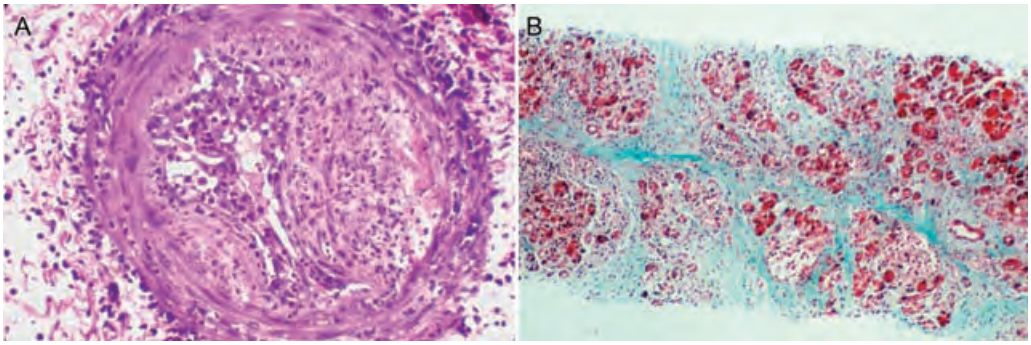

Figure 6: Chronic rejection/graft sclerosis. (A) Artery with severe luminal narrowing due to a combination of acute (intimal arteritis) and active chronic cell-mediated allograft rejection. The latter appears as two 'cushion-like' areas of intimal fibrosis with mononuclear inflammation. (B) Stage II of chronic rejection/graft sclerosis characterized by septal and acinar fibrosis that extends to the center of the acinar lobules.

inflammation affects the exocrine before the endocrine elements of the pancreas in most cases.

Guidelines for processing pancreas allograft biopsies

A methodical and rational approach to the utilization of needle core biopsies can maximize diagnostic yield. A description of routine and ancillary studies recommended is presented in Table 1. In biopsies used to assess hyperglycemia, insulin and glucagon immunostains should be performed to identify selective loss of beta cells (40,61). Masson's trichrome stain is particularly useful to demonstrate inter-acinar fibrosis in the earlier stages of graft sclerosis and to assist in the identification of specific structures or pathological changes (i.e. denuded ducts, fibrinoid necrosis in arterial walls, etc.).

Similar to the kidney, antibody-mediated rejection may be unrecognizable in the absence of C4d staining. There is no general agreement with respect to the best technique for C4d staining. Although the immuno-fluorescence technique is more sensitive, C4d staining of formalin-fixed paraffin-embedded sections is widely used with clinically acceptable results (59). It is currently recommended that the extent of C4d staining (i.e. % of positive inter-acinar capillaries) be reported (Table 2). In cases of C4d positivity, the need for correlation with serological studies (donor-specific antibodies -DSA) should be also stated in the pathology report (59).

Table 1: Recommended guidelines for processing pancreas allograft biopsies. Greater than or equal to 10 slides containing sequential sections are used as follows:

- Three for hematoxylin and eosin staining
- One for trichrome (collagen) staining
- One for C4d immuno-histochemistry
- Five or more intervening unstained sections for additional stains as needed (i.e. insulin and glucagon immuno-histochemistry if the biopsy is done for hyperglycemia; CMV, EBV, etc.).

Histological diagnosis and grading of acute pancreas rejection

The pioneering studies of Nakhleh and Sutherland, in which they compared tissue from failed and functioning allografts, identified intimal and transmural arteritis as features of the more severe forms of acute rejection. This observation led to the first proposal for a rejection grading schema (60). In subsequent years, routine availability of needle core biopsies allowed for the recognition of a broader spectrum of pathological changes in biopsies from functioning as well as rejecting or failing allografts (47–52). Based on a comparison of surveillance versus clinically indicated biopsies, a schema for grading acute rejection with six grades (0 to V) was developed at the University of Maryland (17). The latter schema emphasized progressive changes ranging from lack of inflammation (grade 0), to isolated involvement of fibrous septa (grade I) and septal structures (grade II), to acinar (grade III) and arterial involvement (grade IV). Parenchymal necrosis characterized the most severe form of rejection (grade V). Overall, the Maryland grading schema was shown to have good correlation with ultimate graft outcomes, clinical laboratory parameters and response to treatment (17). However, long-term outcomes and response to antirejection treatment between grades II and III were similar, both likely reflecting grafts with milder forms of acute rejection in contrast to the higher grades (IV and V) (16,17).

In 2005, a multi-disciplinary group of physicians with particular expertise and interest in the field of pancreas transplantation initiated consensus discussions at the 8th Banff Conference on Allograft Pathology (Edmonton, Alberta, Canada), following the successful model used for the development of the Banff schemas for grading rejection in kidney and liver transplantation (59,62). The working proposal presented here was generated after extensive, ongoing, consensus discussions that culminated at the 9th Banff conference on Allograft Pathology in 2007 (La Coruña, Spain).

Table 2: Histological definitions used for the diagnosis of rejection

Septal inflammatory infiltrates: predominantly mononuclear, including 'blastic' (activated) lymphocytes and variable numbers of eosinophils. Eosinophils may be the predominant cell type.
Venulitis: circumferential cuffing of septal veins with sub-endothelial accumulation of inflammatory cells and endothelial damage/lifting.
Ductitis: infiltration of ductal epithelium by mononuclear and/or eosinophillic inflammatory infiltrates and ductal epithelial cell damage. May lead to epithelial denudation.
Neural and peri-neural inflammation: septal inflammatory infiltrates in and around nerve branches (rare finding in needle biopsies).
Acinar inflammation: inflammatory infiltrates with similar characteristics as the septal infiltrates amidst the exocrine acini.
Acinar inflammatory lesion/focus: collection of $\geq$10 lymphocytes/eosinophils within an acinar area.
Focal acinar inflammation: $\leq$2 inflammatory foci per lobule with no evidence of acinar cell injury.
Multi-focal acinar inflammation: $\geq$3 foci of inflammation per lobule with single/isolated acinar cell injury/necrosis. Intervening uninflamed acinar areas.
Severe/extensive acinar inflammation: confluent, diffuse (widespread) acinar inflammation with focal or diffuse multi-cellular/confluent acinar cell injury-necrosis. No or very rare uninflamed acinar areas.
Acinar cell injury/necrosis: cytoplasmic swelling and vacuolization and/or nuclear pyknosis, apoptotic bodies, lytic necrosis leaving empty spaces equaling the size of individual cells (cell drop-out).
Single cell/spotty acinar cell injury/necrosis: only isolated cells are affected, with a vast majority of cells appearing preserved.
Multi-cellular/confluent acinar cell injury-necrosis: acinar cell damage /apoptosis involving multiple acinar cells (clusters).
Minimal intimal arteritis: rare, occasional, clearly defined sub-endothelial (intimal) inflammatory infiltration composed of mononuclear cells but with no evidence of activation or damage of the endothelial lining/intima (see below).
Moderate-severe intimal arteritis: easily identifiable mononuclear cells within the intima of an involved muscular artery and evidence of intimal injury, including any of the following: endothelial cell hypertrophy, activation or sloughing, fibrin leakage, neutrophil margination, macrophage activation, activation/proliferation of intimal myofibroblasts.
Necrotizing arteritis: focal or circumferential fibrinoid necrosis of the arterial wall with or without transmural inflammation.
Transplant arteriopathy: fibrointimal arterial thickening with narrowing of the lumen. Grading is done in the most affected artery as mild, up to 25% of luminal area; >25% but $\leq$50% of luminal area and severe, >50% of luminal area.
'Active' transplant arteriopathy: narrowing of the arterial lumen by a sub-endothelial proliferation of fibroblasts, myofibroblasts and smooth muscle cells with infiltration of the sub-intimal fibrous proliferation by mononuclear cells (T cells and macrophages).
Capillaritis: neutrophil and mononuclear cell margination in dilated inter-acinar and islet capillaries.
C4d semiquantitative grading: diffuse positive, $\geq$50% of inter-acinar capillaries; focal positive, 5–50% of inter-acinar capillaries; minimal positive/negative, <5 % of inter-acinar capillaries. Staining of larger vessels including arterioles is considered nonspecific.

Histological features of rejection and diagnostic categories: specific considerations

The specific histological features utilized in the 2007 Banff working schema are presented in Table 2. The schema consists of six main diagnostic categories, some of which may occur concurrently (Table 3). The severity of the pathological process is graded based on the global assessment of the biopsy. In addition, the extent of fibrosis and parenchymal atrophy are also assessed to determine the 'stage' of graft sclerosis. Reproducibility of the proposed working grading schema has not been yet tested and future studies are warranted to demonstrate its practical usefulness.

Normal: Inflammatory infiltrates are either absent, or very sparse with no features of activation (i.e. small lymphocytes, rare plasma cells). If any inflammation is present, it is focal, mononuclear and confined to the septa with lack of involvement of any of the septal structures such as vessels, ducts or nerves. Acinar inflammation and acinar cell damage are absent.

Normal-appearing biopsies are more often encountered in protocol biopsies of well-functioning grafts (17,49,51,63). An adequate biopsy (see above) with these histological characteristics essentially rules out a diagnosis of acute cell-mediated allograft rejection (ACMR). Thus, even in patients biopsied for graft dysfunction, empirical antirejection treatment has not been shown to be of clear benefit in the presence of a normal biopsy (16,17,63).

'Normal' or near-normal pancreas allograft biopsies may be also encountered under other clinical circumstances (see Table 4). Specifically, in patients biopsied for hyperglycemia, the differential diagnosis of a normal biopsy includes: (i) late phase of recurrent autoimmune disease (DM), that is, after resolution of isletitis and disappearance of beta cells (40,61); (ii) drug toxicity (41), and (iii) acute antibody-mediated rejection, as described in the case reported by Melcher et al. (26).

Indeterminate for Rejection

This category is defined by the presence of focal septal inflammation that displays features of activation (i.e. blastic' lymphocytes, variable numbers of eosinophils), but the overall features do not fulfill the criteria for mild rejection (i.e. partial cuffing of a septal vein or duct but lacking any evidence of endothelial or epithelial involvement, etc.) (Figure 1A). The clear identification of venulitis, and/or ductal inflammation and damage, even if only focal, places the biopsy in the category of grade I/Mild ACMR (see below).

Table 3: Diagnostic categories Banff working grading schema*,[a]

1. Normal. Absent inflammation **or** inactive septal, mononuclear inflammation not involving ducts, veins, arteries or acini. There is no graft sclerosis. The fibrous component is limited to normal septa and its amount is proportional to the size of the enclosed structures (ducts and vessels). The acinar parenchyma shows no signs of atrophy or injury.

2. Indeterminate. Septal inflammation that appears active but the overall features do not fulfill the criteria for mild cell-mediated acute rejection.

3. Cell-mediated rejection

Acute cell-mediated rejection

- Grade I/Mild acute cell-mediated rejection

Active septal inflammation (activated, blastic lymphocytes, ± eosinophils) involving septal structures: venulitis (sub-endothelial accumulation of inflammatory cells and endothelial damage in septal veins, ductitis (epithelial inflammation and damage of ducts). Neural/peri-neural inflammation.

and/or

Focal acinar inflammation. No more than two inflammatory foci^per lobule with absent or minimal acinar cell injury.

- Grade II/Moderate acute cell-mediated rejection

Multi-focal (but not confluent or diffuse) acinar inflammation (≥3 foci^per lobule) with spotty (individual) acinar cell injury and drop-out.

and/or

Minimal intimal arteritis

- Grade III/Severe acute cell-mediated rejection

Diffuse, (widespread, extensive) acinar inflammation with focal or diffuse multi-cellular /confluent acinar cell necrosis.

and/or

Moderate- or severe-intimal arteritis

and/or

Transmural inflammation-Necrotizing arteritis

Chronic active cell-mediated rejection. Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)

4. Antibody-mediated rejection = C4d positivity** + confirmed donor specific antibodies + graft dysfunction

Hyperacute rejection. Immediate graft necrosis (≤1 h) due to preformed antibodies in recipient's blood

Accelerated antibody-mediated rejection. Severe, fulminant form of antibody-mediated rejection with morphological similarities to hyperacute rejection but occurring later (within hours or days of transplantation).

Acute antibody-mediated rejection. Specify percentage of biopsy surface (focal or diffuse). Associated histological findings: ranging from none to neutrophilic or mononuclear cell margination (capillaritis), thrombosis, vasculitis, parenchymal necrosis.

Chronic active antibody-mediated rejection. Features of categories 4 and 5.

5. Chronic allograft rejection/graft sclerosis

- Stage I (mild graft sclerosis)

Expansion of fibrous septa; the fibrosis occupies less than 30% of the core surface but the acinar lobules have eroded, irregular contours. The central lobular areas are normal.

- Stage II (moderate graft sclerosis)

The fibrosis occupies 30–60% of the core surface. The exocrine atrophy affects the majority of the lobules in their periphery (irregular contours) and in their central areas (thin fibrous strands criss-cross between individual acin).

- Stage III (severe graft sclerosis**)**

The fibrotic areas predominate and occupy more than 60% of the core surface with only isolated areas of residual acinar tissue and/or islets present.

6. Other histological diagnosis. Pathological changes not considered to be due acute and/or chronic rejection. e.g. CMV pancreatitis, PTLD, etc. (Table 4)

[a] Categories from 2 to 6 may be diagnosed concurrently and should be listed in the diagnosis in the order of their clinico-pathological significance.

*See Table 2 for morphological definition of lesions.

**If there are no donor-specific antibodies or these data are unknown, identification of histological features of antibody-mediated rejection may be diagnosed as '*suspicious for acute antibody- mediated rejection*', particularly if there is graft dysfunction.

Similarly, sparse- and inactive-appearing inflammation involving acini but not fulfilling the criteria for focal acinar inflammation described in ACMR would also fall into this category (see Tables 2 and 3).

'Indeterminate' histological features can be seen in protocol biopsies of well-functioning grafts as well as in patients biopsied for graft dysfunction. Similar to the 'borderline' category in kidney allografts, these changes might represent early as well as treated acute rejection, or alternatively might be entirely nonspecific (16,49). The treatment of patients with biopsies showing 'indeterminate' features will vary depending on the indication for biopsy, and ultimately depends on clinical judgment. In accordance with the heterogeneous nature of the 'indeterminate' histological changes, clinical response to treatment varies significantly in comparison to biopsies with definite mild ACMR that are usually responsive to treatment (9,16,17).

Table 4: Pathological changes 'other' than rejection in pancreas needle biopsies

Diagnosis	Main histological findings	Clinical presentation
Posttransplant ischemic pancreatitis	Inflammation: neutrophils, foamy macrophages. Location: septal if mild or diffuse if severe Other features: fat necrosis, edema and interstitial hemorrhage. Patchy coagulation necrosis of clusters of acinar cells may be present. No fibrosis, the septa may be expanded due to edema/fat necrosis.	Increase in amylase and lipase in serum. Decrease in urinary amylase.* Hyperglycemia if there is extensive necrosis.
Peripancreatitis/peri-pancreatic fluid collection	Inflammation: mixed (lymphocytes, plasma cells, eosinophils, neutrophils). Location: septa and periphery of lobules Other features: dissecting bundles of active fibroblastic proliferation with obliteration of septal structures, relative preservation of the center of lobules ('cirrhotic appearance')	Local or systemic infectious symptoms, abdominal pain, peri-tonitis. Peripancreatic fluid accumulation.Increase in amylase and lipase in serum.
Cytomegalovirus pancreatitis	Inflammation: mostly mononuclear. Location: septal and acinar, patchy. Other features: cytomegalovirus cytopathic changes in acinar, endothelial or stromal cells.	Increase in serum amylase and lipase. Decrease in urinary amylase.* Systemic symptoms if generalized disease. Other: Duodenal cuff perforation.
Posttransplant lymphoproliferative disorder	Inflammation: ranging from polymorphic with lymphoblasts, plasma cells, eosinophils in low-grade disease, to monomorphic, predominantly lymphoid in high-grade disease (lymphoma). Other features: lymphoid proliferation is nodular, expansive. Necrosis may be present.	Asymptomatic, or increase in serum amylase and lipase. Lymphadenopathy. Tumor mass. May coexist with acute rejection.
Bacterial or fungal infection	Inflammation: variable; acute, chronic, purulent, necrotizing (abscess), granulomatous. Location: random. Other features: same as bacterial and fungal infections in other organs.	Systemic and/or localized infectious symptoms. Peritonitis, duodenal cuff perforation. Increase in serum amylase and lipase.
Recurrent autoimmune disease/diabetes mellitus	Inflammation: islet-centered lymphocytic inflammation (isletitis). No inflammation in late stages after disappearance of beta cells. Other features: immuno-histochemical stains for insulin and glucagon demonstrate absence of insulin producing beta cells in some or all islets depending if early or late disease.	Acute or chronic deterioration in glucose metabolism with increasing need for insulin. Although not pathognomonic, islet cell auto-antibodies typically present (i.e. GAD 65, IA-2, etc.).
Acute calcineurin inhibitor toxicity	Absence of inflammation. Variable degrees of islet cell injury (cytoplasmic swelling, vacuolization, islet cell drop-out. formation of empty spaces (lacunae), apoptotic fragments). Immuno-peroxidase stains: markedly diminished staining for insulin in comparison to controls and to glucagon stain. Electron microscopy: loss of insulin dense core granules with preservation of glucagon dense core granules.	Acute hyperglycemia. High levels of cyclosporine or tacrolimus with return to normoglycemia with adjustment of drug dose or discontinuation.

*In bladder-drained grafts.

Cell-Mediated Rejection

Three grades of acute cell-mediated rejection (ACMR), grade I or mild, grade II or moderate, and grade III or severe, are defined based on the identification of specific lesions that predict progressively worse outcomes (9,15–17,60).

Intimal arteritis and necrotizing arteritis define the more severe forms of ACMR. These are less likely to respond to antirejection treatment and are known to carry an increased risk for immediate and subsequent graft thrombosis/loss and transplant arteriopathy (30). Because affected arteries are not always sampled, the presence and the extent of acinar inflammation (focal vs. multi-focal-diffuse) and the presence of acinar cell injury are also used to define the severity of ACMR because if left untreated or under-treated these findings correlate with development of fibrosis and accelerated graft loss (18).

Inflammation confined to the septa and septal structures (veins, ducts) is typically responsive to antirejection treatment and is therefore less likely to result in irreversible sequelae (9,16,60).

Mild ACMR (grade I)

This grade is defined by predominantly mononuclear septal inflammation that shows features of activation ('blastic' lymphocytes, variable numbers of eosinophils). The inflammation often extends into the sub-endothelial space of veins and inside the basement membrane of pancreatic ducts (Figure 1B and Figure 2). The inflammation can vary from septal area to area, but the presence of venulitis (see Table 2) or any degree of lymphocytic ductitis is sufficient for the diagnosis of grade I/mild ACMR. Inflammation of peripheral nerve branches coursing through the parenchyma is also a feature of rejection, although nerves are rarely sampled in needle biopsies.

Focal acinar inflammation is usually present at the interface between the septal connective tissue and the acinar lobules (e.g. periphery of the exocrine areas). Due to sampling variations, foci of acinar inflammation might be discontinuous from the septal inflammation (i.e. within 'deeper' areas of the lobules) or associated with inconspicuous septal inflammation. In such cases the diagnosis of grade I/mild ACMR depends on mild (focal) acinar cell inflammation and injury, as defined in Table 2. The acinar inflammatory foci are easily identified at low (100 ×) or medium (200 ×) magnification and the composition of the infiltrates is similar to that seen in the septa.

Biopsies with features of grade I/mild ACMR are occasionally found in patients with well-functioning grafts (63) but are significantly more common in patients with graft dysfunction (i.e. increase in serum amylase or lipase levels, or decrease in urinary amylase levels in bladder-drained grafts) (16,49–53). Response to treatment approaches 90% (16). The main histological differential diagnosis is CMV pancreatitis (64).

Moderate ACMR (grade II)

This grade can be defined by two histological features that may be identified either in isolation or concurrently.

The most common presentation consists of multiple foci (≥3 foci per lobule) of acinar inflammation with associated single-cell (individual) acinar cell injury and drop-out (Figures 3 and 4). The inflammatory foci are easily identified at low magnification, although examination at higher magnification is usually needed to exclude confluent acinar cell injury, which increases the grading to 'severe'. In other words, completely un-inflamed acinar/exocrine areas should be easily identified between the inflamed foci. Significant acinar inflammation is always associated with evidence of acinar cell injury (65), but in this grade the latter is spotty (i.e. affects only isolated acinar cells, Table 2).

Alternatively, moderate ACMR is defined by the presence of *minimal intimal arteritis*, recognized by the presence of occasional, clearly identified lymphocytes underneath the arterial endothelium (i.e. within the intima of a muscular artery) but lacking clear evidence of endothelial activation or injury (Figure 1C lower insert, and Table 2).

Biopsies with features of moderate ACMR are typically obtained from patients with graft dysfunction and response to antirejection treatment ranges from 71% to 85% (16).

Severe ACMR (grade III)

This grade is defined by three histological lesions that may be identified either in isolation or concurrently.

Severe acinar inflammation and damage are defined by confluent/diffuse (widespread, extensive) acinar inflammation with associated focal or diffuse multi-cellular/confluent acinar cell injury/necrosis (see Table 2) and (Figure 4B). The inflammation may be predominantly lymphoid or contain abundant eosinophils or variable numbers of neutrophils. Interstitial edema and/or hemorrhage signal severe tissue damage. By definition, there should be no or only rare, focal areas of completely uninflamed acinar/exocrine parenchyma (see Moderate ACMR).

Moderate-severe intimal arteritis: Alternatively, severe ACMR can be defined by easily identifiable intimal arteritis, characterized by mononuclear cells within the intima of an involved muscular artery with additional evidence of intimal injury or response to injury, such as endothelial cell hypertrophy, fibrin leakage, coating neutrophils and/or macrophages, and activation of intimal myofibroblasts, etc. (see Table 2).

Arteritis (vasculitis): Complete or partial circumferential necrosis often secondary to transmural arterial inflammatory infiltrates is also diagnostic of grade III/severe ACMR. On the other hand, arterial fibrinoid necrosis is also associated with antibody-mediated rejection (20,59). Therefore, the identification of this lesion should always trigger staining for C4d and a search for donor-specific antibodies in serum (Figure 1C, upper insert).

Each of the three lesions capable of defining grade III/severe ACMR portends a poor outcome. The short- and long-term impact to the organ will depend on the extent of acinar damage and the size and number of arteries affected by intimal arteritis or necrosis.

Confluent acinar inflammation and necrosis is invariably followed by atrophy or eventual disappearance of the exocrine component in the affected area. Changes of this nature markedly alter the micro-vascular environment of the graft on which the islets depend to maintain adequate function (13,29).

Similar to other solid organ transplants, intimal arteritis is associated with an increased risk of immediate or delayed thrombosis. This lesion is also a precursor of transplant arteriopathy (29). Transmural arteritis/vasculitis is associated with an immediate likelihood of thrombosis and secondary parenchymal infarction.

Biopsies with histological findings corresponding to this category are characteristically associated with graft dysfunction/failure, often including hyperglycemia (16,29,42). Response to antirejection treatment is poor (16,17,60).

Chronic active cell-mediated rejection

This category is defined by the presence of 'active' transplant arteriopathy (Table 2). Although rarely seen in needle biopsies due to sampling issues, this lesion is consistently present in pancreatectomies from failed grafts due to chronic cell-mediated rejection (29).

The entity of active transplant arteriopathy is included in the grading schema because according to clinical and experimental studies, this lesion appears to represent an intermediate stage between intimal arteritis and chronic transplant arteriopathy (Figure 6A). The extent of the histological changes and the amount of inflammatory infiltrates have been shown to correlate with sub-optimal immunosuppression. The identification of this lesion has potential clinical impact, as the process of ongoing cell-mediated vascular injury leading to further arterial narrowing may be halted with optimization of the immuno-suppressive regimen (66).

Antibody-Mediated Rejection (AMR)

This category is poorly characterized in pancreas transplantation. The proposed diagnostic criteria are based on inferences from the few well-documented cases reported in the literature and theoretical analogy to other organs (20,26,27,60). A broad spectrum of clinico-pathological manifestations of AMR are also recognized in the pancreas allograft ranging from fulminant graft failure in the setting of hyperacute rejection to its incidental identification in grafts with stable function.

AMR is characterized by a constellation of histological, clinical and serological features consisting of: (i) complement deposition in vessels (i.e. capillary C4d deposition) that can be accompanied by monocyte/macrophage and neutrophil margination within interstitial capillaries; (ii) graft dysfunction; and (iii) donor-specific antibodies (DSA) in serum.

AMR has been associated with hyperglycemia, suggesting that compromise of the islet micro-vasculature may play a pathogenic role different from cell-mediated injury in which the islets remain largely spared of direct immune damage (20,26,27).

Hyperacute rejection

Routine pretransplant cross-matching to rule out preformed DSA and ABO matching has virtually eliminated this catastrophic form of antibody-mediated rejection. Hyperacute rejection is characterized by extensive, vascular deposition of immune reactants (typically containing IgG), leading to intimal arteritis, arterial necrosis and thrombosis of veins, which in turn, cause widespread hemorrhagic necrosis. Allograft failure occurs immediately (typically $<$60 min) after the vascular anastomoses are completed.

Accelerated antibody-mediated rejection

So-called 'accelerated rejection' or 'delayed hyperacute rejection' is a severe form of AMR that presents clinically as an attenuated form of hyperacute rejection (Figure 5A). The histological findings are, therefore, similar (generalized immuno-globulin and complement vascular deposition, thrombosis and necrosis), but the event occurs within hours or days (rather than minutes) after revascularization of the allograft. The extent of parenchymal involvement is less diffuse in comparison to hyperacute rejection but the prognosis is equally poor. In well-documented cases of accelerated AMR, there has been retrospective documentation of existing DSA despite a negative pretransplant cross-match (29). Accelerated AMR clinically resembles graft thrombosis attributed to 'technical failure' from which it needs to be differentiated by careful histological and immuno-histochemical evaluation (C4d, immunoglobulin staining). Whereas hyperacute rejection is exceedingly rare ($<$0.01% of pancreas transplants), accelerated rejection was found in 2.5% of pancreatectomies in a clinico-pathological study (29).

Acute AMR

Acute AMR manifests typically in the first weeks posttransplantation with the development of allograft dysfunction and the appearance of DSA in the serum (26). On histological evaluation, there is generalized C4d staining of capillaries with no evidence of an underlying chronic injury (i.e. fibrosis). The reported spectrum of associated histological changes varies, from none (normal hematoxylin and eosin [H & E] histology) (26), to widespread thrombosis and parenchymal necrosis (20). Neutrophil and mononuclear cell margination (similar to capillaritis in the kidney) can be seen in association with C4d positivity in inter-acinar capillaries (20) (Figure 5B and C).

In the absence of graft dysfunction or if DSA are not found, a diagnosis of 'suspicious for acute antibody-mediated rejection' may be considered when there is extensive C4d positivity. The immediate- or long-term significance of this finding is currently unknown.

Chronic active antibody-mediated rejection

Humoral mechanisms have been clearly implicated in the development of chronic rejection (28). A diagnosis of

chronic active AMR is applied to biopsies showing features of chronic rejection/graft sclerosis, together with C4d positive staining in parenchymal capillaries. This scenario has been well described in the report of Carbajal et al. (27) (Figure 5D).

Vascular fibrinoid necrosis, with recent or organized thrombosis is supportive of ongoing antibody-mediated rejection. As in all situations in which antibody-mediated rejection is suspected, correlation with the presence of DSA is required for diagnosis (59).

Staging of Graft Sclerosis

The extent of fibrosis on pancreatic needle biopsies correlates with graft survival. The progression of graft sclerosis over time is well documented, particularly in patients with repeated rejection episodes (67). The progressive nature of pancreatic fibrogenesis lends itself to the application of a histological staging schema. Three stages are defined based on the identification of <30%, 30–60% and >60% of fibrosis in the biopsy core (stages I-III, respectively) with corresponding atrophy of the lobular parenchyma directly correlating with the extent of septal fibrosis. In stage I, most of the acinar lobules are preserved and only show focal erosion and irregularities of their contours. With progression of the fibrosis, as is seen in stage II, exocrine atrophy affects the majority of the lobules both in the peripheral (irregular contours) and central areas (Figure 6B). The latter change is best appreciated in collagen stains that show thin fibrous strands crisscrossing between individual acini. Stage III is characterized by diffuse fibrosis with near total or complete absence of functional parenchyma. The proposed staging schema has been shown to be reproducible in needle core biopsies and has significant prognostic value (67).

Transplant arteriopathy (arterial fibrointimal thickening with luminal narrowing) closely parallels the degree of fibrosis. When transplant arteriopathy is identified, this should be graded as mild, moderate or severe using the same morphological criteria applied to kidney biopsies (i.e. Banff cv1–3, Table 2) (68). Despite their major physio-pathological importance, the vascular lesions are not used for staging, because vascular disease is under-represented in needle biopsies (18,29). Similarly, evaluation of endocrine islets is not used for grading because their disappearance does not follow a predictable course in relationship to the overall degree of graft fibrosis (25,67).

Inflammatory infiltrates associated with ongoing acinar cell injury, venulitis and/or intimal arteritis and ductal inflammation indicate active ACMR that should be graded independently based on the key histological features specified above.

Other Histological Diagnosis

Given that acute rejection episodes have become less common under current immuno-suppressive protocols, a variety of other pathological processes are often encountered in pancreas biopsies from patients with graft dysfunction. These include infections, recurrence of autoimmune disease, or graft sclerosis/chronic rejection that may be identified in isolation or concurrently with other diagnostic categories in the schema (Figure 7; Table 4).

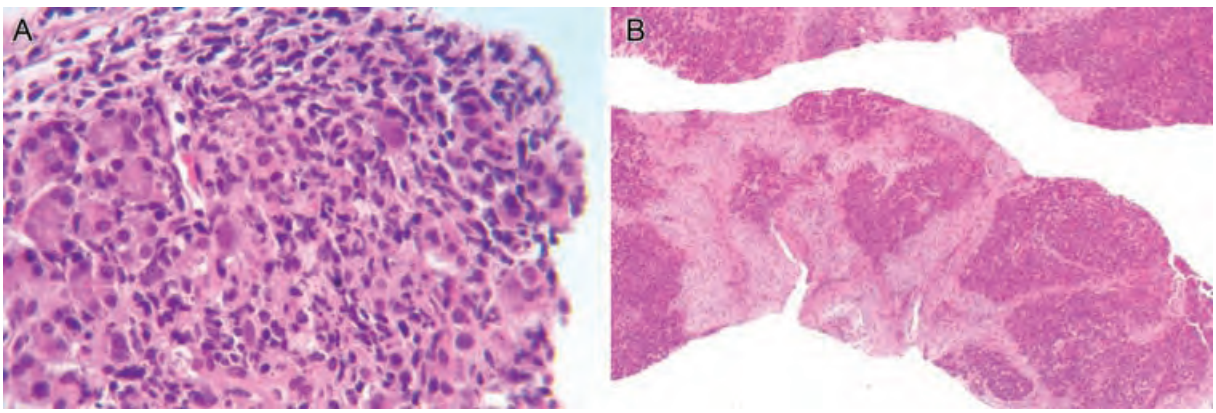

Figure 7: Other pathological processes. (A) Focus of CMV pancreatitis, with acinar inflammatory infiltrates resembling acute cell-mediated allograft rejection. Two cells with viral cytopathic changes (left of center and right above center) have large ground glass nuclei and abundant cytoplasm. (B) Peri-pancreatitis/peri-pancreatic fluid collection occurring in the early posttransplantation period is in the histological differential diagnosis of acute rejection. The pancreatic septa are expanded by an active fibroblastic proliferation that contains mixed inflammation. The septal inflammation and fibrosis push the acinar lobules apart and erodes their periphery, but their central areas are typically not affected (see also Figure 6B).

Concluding remarks

This work summarizes the current knowledge on pancreas allograft pathology and represents the clinical and histopathological cumulative experience of a large number of pancreas transplant centers. Purely morphological classifications of rejection such as this one, are limited by our insufficient understanding of the mechanisms and pathways of allograft injury (69). On the other hand, the availability of generally accepted morphological definitions is an absolutely necessary condition for any further investigational or clinical progress in pancreas transplantation.

The current 'working grading schema' provides specific diagnostic guidelines that should allow for a more accurate diagnosis of rejection, encourage the use of pancreas graft biopsies and should result in improved pancreas transplantation outcomes.

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A SCHEMA FOR HISTOLOGIC GRADING OF SMALL INTESTINE ALLOGRAFT ACUTE REJECTION

TONG WU,[1,3] KAREEM ABU-ELMAGD,[2] GEOFFY BOND,[2] MICHAEL A. NALESNIK,[1] PARMJEET RANDHAWA,[1] AND A. JAKE DEMETRIS[1]

Background. **Histologic evaluation of small bowel allograft biopsies is important for the diagnosis of acute rejection. However, a standard histologic schema to grade the severity of intestinal acute rejection is not currently available. The primary goal of this study was to develop a histologic grading system for the diagnosis of small bowel allograft acute rejection.**

Methods. **We evaluated 3268 small bowel allograft biopsies obtained from adult patients who underwent small bowel transplantation at the University of Pittsburgh Medical Center between 1990 and 1999. A histologic grading system was proposed and validated by retrospective correlation with clinical outcomes.**

Results. **Among the 3268 biopsies, 180 acute rejection episodes were diagnosed (88 indeterminate, 74 mild, 14 moderate, and 4 severe). All four histologically diagnosed, severe acute rejection episodes resulted in graft failure before resolution, despite aggressive immunosuppressive therapy. Four of the 14 moderate acute rejection episodes were associated with unfavorable clinical outcomes. In contrast, the 74 mild and 88 indeterminate acute rejection episodes were not associated with unfavorable clinical outcomes. Statistical analysis for trend revealed that grades indicating more severe acute rejection episodes were associated with a greater probability of unfavorable outcomes ($P<0.01$). In addition, there was good overall agreement among different pathologists regarding the diagnosis of acute rejection using the proposed schema, suggesting that this system is practical.**

Conclusions. **This study provides a reliable predictive schema for assessment of the severity of human small bowel acute rejection.**

Small bowel transplantation is being increasingly performed to treat patients with irreversible intestinal failure or short-bowel syndrome (*1*–*7*). Acute cellular rejection (ACR) is the major cause of intestinal graft failure after transplantation (*8*). If not treated early, intestinal ACR can rapidly increase in severity and cause graft failure and death. In fact, despite aggressive immunosuppressive therapy, most patients with histologically diagnosed severe acute rejection experience progression to graft loss or death. Therefore, accurate diagnosis and treatment of acute rejection are critical for posttransplant patient care.

The diagnosis of intestinal ACR requires close correlation of clinical, endoscopic, and pathologic findings. The clinical symptoms of intestinal ACR include fever, nausea, vomiting, increased stomal output, abdominal pain, and distension. In severe cases, acute rejection may manifest as septic shock, with metabolic acidosis, hypotension, and adult respiratory distress syndrome, which likely results from loss of mucosal integrity and bacterial translocation across the intestinal wall. The endoscopic appearances of intestinal ACR range from edema and hyperemia in mild cases to granularity, loss of the fine mucosal vascular pattern, diminished peristalsis, and mucosal ulceration in more severe cases. The final diagnosis depends on histologic analysis of endoscopy-guided mucosal biopsy specimens. The major histopathologic changes of intestinal ACR were documented in previous studies (*8*, *9*) and include varying degrees of (1) infiltration by a mixed but primarily mononuclear inflammatory population, including blastic or activated lymphocytes; (2) crypt injury (characterized by cytoplasmic basophilia, nuclear enlargement and hyperchromasia, decreased cell height, mucin depletion, and loss of Paneth's cells); (3) increases in the number of crypt apoptotic bodies; and (4) distortion of villous and crypt architecture.

The treatment options for intestinal ACR depend on its severity, which is assessed by histologic grading of the rejection with clinical and endoscopic correlation. For example, whereas relatively mild acute rejection usually requires an increase in basal immunosuppressive drug treatment with close clinical observation, more aggressive immunosuppressive therapy should be initiated for moderate or severe episodes of acute rejection. Therefore, accurate grading of acute rejection is extremely important for successful patient treatment. Histopathologic grading of acute rejection has not yet been addressed in detail, however, and no standard criteria are available for assessment of the grade of intestinal ACR. The major goal of this study was to develop a reliable, practical histologic grading system for pathologic evaluation of human intestinal ACR. On the basis of results from animal intestinal transplantation studies (*10*–*12*) and clinical experience in evaluating thousands of small bowel allograft biopsies in our institution (*8*), we proposed a pathologic grading system for the diagnosis of intestinal ACR. This system was used to retrospectively evaluate 3,268 small bowel allograft biopsies from 52 adult patients who underwent intestinal transplantation between 1990 and 1999 at the Thomas E. Starzl Transplant Institute, University of Pittsburgh Medical Center. The histologic grades determined were then correlated with clinical events, including immunosuppressive therapy and graft and patient outcomes. Our results indicate that the proposed grading system is accurate in the diagnosis

[1] Department of Pathology, Thomas E. Starzl Transplantation Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.
[2] Department of Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.
[3] Address correspondence to: Tong Wu, M.D., Ph.D., Department of Pathology, Thomas E. Starzl Transplantation Institute, University of Pittsburgh School of Medicine, Presbyterian University Hospital C902, 200 Lothrop Street, Pittsburgh, PA 15213. E-mail: wut@msx.upmc.edu.

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of intestinal ACR and is practical for routine histologic evaluation of intestinal allograft specimens.

MATERIALS AND METHODS

Patient Population

During the 9-year period between May 1990 and June 1999, 52 adult patients (26 male and 26 female patients; age range, 19–58 years) underwent orthotopic intestinal transplantation at the University of Pittsburgh Medical Center. The patient demographic characteristics, types of procedures, and causes of intestinal failure are summarized in Table 1. Baseline immunosuppressive therapy consisted of administration of tacrolimus and corticosteroids (*1*). Details of graft procurement, surgical procedures, tacrolimus-based immunosuppressive therapy, and patient treatment were reported previously (*1*, *2*). Surveillance allograft endoscopy was generally performed once or twice per week for the first 3 months and as clinically indicated thereafter. Multiple random, endoscopy-guided biopsies were routinely obtained from the small intestinal allograft (most often from the ileum) for histologic evaluation. Each biopsy specimen consisted of one to five separate mucosal fragments (median of three). The relevant clinical features and course of each patient were retrieved from our computerized database, and missing data were obtained in reviews of patient flow sheets and medical records. Complete follow-up data were available through the completion of the study (June 30, 1999).

TABLE 1. Demographic summary of patients with small intestine transplants

No. of patients	52
Gender (male:female)	26:26
Age range (yr)	19–58
Types of grafts (55 grafts, with 3 cases of retransplantation)	
Isolated intestine	29 (including colon in 8)
Small bowel/liver	16
Small bowel/pancreas	1
Multivisceral	9 (including colon in 4)
Causes of intestinal failure (no. of cases)	
Vascular thrombosis	17
Crohn's disease	12
Abdominal trauma	7
Mesenteric fibromatosis	5
Volvulus	3
Surgical adhesions	2
Radiation-induced enteritis	2
Familial polyposis	2
Pseudo-obstruction	1
Metastatic gastrinoma	1

Histologic Evaluations

All pathologic specimens from the 55 intestinal allografts were reviewed, including 3268 small intestinal mucosal biopsies. The histologic specimens were routinely fixed in formalin and embedded in paraffin, from which 2 to 18 hematoxylin-eosin–stained sections were obtained, from two or more levels in the blocks. Samples were obtained from deeper levels as indicated. For each specimen, the major histologic features, including architectural distortion (villous blunting, as determined in the best-oriented sections), crypt epithelial injury (characterized by cytoplasmic basophilia, nuclear enlargement and hyperchromasia, decreased cell height, and mucin depletion), inflammatory infiltration of the lamina propria and the constituent cell types, presence and cell type of crypt intraepithelial infiltration (cryptitis), lamina propria fibrosis, granulation tissue, and luminal fibrinopurulent inflammatory exudation (pseudomembrane), were semiquantitatively assessed. In addition, the specimens were carefully examined for viral infections, luminal organisms, and submucosal abnormalities. Apoptotic bodies within the crypt epithelium were identified and quantified. Apoptotic bodies were defined as rounded vacuoles containing fragments of karyorrhectic nuclear debris and cytoplasm and were distinguished from small isolated fragments of nuclear chromatin and intraepithelial neutrophils and eosinophils. These bodies were counted by scanning the specimen at medium power, to identify areas of greatest concentration, and then tallying the total numbers in 10 consecutive crypts (regardless of crypt orientation), including more than one level if necessary.

Slides from all biopsy specimens were reviewed at least twice by at least two pathologists. Histologic features relevant to acute rejection were compiled during the initial review; and a list of biopsy features of rejection was recorded by the second pathologist. Ambiguous or difficult cases were further reviewed using a multihead microscope by three or four pathologists. Attention was focused on changes related to rejection (see later discussion).

Histologic Criteria for Grading of Acute Cellular Rejection

The proposed histologic grading system for small bowel allograft biopsies is based on previous animal studies (*10–12*) and our clinical experience in the evaluation of thousands of small bowel allograft biopsy specimens (*8*). The histologic criteria for grading intestinal ACR are summarized (Table 2).

Indeterminate for acute rejection. Indeterminate for acute rejection is defined by the variable presence of the three main features of acute rejection (infiltration by a mixed but primarily mononuclear inflammatory population, including blastic or activated lymphocytes; crypt injury; and increased numbers of crypt apoptotic bodies), which are usually focal and do not meet the criteria for mild acute rejection. The inflammatory infiltrate is usually minimal and localized. Although the mucosa is intact, crypt epithelial injury is often present. There is a variable increase in crypt epithelial apoptosis but usually with less than 6 apoptotic bodies per 10 crypts (Fig. 1). *Indeterminate for acute rejection* should be used only when the biopsy demonstrates

TABLE 2. Histologic criteria for grading of small bowel allograft acute rejection[a]

Grade	Major Histologic Findings
Indeterminate for ACR	Minimal localized inflammatory infiltrate, minimal crypt epithelial injury, increased crypt epithelial apoptosis (usually with <6 apoptotic bodies/10 crypts), no to minimal architectural distortion, no mucosal ulceration, changes insufficient for the diagnosis of mild acute rejection
Mild ACR	Mild localized inflammatory infiltrate with activated lymphocytes, mild crypt epithelial injury, increased crypt epithelial apoptosis (usually with >6 apoptotic bodies/10 crypts), mild architectural distortion, no mucosal ulceration
Moderate ACR	Widely dispersed inflammatory infiltrate in lamina propria, diffuse crypt epithelial injury, increased crypt apoptosis with focal confluent apoptosis, more prominent architectural distortion; possible mild to moderate intimal arteritis; no mucosal ulceration
Severe ACR	Features of moderate ACR plus mucosal ulceration; possible severe intimal arteritis or transmural arteritis may be seen

[a] ACR, acute cellular rejection.

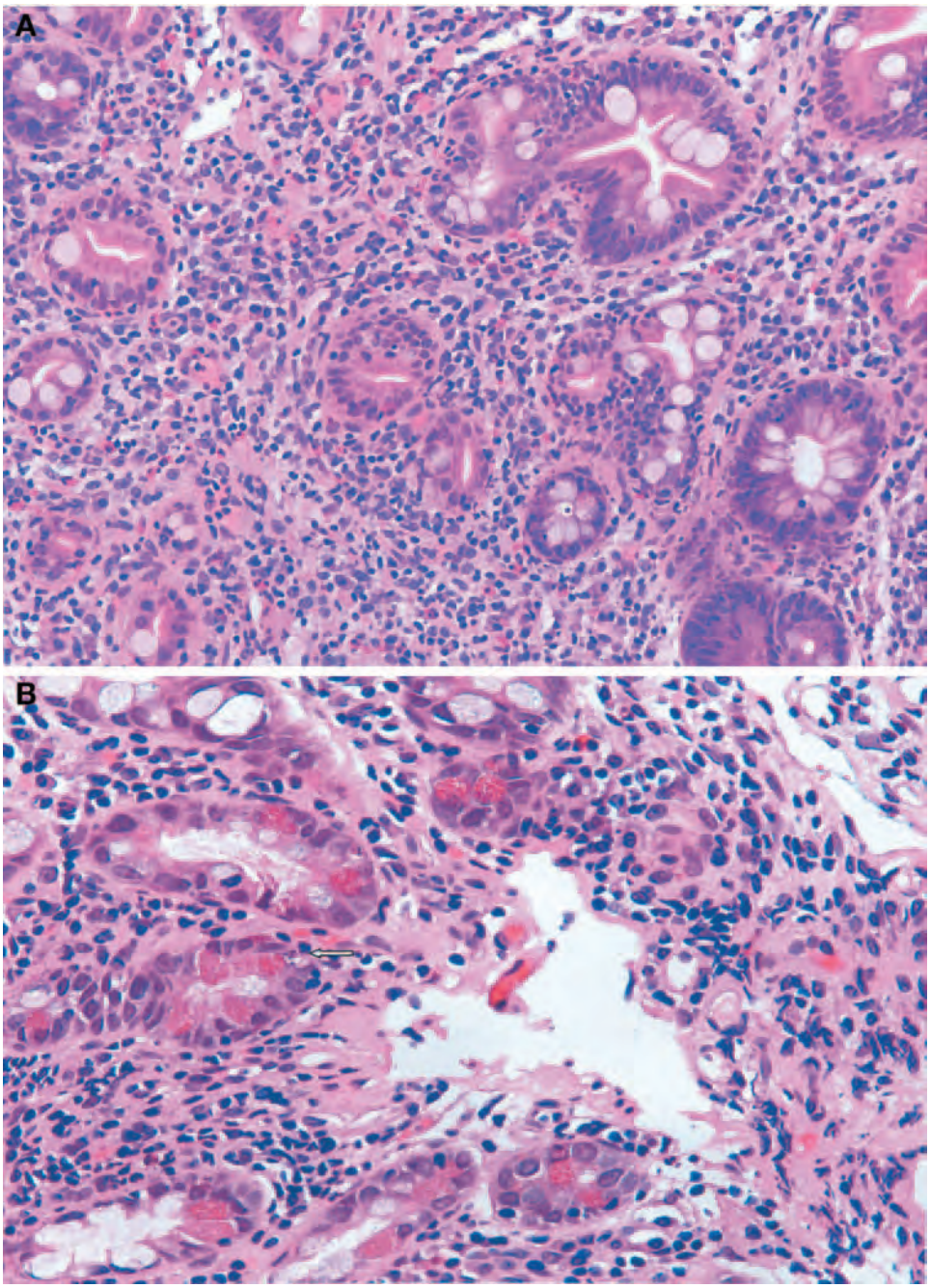

FIGURE 1. Indeterminate for acute rejection. The lamina propria is infiltrated by a heterogeneous population of mononuclear cells composed of blastic and small lymphocytes, plasma cells, and plasmacytoid lymphocytes. There is focal minimal crypt damage and apoptotic bodies (*arrow*) (hematoxylin-eosin; magnification: ×200 in A, ×400 in B). The apoptotic body count is usually less than 6 apoptotic bodies per 10 crypts.

features of acute rejection with degrees of inflammation, epithelial injury, and apoptosis that are lesser than those for mild acute rejection; it should not be applied to nonrejection processes when the diagnosis is not clear.

Mild acute rejection. Mild acute rejection is characterized by a generally mild and localized inflammatory infiltrate, which tends to be concentrated around small venules in the lamina propria. The mucosa is intact, but the crypt epithelium displays evidence of injury, including mucin depletion, cytoplasmic basophilia, decreased cell height, nuclear enlargement and hyperchromasia, and inflammatory infiltration. Crypt epithelial apoptosis is increased, usually with more than 6 apoptotic bodies per 10 crypts. If sampled in the biopsy specimen, preexisting lymphoid aggregates (Peyer's patches) demonstrate an intense accumulation of activated lymphocytes. The villi are variably shortened, and the architectural features may be slightly distorted because of expansion of the lamina propria by inflammatory infiltration (Fig. 2).

Moderate acute rejection. In *moderate acute rejection*, the inflammatory infiltrate is widely dispersed within the lamina propria. Crypt damage is distributed more diffusely than in mild acute rejection, and the villi tend to exhibit a greater degree of flattening. The number of apoptotic bodies is greater than in mild acute rejection, usually with focal "confluent apoptosis." Mild to moderate intimal arteritis may be observed. The mucosa remains intact without ulceration, although focal superficial erosions can be present (Fig. 3).

Severe acute rejection. Severe acute rejection is distinguished by a marked degree of crypt damage and mucosal ulceration. As a consequence of the mucosal destruction, luminal contents gain access to the submucosa, prompting a neutrophil-rich infiltrate and an overlying fibropurulent (pseudomembranous) exudate, with widespread

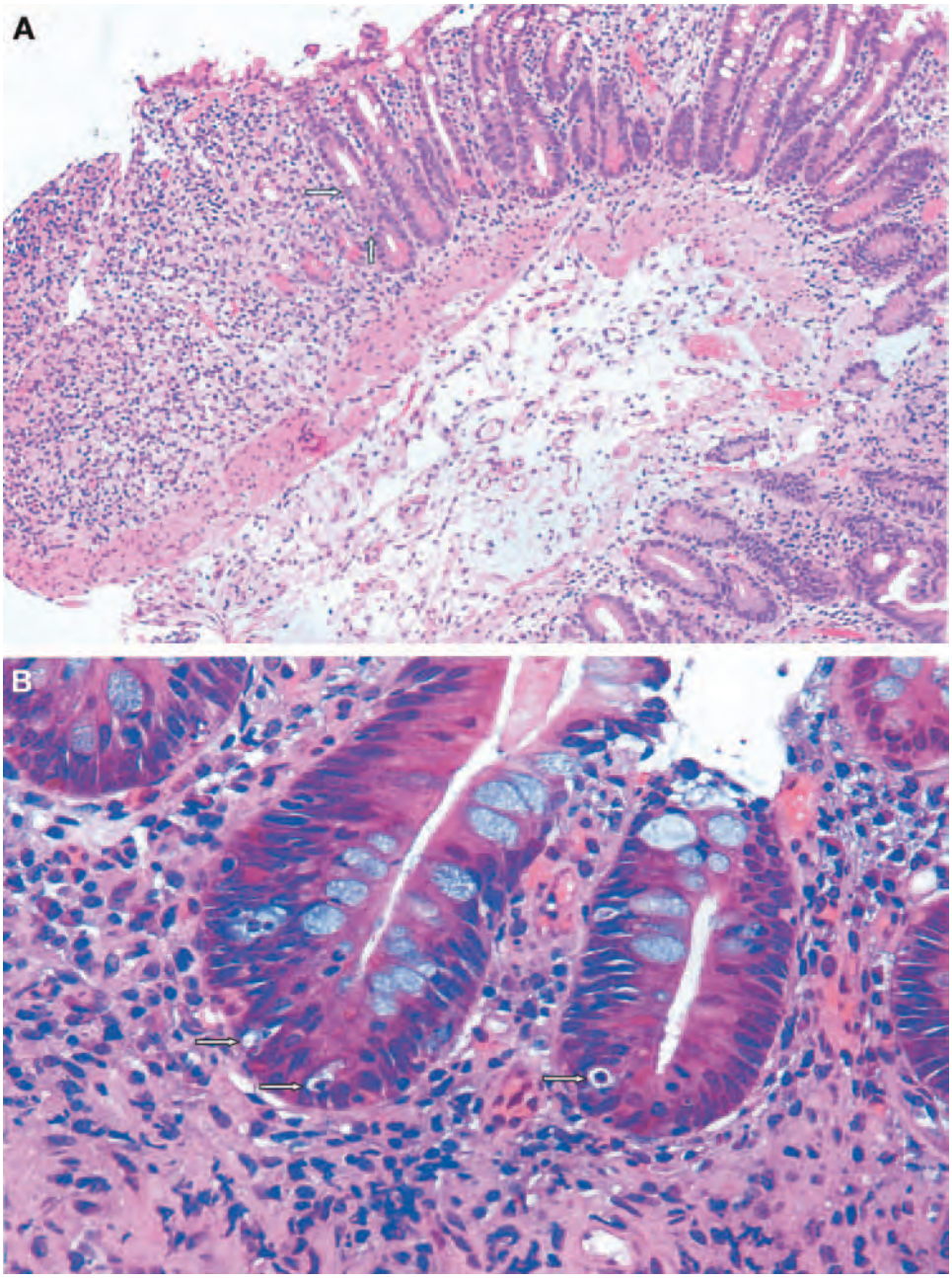

FIGURE 2. Mild acute rejection. (A) The villi are shortened and the architectural features are distorted because of expansion of the lamina propria by the heterogeneous mononuclear cell infiltrate (*left*). The crypts exhibit features of epithelial injury and scattered apoptotic bodies (*arrows*) (hematoxylin-eosin; magnification ×100). (B) Lamina propria mononuclear inflammation, crypt epithelial injury, and apoptotic bodies (*arrows*) (clear spaces with fragmented nuclear debris) (hematoxylin-eosin; magnification ×400). The apoptotic body count in mild acute rejection is usually more than six apoptotic bodies per 10 crypts.

mucosal sloughing as the final result. The adjacent viable epithelium usually exhibits rejection-associated changes, such as crypt epithelial damage and abundant apoptosis (Fig. 4). Severe intimal arteritis or transmural arteritis may be observed.

Prognostic Use of the Grading System

To evaluate the ability of the proposed acute rejection grading system to predict an unfavorable outcome, the histologic diagnoses of acute rejection episodes were retrospectively correlated with the clinical outcomes and treatments. A biopsy was defined as representing an acute rejection episode if the biopsy specimen was the first one to be histologically diagnosed as acute rejection. A new rejection episode was defined by newly developed clinical symptoms and documentation of new histologic features of ACR with at least one normal mucosal biopsy between the rejection episodes. For endpoint analysis, patients were divided into groups with favorable or unfavorable outcomes. Objective unfavorable outcomes were defined by the presence of any one of the following: (1) the rejection resulted in graft failure (death or retransplantation) before resolution; (2) OKT3 or antithymoglobulin was required for the treatment of acute rejection; or (3) complete resolution of the episode failed to occur within 21 days.

Reliability of the Grading System

Sixty-five posttransplantation small bowel biopsy specimens were randomly selected and reviewed by four pathologists. Before reviewing the slides, the pathologists agreed on the histologic grading criteria. Each participating pathologist rendered a final histologic diagnosis on the basis of the standard criteria.

Statistical Analyses

The ability of the grading system to predict an unfavorable outcome was assessed with the chi-square test for trend, using the definitions for unfavorable outcomes. The agreement among pathol-

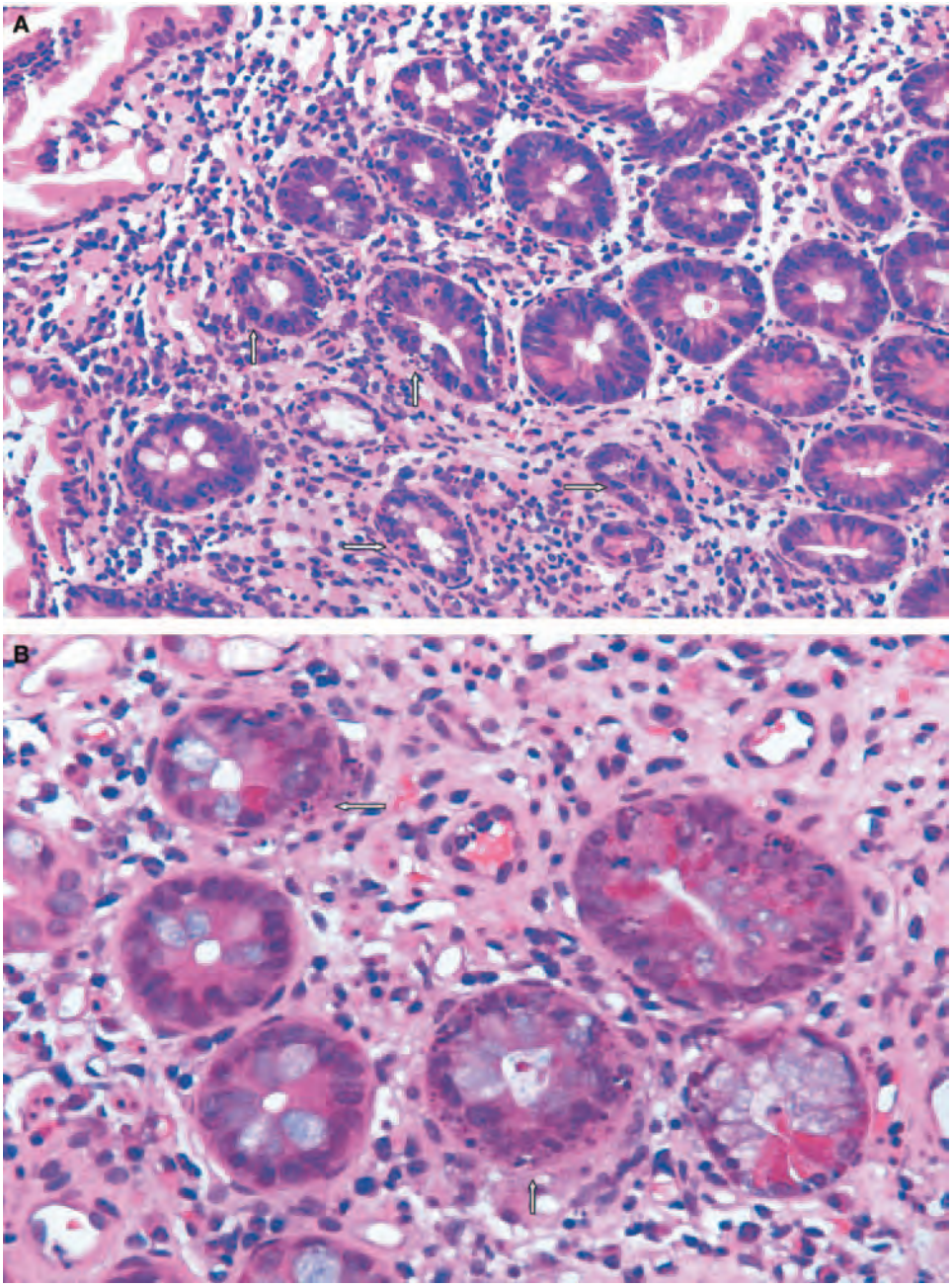

FIGURE 3. Moderate acute rejection. Crypt damage and apoptosis are distributed more diffusely than in mild acute rejection. The number of apoptotic bodies is greater than in mild acute rejection, with focal confluent apoptosis (*arrows*). The mucosa is usually intact, without ulceration (hematoxylin-eosin; magnification ×200).

ogists regarding the histologic diagnosis of ACR was analyzed with multirater kappa analysis.

RESULTS

Histologic Diagnosis of Acute Cellular Rejection

The histologic diagnosis and grading of ACR were performed after careful evaluation of 3268 mucosal biopsies from 55 small intestinal allografts. The initial histologic diagnosis for each biopsy specimen was established by the primary pathologist during the daily signed-out process; each of the biopsies was reevaluated by a separate pathologist (T.W.), and detailed histologic features were recorded. If an ambiguity regarding any histologic feature or a disagreement in diagnoses existed, then the slides were further reviewed under a multihead microscope with two or more additional pathologists, and the consensus opinion was recorded. A biopsy was defined as representing an acute rejection episode if the biopsy specimen was the first one to be histologically diagnosed as acute rejection. A new rejection episode was defined on the basis of newly developed clinical symptoms and documentation of new histologic features of ACR, with at least one normal mucosal biopsy between the rejection episodes. On the basis of the aforementioned criteria, 180 episodes of ACR were histologically diagnosed, among which were 88 (49%) episodes of indeterminate for ACR, 74 (41%) episodes of mild ACR, 14 (8%) episodes of moderate ACR, and 4 (2%) episodes of severe ACR. Among the 180 episodes of histologically diagnosed ACR (including indeterminate for ACR), 85 (47%) episodes occurred within the first 2 months after transplantation, 46 (26%) episodes occurred 2 to 12 months after transplantation, 24 (13%) episodes occurred 1 to 2

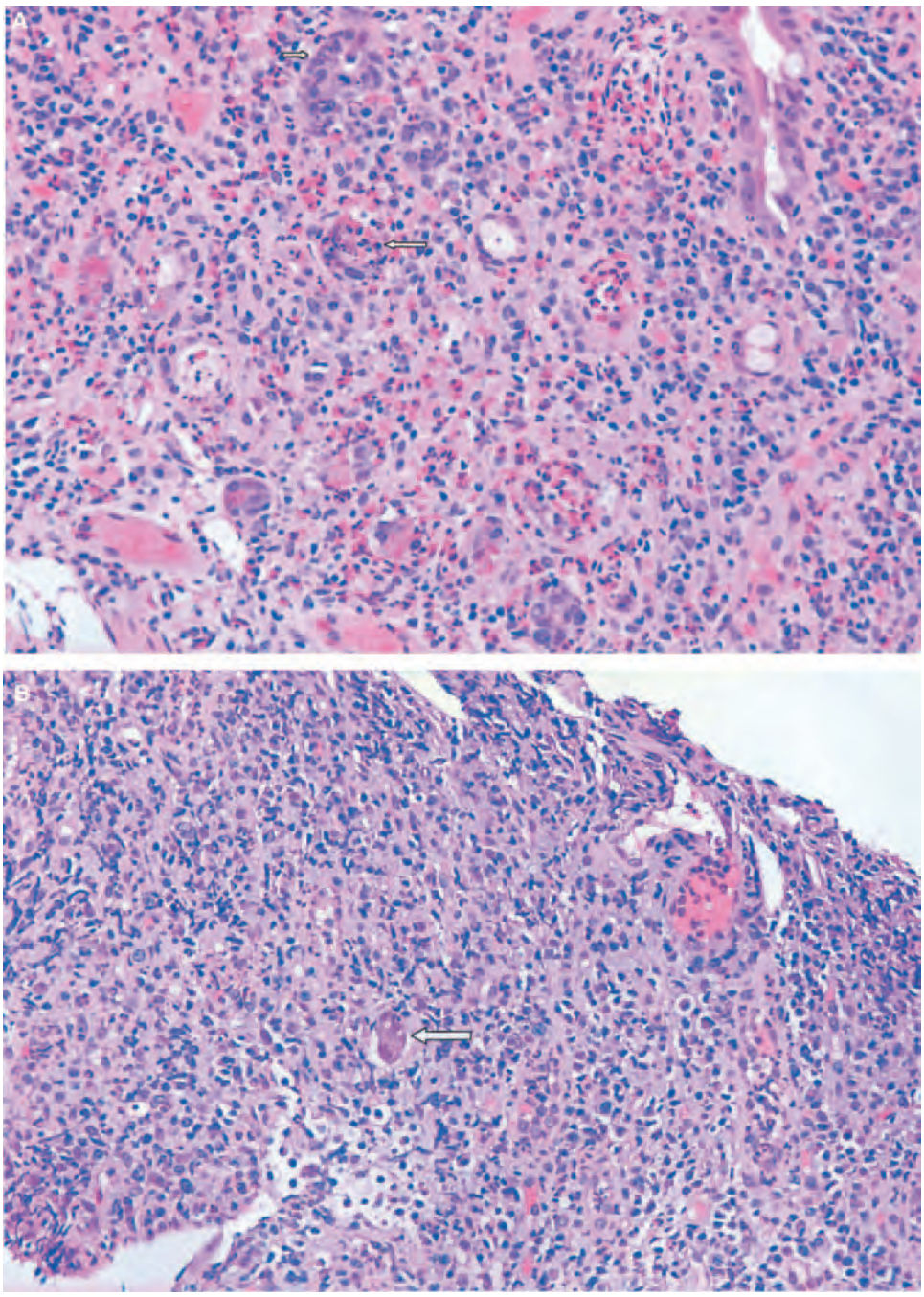

FIGURE 4. Severe acute rejection. There is extensive mucosal destruction, with loss of crypts, mucosal ulceration, and mixed lymphoplasmacytic, eosinophilic, and neutrophilic infiltration. The residual crypts, if present, often exhibit marked epithelial injury and apoptosis (*arrows*) (hematoxylin-eosin; magnification ×200).

years after transplantation, and 25 (14%) episodes occurred more than 2 years after transplantation.

The same histologic grading criteria were used for all biopsies in this study, including biopsies obtained from patients with clinical symptoms and protocol biopsies. The clinical presentations associated with ACR included abdominal pain, nausea, vomiting, diarrhea, fever, and abdominal distention. These symptoms lacked specificity, however, and varied depending on the severity of rejection and the presence of other pathologic conditions, such as acute enteritis, cytomegalovirus (CMV) infection, intestinal obstruction, systemic infection, or posttransplant lymphoproliferative disorder (PTLD). All of the patients with histologic diagnoses of moderate or severe ACR exhibited clinical symptoms, and approximately 95% of the patients with histologic diagnoses of mild or indeterminate acute rejection exhibited symptoms. The remaining 5% of patients with mild or indeterminate acute rejection exhibited no symptoms at the time of the biopsies, and the diagnoses were established with protocol biopsies. Most of the biopsies without histologic evidence of acute rejection demonstrated either normal mucosa or mild nonspecific enteritis; some showed reparative mucosa, CMV infection, Epstein-Barr virus (EBV) infection, or PTLD.

Prognostic Ability of the Grading System

We then wished to analyze the association between acute rejection grades and unfavorable outcomes. For this purpose,

the patients were divided into those with favorable outcomes and those with unfavorable outcomes, according to the aforementioned criteria, and the ability of the grading system to predict an unfavorable outcome was assessed with the chi-square test for trend. The results demonstrated that a grade indicating a more severe rejection episode was associated with a greater probability of an unfavorable outcome ($P<0.01$). In fact, all four of the histologically diagnosed severe acute rejection episodes resulted in graft failure before resolution, despite treatment with OKT3. Of those four grafts, three were removed because of uncontrolled ACR and one patient died as a result of ACR with the graft in place. Of the 14 episodes of moderate acute rejection, 2 episodes required OKT3 treatment and 2 episodes failed to resolve within 21 days with immunosuppressive therapy (other than OKT3). The outcome of one moderate ACR episode could not be determined because of graft removal secondary to chronic rejection, before the resolution of ACR. The remaining nine episodes of histologically diagnosed moderate ACR were not associated with unfavorable outcomes. The outcomes were difficult to assess for 3 of the 74 episodes of mild ACR, because of graft removal in 2 cases (because of chronic rejection and opportunistic infection) and patient death in 1 case (resulting from opportunistic infection) before resolution of the ACR episodes. The remaining 71 mild ACR episodes were not associated with unfavorable outcomes. The 88 indeterminate ACR episodes all resolved within 21 days (spontaneous resolution without treatment, resolution after increased immunosuppressive therapy, or progression to mild ACR that latter resolved with treatment) and were not associated with unfavorable outcomes.

Reliability of the Grading System

A consensus diagnosis was reached by all of the participating pathologists in 60 of the 65 cases (92%), including 4 cases of severe acute rejection, 9 cases of moderate acute rejection, 10 cases of mild acute rejection, 13 cases of indeterminate for ACR, and 24 cases of no acute rejection. Of the five cases for which a uniform diagnosis could not be established, two cases were interpreted as either mild ACR or indeterminate for ACR and three cases were interpreted as either indeterminate or no ACR. There was no disagreement regarding the diagnosis of moderate or severe acute rejection. Multirater kappa analysis demonstrated that there was excellent overall agreement among pathologists regarding the diagnosis and grading of small bowel acute rejection with this grading schema ($P<0.01$). Good intraobserver agreement was noted when the slides were reviewed in a blinded manner by the same pathologist on two separate occasions (with an interval of approximately 6 months).

DISCUSSION

The primary goal of this study was to develop a histologic grading system for the diagnosis of small bowel allograft ACR. To achieve this, we evaluated 3,268 small bowel allograft biopsies obtained from adult patients who underwent small bowel transplantation at our institute during the past decade. On the basis of previously documented major histologic parameters for small bowel allograft acute rejection, the severity of acute rejection was graded as indeterminate, mild, moderate, or severe. This grading system was validated by retrospective correlation with clinical outcomes; more severe rejection episodes were associated with a greater probability of unfavorable clinical outcomes. The excellent overall agreement among different pathologists regarding the histologic diagnosis of acute rejection using the proposed criteria suggests that this system is reliable for the routine pathologic evaluation of small bowel allograft acute rejection. To our knowledge, the criteria in this study represent the first schema for assessment of acute rejection severity in human small bowel allografts.

Several pitfalls in the histologic evaluation of small bowel mucosal biopsies are worth mentioning. We observed that four histologic features are particularly useful for the routine pathologic diagnosis of small bowel allograft ACR, including architectural distortion, crypt apoptosis, crypt epithelial injury, and activated lymphocytic inflammatory infiltration in the lamina propria. These are relatively easily identifiable features that can be reliably quantitatively or semiquantitatively assessed, with a high degree of reproducibility among different pathologists. Because artery sampling is extremely rare in intestinal mucosal biopsies, arteritis has limited diagnostic value in the evaluation of mucosal biopsy specimens, although its presence invariably indicates moderate or severe acute rejection. In this study, arteritis was identified in only 2 of the 3,268 mucosal biopsies. If biopsies are obtained from isolated ulcers or necrotic regions, then an exact histologic diagnosis of acute rejection may be difficult to establish. In such circumstances, careful clinical and endoscopic correlation is particularly important and repeated biopsies from nonulcerated regions are often required.

The quality of the infiltrate (activated lymphocytes mixed with some eosinophils and neutrophils in ACR, compared with nonactivated lymphocytes in nonspecific enteritis) is important in the differentiation of ACR from other conditions. The intensity of the infiltration is generally correlated with the severity of ACR (mild infiltration in mild ACR and intense infiltration in severe ACR). In our experience, the area of infiltration is a less-reliable marker, because the infiltration in low-grade ACR can be diffuse (although less intense). Although eosinophils are frequently observed in intestinal mucosa, significantly increased levels of eosinophils with coexistent activated lymphocytes and crypt apoptosis suggest acute rejection. Peyer's patches are commonly sampled in mucosal biopsies, especially from the ileum. Although localized Peyer's patches without significant lymphoid activation do not indicate acute rejection, Peyer's patches with lymphoid activation (characterized by lymphoid cells with open chromatin, diffuse infiltration into the surrounding mucosa, or mixtures with eosinophils and neutrophils) are frequently associated with acute rejection. The significance of lymphocytic cryptitis (increased numbers of lymphocytes in the crypt epithelium) is unclear. Although cryptitis is present in some cases of acute rejection, it is also observed in biopsy tissues without ACR (such as those exhibiting nonspecific enteritis, viral infections, or PTLD). Statistical analyses in this study failed to demonstrate a correlation between lymphocytic cryptitis and the diagnosis of acute rejection. Acute cryptitis (increased numbers of neutrophils in the crypt epithelium) is usually associated with various causes of acute enteritis and is not a diagnostic criterion for acute rejection.

Adequate tissue sampling is necessary for accurate histologic diagnosis. Because the distribution of acute rejection may be patchy, multiple biopsies (usually three to five) are often required. Biopsies from either the ileum or the jejunum are sufficient for histologic evaluation in most cases, although sampling from both the ileum and the jejunum may be required in some cases with ambiguous diagnoses. The tissue obtained should be fixed in 10% neutral buffered formalin for at least 1 hr before processing, and multiple sections (usually 10–15) should be examined for each biopsy.

Differentiation between indeterminate and mild ACR is important for treatment planning. In our center, most of the histologically diagnosed mild acute rejection episodes were treated with increased immunosuppression (except when rejection occurred in association with opportunistic infections or PTLD), whereas treatment for indeterminate rejections was liberal, based on clinical assessments. A histologic distinction between these two categories can usually be made with this grading system. Among the listed criteria, the number of apoptotic bodies is most helpful (<6 apoptotic bodies per 10 crypts for indeterminate ACR versus >6 apoptotic bodies per 10 crypts for mild ACR), followed by perivenular infiltration (less common for indeterminate ACR and more common for mild ACR). We observed that mild acute rejection was associated with favorable clinical outcomes, which likely reflects successful immunosuppressive therapy. Indeterminate for acute rejection was also associated with favorable clinical outcomes, which likely reflects the minimal activity of acute rejection in this group and the use of immunosuppressive therapy for some of the patients.

Various pathologic conditions must be differentiated from acute rejection, the most common of which include nonspecific enteritis, CMV infection, EBV infection, and PTLD. Acute enteritis is often attributable to bacterial or viral infection and is characterized by neutrophil-rich infiltration in the lamina propria, with acute cryptitis but usually without significantly activated lymphocytes or increased apoptosis. CMV enteritis can sometimes be associated with increased inflammatory infiltration and increased apoptosis, and the diagnosis is made with the identification of characteristic nuclear and cytoplasmic viral inclusions, with confirmatory immunohistochemical staining. EBV infections and PTLD are often associated with significant mononuclear infiltration, and the diagnosis is made with the identification of atypical lymphoid cells on hematoxylin-eosin-stained sections, immunohistochemical staining for T and B cells, in situ hybridization for Epstein-Barr virus-encoded RNA, and clonality analysis. Ischemia-reperfusion injury is generally not a problem in the differential diagnosis, because it usually occurs immediately after reperfusion, with characteristic histologic features that resolve within 2 to 3 days in most cases. For patients with delayed recovery from severe ischemia-reperfusion injury, the diagnosis of early superimposed acute rejection can sometimes be difficult. Under such conditions, the presence of activated lymphocytes and eosinophils, ongoing crypt damage, and significant crypt apoptosis suggests acute rejection.

CONCLUSION

This study provides a reliable, predictive histopathologic schema for assessment of the severity of human small bowel acute rejection. The availability of this grading system should provide important guidance for effective immunosuppressive treatment of patients who undergo small bowel transplantation. It should also facilitate information exchange within and between transplantation centers.

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Current Status of Transplantation of the Small Intestine

Phillip Ruiz,[1,2,3] Tomoaki Kato,[2] and Andreas Tzakis[2]

The evolution of small bowel transplantation has been significant over the past 20 years to the point at which it can now be considered a viable and often successful option in the treatment of many forms of short bowel syndrome. A refinement of surgical techniques, improved immunosuppression, enhanced understanding of gut immunology, and better treatment and prevention of complications have contributed to a marked improvement in graft and patient survival. Whereas this transplant population is still beset with many potential complications after isolated bowel or multivisceral transplantation and long-term graft survival (like with other solid organ transplants) remains a challenge, the future holds promise for a continuation of the current positive trend of improvement in several areas.

Keywords: Small bowel transplantation, total parenteral nutrition, multivisceral transplantation.

(*Transplantation* 2007;83: 1–6)

"Nothing endures but change."

Heraclitus, *from Diogenes Laertius, Lives of Eminent Philosophers*

Greek philosopher (540 BC - 480 BC)

The calamitous and potentially deadly development of short bowel syndrome in adults and children had until the last two decades been treated exclusively with parenteral nutrition (PN) supplementation. Although PN remains a therapeutic mainstay for this group of patients, it can be a limiting treatment with potentially devastating complications. In reality, the complications associated with PN, which include catheter-related morbidity (e.g., sepsis, venous thrombosis), metabolic changes (e.g., hepatotoxicity), psychologic strain, and reduced quality of life, all contribute to a five-year survival rate for all patients on PN of approximately 60% (*1*). The successful emergence of small bowel transplantation as a curative alternative has provided many patients with bowel failure to have an improved quality of life, better nutrition, and reduction in PN-associated complications. Although intestinal transplants have customarily been performed when there was danger to the patient's life, usually as a result of the PN-induced development of liver failure secondary to hepatic scarring and cirrhosis or loss of vascular access for PN, there is now an emerging philosophy of earlier intervention. In this regard, reports of transplants performed at an earlier stage (*2*) have shown encouraging results. This earlier approach is justified because patients awaiting combined liver–intestinal transplantation have the highest mortality rates compared with other transplant candidates (*3*). The gamut of underlying diseases causing short bowel syndrome in patients who have been transplanted is extensive and variable between pediatric and adult populations (Table 1). Generally, nonmalignant conditions are the norm for recipients, although occasional tumors such as desmoids (*4*) have been successfully treated with intestinal transplantation. Recurrence of the native disease in the allograft is typically not a significant issue with this form of transplantation.

Since the initial small bowel transplants were first performed in the 1980s (*5*), there have been technical improvements, novel immunosuppressive agents, better understanding of the immune and gastrointestinal physiology, and increased clinical program experience. All of these factors have contributed to a remarkable improvement in bowel transplant one-year graft and patient survival (estimated 80% and 80%, respectively) compared with only several years ago; these numbers are based on Intestinal Transplant Registry (*6*) data presented at the IX International Small Bowel Transplant Symposium in 2005. Figure 1 shows the most recent data provided by the Intestinal Transplant Registry for graft and patient survival for the worldwide experience in small bowel transplantation at the University of Miami. Still, this highly complex transplant continues to be laden with potential complications and to date remains a relatively uncommon procedure with approximately 1300 transplants performed worldwide according to the International registry, 60% of them for children (*6*).

Surgical

Transplantation of the intestine can be performed as an isolated graft or in combination with other abdominal or-

[1] Department of Pathology, University of Miami, Miami, FL.
[2] Department of Surgery, University of Miami, Miami, FL.
[3] Address correspondence to: Phillip Ruiz, M.D., Ph.D., Department of Pathology, Division of Immunopathology, University of Miami, Holtz Building, Room 2101, Miami, FL.
E-mail: pruiz@med.miami.edu
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TABLE 1. Indications for bowel transplantation in children and adults[a]

	Percentage of worldwide cases
Children	
Gastroschisis	22
Volvulus	17
Necrotizing enterocolitis	12
Pseudoobstruction	9
Intestinal atresia	8
Aganglionosis/Hirschsprung	7
Retransplant	7
Microvillous inclusion	6
Other causes	4
Malabsorption	3
Short gut other	3
Tumor	1
Other motility	1
Adults	
Ischemia	25
Crohn disease	13
Trauma	9
Short gut other	9
Volvulus	8
Motility	8
Desmoids	8
Retransplant	6
Miscellaneous	6
Other tumor	5
Gardner's	3

[a] Data obtained from Intestinal Transplant Registry Data, 2005.

gans, because patients with intestinal failure often experience other complex abdominal pathologies that require organ replacement. As a result, there have been several variants of intestinal transplants, all derivatives of the "cluster" concept originally proposed by Starzl et al. (*7*). Isolated intestinal transplantation (ITx) is transplantation of the small intestine with or without the large intestine and is more commonly performed in adults, whereas combined liver–intestinal transplant (LITx), performed en bloc or separately, is more commonly performed in children. The latter scenario occurs when there is concomitant liver failure (typically PN-induced). With ITx, the entire jejunum and ileum has been transplanted in the majority of cases. With ITx from a living donor and in cases in which reduction of the size of the graft is required, a 200-cm segment (*8*) is usually transplanted. In this regard, it is important to match size because of the need for closure of the abdomen. There is maintenance of as much native bowel as possible, particularly with recent data suggesting that increased residual or allograft bowel provides some protection from PN-associated injury. This is particularly relevant because there may be some supplementation of transplanted patients with PN for a period of time.

When ITx is performed en bloc, the duodenum with a segment (or the entire pancreas) (Omaha technique) may be included to avoid the need for biliary reconstruction. In these cases, the graft duodenum represents a second duodenum (in addition to the native duodenum) and is extraneous, serving as a conduit for bile and pancreatic secretions. The upper gastrointestinal continuity is maintained through the native stomach and pancreaticoduodenal complex, which are retained. In LITx, the intestinal transplant is combined with the liver. These organs are transplanted en bloc or separately. When the liver and intestine are transplanted separately, the two organs can be transplanted contemporaneously or sequentially from the same or a different donor. The great majority of the donors for these two forms of intestinal transplantation are from cadaveric donors, although living donors for ITx have been successfully performed without significant donor morbidity (*9*) and may be an important future source, particularly for pediatric recipients.

Multivisceral transplantation (MVTx) is the removal and replacement of both native foregut and midgut (*10*) in which the native abdominal viscera are resected and the composite graft, which includes the stomach, pancreaticoduodenal complex, and small intestine, are transplanted en bloc and form the new gastrointestinal tract. The liver, kidneys, and large intestine of the donor may or may not be included (Fig. 2) depending on the clinical scenario. This latter variant is reserved for the most extensive abdominal catastrophes and organs are only replaced if there is a suspicion of underlying injury from the patient's general condition. This has typically been used as an alternative for small babies who would have ostensibly received a LITx. Evisceration of the native organs is facilitated by early dearterialization. The latter is achieved by mass clamping of the celiac and superior mesenteric arteries. This can be achieved through a cephalad approach after division of the esophagus or proximal stomach or a caudal approach between the inferior surface of the pancreas and left renal vein. Since 2000, the use of MVTx is increasing and despite the fact that the donors for MVTx are exclusively cadaveric, the one-year graft and patient survival is at least as good as the other forms of intestinal transplantation (*6*). As of mid-2005, an isolated intestinal graft has been performed in 44% of the cases, intestine transplanted in combination with the liver (38%) or multivisceral transplant (18%) (*6*). The decision to use one form of intestinal transplantation versus another is typically determined by the individual patient's particular needs. For example, the type of underlying disorder and surgical history of the patient are important considerations in which type of intestinal transplant is performed, the type and size of the donor, and how much abdominal domain is available to the surgeon. The emergence of promising data suggesting improved survival data and long-term sequelae, as well as possible immunologic advantage for MVTx, is allowing the clinical team more options as it determines which form of transplantation will be recommended.

Immunosuppression

With the advent of clinical intestinal transplantation, it was at once apparent that significant immunosuppression (ISP) was to be necessary to attain the goals of engraftment and graft survival of reasonable duration. Many therapies and combinations thereof have been used, but what remains undefined are the optimal immunosuppression regimens to achieve the aforesaid goals while preserving graft function and not predisposing the recipient to increased infections or malignancy. Although

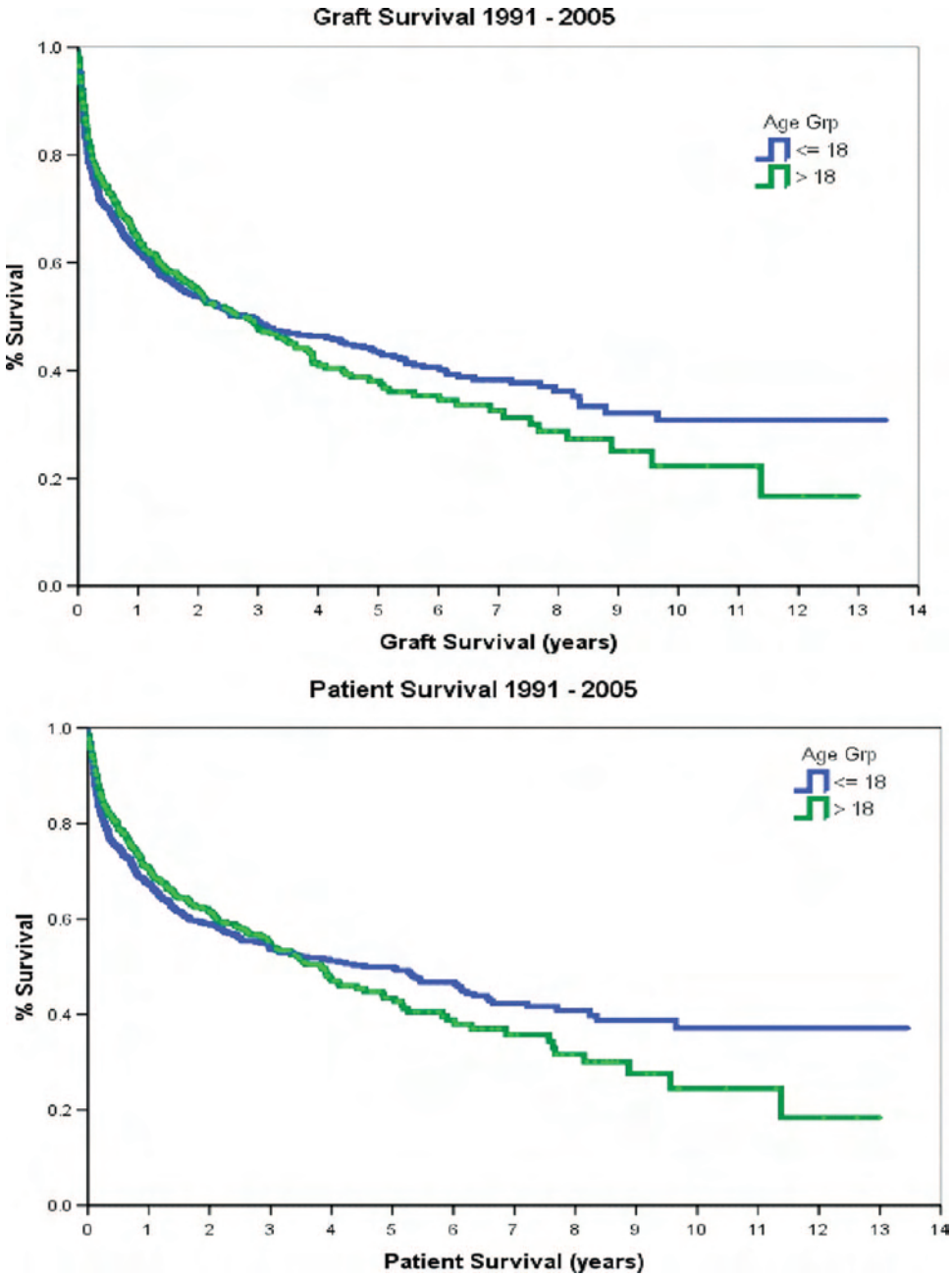

FIGURE 1. Graft and patient survival curves for worldwide adult and pediatric small bowel transplant experience based on data from the Intestinal Transplant Registry.

the first successful cases were reported in the cyclosporine era (*11*), tacrolimus is the drug that allowed development of a consistently successful intestinal transplant series and to date is the maintenance ISP drug of choice. One of the most significant changes to occur with intestinal transplantation is the near ubiquitous use of induction immunosuppression therapy with an estimated 90% of cases now using this as part of the overall regimen. The most common induction ISP agent is anti-IL2-receptor antibody therapy followed by anti-lymphocyte globulin and Campath-1 (*12*, *13*). Their use has been associated with reduction in the incidence and severity of rejection episodes and improvement of survival results, which have allowed maintenance with lower levels of tacrolimus. This latter issue has become important because there is now increasing evidence of calcineurin-inhibitor toxicities in patients receiving nonrenal transplants (*14*). Conversion to noncalcineurin-inhibitor drugs (such as rapamycin), use of steroid-sparing protocols, and a determination as to which ISP therapy best maintains levels of chimeric cells from the donor that promotes graft acceptance remain as new but relatively ill-defined areas in this field of transplantation.

Complications

The technical challenges notwithstanding, one of the most sobering issues continues to be the significant alloimmune response and subsequent rejection of the small intesti-

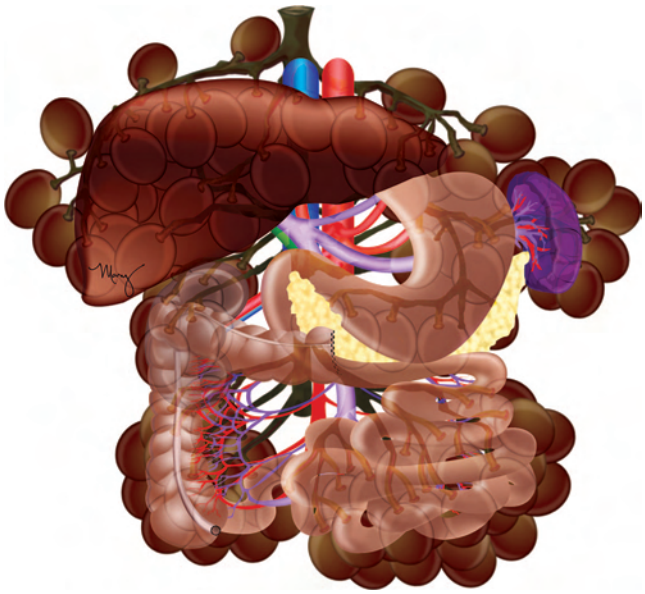

FIGURE 2. Drawing illustrating the organs potentially being transplanted in a multivisceral transplant and how a "cluster of grapes" serves to conceptualize the principal and secondary arterial blood supply to the organ block (Drawing by Mary Campos).

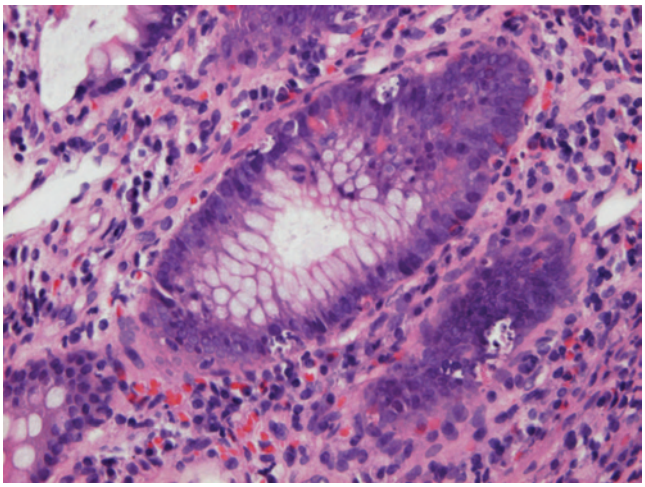

FIGURE 3. Photomicrograph of intestinal allograft from an adult woman approximately 10 years posttransplant. The epithelial structures are undergoing significant apoptosis in the presence of other inflammatory features compatible with acute cellular rejection (hematoxylin & eosin, ×200).

nal graft, an event that occurs more frequently and with greater severity than any other abdominal organ. Indeed, in our experience, rejection of the small intestine occurs with the highest frequency and intensity compared with other transplanted organs; this is best exemplified when comparing rejection rates and severities of the different allograft organs within a given individual who has received a multivisceral transplant (*10*, *15*). The potential reasons for the small intestine to be the nexus of the most vigorous rejection inflammatory response include the heightened immunogenicity and significant donor lymphoid volume in the organ, factors made more significant when considering the large mass of the implanted allograft. For example, small bowel immunogenicity may be enhanced by the type of stimulatory molecules expressed (e.g., major histocompatibility complex class II), the cellular composition (e.g., parenchymal vs. endothelial cells) as well as the donor cell response to injury (e.g., cytokine release).

The attributes and manifestations of the initial wave of alloimmunity to the intestinal graft reveals that the response characterized as "acute rejection" bears some similarities to other solid organ transplants but with unique and poorly understood features. For example, animal and human studies to this point suggest that the archetypical acute rejection response in the bowel is a T-cell-mediated phenomenon (like with other organs) that involves the interstitial and epithelial-lined structures of the organ with kinetics and immune effector cell characteristics that point to a primary immune response. Still, the relationship between the injury that appears to occur in the organ and the clinical manifestations (e.g., fever, increased stool output) remains modestly understood. Acute cellular rejection is now reasonably identifiable by bowel biopsy histology (Fig. 3), and international pathology grading systems have emerged (*16*). In tandem with pathologic changes, improvements in endoscopic monitoring (e.g., magnifying—zoom, capsule endoscopy) help to establish potential sites of rejection (*17*, *18*). For this reason, surveillance endoscopies are now performed two or three times per week or even more frequently during the immediate postoperative period and then at slowly decreasing frequency. A normal endoscopy in the face of pathologic findings suggestive of rejection can simply be repeated the next day and thus avoids overimmunosuppression of the patient.

Disruption in the secretion of products of gastrointestinal and inflammatory cells such as citrulline and calprotectin (*19*, *20*) shows promise as peripheral and adjunctive measurements of altered graft function. Certain molecules such as CD103, like with other bowel diseases (*21*), may be critical cofactors in determining whether immune effector cells can mediate damage to the bowel parenchyma. It is hopeful that measurements of these and other analytes along with biopsy and endoscopy will allow for more efficient screening and identification of acute cellular rejection. The cumulative effect of these advances in prevention, monitoring, and treatment of acute cellular rejection of the intestinal graft has been one of the most important contributors to the significant improvement in patient and graft survival.

Although acute cellular rejection has been reasonably characterized and clinically correlated, other forms of rejection in the bowel remain inadequately defined. In this regard, acute vascular rejection has been recognized infrequently in its most severe form (*22*), although other studies have shown that mild variants and subclinical forms of acute vascular rejection likely exist at a much higher frequency than previously believed (*23*). The role that this humoral-based acute rejection has on long-term graft survival is not known. Chronic rejection (chronic allograft enteropathy) also remains somewhat of an enigma in small bowel transplantation. Diagnosis of this entity is hampered by the lack of specific lesions in the mucosal biopsy, although interstitial fibrosis and other histologic changes considered in the context of the clinical sce-

nario may provide a clue to the presence of chronic allograft enteropathy (*24*, *25*). As patient survival for intestinal transplantation improves, there will likely be better understanding of the frequency of this entity and improved means for its detection and treatment.

Infections in intestinal transplantation, like with other forms of transplantation, have always been a serious problem facing the recipient throughout the posttransplant period. The gamut of viruses, bacteria, and fungi causing morbidity in ITx is similar to other forms of transplant, although occasional unusual microbes (e.g., cryptosporidium) (*26*) involve the allograft. The reemergence and stabilization of the normal bowel flora may have important implications because shifts in the flora toward other atypical microbial residents of the bowel could cause alterations in bowel transit time and may potentiate acute rejection. In our experience, composite organ transplants tend to have fewer infections than isolated bowel (*10*); we suspect that the lower rate of infections (and subsequent less immunosuppression), fewer fistulas, and less complications with arterial anastomosis with MVTx likely all contribute to this finding. However, despite improvements in prophylactic antibiotics, surgical options (e.g., portal venous drainage [*27*]), and earlier identification of infection, sepsis remains the single highest cause of death in this patient population in the short- or long-term posttransplant period (*6*).

Posttransplant lymphoproliferative disease (PTLD) has always been a serious complication in intestinal transplantation (*28*). Interestingly, the rate of PTLD, as defined by the presence of frank malignancy, has remained relatively stable with a frequency of 6% to 8% (although slightly higher in children) despite the introduction of more powerful immunosuppressive agents (*6*). The incidence of the preneoplastic stages of PTLD (e.g., plasmacytic hyperplasia, polyclonal lymphoproliferative changes) remains poorly delineated. PTLD tends to occur approximately at its highest incidence 25 months posttransplantation, but the other precursor forms of PTLD can occur much earlier (*29*). PTLD tends to occur more often with OKT3 or induction therapy, and some forms (e.g., MVTx) of intestinal transplant have a greater risk. Finally, as compared with other solid organ transplants, the gut allograft itself is the most frequent site of early PTLD changes. This causes, at times, a diagnostic dilemma in the allograft biopsy because there are often coexisting inflammatory cells for both acute rejection and early PTLD. Fortunately, current use of rituximab therapy is very useful in the treatment of some forms of PTLD (*30*). The mortality from PTLD has decreased significantly in our experience.

Summary and Future Considerations

Over the past two years, as presented at the 2005 International Bowel Transplant Congress in Brussels, there have been a total of 29 centers performing 323 intestinal transplants. The incidence of 80% one-year graft and patient survival reflects an incredible improvement when compared with results from just several years ago in the year 2000. Positive risk factors for intestinal transplantation include if the center performed greater than 10 transplants and the pretransplant status of the recipient, issues that both reiterate the inherent complexity and morbidity of this procedure (*6*). Unfortunately, most of the gains in patient and graft survival are in the first posttransplant year because long-term survival remains essentially the same as in previous eras of intestinal transplantation. The similarity in the slopes of survival curves with patients undergoing modern-day ITx reflects some of the same problems confronting other solid organ transplants; despite gains in the control of early posttransplant events, there is still a significant decline in graft survival over the subsequent years (*6*). The causes for graft and patient loss over the long term of intestinal transplantation include infections, malignancy, and chronic rejection, similar to other transplants (*6*).

There are numerous areas involving transplantation of the small intestine that hold promise to enhance and improve this procedure so that it will become a cornerstone in the therapy of short bowel syndrome. From a surgical and technical perspective, there continues to be refinement of the three basic techniques (ITx, LITx, and MVTx), but there may be a concomitant role to enhance and lengthen the remaining native bowel (Bianchi procedure, STEP procedure) (*31*). Full-thickness abdominal wall transplantation as an adjunct to small bowel transplantation is now on occasion used to facilitate closure of the abdominal space in certain situations (*32*). Curiously, the skin of the abdominal wall graft shows relatively little acute rejection and is often not synchronized with the changes occurring in the bowel; thus, this graft has had a high rate of success.

Immunologically, the bowel presents an important tool to address the potential relationship between donor cell chimerism (*33*) and immunologic tolerance, because there is a suggestion that the presence of particular cells from the donor may facilitate graft acceptance (*34*). Small bowel allografts may also represent a system to investigate the mechanisms controlling graft-versus-host disease arising from a solid organ allograft, a complication normally in low incidence in bowel transplants (*35*). Little is known regarding the target structures in the bowel for alloimmune cells and the physiological and immunologic restrictions needed for injury to occur. What are the characteristics of the donor lymphoid population mass and repertoire over time and do donor stem cells (*36*) survive long term, possibly serving as a source of chimeric cells?

Will it be possible to supplement bowel allograft surgery with modifications in the native bowel of the host? For example, can intestinal adaptation, a normal phenomenon whereby residual bowel shows compensatory hypertrophy (*37*), be augmented and controlled with the proper growth factors to facilitate engraftment to the transplanted bowel and accelerate healing? Furthermore, as stem cell technology and tissue engineering progress, will there be the capacity to place enterocyte stem cells in matrices on tissue scaffolds (*38*) in the recipient that will eventually generate physiologically capable bowel that will supplement nutrition absorption in the recipient? Modification of intestinal adaptation and gastrointestinal stem cells are among the potential approaches that may assist in recovery from the resection; these areas of investigation share in the fact that they are recipient-derived (thus, not needing immunosuppression) and offering a potentially unlimited supply.

In summary, the field of intestinal transplantation has shown extraordinary growth over the last two decades with a notable level of success so that there is now a realistic alternative for many short bowel syndrome patients over PN.

Although there remain many potential complications and challenges, the upcoming years hold promise of a continuation of our advancement and further improvement with this form of transplantation.

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2007 Banff Schema for Grading of Composite Skin-Containing Allograft Rejection

Based on adequate specimen defined as at least one 4mm skin punch biopsy from most reddened or indurated area of viable inolved skin and containing epidermis, dermis, subcutaneous tissue, adnexa and vessels, and minimally stained with H&E and PAS stains.

Acute Cell-Mediated Rejection

Grade	Histopathology	Comments
0	No inflammation OR rare inflammatory infiltrates	1. Essentially normal looking skin
I (Mild)	Mild perivascular inflammation WITHOUT involvement of the overlying epidermis.	1. Mainly lymphocytic, begins in upper dermis. 2. Nonspecific, may be seen with viral exanthems, etc. 3. Diagnosis of rejection remains "somehow tentative" at this stage.
II (Moderate)	Moderate to severe perivascular inflammation WITHOUT epithelial dyskeratosis and/or apoptosis and/or keratinolysis. MAY HAVE mild spongiosis and/or exocytosis of epidermis and/or adnexae	1. Typically inflammation in upper and mid-dermis, may have mild interface dermatitis, rarely vesicle formation. 2. Differential diagnosis includes viral/drug eruptions (often with some extravasated red cells), contact dermatitis, insect bites (may contain more eosinophils), dermatophyte infections (eczematoid epidermal changes, PAS-positive organisms).
III (Severe)	Dense inflammation and epidermal involvement WITH epithelial apoptosis	1. Dermal infiltrates form perivascular and periadnexal nodules, epidermis may have

	and/or dyskeratosis and/or keratinolysis	lichenoid changes. 2. Differential diagnosis includes pseudolymphoma (T cells, B cells, variable eosinophils and histiocytes), Drug rash with eosinophilia and systemic symptoms (DRESS syndrome), PTLD/B cell lymphoma (B cell predominance, may contain EBV, clonal- note: no examples yet described)
IV (Necrotizing)	Frank necrosis of epidermis or other skin structures	1. Inflammation mainly lymphocytic, may have numerous eosinophils. 2. Differential diagnosis includes drug eruption, i.e., toxic epidermal necrolysis (less dermal inflammation than necrotizing rejection), pseudolymphoma, insect bites, eosinophilic cellulitis.
Chronic Rejection		
Grade	Histopathology	Comments
Insufficient data at present for classification of chronic rejection	Likely correlates include vessel narrowing, loss of adnexa, atrophy of skin and muscle, myointimal proliferation, deep tissue fibrosis, nail changes.	1. Likely to reflect both chronic rejection-associated and/or nonimmune events in individual cases. Further study needed.
Antibody-Mediated Rejection		
Category	Histopathology	Comments
Insufficient data at present for classification of antibody mediated	Likely histopathologic correlates would include C4d deposition, vasculitis, neutrophil margination, vascular	1. Recommended to gather this information along with donor-specific antibody levels, PRA, cross-match results including both T and B cell, transfusions, pregnancies, prior allografts,

rejection	thrombi, and/or necrosis.	autoantibody levels in order to prospectively assess the pathobiology and diagnostic criteria appropriate for antibody-mediated rejection of composite allografts.

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The Banff 2007 Working Classification of Skin-Containing Composite Tissue Allograft Pathology

L. C. Cendales[a,*], J. Kanitakis[b], S. Schneeberger[c], C. Burns[d], P. Ruiz[e], L. Landin[f], M. Remmelink[g], C. W. Hewitt[h], T. Landgren[i], B. Lyons[j], C. B. Drachenberg[k], K. Solez[l], A. D. Kirk[m], D. E. Kleiner[n] and L. Racusen[o]

[a] *Emory Transplant Center, Emory University, Atlanta, GA*
[b] *Department of Dermatology and Dermatopathology, Ed. Herriot Hospital, Lyon, France*
[c] *Department of General and Transplant Surgery, Medical University Innsbrück, Innsbrück, Austria and Division of Plastic and Reconstructive Surgery, UPMC, Pittsburg, PA*
[d] *Jewish Hospital Pathology Department, Jewish Hospital and St Mary's Healthcare, Louisville, KY*
[e] *Department of Pathology and Surgery, University of Miami, Miami, FL*
[f] *Reconstructive Surgery Unit, Pedro Cavadas Foundation, 'La Fe' University Hospital, Valencia, Spain*
[g] *Department of Surgical Pathology. CUB-ULB Hôpital Erasme, Brussels, Belgium*
[h] *Robert Wood Johnson Medical School, Camden, UMDNJ Cooper Health System, Camden, NJ*
[i] *Department of Anatomical Pathology, Melbourne Health Pathology, Royal Melbourne Hospital, Victoria, Australia*
[j] *Department of Histopathology, Derriford Hospital, Plymouth, United Kingdom*
[k] *Department of Pathology, University of Maryland School of Medicine, Baltimore, MA*
[l] *Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada*
[m] *Emory Transplant Center, Emory University, Atlanta, GA*
[n] *Laboratory of Pathology, National Cancer Institute, Bethesda, MA*
[o] *Department of Pathology, The Johns Hopkins University School of Medicine, Department of Pathology, Baltimore, MA*
* *Corresponding author: L. C. Cendales, lcendal@emory.edu*

Composite tissue allotransplantation (CTA) is a recently introduced option for limb replacement and reconstruction of tissue defects. As with other allografts, CTA can undergo immune-mediated rejection; therefore standardized criteria are required for characterizing and reporting severity and types of rejection. This article documents the conclusions of a symposium on CTA rejection held at the Ninth Banff Conference on Allograft Pathology in La-Coruña, Spain, on 26 June 2007, and proposes a working classification, the Banff CTA-07, for the categorization of CTA rejection. This classification was derived from a consensus discussion session attended by the first authors of three published classification systems, pathologists and researchers from international centers where clinical CTA has been performed. It was open to all attendees to the Banff conference. To the extent possible, the format followed the established National Institutes of Health (NIH) guidelines on Consensus Development Programs. By consensus, the defining features to diagnose acute skin rejection include inflammatory cell infiltration with involvement of epidermis and/or adnexal structures, epithelial apoptosis, dyskeratosis and necrosis. Five grades of severity of rejection are defined. This classification refines proposed schemas, represents international consensus on this topic, and establishes a working collective classification system for CTA reporting of rejection in skin-containing CTAs.

Key words: Anitbody-mediated rejection, Banff, Banff schema, chronic rejection, composite tissue allograft, humoral rejection, rejection, skin allograft, transplant

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Background and Goals

Composite tissue allotransplantation (CTA) is an emerging discipline for the treatment of functionally significant tissue or limb defects. In contrast to solid organ transplants, CTAs often include skin as well as tissues of diverse embryological origin. Most CTA recipients have experienced reversible episodes of acute rejection (1) but to date, no universally accepted criteria for CTA rejection reporting has been established. Histopathology plays a key role in diagnosis of rejection, in understanding the physiopathology of rejection and in facilitating management. Currently, four classification systems have been published and as such, a universally accepted grading scheme for ranking pathological severity of rejection is needed. Standardization is necessary for reporting clinical results and to establish objective end points for clinical trials. Recognizing that a dispersed and unstandardized development of CTA would present a major barrier for progress and reporting, a collaborative relationship was established with investigators with experience in clinical CTA worldwide to initiate the groundwork for a universally accepted histological classification. In addition, as immunomodulatory regimens are minimized, CTA

will experience a growth period in the near future. This article describes a consensus schema for the stardardization of clinical reporting for the advancement of the study of the histopathology in CTA-containing skin for dissemination to the health care practice and medical community. As a working classification, the schema will continue to be refined in subsequent meetings as more clinical and experimental data become available for skin and other tissues used in CTAs.

Investigators in the field of CTA including representatives from multiple sites reporting a clinical CTA in the past decade were invited to a consensus discussion on CTA histopathology at the Ninth Banff Conference on Allograft Pathology. In keeping with established National Institutes of Health (NIH) guidelines on Consensus Development Programs (2), this conference included: (1) a broad-based nonadvocacy, independent panel gathered to give balanced, objective and knowledgeable focus to the topic, (2) freedom from scientific or financial conflict of interest from the speakers, (3) predetermined questions defining the scope and the direction of the conference, and (4) a systematic literature review of the topic. The presenters included the three first authors of the four classification systems published and investigators who have actively followed CTA patients from a clinicopathological view and/or published reports on CTA rejection. A pathologist from the center where the fourth classification system was published was also invited, provided a presentation and participated in the discussions. Six out of six western international centers with reported experience in hand transplantation at the time of the call were invited and five centers were represented. Furthermore, two out of two centers with experience in other CTA's-containing skin were represented (i.e. face and abdominal wall). All published scoring systems for CTA were reviewed (3–7). In addition, a senior investigator in CTA was invited to provide a historic perspective. Each presenter provided data followed by a discussion. Of the presenters, five were clinical pathologists, three were surgeons and one was a basic investigator. The session was open to the public and all attendees of the Banff Conference. A total of 20 attendees provided oral and/or written comments to the questions posed.

To date, 41 patients receiving skin-containing CTA's have been reported; 28 have received hands, three faces, one knee with a skin island and nine abdominal walls. Essentially, all patients have experienced episodes of rejection (1,8,10). Clinical manifestations of rejection have been characterized by cutaneous changes including mild pink discoloration, gradual erythema, macules progressing to red infiltrated lichenoid papules with or without limb edema and onychomadesis in advanced rejection (1,11). Histological findings disclose predominantly lymphocytic inflammatory-cell infiltrate of variable density, epithelial intracellular edema (spongiosis), lymphocyte exocytosis and keratinocyte apoptosis (1,12). Macroscopic skin changes in a case reported after steroid resistant rejection showed blisters in the superficial layers with epidermal desquamation. Histology revealed dermal and epidermal lymphocytic infiltration with apoptotic and necrotic keratinocytes (13).

The four published systems on grading CTA skin rejection ranked the degree of rejection based on evaluation of morphologic features (3–7). All systems illustrated substantial agreement on the basic grade stratification for acute rejection. All agreed that perivascular lymphocytic infiltrates become progressively denser and involve more vessels as the severity of rejection increases. The inflammation then extends to involve dermal stroma, epidermis (including the basal cell layer) and adnexa at moderate to marked grades of rejection. Epidermal apoptosis/necrosis is a marker of severe rejection in all of the published systems where it was observed. The classification based on full thickness, vascularized, myocutaneous-free flaps for closure of abdominal defects (3) stratified rejection based on the extent of vessels infiltrated, from $<10\%$, to 11–50% in mild, and to more than 50% in moderate and severe rejection. Severe rejection of abdominal wall grafts showed dyskeratosis and spongiosis.

The discussion initiated with the following predetermined questions chosen by the CTA session committee chair in conjunction with investigators in the field: (1) Specimen and Slide Preparation: which structures are required to constitute an adequate sample? How will the biopsy be taken to appropriately reflect the clinical involvement? How many samples are required? What are the stains besides hematoxylin and eosin (H&E) that should be applied? (2) Scope of disease-acute: What are the basic features to diagnose rejection? What other features should be recorded and how? What should be excluded from acute rejection? (3) Lesion scoring-acute: How will severity be graded? (4) Scope of disease-chronic: What are the defining features of chronic injury? (5) Scope of disease-humoral: What information should be collected to define this effector mechanism in CTA?

The questions were provided to the participants in both oral and written formats. Oral and written comments were collected throughout the consensus discussion session. This article represents the recompilation of the discussions including all oral and written comments.

Specimen Adequacy

Allografts that include skin are distinctive in that rejection can be recognized by visual inspection. To include this unique feature of CTA, the clinical involvement as assessed visually at the time of biopsy or rejection should be reported as no visible changes, $<10\%$, 10–50% and $>50\%$ of the CTA. Features include but are not limited to rash, edema, erythema, vesiculation, desquamation, necrosis and/or ulceration. To diagnose and classify skin rejection, specimen adequacy is defined as at least one 4-mm punch biopsy

taken from the most reddened and/or indurated but apparently viable area of involved skin. Only one biopsy is required for diagnosis, to avoid unnecessary scarring, especially with multiple episodes of rejection. The structures required to constitute an adequate sample are the epidermis and its adnexa, dermis, subcutaneous tissue and vessels. The recommendations for slide preparation are hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stains. Immunohistochemical stains are also recognized as potentially important and are thus recommended "as needed" based on H&E findings and/or for research purposes. These included but are not limited to CD3, CD4, CD8, CD19, CD20 and CD68, as well as HLA-DR, CMV and C4d. The use of trichrome stain is not considered mandatory at this time but could be performed if desired.

Acute Cell-Mediated Rejection

The basic features to diagnose and classify rejection requiring specific comment in diagnostic reports are immune cell infiltration, and epidermal and/or adnexal involvement namely spongiosis, apoptosis, dyskeratosis and necrosis. The cellular infiltrate can be mixed (e.g. including neutrophils) and not limited to lymphocytes. The pattern of the infiltrate should be characterized as perivascular or interstitial, focal or diffuse and dermal and/or hypodermal. Early signs of rejection may include the presence of scattered dermal infiltrates. Interface inflammation/dermatitis is an important feature to identify, as this may relate to the severity of the rejection or may signal a nonrejection etiology. Infiltration of eosinophils should be recorded descriptively but is not included in the current classification. This will allow the study of its significance in the future.

As in other pathologies in which ulceration or necrosis develops, vasculitis may be either primary or secondary to the ulceration. Indications of rejection-related vasculitis include: absence of a history of trauma; involvement of vessels distant from the ulcer; multi-focality of the necrotizing process within the affected vessel; and involvement of several vessels within the biopsy, particularly vessels of various sizes and depths within the dermis. The pathologic and clinical features of immune and nonimmune processes are potentially overlapping and will require further study. Because there is insufficient data to absolutely exclude nonimmune conditions from a particular CTA biopsy, a descriptive observation is currently the appropriate format for reporting findings. As with solid organ transplants, other inflammatory, infectious or neoplastic processes may coincide with acute rejection.

The Banff CTA 2007 Classification for Cell-Mediated Acute Rejection of Skin

The acute/active skin rejection scoring system was divided in five grades, based on intensity and localization of infiltrates. The rejection classification is shown in Table 1.

Chronic Rejection

Currently, insufficient data are available to define specific changes of chronic rejection in a CTA. Chronic changes and injury to an allograft evolve over time with persistent immune insult and are likely to be altered in tempo and character by concomitant treatment. Fibrosing changes can also be caused by nonimmune events, and in certain circumstances both can overlap. Histologic and clinical features highlighted as indicative of chronic injury in a CTA include vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes. As with other solid organs, it is likely that chronic/persistent injury begets a common histological phenotype through a variety of nonexclusive mechanisms. A possible correlation between graft-versus-host disease (GVHD) and CTA-skin was noted.

Antibody-Mediated Rejection (AMR)

There is not enough information to draw any conclusions regarding AMR. However, several pieces of histologic and clinical information should be gathered in order to define AMR in CTA. These include the presence of C4d deposition and its relationship with donor-HLA-specific antibodies as well as the presence of vasculitis, neutrophilic margination, thrombi and necrosis. A complete history including patient sensitization (e.g. PRA, cross-match results, transfusions, pregnancies and previous allografts) as well as the presence or absence of autoantibodies and T- and B-cell crossmatch is to be performed before transplantation. The correlation between graft dysfunction and rejection in CTAs has not been established. Therefore, clinical evidence of graft dysfunction is not included at this point.

Table 1: The Banff 2007 working classification of skin-containing composite tissue allograft pathology

Grade 0. No or rare inflammatory infiltrates.
Grade I. Mild. Mild perivascular infiltration. No involvement of the overlying epidermis.
Grade II. Moderate. Moderate-to-severe perivascular inflammation with or without mild epidermal and/or adnexal involvement (limited to spongiosis and exocytosis). No epidermal dyskeratosis or apoptosis.
Grade III. Severe. Dense inflammation and epidermal involvement with epithelial apoptosis, dyskeratosis and/or keratinolysis.
Grade IV. Necrotizing acute rejection. Frank necrosis of epidermis or other skin structures.

Table 2: Differential diagnosis in skin allograft biopsies

Infections
Posttransplant lymphoproliferative disorder (PTLD)/lymphoma
Insect bites
Drug reactions/toxicity
Eosinophilic dermatitis
Graft vs. host disease
Allergic or irritant contact dermatitis
Other

Related/Nonrejection Pathology

It was recognized that skin changes in a CTA are not limited to alloimmune injury (8). Specific differential diagnoses to consider include; infections (particularly fungal); drug toxicity (e.g. topical steroids or other drugs); posttransplant lymphoproliferative diseases (PTLD)/lymphoma; insect bites; GVHD; allergic or irritant contact dermatitis and eosinophilic cellulitides (see Table 2). Detailed description of these processes are beyond the scope of this manuscript, but should be identified in reporting CTA pathology.

Observations

With this international effort, we have initiated an international consensus approach that will progress over time. Standardized reporting of results should advance research related to CTA. Common methods of data collection facilitate clinical interpretation, communication between clinicians and pathologists and prospective data compilation for future studies. As a working classification, the schema will continue to evolve and develop as more scientific information becomes available for skin and other tissues included in CTA.

This new international classification follows the published systems, which adopted a tiered approach to grading rejection. Tiered systems tend to differentiate levels of severity by the addition of a new lesion at each level. In this classification, the first lesion to appear is perivascular inflammation, which is usually mild and focal. In grade II lesions there is expansion of the infiltrate accompanied by involvement of epidermis or adnexa but without dyskeratosis or epidermal apoptosis. Grade III adds these latter features of individual cell injury, while grade IV adds frank necrosis.

Both dermal edema and spongiosis reaction are found in a wide range of disorders including those associated with immunologic abnormalities, infections and neoplasia. Some form of microvascular injury in the dermis is thought to be the initiating phenomena withdermal fluid transiting to the epidermis producing spongiosis. Outside the field of transplantation, cell-mediated and antibody-mediated processes have been implicated in the reaction along with other causes of endothelial/vessel wall injury. Edema (dermal and epidermal spongiosis) is likely to be part of many reactions in the allograft and is probably nonspecific, though the severity and extent of edema may assist in the interpretation of the process. However, further studies are needed in this area.

Consideration was given to the histopathology of cutaneous GVHD as a parallel to alloimmune injury in skin-containing CTA. In 2006, the National Institutes of Health Consensus Development Project Pathology Working Group presented requirements for the diagnosis of chronic GVHD (14). The report presented the progression of histologic changes from acute-to-chronic cutaneous GVHD including four chronic forms: skin lichen planus-like, sclerotic, morpheic and fascitis. The sclerotic stage is characterized by a zone of relatively avascular collagen, which replaces the papillary and upper reticular dermis. Microscopically, there is hyperkeratosis with flattening of rete ridges, vacuolar changes of the basal cell layer and lymphocytic infiltration and epidermal melanin incontinence. The report highlighted the histologic changes in chronic GVHD related to immunosuppressive therapy and underlined the need for studying the significance of perivascular lymphocytic inflammation or persistent vacuolar degeneration after treatment. It is possible that chronic skin changes in CTA might parallel those of chronic cutaneous GVHD and 'chronic rejection' in other types of transplants. Clinicopathologic correlation from prospective data will aid in the assessment of chronic changes in CTA not only for skin but for all tissues involved in a CTA.

The implications of several pathological changes unique to limb transplantation remain to be determined. These include changes associated with the nail bed, which have been suggested to be evidence of chronic persistent immune injury but could also be precipitated by more acute inflammatory invasion of the nail matrix. While the Banff CTA 2007 scoring system focuses on the rejection-related changes, there are a number of other immune and nonimmune mediated processes that must be recognized and contemplated in the differential diagnosis (Table 2) (8).

The Banff CTA 2007 grading scheme for acute rejection is developed enough to have immediate clinical utility. It is likely that, similar to solid organ transplantation, CTAs will undergo indolent chronic changes and a grading of severity of chronic rejection will evolve in this working classification. CTAs that contain skin are unique in that rejection-related changes can be directly observed. Indeed, it has been shown that significant perivascular infiltration appears coincident with a skin rash (13,15–16). Areas of scleroderma resulting from chronic infiltration and injury have been noted at times in the absence of significant infiltrates (Charles W. Hewitt, personal communication, La Coruña, Spain, 26, June 2007). However, given the heterogeneity of CTAs, and the potential that repeated

inflammation could manifest as chronic dysfunction, it is important to begin cataloging the histology of CTA rejection in objective terms. The visualization of skin changes can be used as a clinical indicator of rejection; however, the sensitivity and specificity of rash and/or other sign as markers of rejection remains to be established, as does the evidence of histological response to therapy. To this end, the clinical percentage of gross graft involvement has been added as a starting point for correlation between clinical presentation and severity of rejection.

The fundamental biology underlying CTA is sufficiently similar to that of other solid organs that additional phenotypes of rejection will appear as the clinical experience grows, including AMR, chronic fibrosis/atrophy and vascular rejection. These will be addressed at subsequent congresses and characterized based on accumulated experience in the field. As an emerging field, many questions remain unanswered, and there is ample opportunity for clinical and basic investigation. Future directions include the characterization of the infiltrating cells and their function, the study of accommodation, chronic injury and AMR, the utility of molecular studies and the inflammatory response in this complex transplant. This Banff CTA-2007 classification is an international effort to lay the groundwork to advance the understanding of CTA pathology. It will enhance the communication between investigators and will contribute to clinical analysis.

Acknowledgments

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(updated April 19, 2005)

World Health Organization PTLD Classification of 2001						
Category	**Examples**	**Histopathology**	**Immunophenotype**	**Clonal Status**	**Oncogene, Tumor Suppressor Gene Changes**	**Comments**
"Early" lesions	Reactive plasmacytic hyperplasia (PH) infectious mononucleosis-like PTLD	Some architectural preservation; numerous plasma cells and lymphocytes;variable paracortical expansion; many immunoblasts may be present; atypia slight; some cases may have overlapping features of PH and IM-PTLD	Polyclonal B cells, plasma cells and T cells. Immunoblasts often EBV-positive	Polyclonal; EBV present in most case of PH- IM cases typically EBV positive, may have minor monoclonal or oligoclonal bands	(None)	Often regress with reduced immunosuppression, severe cases may be fatal Examples of posttransplant plasmacytic hyperplasia without EBV should not be considered as PTLD.
Polymorphic PTLD	Polymorphic B cell hyperplasia, Polymorphic B cell lymphoma	Destruction of underlying architecture, full range of B-cell maturation seen, may have necrosis, scattered large bizarre cells (atypical immunoblasts), frequent mitoses, may have monomorphic areas	Mixture of B and T lymphocytes, surface and cytoplasmic Ig polytypic or monotypic; most cases EBV positive	Monoclonal; Rare cases may be polyclonal	None	Overall impression of mixed small and large cell lymphoma or polymorphous immunocytoma; may be multiple; Some cases regress with reduced immunosuppression, others may progress
Monomorphic PTLD	**B-cell neoplasms:** Diffuse large B-cell lymphoma, Burkitt's or Burkitt-like lymphoma, plasma cell myeloma, plasmacytoma-like lesions **T-cell neoplasms:** Peripheral T-cell lymphoma, not otherwise specified; other types	Morphological lymphomas; classify according to current lymphoma categorization; most to all cells transformed, blastic (plasma cell leisons excepted); most look like diffuse large B-cell lymphoma, other types less common; Monomorphic T-cell PTLD probably inlcudes most or all types of T-cell neoplasms	B cell PTLD show CD19, 20, 79a; monotypic Ig expressoin in 50%; Many express CD43, CD45RO (due to upregulation of these T cell markers in B cells harboring EBV); CD30 often postive; most EBV pos. T cell PTLD may express CD4 or 8, CD56, CD30, and alpha-beta or gamma-delta T-cell receptors	Monoclonal Ig genes in B cell PTLD; EBV pos. cases also have clonal EBV; T cell PTLD usu. have clonal T cell receptor; 25% with clonal EBV	Present in some cases	Recommended that these be classified according to standard lymphoma classification, with term " PTLD" added; Monomorphism means that most cells are transformed-cellular monotony may be present but is not required; Regression possible but uncommon compared to early lesions and polymorphic PTLD. Overall mortality 60% solid organ, 80% marrow

						recipients.
Hodgkin lymphoma and Hodgkin lymphoma-like PTLD	Classic HL; Hodgkin-like PTLD	Reed Sternberg cells in appropriate backgound (see comments)	Classic HD CD15, CD30 pos; HD-like PTLD more atypical phenotype, usu B cell antigens expressed; all or almost all cases EBV pos (HD and HD-like)	--	--	Since Reed-Sternberg-like cells can be seen in polymorphic PTLD, diagnosis requires appropriate morphologic and immunophenotypic features

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The Clinicopathologic Spectrum of Posttransplantation Lymphoproliferative Disorders

Lawrence Tsao, MD; Eric D. Hsi, MD

• ***Context.*—Posttransplantation lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphoid proliferations occurring in the setting of solid organ or bone marrow transplantation. They show a clinical, morphologic, and molecular genetic spectrum ranging from reactive polyclonal lesions to frank lymphomas. The close association with Epstein-Barr virus has been established and the pathogenetic role of this virus is becoming better understood. Although they are relatively uncommon, PTLDs are a significant cause of morbidity and mortality in transplant patients.**

***Objective.*—To review the incidence, risk factors, clinical features, pathogenesis, and classification of PTLDs.**

***Data Sources.*—We reviewed relevant articles indexed in PubMed (National Library of Medicine), with emphasis on more recent studies. The classification of PTLDs is based on the most current World Health Organization classification text.**

***Conclusions.*—Posttransplantation lymphoproliferative disorders are a heterogeneous group of disorders showing a wide clinical and morphologic spectrum. Although relatively uncommon, PTLDs represent a serious complication after transplantation. Many risk factors for PTLD are well established, including transplanted organ, age at transplant, and Epstein-Barr virus seronegativity at transplant. However, other factors have been implicated and still require additional examination. Recent studies are shedding some light on the pathogenesis of PTLDs and defining relevant pathways related to Epstein-Barr virus. As the pathogenesis of PTLDs is further elucidated, the classification of PTLDs will most likely evolve.**

(*Arch Pathol Lab Med.* 2007;131:1209–1218)

Posttransplantation lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphoid proliferations occurring in the setting of solid organ or bone marrow transplantation. It has long been known that intact immune systems are required for antitumor surveillance. The occurrence of lymphoma in immunosuppressed transplantation patients was first recognized in 1968 and its close association with Epstein-Barr virus (EBV) infection followed.[1,2] Today we recognize a spectrum of lymphoid proliferations ranging from reactive polyclonal lesions to frank lymphomas. The close association with EBV is well described and the pathogenetic role of this virus is beginning to be understood. However, not all PTLDs are EBV driven, and a significant subset of EBV-negative PTLDs have been identified.[3,4] Although PTLDs represent a relatively uncommon complication in transplant patients, they are a significant cause of morbidity and mortality in these patients. Because of variability in clinical, histopathologic, and immunophenotypic presentations, the diagnosis and classification of PTLDs can be difficult. In this review, we will consider the incidence, risk factors, clinical features, pathogenesis, and histopathology of this group of lymphoproliferative disorders.

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From the Department of Pathology, University of New Mexico, Albuquerque (Dr Tsao); and the Department of Clinical Pathology, Cleveland Clinic, Cleveland, Ohio (Dr Hsi).

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Reprints: Eric D. Hsi, MD, Department of Clinical Pathology, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195 (e-mail: hsie@ccf.org).

EPIDEMIOLOGY

Incidence

Although there is significant variation in the reported incidences of PTLD after solid organ and bone marrow transplantation, the overall incidence is less than 2% of transplanted patients.[5] There is a clear association between the incidence of PTLD and type of transplantation, with the highest incidence in the first year after transplantation.[6] Among the most commonly transplanted solid organs, cardiac, lung, and hepatic transplantation show the highest incidences of PTLD, ranging from 2% to 5%,[7–9] 2% to 3%,[10,11] and 2% to 5%,[12–14] respectively. The incidence after pancreatic transplantation was recently reported to be 2.1%.[15] Renal transplants show a much lower incidence of PTLD at approximately 1%.[16,17] This may be because of the generally lower intensity of immunosuppression required compared with that of other vital organs. The incidence of PTLD after bone marrow transplantation ranges from 0.5% to 1.0%.[18,19] In recent larger series, the incidence of PTLD appears to be lower than previously cited, possibly the result of better management of immunosuppression (Table 1). Because of the higher risk of PTLD in children (discussion follows), studies examining pediatric populations will generally report incidences 2- to 3-fold higher than in adults.

Risk Factors

Several risk factors for the development of PTLD have been identified (Table 2). These include type of organ

Table 1. Incidence of Posttransplantation Lymphoproliferative Disorders in Organ Transplant in Selected Recent Studies

Organ	Incidence, %	Source, y
Kidney	~1	Caillard et al,[16] 2006
Pancreas	2.1	Paraskevas et al,[15] 2005
Heart	2.3*	Swerdlow et al,[7] 2000
Liver	4.3†	Jain et al,[14] 2002
Lung	2.5	Reams et al,[10] 2003

* Up to 5% in pediatric populations.[8]
† Up to 10% in pediatric populations.[14]

Table 2. Risk Factors for Posttransplantation Lymphoproliferative Disorders

Transplanted organ (multivisceral > lung > liver > heart > kidney)
Pediatric age group
Epstein-Barr virus seronegativity
Immunosuppressive drugs/regimen (OKT3)
Underlying host disease
Cytokine gene polymorphisms

transplanted, immunosuppressive drugs, age of the patient, and EBV status pretransplantation. As previously noted, the incidence of PTLD varies by transplanted organ, with the renal transplants having the lowest incidence, heart transplants having intermediate incidence, and heart-lung or multivisceral transplantation generally having the highest incidence.[20] Large collaborative databases have defined relative risks of PTLD for major organ types.[6] Specific biologic factors may account for these differences. For example, lung and intestinal transplants typically include the highest amount of lymphoid tissue, which may increase the EBV infection rates. Relative ease of mucosal biopsies in these sites may also raise the incidence of early PTLD detection.

The variation in the incidence between the different types of transplanted organs may also be related to varying degrees of immunosuppression necessary for each organ. Specific drugs have been implicated as high-risk factors. In the early days of transplant, use of the potent immunosuppressive OKT3 resulted in a marked increase in PTLDs in cardiac transplant.[21] Use of cyclosporine also increased the incidence of PTLD; however, this could be reduced by careful therapeutic monitoring to avoid overimmunosuppression.[22,23] Although immunosuppression is a major risk factor, it is still unclear whether the contribution is due to the cumulative dose or peak levels of drugs. Some studies, unable to identify any specific agents, have suggested the cumulative immunosuppressive dose to be the contributory factor.[24–27] In bone marrow transplantation, T-cell depletion of the donor bone marrow is a well-known risk factor for PTLD.[28–30] However, studies of newer immunosuppressive agents targeting T cells have not always conclusively demonstrated similar increased risk in solid organ transplantations.[24] Experience with these newer immunosuppressive agents may help define the magnitude of risk for a PTLD associated with their use.

Mismatch of EBV status in the recipient and donor (seronegative recipient with seropositive donor) is another well-known risk factor for PTLD and is intimately associated with the pathogenesis of PTLD.[26–33] In one striking study of a single institution's experience with solid organ transplantation, seronegative patients had a 76-fold risk of PTLD compared with seropositive patients.[34] The higher risk associated with EBV-naive patients also explains, to some extent, the higher incidence of PTLD among pediatric transplant patients.[35] Not surprisingly, EBV-naive patients will frequently present initially with EBV-associated PTLD of the early lesion or polymorphic type, possibly representing an abnormal primary EBV response in these immunosuppressed patients.

A patient's underlying disease has been suggested in some series to be a risk factor for PTLD. Primary immunodeficiency showed a 2.5-fold increased risk in one bone marrow transplant series.[18] Patients with hepatitis C infection,[9,36] autoimmune hepatitis,[37] cystic fibrosis,[38] and Langerhans cell histiocytosis[39] have also been suggested to be at higher risk for PTLD. Other infectious agents including cytomegalovirus,[27,40] human herpes virus 8,[41] and, recently, simian virus 40[42] have all been reported in cases of PTLD and may contribute to increased risk. The number and severity of rejection episodes and degree of HLA mismatching have also been examined as risk factors. However, the magnitude of risk these factors pose is still controversial.

Innate host immune responses may also play a role in the development of PTLD. Cytokine gene polymorphisms associated with regulation of cytokine production during immune responses are being examined. Specifically, there is some evidence that low interferon gamma production may be associated with increased risk of PTLD in liver and renal transplant patients.[43,44]

CLINICAL FEATURES

The clinical presentation of PTLD is highly variable, depending on the type of immunosuppression, type of allograft, and histologic type of PTLD (early lesions, polymorphic PTLD, or monomorphic PTLD). Patients may present with infectious mononucleosis-like symptoms. There is frequent involvement of the tonsillar tissue and Waldeyer ring, especially in pediatric patients. Such PTLDs often have the histology of so-called early lesions. Monomorphic PTLD, like lymphoma, can present with constitutional symptoms, lymphadenopathy, and mass lesions. Up to 25% of patients may present with allograft failure due to involvement by PTLD. In these patients, the clinical presentation can mimic allograft rejection. In bone marrow transplants, widespread involvement is common and may simulate graft-versus-host disease. Bone marrow involvement may present with new-onset or persistent cytopenias. Polymorphic PTLD may present with features overlapping early lesions and monomorphic PTLD. As a result of the variability of presentation, a high index of suspicion must be present in any patient with a history of transplantation.

PATHOGENESIS

Investigations have yielded insight into the pathogenesis of PTLD. Phenotypic and immunoglobulin mutational studies have resulted in a model of histogenesis for PTLD. Molecular studies have supported this model and have identified several genes thought to be important in molecular pathogenesis. Epstein-Barr virus infection, of course, plays a central role in development of PTLD and recent work has also elucidated important mechanisms of oncogenesis relevant to these proliferations.

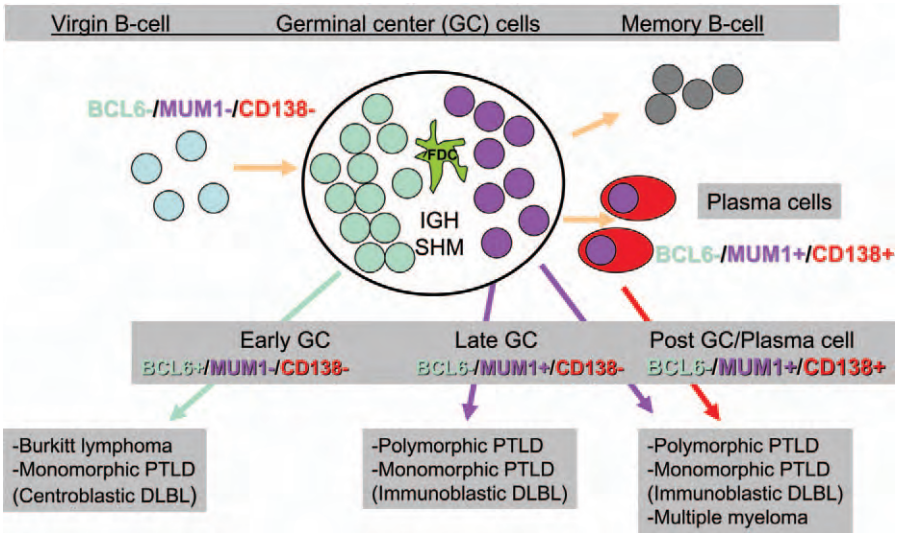

Figure 1. *Model of posttransplantation lymphoproliferative disorder (PTLD) histogenesis. Adapted from Capello et al[46] with permission from John Wiley & Sons Limited, 2005.*

Histogenesis

Like B-cell non-Hodgkin lymphomas, molecular and phenotypic features of PTLD have been compared with normal B-cell counterparts. Analysis of immunoglobulin heavy chain variable (*IGHV*) genes is a powerful tool in determining the maturational state of B cells. According to this model, unmutated (germline) genes represent antigen-naive (pregerminal center or virgin) B cells, and B cells harboring somatic hypermutation have been exposed to the germinal center (GC) microenvironment and thus represent GC or post-GC B cells.[45] Approximately 25% of polymorphic PTLDs and 10% of diffuse large B-cell lymphomas (DLBLs) have unmutated (germline) *IGHV.* Burkitt lymphoma (BL) and 25% of centroblastic DLBL show ongoing somatic hypermutation, consistent with GC B cells. The majority of polymorphic (75%) and monomorphic (65%) PTLDs show somatic hypermutation that is stable among clones, suggesting a late GC or post-GC phenotype. Thus, most PTLDs derive from GC or post-GC cells.[46] The few PTLDs that lack somatic hypermutation appear to arise early after transplantation and are EBV-associated. They may derive from true pre-GC cells or cells that are incapable of undergoing the GC reaction.[46] A recent study that analyzed both immunoglobulin heavy and light chain genes showed that 94% of PTLDs had somatic hypermutation.[47]

Further phenotypic characterization into GC and post-GC stages using Bcl-6 (GC marker), MUM1 (late GC and post-GC), and CD138 (post-GC, terminal differentiation) has resulted in the model shown in Figure 1.[46] The Bcl-6$^+$/MUM1$^-$/CD138$^-$ PTLDs derive from cells experiencing the GC reaction. They harbor ongoing mutations and morphologically correspond often to centroblastic types of DLBL or BL. A Bcl-6$^-$/MUM1$^+$/CD138$^-$ phenotype corresponds to PTLDs that derive from B cells that have completed the GC reaction and include most (65%) polymorphic and some (30%) monomorphic PTLDs, particularly DLBL with immunoblastic features. This phenotype is uncommon in human immunodeficiency virus–related lymphoma.[48,49] A third phenotype, Bcl-6$^-$/MUM1$^+$/CD138$^+$, represents post-GC cells and includes polymorphic or monomorphic PTLDs showing immunoblastic DLBL morphology or plasmacytic differentiation.

Antigen Stimulation and Viral Oncogenesis

Analysis of immunoglobulin gene usage provides evidence that specific antigen stimulation and selection may not play a major role in the pathogenesis. In an analysis of 50 PTLDs, no preferential use of *IGHV* family genes was noted, suggesting a lack of specific pathogenetic antigen. Evidence of antigen selection in tumor cells based on replacement mutations in the complementarity determining regions of the immunoglobulin heavy chain gene (*IGH*) was seen in less than 30% of cases.[47] In fact, up to 50% of PTLDs have lost the ability to express functional immunoglobulin.[47–51]

Epstein-Barr virus infection, in the setting of immunosuppression, has a central role in the pathogenesis of PTLD. Several lines of evidence can be offered. It is present in almost all PTLDs that occur early after transplant.[52,53] It is also frequently clonally integrated in tumor cells of polymorphic and monomorphic PTLDs, indicating that it was present at the time of malignant transformation.[54] Increasing EBV titers can also be detected in the blood of patients prior to development of PTLD and treatment with EBV-specific T cells can result in tumor reduction.[55–59] Finally, EBV latent genes have transforming activity in B cells. In fact, EBV has been found to transform GC cells lacking immunoglobulin.[60] The exact mechanism of viral oncogenesis is yet to be elucidated; however, EBV latent membrane proteins (LMP), LMP-1 and LMP-2A, have been the focus of attention. These oncogenic proteins activate intracellular signaling pathways, mimicking CD40 (a member of the tumor necrosis factor receptor family) and B-cell receptor signals.[61] Latent membrane protein 1 has been shown in PTLD tissue to mimic activated tumor necrosis factor receptor family members through tumor necrosis factor receptor-associated factors. This results in downstream activation of NFκB, an important transcription factor that activates prosurvival genes.[62,63]

Although most PTLDs are EBV related, approximately 20% of patients with PTLD will lack evidence of EBV in their tumors, and the incidence may be increasing.[3] The EBV-positive and EBV-negative PTLDs show differences in clinical course and may represent independent entities.[3,4] Specifically, EBV-negative PTLDs appear to occur late after transplantation, are more often classified as

monomorphic compared with EBV-positive PTLDs, and generally have an aggressive course. However, some will still respond to decreased immunosuppression. Given the relative rarity of these tumors, the pathogenesis of these EBV-negative PTLDs is still poorly understood. Currently, the question of whether these are better considered coincidental lymphomas or part of the heterogeneity of PTLDs remains unanswered.

Genetic Alterations

Several genetic alterations in oncogenes or tumor suppressor genes have been found in PTLDs. These include *MYC, BCL6, NRAS,* and *TP53.*[64–66] Chromosomal translocations involving *MYC* and mutations in *MYC, BCL6, NRAS*, and *TP53* have been described.[64–66] Alterations in *MYC, NRAS*, and *TP53* are uncommon and seen only in monomorphic (immunoblastic lymphoma histology) or multiple myeloma types of PTLDs and are never present in polymorphic lesions.[66] Rearrangement of *BCL6* is very uncommon in PTLD as opposed to DLBL in immunocompetent patients. However, *BCL6* mutations are common (approximately 50%), and have been associated with shorter survival and nonresponsiveness to reduced immunosuppression.[64] Rearrangements of *MYC* have also been associated with more aggressive disease and poor outcome.[67] Microsatellite instability has been described in a higher proportion of PTLDs than in non-Hodgkin lymphoma from immunocompetent hosts, corresponding to the high degree of genetic instability in PTLDs.[68]

Recently, epigenetic alterations have been examined. In particular, hypermethylation of O6-methylguanine-DNA methyltransferase (*MGMT*), a DNA repair gene, has been found in 60% of monomorphic PTLD. Inactivation of *MGMT* has been shown to be lymphomagenic in knockout mice and may promote genetic instability with acquisition of *TP53* and *RAS* mutations.[69,70] Other genes identified as abnormally methylated include death-associated protein kinase (*DAPK1*), a proapoptotic molecule, and *TP73*, a putative tumor suppressor gene related to *TP53.*[69] Much work remains to be done and new tools such as array-based comparative genomic hybridization studies have identified other abnormalities.[71] However, the exact role of these abnormalities in the development of PTLD remains largely unknown.

Donor Versus Host Origin

Studies on the cell of origin of PTLD have shown that at least 90% of PTLDs originate from host B cells in solid organ transplantation.[72] The converse is true for bone marrow transplantation.[73] Although donor-derived PTLDs have been reported with increased incidence in liver and lung transplants, with suggestions of predilection for involving the graft, recent studies have been controversial.[72–76] The prognostic significance of donor versus host-derived PTLD is unclear.[76] In addition, there have been no large-scale studies examining T-cell and natural killer (NK) cell PTLDs.

PATHOLOGIC FEATURES AND CLASSIFICATION

Classification of PTLD is currently based on the World Health Organization (WHO) system for classifying hematopoietic neoplasms.[77] The key morphologic, immunophenotypic, and molecular characteristics of each type of PTLD are listed in Table 3. The WHO divides PTLD into 4 major categories: early lesions, polymorphic PTLD, monomorphic PTLD, and Hodgkin lymphoma (HL) and HL-like PTLD. Early lesions, polymorphic PTLD, and monomorphic PTLD represent a pathologic spectrum that can be observed synchronously or metachronously within a single specimen or within multiple specimens from a single patient.

Early Lesions

Early lesions consist of 2 morphologic types: plasmacytic hyperplasia and infectious mononucleosis–like PTLD. The common defining characteristic of early lesions is some degree of preservation of the underlying architecture of the involved tissue (Figure 2, A). Plasmacytic hyperplasia is a lesion characterized by numerous plasma cells with rare immunoblasts. Infectious mononucleosis–like lesions resemble typical infectious mononucleosis, with marked paracortical expansion by a mixed T-cell and plasma cell infiltrate and a prominent immunoblastic proliferation. Some early lesions may show overlapping features between plasmacytic hyperplasia and infectious mononucleosis–like lesions.

Immunophenotyping of early lesions is of limited diagnostic utility as it will confirm the morphologic impression of variable mixtures of B cells, T cells, and plasma cells with polytypic light chain expression. Immunoblasts will frequently show evidence of EBV infection using in situ hybridization for EBV-encoded RNA (EBER) or EBV LMP-1 immunohistochemical stain. Other EBV-associated nuclear antigens (ie, EBV-encoded nuclear antigen, LMP) are not reliably expressed.[78] As the name implies, early lesions represent the earliest morphologic and genotypic changes of PTLD.[66] These lesions occur early (<1 year) in the course of transplantation and are more common in EBV-naive pediatric and adult transplant recipients. Analysis of *IGH* and episomal EBV genome will frequently yield polyclonal or oligoclonal patterns. Occasionally, a minor clone is seen, but is of no clinical significance. Clonal cytogenetic changes are rare in early lesions.[67,79]

Polymorphic Lesions

Polymorphic PTLD is characterized by a mixed lymphoproliferation consisting of immunoblasts, plasma cells, and intermediate-sized lymphoid cells. In contrast to early lesions, polymorphic PTLD is characterized by destruction of the underlying architecture of the involved tissue (Figure 2, B). However, in contrast to monomorphic PTLD, polymorphic PTLD shows a full spectrum of B cells from small to intermediate-sized lymphocytes to immunoblasts and mature plasma cells (Figure 2, C). Atypia, necrosis, and numerous mitotic figures are all acceptable. In the past, these features of ''malignancy'' were used to distinguish ''polymorphic lymphoma'' from ''polymorphic hyperplasia.''[80] However, subdividing polymorphic PTLD is no longer necessary under the WHO classification because recent findings revealed that morphologic subdivision does not reliably predict clinical behavior.[66,81] Immunophenotyping of polymorphic PTLD will show variable mixtures of B cells and T cells. Analysis of surface or cytoplasmic immunoglobulin expression is useful for identifying monotypic B-cell populations. However, B cells may show polytypic immunoglobulin expression in polymorphic PTLD. Most polymorphic PTLDs will show EBV latency II and III patterns, expressing EBER and EBV–LMP-1 with variable expression of EBV-encoded nuclear antigen 2 and other viral antigens.[78] Although immuno-

Table 3. Summary of Pathologic Features of Posttransplantation Lymphoproliferative Disorders (PTLDs)*

Subtype	Morphology	Immunophenotype	Molecular	EBV Status
Early lesion	Preservation of the underlying architecture	Mixture of B, T, and plasma cells	IgH: polyclonal or oligoclonal EBV: polyclonal or oligoclonal	(+), virtually all
IM-like	Increased numbers of immunoblasts	$CD30^+$ immunoblasts will be present	See early lesion	(+), virtually all immunoblasts $EBER^+$
Plasma cell hyperplasia	Large aggregates and sheets of plasma cells	κ and λ show polytypic plasma cells	See early lesion	(+), majority; occasionally can be (−)
Polymorphic	Some degree of effacement of underlying architecture with a spectrum of lymphoid cells ranging from small lymphocytes to intermediate to immunoblasts	B-cell markers may highlight the spectrum of B cells present CD30 will highlight immunoblasts	IgH: clonal EBV: clonal	(+), majority; variable numbers of $EBER^+$ cells
Monomorphic	Effacement of underlying architecture with cytologic atypia sufficient for a lymphoma	Varies with lineage	Varies with lineage	Varies with lineage
B cell	Majority will resemble diffuse large B-cell lymphoma A subset may resemble Burkitt lymphoma	Positive for B-cell markers, but can show abnormal phenotype (ie, aberrant expression or loss of antigens) Burkitt immunophenotype ($CD20^+$, $CD10^+$, $CD43^+$, Bcl-6^+, Bcl-2^-, Ki-67: ~100%)	IgH: clonal EBV: clonal	(+), majority; large numbers of $EBER^+$ cells
T/NK cell	Varies with type	Varies with type (WHO T-cell lymphomas) Pan–T-cell antigens should be evaluated for aberrant loss	T cell: TCR: clonal NK cell: TCR: germline EBV: clonal (if present)	(−), majority of T cell (+), virtually all NK cell
Plasma cell myeloma Plasmacytoma	Sheets of plasma cells Must be differentiated for early lesion	Positive for plasma cell markers κ and λ show monotypic plasma cells	IgH: clonal EBV: clonal (if present)	Variable
HL and HL-like	RS cells in the classic HL milieu	HL: classic HL immunophenotype ($CD30^+$, $CD15^+$, $CD45^-$, $CD20^{-/+}$, $CD3^-$, weak PAX-5) HL-like: aberrant immunophenotype (ie, $CD20^+$)	IgH: varies EBV: clonal (if present)	(+), majority; RS cells $EBER^+$
MALT-type PTLD	Lymphoid infiltrate of small, mature-appearing lymphocytes expanding underlying mucosa and submucosa Lymphocytes show slightly irregular nuclei with moderate amounts of pale cytoplasm	Similar to MALT-type lymphomas in immunocompetent patients ($CD20^+$, $CD5^-$, $CD10^-$, $CD43^{+/-}$)	IgH: clonal	(−), majority *H pylori* associated

* EBV indicates Epstein-Barr virus; IgH, immunoglobulin heavy chain; IM, infectious mononucleosis; EBER, EBV-encoded RNA; NK, natural killer; WHO, World Health Organization; TCR, T-cell receptor; HL, Hodgkin lymphoma; RS, Reed-Sternberg; MALT, mucosa-associated lymphoid tissue; *H pylori, Helicobacter pylori*.

phenotyping may appear polytypic, molecular analysis of *IGH* or episomal EBV genome will usually show a clonal pattern.[52,81] Clonal cytogenetic changes may be present.[67,79]

Monomorphic Lesions

Monomorphic PTLDs are characterized by architectural and cytologic atypia sufficient to be classified as a lymphoma based on morphologic features.[77] In general, monomorphic PTLDs show invasion and architectural effacement by large aggregates and confluent sheets of transformed cells with large nuclei with prominent nucleoli (Figure 2, D and E). The neoplastic cells can show marked pleomorphism or plasmacytoid/plasma cell differentiation. These cases of monomorphic PTLD generally are not diagnostically problematic. However, occasional cases of PTLD may span the spectrum of polymorphic PTLD and monomorphic PTLD. These cases are difficult to classify within a single category. The presence of areas of monomorphic PTLD, however, should always be clearly indicated.

Monomorphic PTLDs are divided according to B-cell or T-cell lineage and further subclassified according to the WHO classification of lymphomas in the nontransplant population.[77] It is beyond the scope of this review to include a detailed description of the WHO classification of lymphomas, so only a general description will be included with areas of difficulty highlighted.

Monomorphic B-cell PTLD (B-PTLD) is the prototypic monomorphic PTLD. The majority of the B-PTLDs will resemble DLBL in nontransplant patients. Morphologic variants include immunoblastic, centroblastic, and, less commonly, anaplastic morphology. However, as with DLBL, there does not appear to be any clinical significance

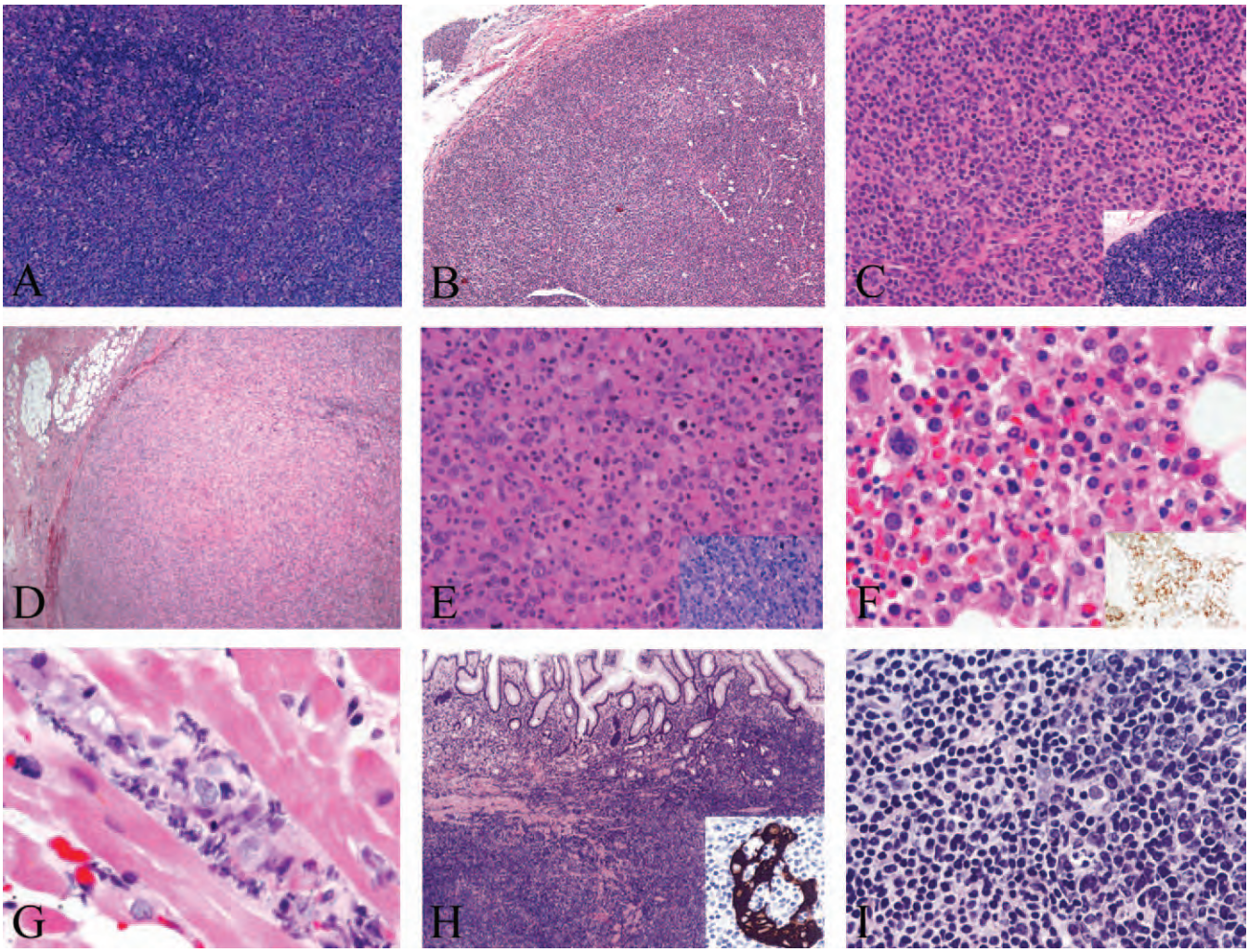

Figure 2. *A, Infectious mononucleosis–like early lesion with marked paracortical expansion surrounding a follicle (hematoxylin-eosin [H&E], original magnification ×20). B, Polymorphic posttransplantation lymphoproliferative disorder (PTLD) showing effacement of the underlying lymph node architecture (H&E, original magnification ×20). C, High magnification of B showing lymphoid cells with minimal cytologic atypia and showing a spectrum of sizes (H&E, original magnification ×200). There are small lymphocytes, scattered immunoblasts, and numerous intermediate-sized cells, which is characteristic of polymorphic PTLDs. The inset shows Epstein-Barr virus (EBV)–encoded RNA in situ hybridization (EBER-ISH) highlighting the numerous EBV-positive cells (original magnification ×200). D, Monomorphic PTLD also showing a destructive pattern, similar to polymorphic PTLDs (H&E, original magnification ×20). E, However, high-power magnification of D shows a predominance of large pleomorphic cells with marked cytologic atypia, characteristic of monomorphic PTLDs (H&E, original magnification ×400). Inset of EBER-ISH also shows EBV in most neoplastic cells (original magnification ×200). F, Plasma cell myeloma PTLD with numerous infiltrating plasma cells in a bone marrow biopsy (H&E, original magnification ×400). Inset of CD138 highlights the infiltrating plasma cells (original magnification ×40). G, T-cell PTLD involving the allograft as a perivascular lymphoid infiltrate with marked cytologic atypia (H&E, original magnification ×400). Immunophenotyping (not shown) is necessary to confirm the T-cell lineage. H, Gastric low-grade muscosa-associated lymphoid tissue type of PTLD expanding the mucosa and submucosa (H&E, original magnification ×20). Inset of a cytokeratin stain highlights characteristic "lymphoepithelial" lesions (H&E, original magnification ×400). I, Marginal zone cells (left) with slightly irregular nuclei and moderate amounts of pale cytoplasm adjacent to and infiltrating a reactive germinal center (right) (H&E, original magnification ×400).*

associated with these morphologic variants. Morphologic resemblance to BL or atypical BL is diagnostically significant and should be confirmed with immunophenotypic and cytogenetic studies. Immunophenotypic analysis will show expression of B-cell antigens or a BL phenotype in cases of BL or atypical BL. Antigens aberrantly expressed by conventional DLBL (ie, CD43, Bcl-2) may be present. Surface immunoglobulin expression may be monotypic or absent. Currently, the evaluation of GC or post-GC phenotype is not required because the clinical and prognostic implications are still uncertain.[45–48] The majority of B-PTLDs show presence of EBV infection within the transformed cells, with variable latency patterns.[82] Virtually all cases show a clonal pattern of *IGH* rearrangement and, if present, episomal EBV genomes. Cytogenetic evaluation will show clonal karyotypic abnormalities, which can include trisomies 9 and/or 11 and abnormalities of 8q24.1, 3q27, and 14q32.[67]

Rare cases of B-PTLD are morphologically and immunophenotypically identical to plasma cell neoplasms (Figure 2, F).[83,84] Plasma cell myeloma and plasmacytoma-like PTLD can also be EBV associated in about 50% of the cases reported.[83,84] Clinically, these can present as rare extramedullary plasmacytic neoplasms similar to plasmacytomas or plasma cell myeloma. Plasma cell PTLDs need to be differentiated from plasmacytic hyperplasia, a non-

destructive early lesion, and DLBL with marked plasmacytic differentiation, a monomorphic PTLD. Because of the rarity of plasma cell PTLD, it is currently unclear if plasma cell directed, B-cell directed, or both, is the most effective therapy. The evaluation for urine and serum M components, serum immunoglobulin levels, and lytic bone lesions, although not always conclusive, can be helpful in the diagnosis of plasma cell myeloma-PTLD.[83] Immunophenotypic evaluation of B-PTLD should include B-cell and plasma cell–associated antigens.

T-cell PTLDs (T-PTLDs) are all classified as monomorphic PTLDs and must show a similar degree of architectural and cytologic atypia required for B-PTLD (Figure 2, G). The T-PTLDs are subclassified according to the WHO classification for T-cell neoplasms in the nontransplant setting. Immunophenotyping is essential for diagnosis and subtyping of T-PTLD. Depending on the subtype, the immunophenotype will vary. Evaluation of pan–T-cell antigens, although not always conclusive, is useful for demonstrating any aberrant losses of expression. CD4 or CD8 expression and αβ or γδ T-cell receptor expression will follow what is generally known for T-cell lymphomas in nonimmunosuppressed patients. Markers of immaturity (CD1a, TdT, and CD34) can be seen in cases of precursor T lymphoblastic lymphoma. CD30 expression can be present, especially in the anaplastic large cell lymphoma subtypes. CD56 and cytotoxic markers can be expressed by T-PTLD. Most (60%–80%) T-PTLDs lack EBV; however, a minor subset may be EBV positive.[85] Molecular analysis of the T-cell receptor (*TCR*) gene should show a clonal pattern. Analysis of episomal EBV genome is usually not indicated, but will show a clonal pattern when EBV is present. True NK-cell PTLD will frequently express CD56 and cytotoxic markers, but must lack surface CD3. Variable expression of pan–T-cell antigens, CD2 and CD7, can be seen. Unlike T-PTLD, the vast majority (80%–90%) of true NK-cell PTLD shows EBV infection with clonal episomal EBV genome.[86] Molecular analysis of *TCR* must show a germline pattern to be diagnosed as a true NK-cell PTLD.

Hodgkin Lymphoma and Hodgkin Lymphoma–like Lesion

Hodgkin lymphoma and HL-like PTLD is a rare category of PTLD that is classified independently from other monomorphic PTLDs. Hodgkin lymphoma PTLD shows the morphologic features characteristic of classic HL in nontransplant patients. These include the proper background inflammatory infiltrate and Reed-Sternberg cells. Hodgkin lymphoma–PTLD must be distinguished from polymorphic PTLD with Reed-Sternberg–like cells. Hodgkin lymphoma–PTLD has Reed-Sternberg cells with the classic HL phenotype ($CD45^-$, $CD3^-$, $CD20^-$/$weak^+$, $CD15^{+/-}$, $CD30^+$). These cases usually arise late in transplantation and frequently show evidence of EBV infection. Although currently HL and HL-like PTLD are considered similar, there is evidence suggesting that HL-like PTLD may be more related clinically and pathologically to a monomorphic B-cell PTLD.[87] The HL-like PTLD frequently shows an atypical immunophenotype for HL such as strong expression of CD20.

Low-Grade B-Cell Lymphoproliferative Disorders

The current WHO classification does not recognize low-grade B-cell lymphoproliferative disorders as PTLD. However, they do occur in the posttransplant setting. Extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type occurring as PTLDs morphologically and immunophenotypically resemble their counterparts in immuncompetent patients (Figure 2, H and I).[88,89] These MALT lymphomas do not show evidence of EBV, but are frequently associated with *Helicobacter* organisms, especially in gastric sites.[88] Molecular analysis of *IGH* will show a clonal pattern.[89] Other low-grade B-cell lymphoproliferative disorders reported after transplantation include hairy cell leukemia.[90] This is extremely rare. Clinically, morphologically, and immunophenotypically, these cases are identical to those seen in the nontransplant setting and may represent coincidental events.

Clinical Course

The clinical course of PTLD is highly variable and dependent on the type of PTLD, the lineage of the PTLD, and the association with EBV. Virtually all early lesions regress with reduction in immunosuppression and generally show good prognosis, especially in pediatric patients.[81,91] About half of polymorphic PTLDs regress with reduction of immunosuppression; however, some will progress, requiring chemotherapy.[19,81] Of those progressing, more than half will respond to therapy.[81] Some studies have found the presence of *BCL6* gene mutations to predict poor response to reduction of immunosuppression.[64] The majority of monomorphic PTLDs do not regress with reduction of immunosuppression alone. In addition, some monomorphic PTLDs do not show good response even to chemotherapy.[81] Among the monomorphic PTLDs, EBV-associated PTLDs consistently have a better prognosis when compared with EBV-negative PTLDs.[3,4] However, a minor subset, up to one-third, of EBV-negative PTLDs have been reported to regress with reduction of immunotherapy.[3] Monomorphic PTLDs of T-cell/NK-cell lineage almost never regress with reduction of immunosuppression alone and respond poorly to chemotherapy.

Hodgkin lymphoma and HL-like PTLDs usually arise late after transplantation (>1 year). The prognosis of HL and HL-like PTLDs appears relatively good. In one large study, none of 60 patients developing HL and HL-like-PTLD died of PTLD-associated causes.[84]

The low-grade B-cell lymphoproliferative disorders, specifically MALT lymphomas, are also usually seen late after transplantation (>1 year).[88,89] Clinically, the MALT lymphomas behave indolently. The majority of cases can be managed by eradication of the *Helicobacter* organisms and conservative management (localized radiation, surgery, and single-agent chemotherapy).[88,89] The rare cases of hairy cell leukemia reported after transplantation have shown an indolent course and excellent response to conventional hairy cell leukemia therapy.[90]

DIAGNOSIS

The timely and accurate diagnosis of PTLD is essential for early intervention. However, a high clinical index of suspicion is required. Recently, monitoring and quantification of EBV viral load in peripheral blood has been shown to be helpful in predicting the development of PTLDs. Although clear guidelines have yet to be established regarding laboratory procedures and management, the trends are clear. Persistently low EBV viral load has good negative predictive value for development of EBV-positive PTLD. The EBV levels appear to increase prior to PTLD and fall after successful therapy.[92] In an attempt to

define important thresholds, a level of 200 copies/10^5 leukocytes was shown to correlate with symptomatic EBV infection or PTLD in pediatric transplant patients.[93,94] Some investigators have also suggested preemptive therapy with agents such as rituximab.[95,96] However, because not all patients with elevated levels develop PTLD and EBV-negative PTLDs cannot be predicted with such a test, further work is needed to precisely define the role of EBV viral load testing in transplant patient populations.[97,98] Recent studies have suggested using cytokine genotyping in addition to EBV viral loads to increase the predictive value for PTLDs.[99]

PATHOLOGIC EVALUATION: WHAT NEEDS TO BE DONE?

Practically speaking, excisional biopsies of masses or enlarged lymph nodes are preferred because one of the characteristic differentiating features between early lesions and polymorphic and monomorphic PTLD relies on the ability to document preservation of underlying architecture. Extranodal disease is common. Involved sites may include the gastrointestinal tract, liver, lung, and bone marrow. If endoscopic or needle biopsies are used, several biopsies or passes are advised to obtain adequate tissue for ancillary studies.

When multiple sites of involvement are present, sampling of several lesions should be considered as early, polymorphic, and monomorphic lesions can be synchronously present in different sites. In addition, because synchronous lesions may actually represent different clonal proliferations, separate work-up at the genetic level (ie, molecular analysis of *IGH* gene) may be of interest for follow-up purposes.[100] In patients with allograft involvement where rejection enters into the clinical differential diagnosis, allograft biopsies can help differentiate rejection from PTLD. Assessment of EBV can be helpful because PTLDs are often positive, whereas EBV is absent in rejection. Overall, focusing the diagnostic evaluation on the basis of organ dysfunction or a mass lesion provides the highest yield for obtaining adequate tissue for diagnosis. Screening blood or bone marrow evaluations in patients suspected of PTLD is usually of low diagnostic yield.

Immunophenotyping PTLDs is essential because of the significant differences in prognosis and therapy between B-cell and NK/T-cell lymphomas. Evaluation for presence of EBV by immunohistochemical or molecular techniques is also essential because of the differences in prognosis between the EBV-positive and EBV-negative cases. The EBER in situ hybridization is preferred, given its presence in all latency patterns. Although not absolutely required for diagnosis in the majority of cases, testing for clonality (usually by antigen receptor-rearrangement studies) is also helpful for complete characterization and can be used for comparison to simultaneous or future PTLDs. A distinct clone at a later date would suggest a new independent PTLD rather than a relapse. Cytogenetic studies, also not necessary, similarly may be helpful. Assessment of oncogene mutations or translocations, by molecular or cytogenetic techniques, is currently not routinely performed. Diagnostically, the type of PTLD, lineage of the PTLD (if a monomorphic lesion), clonal status, and EBV status should be clearly indicated in the pathology report.

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Conference Report

Tumors and Solid Organ Transplantation: Intersections at Multiple Levels

Highlights from American Society of Transplant Surgeons 3rd Annual Winter Symposium: Tumors and Transplantation; Miami Beach, Florida; January 24-26

Michael A. Nalesnik, MD

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Introduction

Malignancy is a problem that requires careful consideration in the evaluation of organ donors and transplant recipients both before and after transplantation. Utilization of organs from donors with malignancy, allocation of organs to transplant candidates with a history of malignancy, and factors related to development and treatment of recurrent or *de novo* posttransplant neoplasias serve as referential points from which to raise a number of issues. The application of immunotherapy, particularly cell therapy, to both infectious disease and neoplasia in the solid organ transplant population is undergoing a period of rapid development. These and additional topics were discussed and debated at this symposium.

Neoplasia in the Donor

Cancer may be transferred from an organ donor to a recipient as a result of occult malignancy in the donated organ or misdiagnosis, in a known situation such as when a small renal cell carcinoma occurs in the donor kidney and undergoes wide excision, in a low-risk situation such as when the donor has a known skin malignancy or carcinoma in situ of the cervix, or when a known cancer was believed to have been cured. In addition, *de novo* malignancy may arise from either graft parenchymal cells or passenger cells in the graft. Transmission of donor-related malignancies was examined from multiple perspectives, beginning with insight by Joseph Buell, MD,[1] of the University of Cincinnati, from the Israel Penn International Transplant Tumor Registry (IPITTR).[2] This voluntary registry was instituted in 1969 and provides both current and historical perspectives.

In the past, many types of tumors have been considered absolute contraindications to organ donation. Today, however, 2 tumors -- choriocarcinoma (93% transmission, 64% mortality) and melanoma (74% transmission, 60% mortality) -- fall into this category. In addition, since lung cancers and sarcomas, particularly high-grade variants, also behave aggressively, procuring organs from donors with these tumors is not recommended.

Renal Cell Carcinoma

A different picture emerged from the data on renal cell carcinoma (RCC) in the overall IPITTR

series. Recipients had a high (61%) rate of tumor development, but a lower mortality rate (23%), if they received a graft from a patient with renal cell carcinoma. Furthermore, 14 cases of renal transplantation in which tumors in the donors were identified and excised prior to transplantation were reported. All tumors were </= 4 cm in size (median 2 cm) with negative resection margins and Fuhrman grade I-II/VI histology based on rapid permanent histologic sections. No tumor development had been reported at a median follow-up time of 69 months.

Renal cell carcinomas are more frequently detected as small lesions; this allows for the resection of earlier-stage tumors and improved survival. It was also noted that some renal tumors such as oncocytomas or angiomyolipomas are benign, regardless of size, and may be excised, whereupon the remaining kidney can be used for transplantation.[3]

The reason that donors with cancer are even considered is the current organ shortage, according to L.Thomas Chin, MD,[4] of the University of Wisconsin, Madison, and Arthur I. Sagalowsky, MD,[5] of the University of Southwestern Texas, Dallas. The risk of cancer recurrence (in the donor) following partial nephrectomy for tumors < 4 cm in diameter was estimated at 0% to 3%. The incidence of bilateral involvement was estimated at 10% to 20% for familial or papillary RCC, and 2% to 4% for sporadic clear cell RCC (Fuhrman nuclear grade I-II).

Metastatic RCC may occur in tumors < 3 cm in diameter, and may occur in the donor as late as 20 years after the primary disease.[4,5] Most investigators suggest that donors should be disease-free for at least 2-5 years, and potential recipients with prior RCC should be disease-free for at least 2 years before transplantation.

Central Nervous System (CNS) Tumors

CNS tumors accounted for 21% of all cases in the IPITTR. Four risk factors for tumor transmission were identified: high-grade (grade III-IV) histology, previous surgery, radiation therapy, and chemotherapy. The rate of tumor transmission was 7% in the absence of risk factors, but rose to 30% to 40% in the presence of 1 or more of these conditions. Development of CNS tumors in recipients was associated with a high mortality rate; the Cincinnati group advocates limited autopsies of donors with intracerebral hemorrhage of unknown etiology to rule out CNS tumors.

Interpretation of the literature on CNS tumors is difficult for a number of reasons: different pathologic terms may be applied to the same tumors, some series do not report histologic types of CNS tumors, and some tumors may show different grades of differentiation either in a synchronous or metachronous fashion. The spread of glioblastoma multiforme (GBM) recapitulates normal glial cell development, and the tumor invades not by lymphatic or vascular spread, but by migration of individual cells within the CNS, observed Eric C. Holland, MD, PhD,[6] of Memorial Sloan-Kettering Cancer Center, New York, NY. Mass lesions disrupt the blood-brain barrier, which in turn raises the question of spread of tumor cells outside of the CNS even prior to surgery or other forms of therapy. Almost all GBM have Ras activation and 70% have hyperactivity of Akt and downstream activation of mTOR, thought to promote tumor cell survival. Sirolimus and its derivatives have potent mTOR inhibitory activity, and blockade of the Akt-mTOR pathway has antitumor activity in other models. The possibility of a beneficial side effect of sirolimus in this circumstance was raised, although animal models suggest that this approach alone will likely not be curative for these tumors.

The antiangiogenic effects of sirolimus in relation to its mTOR inhibitory function were discussed by Edward K. Geissler, PhD,[7] of the University of Regensburg, Germany.[8] He indicated that this antitumor effect was active at immunosuppressive doses, and suggested that sirolimus may be an exception to the rule that immunosuppressive drugs favor tumor development.

A wide variation (0.5% to 18%) in transmission rates for GBM is reported. The possibility that rates may also vary among different organs was raised as a point worthy of study. The present unfortunate circumstance is that approximately 130,000 patients die annually from GBM in the United States. Continued exploration of specific circumstances associated with minimal risk of tumor transmission may allow some of these individuals to serve as organ donors in the future.

Breast and Colon Cancer

Using recent cancer screening figures[9] and current donation rates, Reid B. Adams, MD,[10] of the University of Virginia, Charlottsville, estimated that inclusion of patients with stage 0-1 breast or colon cancer would result in a mere 9-10 additional donors per year. It was pointed out that a higher proportion of cancers might be detected at early stages in the future, potentially increasing the number of potential donors.

Whether or not organs from such patients are suitable for transplantation is currently unknown, and both historical registry data and data concerning stage-specific cancer survival are starting points from which to estimate the risks of tumor transmission. Older series, comprised mainly of patients with advanced-stage cancers, indicated a transmission rate of 6% for breast cancer and 25% for colon cancer.

On the basis of more recent figures, it was suggested that patients with stage 0 or 1 colon cancer might be considered as organ donors following definitive treatment resolution of their tumors. White males could donate immediately after therapy, whereas an interval of 5 years might be required for females or black males, as predicated by current survival figures.

In the case of breast cancer, patients with stage 0 tumors, excluding those with high-risk features such as extensive carcinoma in situ, might be considered for organ donation at any time following definitive therapy. Patients with stage 1 T1a or T1b tumors could be considered after 10 years of disease-free follow-up. Patients with T1c or higher-stage breast cancer are not considered acceptable donors.

Prostate Cancer

Prostate cancer in the donor was addressed by Stephen C. Jacobs, MD,[3] University of Maryland, Baltimore. The use of widespread prostate specific antigen screening and early biopsy has led to prolonged survival in patients with prostate carcinoma, particularly in the case of low-grade histology. Dr. Jacobs observed that, given the near universal occurrence of this cancer with increased age, many transplants are undoubtedly performed using organs from donors with occult prostate carcinoma. If the patient with prostate cancer is a renal transplant candidate, therapies such as brachytherapy or cryosurgery that spare the bladder from radiation exposure might be considered. There have been no cancer-related deaths at this time in his series of renal transplant recipients who developed prostate carcinoma.

Metastatic Disease

The use of organs from donors with metastatic carcinoma of any type is associated with a high rate of tumor transmission and should be avoided, noted H. Myron Kauffman, MD,[11] consultant for the United Network for Organ Sharing (UNOS), in his perspective on donor malignancy based on UNOS Tumor Registry data.[12] An analysis from this database revealed that the frequency of tumors in donors was 0.04% with an overall transmission rate of 0.016%. It was noted that, even in the case of good-prognosis RCC, the risk for recurrence in the nonimmunosuppressed population was 9.6% after 5 years. This figure has implications for both donors and potential recipients with a history of this tumor. Similarly, the recurrence rate for melanoma in the original

host was up to 1% after 15 years. The risk of cancer transmission must be balanced against the need for life-saving transplantation, but UNOS cautions against using donors with a history of certain types of cancer including choriocarcinoma; melanoma; lymphoma; GBM; medulloblastoma; or cancers of the lung, kidney, breast, or colon.

Recommendations

The risk of tumor transmission must be weighed against the risk of death without transplantation and the benefits of organ transplantation on a case-by-case basis. The patient must be fully informed and involved in the decision to consider use of an organ from a donor with a possible malignancy. Full disclosure to and involvement of the patient at every step of this process was a theme echoed by a number of the speakers.

Neoplasia in the Transplant Candidate and Recipient

Solid organ transplant recipients are at particular risk for the development of cancer (recurrent and *de novo*) after transplantation; a major factor is the effect of immunosuppressive drug therapy necessary to prevent organ rejection. The overall recurrence rate of tumors in transplant recipients with preexistent cancer is 21%, according to data from the IPITTR by E. Steven Woodle, MD, of the University of Cincinnati, Ohio.[13] Cancers with a low (</= 8%) recurrence rate include uterine, cervical, testicular, thyroid, and early RCC. "Incidental" asymptomatic RCC had a recurrence rate of 8% with a 3% mortality rate, whereas symptomatic RCC had a 43% recurrence and 26% mortality rate. Patients with RCC had a significant rate of recurrent disease even after a 5-year disease-free interval prior to transplantation. Patients with prior prostate cancer had a recurrence rate of 18% and a tumor-related mortality rate of 7.8%. The risk of recurrence of stage 3 cancer was more than double the risk of stage 1 or 2 cancer. The recurrence rate for bladder cancer was 18% with a mortality rate of 12%. The overall recurrence rate of breast cancer was 14% with a mortality rate of 8%. Patients with stage 1 or 2 disease had favorable survival compared with patients with stage 3 disease. The recurrence rate of vulvar cancer decreased after a 10-year wait, but the same was not true for cervical cancer. The overall recurrence rate of melanoma was 21%, with lower frequency of recurrence for lower-grade tumors. The tumor recurrence rate in patients with colon cancer (23%) was higher in thoracic vs nonthoracic organ transplant recipients. It was suggested that this might relate to differences in immunosuppression levels.

Data on the increased risk of cancer in transplant recipients from the Surveillance Epidemiology and End Results registry and the Scientific Registry of Transplant Recipients data for Southeast Michigan were presented by Friedrich K. Port, MD, MS,[14] of the University of Michigan, Ann Arbor. The standardized incidence ratio (SIR) was defined as the observed/expected numbers of tumors in transplant recipients compared with age-matched and region-matched controls. By this definition, there was a 2-fold increase in solid tumors (confidence interval [CI] = 1.6-2.4) and a 4.3-fold increase in lymphomas (CI = 2.4-7.0) in transplant recipients compared with lymphomas and leukemia in controls. There were no significant differences in tumor incidences when analyzed by allograft type. Among solid tumors, those arising in the kidney, vulva, or colon appeared to have a high SIR.

Evaluation of Transplant Candidates

UNOS data show that the overall cancer recurrence rates in kidney, liver, and heart transplant recipients are 1.1%, 6.5%, and 2%, respectively. For all organ types, the risk is lower than the risk of developing *de novo* tumors, which is 8%, 5%, and 14%, respectively, in these groups.

Integrating cancer screening into the evaluation of transplant candidates was advocated by

Sundaram Hariharan, MD,[15] of the Medical College of Wisconsin, Milwaukee. While many types of cancer cannot be screened for or detected at an early stage, the major types of cancer encountered after transplantation can be screened for. At this time it is not clear what constitutes optimal screening or the optimal periods of time between cancer treatment and transplantation. However, disease-free delay periods were suggested, taking into consideration probable times of relapse:

- No delay for incidental RCC;
- Delay of 0-2 years for nonmelanoma skin cancer;
- Delay of 2 years for cancers of the bladder, prostate, uterus, melanoma, or Wilms' tumor with appropriate cytogenetic support;
- Delay of 2-5 years for cervical cancer, breast cancer, RCC (2-5 cm in diameter), and lymphoma; and
- Delay of 5 years for colorectal cancer or RCC > 5 cm in diameter.

Despite the availability of reliable and relatively simple and inexpensive screening tests for early detection, advanced cancer represents a major health risk after transplantation.

Skin Cancer

Forty percent to 70% of patients develop skin cancers within 20 years after transplantation, according to Clark C. Otley, MD,[16] of the Mayo Clinic, Rochester, Minnesota. The most common skin cancer, squamous cell carcinoma (SCC), has a 7% metastatic rate and 56% 3-year survival following metastasis. Integration of dermatologic consultation into patient follow-up could aid in identification of high-risk patients, early diagnosis of cancerous and precancerous lesions, and effective patient education regarding preventive measures. A variety of therapies, including topical retinoids, serial chemotherapy, biological response modifiers, and Mohs surgery, can be used in individual cases. Dr. Otley encouraged physicians and other transplant professionals to use the International Transplant Skin Cancer Collaborative as a resource for information and consultation on skin cancer in their patients.[17]

The implications of skin tumors in organ transplant recipients were further elucidated by Stuart J. Salasche, MD,[18] University of Arizona, Tucson. Since transplant recipients have a tendency to develop multiple and aggressive forms of SCC more often than the general population does, a high degree of vigilance was recommended. Fair skin, ultraviolet exposure, duration of immunosuppression, human papillomavirus (HPV) infection, and less common conditions such as osteomyelitis are risk factors for development of skin cancer. Patients with preexistent SCC may be at increased risk for multiple recurrence and metastasis. Delaying transplantation for 2-3 years in patients at high risk for metastatic SCC was suggested, given the poor prognosis associated with this type of cancer.

A similar argument was made in general for melanoma, which is associated with a disproportionately high percentage of deaths. However, early-stage disease is associated with good survival. Stage 1 disease may be curable, but a small percentage of patients have demonstrated recurrent disease up to 15 years after transplantation. Individual prognostic factors need to be weighed when considering the option of organ transplantation in the patient with melanoma. Merkel cell carcinoma is also prone to recurrence and metastasis after transplantation. It was recommended that patients be carefully assessed, preferably with dermatologic consultation. It has been observed that, unfortunately, only 14% of renal transplant recipients receive dermatologic follow-up.

Hepatocellular Carcinoma (HCC)

Assessment of patients with HCC for liver transplantation in the setting of the Model for End-Stage Liver Disease (MELD) criteria[19] was discussed by Richard B. Freeman, MD,[20] of Tufts University, Boston, Massachusetts. This scoring system went into effect in the United States on February 27, 2000, and incorporates measures of bilirubin, INR (international normalized ratio for coagulation testing), and serum creatinine. It results in a score of 6-40, is predictive of death within 3 months, and is modified for pediatric patients (Pediatric End-Stage Liver Disease [PELD]).

In most cases, liver transplantation is preferable to resection of HCC due to underlying cirrhosis, but a significant problem is tumor progression and/or death while awaiting transplantation. Using the Milan criteria,[21] 3-year posttransplant survival was 83% with only 8% recurrence if transplantation was performed for a single HCC < 5 cm in diameter, or for up to 3 separate HCC lesions, each < 3 cm in diameter. In response, the UNOS/Organ Procurement Transplant Network Liver Committee assigned a priority MELD weight of 24-29 points to patients with HCC who met the Milan criteria while awaiting transplantation. Under this system, transplantation in patients with HCC increased 3.5-fold over a corresponding time interval from the prior year; 86% to 91% of patients with HCC received a transplant within 3 months of being issued a priority MELD score based on a combination of liver disease and tumor. There has been no detectable trend toward increased use of priority scores to obtain transplants on a preferential basis. Analysis of the Milan criteria and other systems will continue in an effort to refine criteria for entry of patients with HCC into the liver transplant waiting list. The continuation of studies such as this is necessary to assure the most equitable system of organ distribution possible.

A model for staging HCC based on fraction of allelic loss to define the probability of posttransplant recurrence of HCC in liver transplant candidates with preexistent HCC was described by Wallis Marsh, MD,[22] of the University of Pittsburgh, Pittsburgh, Pennsylvania. Using this approach, Dr. Marsh and colleagues were able to categorize 91 of 103 patients; the model was 100% accurate in 81 evaluable patients. This model has potential to replace the staging system for HCC based on differentiation status. It appears to be an excellent way to determine who will and who will not have recurrent HCC, and this information could be used to decide who and who not to transplant.

Cholangiocarcinoma

Liver transplantation of patients with cholangiocarcinoma has historically been controversial. Charles B. Rosen, MD,[23] of the Mayo Clinic, Rochester, Minnesota, and C. Wright Pinson, MD,[24] of Vanderbilt University, Nashville, Tennessee, debated this issue. Dr. Rosen (pro) reported on a subset of patients with early-stage disease enrolled in a protocol of pretreatment by radiation therapy and chemosensitization between 1993 and 2001. Fifteen of 41 enrolled patients survived 1-9 years after transplantation, 14 of these disease-free. According to Dr. Rosen, hilar cholangiocarcinoma is emerging as an indication for liver transplantation in patients receiving neoadjuvant therapy. Dr. Pinson (con) countered that transplanting patients with cholangiocarcinoma is a misappropriation of an already taxed donor supply, with limited return. Further objections included the high rate of patient dropout from the treatment protocol cited due to advanced disease or toxicity, the similarity of survival rates with those of resection for early-stage cholangiocarcinoma in some series, and the added variable of posttransplant immunosuppression. Despite these reservations, Dr. Rosen and Dr. Pinson concurred that continued development of this investigational protocol in selected expert centers is warranted in efforts to improve the survival for patients with these tumors.

Lung Cancer

The cure rate of resected stage 1 lung cancer is 70% to 80%, but only 15% of patients with lung cancer are diagnosed at this stage, and the overall cure rate is only 12%. Low-dose helical

computed tomography (CT) scan detects early-stage lung tumor nodules at least 3 times more frequently than chest x-ray, noted J. Michael DiMaio, MD,[25] University of Texas/Southwestern Medical School, Dallas. Dr. DiMaio's group performs annual CT scans in heart transplant recipients with a >/= 10 pack-year smoking history. They noted that enforcement of smoking cessation in transplant candidates prior to transplantation is one method of reducing a known risk factor for cancer, and this may have benefit as lung cancer typically arises a number of years after transplantation.

Cancer Screening

Prevention and early detection of cancer in transplant recipients is not a routine part of posttransplant care. Bryce Kiberd, MD,[26] of Dalhousie University, Halifax, Nova Scotia, and William M. Bennett, MD,[27] of Oregon Health Sciences University, Portland, encouraged transplant clinicians to incorporate American Cancer Society guidelines on screening and surveillance for cancer into patient follow-up. Questions were raised, however, regarding the effectiveness and potential harm of cancer screening in transplant recipients with limited life expectancy, since it can take at least 5 years before screening has an impact on survival. Invasive procedures such as colonoscopy might have a higher morbidity in immunocompromised patients, and screening tests such as stool guaiac could have a higher false-positive rate in patients with multiple sources of blood loss. On the basis of these concerns, it was suggested that no screening was required for patients with a life expectancy less than 5-7 years, but that screening be recommended for those with life expectancies exceeding 10-12 years. Patients in between should be informed of the relative risks and benefits of particular screening procedures. Cancer-specific rates of death are required to develop more objective criteria for refining cancer screening recommendations for transplant recipients.

Immunity, Viruses, and Neoplasia

T lymphocytes are a central component of the host immune response to viral infection and some cancers, and viruses are important cofactors in the development of some cancers, particularly in the immunosuppressed patient. Work in the area of T cell-based therapy of cytomegalovirus (CMV) and melanoma was summarized by Philip D. Greenberg, MD,[28] of the University of Washington, Seattle.[29] His group has taken the approach of expanding antigen-specific T-cell lines in vitro and infusing these into immunocompromised patients to provide cellular immunity against specific targets. This therapy has been applied primarily to hematopoietic stem cell transplant recipients, who may develop fatal CMV disease during periods of immunodeficiency.

T cells derived from the original donor are raised against CMV over a 6- to 8-week period and can provide protective immunity against CMV infection when infused into the recipient. Cytotoxic $CD8^+$ cells provide immune effector activity, and long-lived immunity requires support from $CD4^+$ T cells. In Dr. Greenberg's study, side effects were mild. Current studies seek to modify the glucocorticoid receptor of the infused cells, as high-dose corticosteroids (such as might be given for rejection or graft-vs-host disease) normally cause lysis of lymphocytes leading to loss of effector function. Modification of T-cell receptors to introduce antiviral specificities into T cells from CMV-seronegative organ transplant recipients is also developing as a viable strategy to generate a rapid and specific antiviral response.

A modified approach is necessary for antitumor immunity, as tumors may actively downregulate or even destroy invading T cells. Using the melanoma model, this group has shown that CD8+ T-cell activity can be enhanced and prolonged in the setting of concurrent low-dose interleukin (IL)-2 support as a surrogate for CD4+ help. Despite initially effective killing, the tumor undergoes phenotypic evolution to shed the target antigen, leading to an antigen-deficient subclone. One strategy to circumvent this tumor evasion is to identify antigens that are indispensable to the malignant phenotype. Several such potential targets have been identified, but these often have

poor immunogenic capacity. To counter this, modified T-cell receptors exhibiting a higher antigen affinity for these targets, together with modified granulocyte macrophage colony-stimulating factor receptors capable of generating a signal for IL-2 production, have been developed and inserted into CD8+ T cells in vitro. Other changes that lower the energy of activation of the T-cell receptor also lead to endogenous IL-2 production. Such approaches can lead to the development of antitumor T-cell reagents with predefined specificities, and may ultimately result in antitumor reagents that could be produced in advance and administered directly at time of therapy.

James E. Sligh, MD, PhD,[30] of Vanderbilt University, Nashville, reviewed the association of HPV and skin cancer. HPV normally causes cutaneous warts. However, certain conditions, such as epidermodysplasia verruciformis, are associated with an antiviral immune defect and lead to numerous warts that can progress to malignancy, particularly in areas exposed to ultraviolet light. HPV with a high risk for cancer development (most commonly type 16 or 18) integrates its DNA into the host genome, in contrast to low-risk HPV, which persists as separate episomes. This may underlie differences in expression of the viral oncoproteins E6 and E7. These proteins have been shown to immortalize human keratinocytes in vitro and interfere with the tumor suppressor activity of the cell cycle proteins p53 and Rb. E6 protein may also protect against UV-induced apoptosis. Approximately half of all organ transplant recipients will develop warts by 5 years posttransplantation. This patient population also carries high-risk papillomavirus strains in the lower genital tract, more commonly than do nonimmunocompromised individuals. Thus, screening for premalignant lesions of the skin and genital tract was emphasized as a routine component of transplant recipient follow-up.

Donald Ganem, MD,[31] of the University of California, San Francisco, provided insight into the pathogenesis of Kaposi's sarcoma (KS) by contrasting the etiologic agent (KSHV, HHV-8) with other transforming gamma herpes viruses. He noted that KSHV is likely not a fully immortalizing virus and could even be lost from infected cells. He proposed that the lytic portion of the life cycle might contribute to KS by providing paracrine factors to stimulate angiogenesis and inflammation, by recruiting additional infected cells to the lesion to replace apoptotic cells, and by reinfecting cells that had lost their viral episomes.

Murine models of hepatocellular carcinogenesis, particularly transgenic mice with expression of either hepatitis virus proteins or growth factors, were reviewed by Ravi S. Chari, MD,[32] of Vanderbilt University. Numerous strains transgenic for hepatitis B virus (HBV) proteins have been developed, and these animals show variation in tumor characteristics. Tumors are also associated with mice transgenic for expression of hepatitis C virus proteins. Resultant tumors show expression of viral core, but not envelope proteins, suggesting the former as important for carcinogenesis. Mice transgenic for hepatocyte growth factor or transforming growth factor alpha (TGF-alpha) also develop HCC with a high frequency. In the case of TGF-alpha, mice transgenic for both this gene and for either c-myc or HBV surface antigen show increased hepatocarcinogenesis indicating synergism between the genes and likely mimicking the clinical condition more closely. P53 knockout mice develop liver tumors when treated with diethylnitrosamine, but these are largely angiosarcomas.

Posttransplant Lymphoproliferative Disorders (PTLD)

Cliona Rooney, PhD,[33] of Baylor College of Medicine, Houston, Texas, used the biology of the Epstein-Barr virus (EBV) as a foundation for understanding PTLD and other EBV-related neoplasias. This infection normally reaches an asymptomatic steady state in which largely latent viral-infected cells are controlled by a combination of virus-specific T cells and neutralizing antibody. In immunosuppressed transplant recipients, T-cell immunosuppression can allow outgrowth of viral-infected cells that express a wide range of EBV latency-associated proteins. Dr. Rooney's group has used donor-derived EBV-specific T cells to prevent and treat PTLD in bone

marrow and stem cell recipients.[34]

Hodgkin's disease in nonimmunosuppressed patients is an example of an EBV-associated neoplasia in which tumor cells circumvent the immune response by downregulating a number of viral antigens and by producing locally immunosuppressive molecules. Some genetic modifications to counteract this include: engineering T cells to contain an immunodominant TGF-beta receptor to allow function in the presence of normally inhibitory levels of TGF-beta, and the use of IL-12-secreting T cells to counteract the local Th2 microenvironment produced by tumor secretion of IL-13 and the chemokine thymus and activation-regulated chemokine (TARC).

The pathologic classification of PTLD was outlined by Michael A. Nalesnik, MD,[35] of the University of Pittsburgh. The evolution of the classification systems was traced, leading up to the present World Health Organization (WHO) 2001 classification that divides PTLD into 4 categories: (1) early lesions (including infectious mononucleosis-like and reactive plasmacytic hyperplasia lesions), (2) polymorphic PTLD, (3) monomorphic or lymphomatous PTLD (including B- and T-cell neoplasms), and (4) Hodgkin lymphoma/Hodgkin-like PTLD. For additional information on these classifications, go to the Transplant Pathology Internet Services Web site.[36] "PTLD" is a generic term, and subclassification is crucial for appropriate selection of therapy. Evaluation should include histopathologic, phenotypic, clonal, and virologic assessment. The majority of polymorphic PTLDs are clonal proliferations, but may still be capable of regression with reduced immunosuppression, noted Dr. Nalesnik.

There has been an increase in the relative frequency of EBV-negative PTLD in recent years. In some instances, these have been linked to *Helicobacter* infection, and treatment may lead to resolution of the lymphoproliferation. Recurrent PTLD may represent true recurrence or separate tumors, and biopsy is desirable in this setting. Douglas W. Hanto, MD,[37] of the Harvard Medical School, Boston, Massachusetts, focused on the polyclonal-to-monoclonal transition that occurs in the development of PTLD, and classified PTLD into 4 conditions: (1) posttransplant infectious mononucleosis, (2) benign polyclonal B-cell hyperplasia, (3) early malignant transformation in polymorphic B-cell lymphoma, and (4) monoclonal polymorphic B-cell lymphoma; these correspond to the first 2 general categories of the WHO classification. Dr. Hanto stressed the need for a multimodal approach to diagnosis, in particular emphasizing the importance of CD20 and EBV assessment. He noted that mononucleosis might resolve without therapy in some cases, but requires antiviral therapy and reduced immunosuppression in others. Therapy must be individualized, and combinations of reduced immunosuppression, antiviral agents, and intravenous immune globulin in polyclonal disease were suggested. In some cases, CD20 monoclonal antibodies (mAb) and alpha interferon might have a role. He recommended reduced immunosuppression and anti-CD20 mAb or cytotoxic chemotherapy in cases of monoclonal disease, with surgical resection and radiotherapy used as appropriate. Prospective multicenter studies are required for development of optimal treatment algorithms.

Current applications of therapeutic algorithms for PTLD were described by Steven A. Webber, MD,[38] of the University of Pittsburgh. Dr. Webber underscored the role of reduced immunosuppression and pointed out the potential shortcomings such as rebound acute rejection and early chronic rejection. The advocacy of anti-CD20 mAb and/or chemotherapy as initial therapy requires prospective clinical trials for evaluation. Since a proportion of monomorphic PTLD may regress under the proper conditions, there is a need to define markers to separate these from other monomorphic tumors likely to require chemotherapy for resolution.

Two additional therapies that are used without proof of clinical efficacy are antiviral and immune globulin agents. He advocated continued use of these agents based on clinical experience, until objective criteria for their use are established. Questions regarding the extent and length of immunosuppression reduction as well as reinstitution of antirejection therapy are currently based largely on clinical judgment by the individual physician. In the EBV-positive PTLD of pediatric

patients, EBV genomic titers in peripheral blood are a useful marker of disease activity. However, few data exist in the adult population, and this marker is likely of little value in EBV-negative tumors.[39] Given the promising results of anti-CD20 mAb, a prospective multicenter trial addressing the role of this agent at the time of initial diagnosis is being planned.

The use of chemotherapy in the treatment of PTLD was summarized by Thomas G. Gross, MD, PhD,[40] of Ohio State University, Columbus. He observed that in most published series, regimens designed for conventional non-Hodgkin's lymphomas (cyclophosphamide, doxorubicin, vincristine and prednisone [CHOP]; ProMace-CytaBOM; etc.) were used for refractory disease or in patients in whom immunosuppression could not be reduced. He estimated a 50% long-term relapse-free survival using this approach. In order to reduce systemic toxicity and minimize the effect of chemotherapy on antiviral immunity, his group used low-dose cyclophosphamide (600 mg/m^2) and prednisone (2 mg/kg/day) for 5 days in a multicenter pilot study of treatment of refractory PTLD in children.[41] This regimen, termed "CHOP-Lite", led to complete remission in 77%, which rose to 84% if patients with fulminant disseminated disease (which does not respond to any intervention at present) were excluded from analysis. However, the relapse rate was 18% with a 2-year relapse-free survival of 73%. Those patients who did not develop normal anti-EBV immunity appeared to be at greater risk for relapse. He recommended additional studies to optimize this regimen, particularly for fulminant disease, and to evaluate its efficacy in the adult population.

Cellular therapy for the treatment of EBV-associated PTLD[42] was proposed as a treatment by Malcolm K. Brenner, MD,[43] of Baylor College of Medicine. He observed that anti-CD20 antibody appeared to be a significant advance in therapy, but relapses were common in the solid organ transplant population, and in some cases recurrent disease evolved into a CD20-negative phenotype, rendering the antibody treatment ineffective. He reported a greater than 98% success rate in establishing anti-EBV cytotoxic T-cell lines from a series of 300 stem cell or solid organ transplant recipients. He noted that such lines could even be developed from patients who were EBV-seronegative at time of transplantation. Infused cells behave differently in different patient subpopulations, expanding rapidly in stem cell recipients, while growing more slowly in organ transplant recipients. His group has taken the approach of monitoring EBV levels at 2- to 4-week intervals early posttransplantation and utilizing anti-CD20 antibody in cases of PTLD while simultaneously generating cytotoxic T-cell lines, a process that takes 4-6 weeks. The cells are then administered as definitive therapy. With this approach they have had no fatalities from EBV disease in a series of more than 600 solid organ and stem cell transplant recipients.

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Suggested Readings

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Michael A. Nalesnik, MD, Division of Transplantation Pathology, Thomas E. Starzl Transplantation Institute, Pittsburgh, Pennsylvania