Resident Handout

Division of Transplantation Pathology
University of Pittsburgh Medical Center
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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 native 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 potential donor organs and handles all native liver biopsy specimens.

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 and performs frozen sections by clinical request on a 24/7 basis.

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 **9:00 AM** and permanent H&E slides are ready for review by **3:00-3:30 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 (Monday through Friday) for Stats and Quicks and handles all frozen sections between 7:30 AM and 5:00 PM. A second staff pathologist is on "Big" service, which runs from Monday through the following Sunday. This pathologist covers all Consults in the Division (except for consults that are specifically addressed to an individual pathologist), Big specimens and Native kidney specimens from Monday through Friday and covers Frozen sections from 5:01 PM to 7:29 AM the following morning. This pathologist also covers all specimens on the weekend, regardless of type, and is on call for frozen sections throughout the weekend. Holidays are treated like a weekend, in that the pathologist on "Bigs" covers all casework and has call responsibility.

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. It has been the experience of junior residents that attempting to sit in on all signouts of "Bigs", "Quicks", and "Stats" can be a bit overwhelming in light of the short (usually 3 weeks) rotation schedule. The purpose of the rotation is to give you hands on experience with grossing transplant specimens, and with interpreting both native and allograft-based pathology. You are not expected to workup every case that comes through during this time, and your particular schedule will be worked out with the staff at the beginning of the rotation.
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 9:00-9:30 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."

Since the Department has moved to a "Centers of Excellence" format, material from the Transplant Division has been submitted to the Departmental website in accordance with their requirements. This material may be accessed at:
http://aplis.upmc.edu/intranets/COE/xplant/indexXplant.htm.
As part of this initiative, we are required to test the residents at the end of their rotations. The standardized test questions are found online at the above web page. In addition, we give a written and slide test at the end of the rotation. This is not used to grade the resident, it is used as a form of feedback for the resident to identify particular strengths and weaknesses.

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. The cases are maintained by Ms. Jill March (E-736 MUH, 647-9509) and may be signed out while the resident is on this rotation.

We also encourage residents to attend our divisional research conferences and to discuss their research interests and projects with our staff.

Transplantation Pathology on the World-Wide Web and Telepathology

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. 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.

For the past several years, we have been supporting pathologists at Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) in Palermo, Italy through telepathology. More than 300 cases, most involving transplant pathology, have been consulted so far. Centers in Kyushu, Japan and Jerusalem, Israel are now connecting to the system to share cases. The telepathology system was created to meet the specific requirements of transplant pathology, which requires coverage 24 hours day, 7 day a week for frozen sections and organ transplant specific data elements. There are advantages to such a transplantation telepathology system including, increased confidence in the primary diagnosis, access to an expert knowledge base, and access to experience in similar difficult situations. This particular telepathology system is easily adaptable as a core structure for a transplantation telepathology consortium. The Division has initiated the start-up of such a consortium, which would be ideal for sharing interesting cases and information, disseminating useful information, conducting continuing education and coordinating multi-center trials evaluating the efficacy of immunosuppressive and anti-viral drugs where histopathology is used as an endpoint. If you are interested in learning more about this aspect of our practice, you may speak to any of the staff and we would be happy to demonstrate the current system.

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 a partial weekly work schedule. Bigs and consult signouts are more variable and are not listed here. All signouts occur in the signout room, E-733 MUH. 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 signout times are estimates and this schedule may be modified by other conferences and commitments.

Day	Time	Room	Activity
Monday	3:00 – 5:00 p.m.	E-733	Stat signout
	3:00 – 4:00 p.m.	E-724	**Pancreas Transplant Conference**
Tuesday	9:00 – 9:30 a.m.	E-724	**AM Slide Review Conference**
	10:30 – 11:30 a.m.	E-733	Quicks signout
	1:00 – 1:30 p.m.	E-724	**Heart Transplant Conference**
	3:00 – 5:00 p.m.	E-733	Stat signout
	3:00 – 4:00 p.m.	E-724	**Kidney Transplant Conference**
Wednesday	9:00-10:00 a.m.	E-724	**Research Conference** (monthly)
	10:30 – 11:30 a.m.	E-733	Quicks signout
	3:00 – 5:00 p.m.	E-733	Stat signout
	3:00 – 4:00 p.m.	E-724	**Liver Tumor Conference**
Thursday	9:00 – 9:30 a.m.	E-733	**AM Slide Review Conference**
	10:30 – 11:30 a.m.	E-733	Quicks Signout
	3:00 – 5:00 p.m.	E-733	Stat signout
	3:00 – 4:00 p.m.	E-724	**Liver Transplant Conference**
Friday	10:00 – 11:00 a.m.	E-724	**Intestinal Transplant Conference**
	10:30 – 11:30 a.m.	E-733	Quicks signout
	3:00 – 5:00 p.m.	E-733	Stat signout
Saturday	9:00 – 11:00 a.m.	E-733	Bigs, Quicks signout
	2:30 – 3:30 p.m.	E-733	Stats signout
Sunday	10:30 – 11:00 a.m.	E-733	Quicks signout (Staff only)
	2:30 – 3:30 p.m.	E-733	Stats signout (Staff only)

Staff Locations and Telephone Numbers

	Name	Office	Phone	Page	Fax
STAFF	Askren, Linda	E-742 MUH	647-2067		647-2084
	Cappella, Nickie	E-735 MUH	647-8375	13322	647-5237
	Demetris, MD, A. Jake	E-741 MUH	647-2072	2237	647-2084
	Duquesnoy, PhD, Rene	W-1552 BST	624-1075		624-6666
	March, Jill	E-736 MUH	647-9509		647-5237
	Marcoz, Joyce	E-733 MUH	647-7645		647-5237
	Nalesnik, MD, Michael	E-738 MUH	647-2094	2006	647-5237
	Ochoa, MD, Erin	E-739 MUH	647-9568	7952	647-5237
	Randhawa, MD, Parmjeet	E-737 MUH	647-7646	2798	647-5237
	Wu, MD, PhD, Tong	E-740 MUH	647-9504	2795	647-5237
	Zeevi, PhD, Adriana	W-1551 BST	624-1073	2024	624-6666
OTHER					
	Transplant Signout Room	E-733 MUH	647-7645		647-5237
	Transplant Resident/Fellow Office	E-732 MUH	647-7641		647-5237

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

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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

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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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History of Transplant Immunobiology (Part 1 of 2).

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Introduction

During the past quarter-century, transplant immunobiology has established itself as a scientific discipline to study the mechanisms by which a recipient rejects or accepts a transplant from a genetically different donor. In the early history of transplantation, five separate disciplines of investigators approached the problem of graft rejection. They are the surgeons, the tumor specialists, the Mendelian geneticists, the biologists and finally the immunologists. Each had their own agenda and a lack in communication prevented the recognition and application of conceptional advances in the other disciplines. Nevertheless, most laws of transplant immunity had already been defined during the first two decades of the twentieth century. During the late 1960s, largely because of the work of Sir Peter Medawar, transplant immunobiology became established as a multidisciplinary science. This historical overview deals with progress made in the different disciplines before that time. Part 1 summarizes events before World War II and Part 2 deals with milestones from the 1940s through the 1960s. Much of the information have been extracted from seven historical reviews by noted investigators who offer additional perspectives. These references are listed at the end of this article.

The Transplant Surgeons

For several milennia, the replacement of diseased or injured organs with healthy ones has stimulated the imagination of humankind. In the mythological world, chimeric gods and heros have been transplanted with heads and other organs mostly from different species (these are examples of xenotransplants). In the early biblical times the prophet Ezekiel refers to cardiac transplantation:

"A new heart also I will give you, and a new spirit will be put within you; and I will take away the stony heart out of your flesh, and I will give you a heart of flesh".

The New Testament mentions transplant cases like Jesus of Nazareth restoring a high priest servant's ear cut off by Simon Peter's sword. Later on Saint Peter replanted the breasts of Saint Agatha pulled off during torture and Saint Mark replaced an a soldier's hand amputated during battle. These are examples of autotransplants.

In the fifth century BC, the legendary Chinese physician Pien Ch'iao exchanged the hearts between a man with a strong spirit but a weak will and a many with the opposite personality to cure the unbalanced equilibrium of the two men's energies.

A famous example of a cadaveric allograft is described in Jacopo da Varagine's *Leggenda Aura* in 348 CE. In the "miracle of the black leg", the twin brothers Saints Cosmas and Damian succesfully replaced the gangrenous leg of the Roman deacon Justinian with a leg from a recently buried Ethiopian Moor.

While it seems unlikely, that proper surgical techniques were available to perform these transplants, the practice of skin grafting has been known for many centuries. During the second century BC, the Indian surgeon Sushruta pioneered the skin grafting procedure for rhinoplasty, i.e. plastic surgery whereby the patient's own skin is used to do reconstruction of the nose. In

those days, the cutting noses was a common practice to punish criminal offenders and of course, we should not discount the fights with knives and swords.

During the Renaissance, the Gaspare Tagliacozzi, a famous surgeon and anatomist from Bologna, Italy, used a flap from the upper arm to do reconstructive surgery on a person who had lost his nose. While autografts were generally successful, virtually all allografts failed. The practice of donation consent did not exist, since slaves were used as skin donors, and often they suffered serious (infectious) complications. Such cases of the "sympathetic nose" were criticized by Voltaire and other writers. In his play *Hudibras* , Samuel Butler states

"When the date of Nock was out, off dropt the sympathetic Snout".

Taglicozzi was well aware of the limitations of the allograft procedure. In 1596, in his treatise "*De Curtorum Chirurgia per Insitionem",* he concluded that

"The singular character of the individual entirely dissuades us from attempting this work on another person. For such is the force and power of individuality, that if any one should believe that he could achieve even the least part of the operation, we consider him plainly superstitious and badly grounded in physical science".

He seemed to have recognized the concept that individual differences were responsible for these allograft failures. Almost nothing was known about genetics until Mendel did his pioneering studies 250 years later.

Other early transplant-related activities dealt with the grafting of teeth; this was done on humans already in the 17th century. Around 1800, the renowned English surgeon John Hunter, reported successful transplants of human teeth into the highly vascularized comb of a cock (a xenograft). He also grafted a cock's spur into its comb and also a cock testes into a hen (*"without altering the disposition of the hen")*. Hunter concluded that

"Transplantation is founded on a disposition in all living substances to unite when brought into contact with each other".

This view seems compatible with the modern concept about the relation between microchimerism and allograft acceptance.

In 1804, G. Baronio in Milan, reported successful skin transplants between sheep and other animals of the same and from different species. Other investigators were much less successful with such allografts and xenografts. In Paris, Paul Bert, a pupil of Claude Bernard, described in his 1863 thesis *"De la Greffe Animale",* many kinds of allogeneic and xenogeneic skin transplants. He could not duplicate Baronio's results. In parabiosis experiments, Bert established a cross circulation between rats by using belladonna injections (*"la greffe siamoise"*)

The techniques of Reverdin (1869) and Thiers (1874) for covering granulating surfaces with small pieces of epidermis lead to therapeutically acceptable skin grafting procedures. There were no indications of long-term graft survival. Successful allogeneic skin transplants have been reported. One case involves Sir Winston Churchill, who during the Sudanese war in 1898, was asked to donate a piece of skin for an injured fellow officer. The doctor, a 'great raw-boned' Irishman, spoke to Churchill:

"*Oi'll have to take it of you, Ye've heeard of a man being flayed aloive?*

Well this is it what it feels loike."

(This is quite a unique approach for obtaining informed donor consent!).

And Churchill wrote:

" A piece of skin and some flesh about the size of a shilling from the inside of my arm. This precious fragment was grafted to my friend's wound. It remains there to this day and did him lasting good in many ways. I for my part keep the scar as a souvenir."

Evidently, an example of a long-term success of a skin allograft!

Corneal transplant procedures were developed during the 19th century. In 1837, the Irishman Samuel Bigger performed a successful transplant of a full-thickness cornea into the blind eye of a pet gazelle. Continuing improvement in the grafting procedure and increasing success rates in experimental animals led to the first successful human corneal transplant in 1906. Corneal transplantation became a standard procedure in ophthalmology practice, its success was in marked contrast to the high failure rate of skin grafts.

Because of the advances in suturing techniques towards the end of the 19th century, surgeons began to transplant organs, especially kidneys between dogs. Several reports claimed success and long-term graft survival. In Lyon, the French surgeon Jaboulay tested pig and goat kidney transplants in humans. Alexis Carrell perfected the vascular anastomosis technique and this led to all kinds of experimental transplants including the grafting of a dog's head onto the neck of another dog. All these allogeneic and xenogeneic transplants were invariably unsuccessful as was the first human cadaveric kidney transplant performed in 1933 by the Ukranian surgeon Voronoy.

Although nobody understood the reasons for the high failure rate, the contention was that the major problems had been solved and that little work remained to perfect transplantation. C.C. Guthrie, who worked with Carrell, noted that

"...The outlook is by no means hopeless and the principles of immunity, which yield such brilliant results in many other fields, would seem to be worthy of being tested in this case".

Indeed, the field of immunology had undergone a dramatic expansion during the past few decades as illustrated by the following examples:

- Pasteur: vaccination against cholera, anthrax and rabies
- Ehrlich: antibodies and antigens
- Koch: tuberculin hypersensitivity
- Von Behring: therapeutic potential of antitoxins
- Bordet and Gengou: complement activity
- Pfeiffer: immune bacteriolysis
- Belfanti and Carbone: immune hemolysis
- Landsteiner: ABO blood groups
- Portier and Richet: systemic anaphylaxis
- Von Pirquet and Shick: serum sickness
- Arthus: local antibody-mediated reaction
- Donath and Landsteiner: autoimmune disease
- Metchnikoff: phagocytic theory of host resistance

It should be noted that most immunological concepts in those days pertained to humoral immunity and nothing was known about cellular immunity and lymphocyte function.

During the first quarter of the 20th century, a relatively few number of studies were reported on the immune basis of skin graft failures. Underwood (1914) suggested that an "anaphylactic hypersensitivity" was responsible for allograft rejection. With repeat skin transplants on children, Holman (1924) reported that a "second set" of transplants from the same donor "did not take but disappeared simultaneously with the first group of isografts". Davis (1917) and Shawan (1919) suggested that blood groups might play a determining role in allograft success or failure.

The Tumor Specialists

Most of the information about the immune basis of allogeneic transplant failures would come from the studies of the tumor specialists. Stimulated by the rapid advances in vaccination against microbial agents, the tumor researchers attempted similar approaches for the treatment of cancer. However, *p*reventive immunization or serum therapy of naturally occurring tumors was generally unsuccessful. This led to the development of transplantable tumor lines in experimental animals. In 1912, Georg Schöne's book: "*Heteroplastische und Homoplastische Transplantation"* summarized the experimental work reported in about 500 publications during the first decade. He coined the term: ***"Transplantationsimmunität"*** and formulated the following rules:

- Heteroplastic (xenogeneic) transplants invariably fail
- Homoplastic (allogeneic) transplants usually fail
- Autografts are almost always successful
- There is an initial take of a first allograft which is then followed by rejection
- Second grafts undergo accelerated rejection if recipient has previously rejected a graft from the same donor or, if recipient has been preimmunized with material from tumor donor
- Graft success is more likely when donor and recipient have a closer "blood relationship"

As Silverstein points out in his 1989 book "*A History of Immunology"*, the "laws of transplantation" were substantially defined already in 1912. On the other hand, Leslie Brent concludes in his recent book *"A History of Transplantation Immunology"* that this credit to Schöne is not wholly justified. A subsequent review published in 1916 by Tyzzer on *"Tumor Immunity"* confirmed Schöne's findings . Tyzzer further pointed out that

- Presensitization for second set rejection requires living cells
- Cytotoxic antibodies cannot be found
- The delayed reaction is difficult to explain except that an 'immune body' has been produced
- Lymphocytes predominate at rejection site: the reaction is not merely exudative but is proliferative as well
- There is no tissue specificity, but rather a racial specificity with respect to the genetic origin of the antigens

In 1929, Woglom's book *"Immunity to Transplantable Tumors"* represents a review of 600 reports published since Schöne's book. His additional conclusions include

- All living tissues can immunize for accelerated rejection
- Whole blood but not washed erythrocytes can immunize, activity in leukocytes
- Transplantation immunity is systemic, but certain sites (brain) are exempt
- Newborns from sensitized mothers are not immune to tumors
- Passive transfer of tumor immunity cannot be done with serum

While considerable evidence had accumulated for an immune basis of tumor allograft rejection, only humoral mechanisms were considered. However, serum antibodies were never effective in

controlling in vivo tumor growth. In those days, the concept of cellular (i.e. lymphocyte-mediated) immunity was not recognized although Da Fano (1910) reported that rejecting tumor allografts contained large numbers of lymphocytes rather than polymorphonuclear leukocytes. These findings are similar to those of Tyzzer who also noted that the lymphoid response was infiltrative and proliferative.

Murphy and Rous (1912) described the histological predominance of lymphocytes in fowl sarcoma rejection model. Injection of sarcoma cells in chicken (as well as duck and pigeon) embryos resulted in uninhibited growth during the early days of gestation. Thereafter, the tumors were rejected and this coincided with the appearance of lymphocytes. Tumor rejection occured also after transfer of adult lymphocytes into the embryo. These data indicated for the first time a relation between the ontogeny of the immune response and transplant rejection. Further studies by Murphy have shown that lymphopenia inhibits tumor rejection and that X-irradiation causes lymphopenia and depresses antibody formation. Unfortunately, the functional role of the lymphocyte remained a mystery until the late 1950s.

It should be noted that other investigators did not ascribe to the immune basis of tumor allograft rejection. In his 1930 Book *"Transplantation and Individuality",* the prominent biologist Leo Loeb recognized the genetic basis of individual differences and transplantation incompatibility. Rather than considering immune mechanisms, he argued that rejection resulted because the graft could not make the connections necessary to the survival in the new environment of the recipient. His concepts were based on Ehrlich's "athrepsia" theory which considers nutritional needs of tissues and cells

The Mendelian Geneticists

In 1903, the Danish geneticist Jensen first demonstrated with a breeding stock of albino mice that genetic differences control rejection of transplantable tumors. Loeb (1908) and Tyzzer (1909) reported similar findings with inbred "Japanese waltzing mice". Clarence C Little (1916) used inbred mouse strains by brother-sister mating and concluded in 1924 that

"...The genetics of tissue transplantation is likely to become in the not distant future of far greater importance".

In 1929 he founded the Jackson Memorial Laboratory at Bar Harbor, Maine and George D Snell was hired in 1935. As the editor of the book "The Biology of the Laboratory Mouse" , Snell was inspired by Little's chapter "The Genetics of Tumor Transplantation" to pursue a research carreer in mouse genetics which lead him to the discovery of the H (or histocompatibility) locus that controls tumor graft rejection.

During the late thirties, the Englishman Peter A Gorer performed serological studies with sera from rabbits immunized with mouse red blood cells which led to the discovery of antigen II expressed by certain mouse strains. Grafting of albino mouse sarcoma cells induced the development of anti-antigen II antibodies by the tumor-resistant Auguti and Black mice. All tumor-susceptible cross-bred mice expressed antigen II and specific antibodies killed tumor cells in vitro. In 1946, Gorer visited Snell and H and II were combined as H-2, " a Major Histocompatibility Gene" and nine alleles were identified. These investigators developed a highly productive research collaboration which established the major strains of inbred laboratory mice and more than twenty so-called congenic-resistant mouse lines that differ only at H-2, most of them are still being used in immunology research.

Progress in Transplantation Immunology during the Nineteen-Thirties

The nineteen-thirties was a period of decline of transplantation immunology-related research. The surgeons concluded that, except for corneal grafting, all attempts at skin and organ transplants will fail due to rejection. Immunosuppression by X-irradiation turned out not to be practical, and the immunity hypothesis of rejection was largely discarded. The tumor researchers had lost faith in the approach of treating cancer via vaccination or transplantation. The geneticists shifted their interest towards "pure" genetics by breeding inbred strains of mice and by studying gene polymorphisms.

And then came World War II (article to be continued in part 2).

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Early History of Transplantation Immunology (Part 2 of 2)

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The Biologists

The bombing of cities during the war caused a marked increase in the number of burn victims for whom a skin autograft was not feasible. The application of skin homografts (a old term for allograft) was known for its high failure rate due to rejection. The 'War Wounds Committee' of the British Medical Council assigned a young, Oxford-educated zoologist named Peter Medawar to investigate the problem of homograft rejection and how to circumvent it. Medawar worked first in a clinical setting with Thomas Gibson at the Burn Unit at Glasgow Infirmary. In 1943, they published a detailed *report "The Fate of Skin Homografts in Man"* on a single burn victim with multiple 'pinch grafts' of skin. Their comprehensive analysis of serial biopsies led to the following conclusions:

1 Autografts succeed, but allografts fail after an initial take
2 "Second-set" grafts undergo accelerated rejection.
3 The breakdown of foreign skin epithelium is not due to "a local reaction" (a term used by Loeb) on the part of lymphocytes or other mesenchyme cells.
4 The destruction of the foreign epidermis is brought about by a mechanism of active immunization

This report shows the Medawar's awareness of the immunity hypotheses of Schöne, Holman, Woglom, and others to explain graft rejection. He returned to Oxford University to study the homograft rejection in laboratory animals and to prove that this was an immunologic phenomenon. A series of carefully designed and stringently controlled experiments with a rabbit skin graft model were described in two reports to the War Wounds Committee published in the Journal of Anatomy in 1944 and 1945.

Medawar concluded that the mechanism by which foreign skin is eliminated belongs to the category of *actively acquired immune reactions*. His early insight into the mechanism of transplant rejection is reflected by statements such as *"The accelerated regression of second-set homografts argues for the existence of a systemic immune state*". "*The inflammation has in all likelihood the character of a local anaphylaxis"* (he implicates humoral immune responses), but "*yet, the reaction is atypical; for the lymphocyte takes the place of the polymorph in the "classical picture*". He postulated that the graft-infiltrating cells are "*directly concerned in the manufacture of antibodies"*, and that *"The homograft reaction is governed by the operation of at least 7 antigens freely combined*". In those days, the immunological community was almost solely focused on the humoral immune response, although some investigators, notably Merrill Chase (and Landsteiner) had obtained evidence that cutaneous hypersensitivity to picryl chloride and tuberculin could be transferred by lymphoid cells and not by antibodies.

Cellular Immune Basis of Transplant Immunity

Medawar recognized the significance of donor leukocytes in inducing transplant immunity and accelerated rejection of skin grafts. Burnet and Fenner pointed out in 1949, the analogy between transplantation immunity and delayed-type hypersensitivity, a immune response type exemplified by the tuberculin reaction. Both phenomena showed the absence of detectable antibodies, and the systemic nature of the sensitization process induced by intradermal immunization with leukocytes. A few years later, Mitchison (1954) showed that passive transfer of lymphoid cells from sensitized donors induced immunity to transplanted allogeneic tumors. Soon afterwards, Billingham, Sprent and Medawar conducted similar studies on the adoptive transfer (a term

coined by Medawar) with lymph node cells in a skin allograft model and their findings firmly established a cellular basis of transplant immunity. The functional role of lymphocytes remained a mystery until at least ten years later. Most investigators considered the lymphocyte an unimportant cell which with its paucity of cytoplasm, could not have any significant functional activity. Apart form the non-immunologic functions considered by some investigators, others thought that lymphocytes were hematopoietic stem cells. Chase and others interpreted their findings that lymphocytes were involved with antibody formation although the actual production of antibodies was never established.

Gowans provided the first major step towards the understanding of lymphocytes. He demonstrated with radiolabeled thoracic duct cells, that lymphocytes recirculate from blood to lymph by crossing the endothelial walls of specialized blood vessels known as post-capillary venules. Medawar had begun to focus his efforts on the concept that lymphocytes were "*immunologically competent*" cells. Paul Terasaki then a post-doctoral fellow with Medawar, demonstrated that injection of small lymphocytes into newly hatched chicks induced graft-versus-host (GVH) reactions. Morton Simonsen in Copenhagen has been credited as the discover of GVH disease caused by inoculation of adult lymphoid cells in the chick embryo and manifested by severe hemolytic anemia, splenomegaly and soon death. The powerful GVH effects of allogeneic lymphocytes were also noted by Billingham and Brent in neonatal mice who developed *"runt disease*" and in other experimental models such as "*parabiosis intoxication*" in parabiotically attached animals , "*secondary disease*" in irradiated mice injected with allogeneic bone marrow cells (Trentin) and " *F1 hybrid disease*" in F1 hybrids given parental lymphocytes (Gowans). Although it was generally believed that time that lymphocytes participated in the allograft response as carriers "cell-bound" antibody, several investigators began to elucidate the functional roles of these cells in transplant immunity. During the late fifties, Govaerts in Belgium demonstrated that lymphocytes taken from dogs with rejected kidney allografts had a specific cytotoxic effect on renal cells from the donor. Rosenau and Moon showed a similar *in vitro* cytotoxic effect of sensitized mouse lymphocytes and it required a close contact with the allogeneic targets. Wilson in Philadelphia introduced the "single-hit" mechanism of cytotoxicity and in quantitative inhibition assays with a 6-mercaptopurine derivative, he showed that the cytotoxic effect of sensitized lymphocytes required RNA-dependent protein synthesis. During the sixties, Ginsburg in Israel applied time-lapse cinematography to show the movements of large lymphoblasts (called lysocytes) from one target to another on a cultured cell monolayer. Shortly afterwards. Brunner and Cerrottini in Switzerland developed the classical cell-mediated lympholysis (CML) assay that has been used in so many studies to increase our understanding of cytotoxic lymphocytes. For example, this assay was used in 1974 by Zinkernagel and Doherty to elucidate the role of the major histocompatibility complex (MHC) restricted nature of T lymphocyte cytotoxicity against virus-infected cells.

The Mixed Lymphocyte Culture (MLC) assay became another important tool for studying lymphocytes. Bain, Vas and Lowenstein in Montreal reported in 1963 the transformation of large immature basophilic cells from lymphocytes cultured together from two unrelated individuals. Such cells can synthesize DNA and undergo mitosis. These investigators coined the term Mixed Leukocyte Reaction (MLR) and they suggested that this reaction might be related to homograft immunity and that this test seems useful as an indicator of compatibility between siblings. Around the same time, Bach an Hirschhorn in New York developed the one-way MLC assay whereby recipient cells were studied for their response to mitomycin C-treated cells from the donor. These responses were measured after seven days by microscopic examination of fixed smears for blast cell transformation and mitosis. Bach provided first evidence for the role of histocompatibility antigens and he suggested that the MLC might be a useful typing test for transplantation. This led to an active research endeavor that resulted in the definition of the so-called lymphocyte defined histocompatibility antigens during the seventies. For many years, MLC has been used as a critical test to determine donor-recipient compatibility in bone marrow transplantation. Hayry and Defendi found that mouse lymphocytes generated from the MLR were cytotoxic to stimulator cells and Solladay and Bach reported the same phenomenon with human lymphocytes. The MLR was now considered as representing an *in vitro* alloactivation model of

the allograft response and the CML as an *in vitro* model of an effector mechanism of cellular transplant immunity.

During the early sixties, independent studies by JFAP Miller in London and Robert A. Good in Minneapolis established the significance of the thymus gland in cellular immunity. Neonatal thymectomy of mice caused a severe immunodeficiency characterized by lymphopenia, a wasting syndrome and early death. Bruce Glick at the University of Wisconsin made the serendipitous discovery that early removal of the bursa Fabricius, a cloacal organ in the chicken, led to an antibody deficiency state and low levels of serum gamma globulins. These findings provided the basis to differentiate between thymus (or T)-dependent and bursa (or B)-dependent lymphocytes. Henry Claman at the University of Colorado provided crucial evidence that antibody formation required the interaction between B and T cells. These different types of lymphocytes could soon be distinguished through the expression of cell surface markers like immunoglobulins on B cells and theta markers on T cells (Reif and Allen; Raff). Furthermore, Harvey Cantor and Ed Boyse in New York identified three genetic loci Ly-1, Ly-2 and Ly-3 that could differentiate between murine cytotoxic (CD8) T lymphocytes and helper (CD4) T lymphocytes.

The "Passenger Leukocyte" concept represented another important step towards a better understanding of the allograft response. Snell noted already in 1957 from data obtained by Stork in 1953, that donor leukocytes may play a role in the induction of transplant immunity, because donor organs transplanted from cortisone treated rats had longer survivals and that such organs had reduced numbers of leukocytes. Ten years later, Steinmuller reported a series of seminal experiments whereby he first induced neonatal tolerance in mice with allogeneic hybrid spleen cells and transplanted skin from tolerant animals to syngeneic recipients. As expected, such transplants survived indefinitely but they sensitized the recipients to skin from the spleen cell donors. Steinmuller concluded that allogeneic leukocytes from the tolerizing inoculum had migrated to the skin of the tolerant mice in sufficient numbers to induce an allograft response in syngeneic recipients. He hypothesized that a "leukocyte containment" and raised the question whether the immunizing ability of skin grafts is dependent on leukocytes. The term "passenger leukocyte" was coined later by Elkins and Guttmann who in an elegant series of experiments showed that local GVH reactions could be induced by syngeneic spleen cells inoculated under the capsules of transplanted kidneys in F1 hybrid rats.

Transplantation Tolerance

Although it had become clear many years ago that the rejection problem presented a formidable barrier to successful transplantation, investigators began to wonder whether the immune system be rendered unresponsive to the transplant. Some early studies had shown the feasibility of immunological tolerance. Felton (1926) reported that high doses of pneumococcal polysaccharide can induce unresponsiveness upon rechallenge with this antigen. Landsteiner and Chase (1937) found that *per os* administration rather than percutaneous application of chemicals evoked unresponsiveness rather than delayed-type hypersensitivity. First evidence for transplant tolerance was however, obtained by embryologists.

During the first few decades of the twentieth century, many experimental embryologists conducted tissue differentiation studies on transplanted xenogeneic grafts in avian or amphibian embryos. These grafts were often quite successful in these hosts, but these studies never addressed any immunological implications. A notable exception is the work by Rous and Murphy who around 1912, studied the growth of rat sarcoma cells in the chorioallantoic membranes of chick embryos. These tumor cells grew quite well during the incubation period of the eggs but were rejected around the time of hatching and this was accompanied by the appearance of lymphoid cells around the graft. These data indicated for the first time a relation between the ontogeny of the immune response and graft rejection

The discovery of neonatal transplant tolerance has been credited to Ray Owen, a geneticist at the University of Wisconsin who studied the inheritance of red blood cell antigens in cattle. He reported in 1945 that dizygotic twins had mixtures of their own cells and their twin partner cells.

Thirty years earlier, Lillie had observed that bovine dizygotic twins develop a fusion of their placenta during embryonic life. This results in a common intrauterine circulation and the passage of sex hormones from the male embryo to the female twin impairs the sexual development in the female twin and the result if a sterile so-called "free-martin". Owen recognized that the common intrauterine circulation also leads to an exchange of hematopoietic stem cells during embryonic life and the establishment of the chimeric state of red cells. Moreover, these calves did not have isoantibodies to their twin partners.

A few years later, Burnet and Fenner acknowledged in their influential book "*The Production of Antibodies"* the importance of Owen's findings and they proposed their famous *"self-nonself"* hypothesis for immune development. Burnet had concluded that chick embryos and fetal animals are incapable of antibody production and that the development of immunocompetence is a slow process in young animals. He postulated that during embryonic development, *"a process of self-recognition takes place"* and "*no antibody response should develop against the foreign cell antigen when the animal takes on independent existence*". Owen's red cell chimeric model in the dizygotic cattle twins seemed similar to the phenomenon induced by inoculation of foreign embryonic cells in the chick embryo and Burnet hypothesized a *"tolerance acquired by fetal exposure to 'nonself' constituents"*. Interestingly, two Russian scientists Lopashov and Stroyeva published independently in 1950 a paper (in Russian) reflecting similar concepts about the embryonic development of the immune response to transplants.

Medawar predicted that an exchange of skin grafts between dizygotic calves could verify Burnet's hypothesis and together with his post-doctoral fellow Rupert Billingham, he performed a series of grafting experiments that provided direct support for the concept of neonatally acquired transplant tolerance. Moreover, subsequent experiments by Billingham and Leslie Brent, then a doctoral student with Medawar, demonstrated in 1953 that neonatally acquired transplant tolerance could be achieved in mice by inoculation of embryos or intravenous injection of newborn mice with allogeneic cells. Thus, "*the exposure of animals to antigens before the development of the faculty of immune response should lead to tolerance rather than to heightened resistance".* It should be noted that the concept of neonatal tolerance was still solely based on antibody production, because the knowledge of cellular immunity was virtually nonexistent.

At the same time, Milan Hasek in Prague demonstrated that parabiosis of different strain chick embryos induced a immune hyporesponsive state to each other's red cells. Under prevailing Soviet scientific ideologies promoted by Lysenko and Michurin, the early publications from Hasek's laboratory (in Czech and Russian journals) explained these findings that the exchange of blood elements between chick embryos of different breeds reflected a "*vegetative hybridization"*, a condition in which the parabionts were expected to display some of the characteristics of their partners. Later publications reflected a departure from Soviet-dominated ideologies towards immunological interpretations and Hasek and many coworkers (including T. Hraba, J. Sterzl, J. Klein, P. Ivanyi and P. Demant) have made numerous fundamental contributions to transplant immunology.

Besides neonatally induced tolerance, some investigators began to note that transplant unresponsiveness could be induced in young animals provided certain experimental conditions reflected the so-called "null" or "neutral" period concept proposed in 1956 by Billingham, Brent and Medawar. According to this concept, the immunological development of a young animal is such that exposure of antigen will neither induce immunity nor tolerance. This concept was prompted by findings that intraperitoneal inoculation of allogeneic tissues to newborn mice led to tolerance in a few and that many mice became neither immune nor tolerant. While the administration of cortisone seemed to promote tolerance, it became also apparent from data from Billingham and Brent and from Robert Good's laboratory, that the intravenous injection of adult spleen cells into newborn mice can produce long-term tolerance depending on the donor-recipient strain combination and the cell dose and timing. Few studies on blood transfusions of newborn infants had shown however, that human neonates have already a highly developed immune system and Medawar concluded that neonatally induced tolerance had no clinical applicability.

The concept of acquired tolerance had nevertheless, become deeply imbedded in the minds of transplant immunologists. Tolerance could be induced to specific histocompatibility antigens and tolerance maintenance required the continuous presence of the tolerogenic antigen as had been first demonstrated in Ray Owen's chimeric cattle model. Silvers and others reported that lymphoid cells from tolerant mice could not induce GVH reactions in donor mice and they lacked donor reactivity in MLR cultures. These findings supported the clonal deletion concept as a tolerance mechanism and Byron Waksman's studies linked the thymus gland with the events leading to the induction of tolerance to protein antigens such as bovine gamma globulin. Moreover, several experimental models showed the feasibility of tolerance induction in adult animals and other mechanisms were proposed such as "blocking" antibodies (Hellstrom) and "suppressor cells" (Gershon) and transplant immunologists began to apply the phenomenon of antibody-mediated "immunologic enhancement" of the growth of transplantable tumors in allogeneic hosts immunized with lyophilized preparations of tumor cells (Snell, Kaliss). These newly emerging concepts created a wave of investigations of many experimental models for prolonging graft survival and to define the factors responsible for these phenomena. Although these studies generated often contradictory observations that were difficult to interpret, they provided the basis of modern research efforts to unravel the complexity of the regulatory mechanisms of the immune response.

Immunosuppression.

The success of clinical transplantation depends on the control of graft rejection by immunosuppressive agents. Until the 1950s, immunosuppression in organ transplantation consisted primarily of whole-body X-irradiation. Since the beginning of the century, it was well-known that irradiation inhibited antibody responses and caused leukopenia and Dempster and co-workers in London reported in 1950 that irradiation inhibited skin allograft rejection and delayed-type hypersensitivity reactions. After the atomic bombing during worldwar II, an upsurge in radiation biology research led to an understanding of radiation-induced tissue damage and that administration of bone marrow cells provided a protection through the "generation of new areas of hematopoiesis" (Lorenz and Uphoff). These findings were important in the development of bone marrow transplantation protocols (pioneered by Donnall Thomas in Seattle) to treat leukemia patients.

During the fifties, several surgeon teams in Boston (Merrill, Murray, Hume), France (Kuss, Hamburger) and elsewhere began to transplant kidney between related individuals. Total body irradiation protocols combined later on with bone marrow infusions, produced unsatisfactory results and virtually every case failed. A 1960 editorial in the British Medical Journal concluded that "*true homografts of the kidney may be expected to fail…for immunological reasons*". Fortunately, basic immunologists discovered a number of drugs with immunosuppressive properties. For instance, Baker reported in 1952 the immunosuppressive effects of nitrogen mustard, but this agent was too toxic. Most promising results were obtained with an anti-mitotic agent 6-mercaptopurine synthesized by Elion and Hitchings. This drug interferes with nucleic synthesis and which had been tested to treat cancer. Schwartz and Dameshek discovered in 1959 that 6-mercaptopurine suppressed the antibody responses of rabbits to bovine serum albumin and prolonged skin allograft survival. Several investigators began to study 6-mercaptopurine in various experimental transplant models, but this drug turned out too toxic for clinical use. During his studies in Boston, the English transplant surgeon Roy Calne identified an imidazole derivative of 6-mercaptopurine (BW 57-322) which was an effective immunosuppressive drug without major adverse side effects. Also called Imuran (or Azathioprine), it became widely used as the primary anti-rejection drug in organ transplantation until the application of cyclosporine during the early 1980ties.

Azathioprine could however, not be used the sole immunosuppressive agent in transplant recipients. While additional treatments included actinomycin D, azaserine and low radiation doses, the application of corticosteroid hormones produced the best results. Billingham showed in 1951 that daily administration of cortisone acetate to rabbits prolonged skin allograft survival. Thomas Starzl made the seminal observation in 1963 that large doses of prednisone can reverse rejection episodes and stabilize kidney graft function. Moreover, the combined use of

azathioprine and prednisone became established procedures to manage transplant recipients until the eighties when the cyclosporine era began.

Another method of immunosuppression is the use of anti-lymphocyte antibodies raised against lymphocytes from another species. Many investigators including Metchnikoff, Flexner and Funck had already demonstrated during the early part of the century, that sera from immunized xenogeneic hosts had cytotoxic effects on leukocytes and lymphoid tissues. During the late nineteen thirties, Chew and co-workers as well as Cruickshank studied the effects of anti-lymphocyte sera raised in rabbits. These data provided the basis of the investigations two decades later, by Byron Waksman in Boston and Michael Woodruff in Edinburgh, that anti-lymphocyte serum prolonged skin allograft survival in rats. These findings were confirmed in large animal models and Starzl reported in 1968 a favorable effect of anti-lymphocyte globulin on human kidney transplant outcome. While the immunogenicity of anti-lymphocyte and anti-thymocyte globulin limits its long-term use in a clinical setting, these agents have been successfully been used to treat rejection episodes of transplant patients.

Histocompatibility Testing

Karl Landsteiner is the discoverer of the ABO red blood cell antigen system. In 1901, he published a paper on the serological reactions between sera and erythrocytes from normal individuals and he recognized two types of naturally occurring agglutinating antibodies: anti-A and anti-B. Many tissues express ABO antigens that will react with these hemagglutinins and ABO incompatibility has been avoided in organ transplantation

The Major Histocompatibility Complex controls potent transplantation antigens that elicit the rejection process. The earliest studies on the Human Leukocyte Antigen (HLA) complex (H-2 is the mouse equivalent) were done by red blood cell serologists in the 1950s. Jean Dausset in Paris recognized the immunological origin of the agglutination of white blood cells by sera from transfused patients. He identified in 1953 the first leukocyte specificity Mac, which is now called HLA-A2. Rose Payne at Stanford reported in 1958 the appearance of similar leukoagglutinating antibodies in multiparous women. Independently, Jon van Rood (Leiden, The Netherlands) made similar observations and he used computer programs for leukocyte antigen grouping from clusters of leukoagglutinating antibodies.

Since Bernard Amos had shown in 1953 that mouse H-2 antigens can be detected by leukoagglutinins, human leukocyte antigens were suspected to play a role in transplantation. Clinically, van Loghem (Amsterdam) showed in 1956, that such antibodies were associated with nonhemolytic transfusion reactions. In 1962, Felix Rapaport reported the accelerated rejection of skin grafts with leukocyte antigen mismatches.

During the early 1960s, a growing group of investigators attempted to define leukocyte antigen groups with serological techniques such as leukoagglutination and complement fixation on platelets. While these assays were lacking reproducibility, another problem was the extreme complexity of the genetics of leukocyte antigens. A turning point in the history of leukocyte typing was the intensive international collaboration that began as the First Workshop and Conference on Histocompatibility organized by Bernard Amos (Durham, NC) in 1964. This was a laboratory bench study whereby the participants compared the reactivity of their sera with various techniques. The results were so discordant that they could not be published. The Second Workshop held the following year in Leiden, yielded more coherent results and several serological specificities emerged clearly. The concept was forwarded that all of them belonged to a single, complex antigenic system analogous to the H-2 system of the mouse. Paul Terasaki and John McClelland at UCLA introduced the complement-dependent microlymphocytoxicity technique which has remained the standard serological test for HLA typing. The Third Workshop organized by Ruggero Ceppelini (Torino, Italy) in 1967, clearly established the HLA system and two segregant series of specificities (now called HLA-A and HLA-B) were recognized. The success of this international collaboration has assured the continuation of the histocompatibility workshops (Los Angeles,1970; Evian, France, 1972; Aarhus, Denmark, 1975; Oxford, 1977; Los Angeles, 1980; Munich, 1984; New York, 1987; Yokohama, 1991, and St Malo/Paris, 1996). After each workshop, a nomenclature committee has incorporated salient findings towards the

definition of HLA polymorphisms including the identification of additional class I loci such as HLA-C (Thorsby, 1970), and the class II loci HLA-DR (1977) and a few years later HLA-DQ (formerly called MB) and HLA-DP (formerly SB). The latter comprise the HLA-D region which was first recognized as the MLC locus by Amos and Yunis in 1970 as a genetic system responsible for T-cell activation in the mixed leukocyte culture.

The influence of HLA matching on kidney transplant outcome was first indicted by the higher success rates of kidney transplants from HLA-identical sibling donors. During the late sixties, Terasaki, and co-workers presented early data indicating the potential beneficial effects of HLA matching on cadaveric kidney transplant survival, although these findings were based on typing information with a limited set of rather crude anti-HLA antisera. Furthermore, van Rood's group, Batchelor and Joysey, and other investigators also found that matching for HLA improves kidney and skin graft survivals. In those days, serological typing had many problems of reproducibility and lack of reagents. The conflicting presentations by Terasaki's group at the Third International Congress of the Transplantation Society in The Hague in 1970 produced considerable controversies about the significance of histocompatibility matching in kidney transplantation. Many well-matched kidney transplants failed early and conversely, badly matched kidneys did often enough function quite well. Other investigators had noted that same experience and it was not really surprising that many transplant surgeons chose to ignore tissue typing results. Of course, all these controversies arose when HLA matching was limited to an incomplete set of HLA-A and HLA-B antigens; there was no typing for HLA-DR and the available serological tests had a rather low level of reproducibility. Because of improved serological procedures and especially, the application of DNA-based techniques, HLA compatibility can now be much better defined and there remains no doubt that HLA matching correlates with less rejection and prolonged kidney transplant survival.

Histocompatibility testing for organ transplantation requires usually a crossmatch test between recipient serum and donor cells. Starzl and co-workers reported in 1965 the first case of a patient with complement-dependent anti-donor antibodies. This patient rejected almost immediately a kidney transplant from this donor and the tissue pathology suggested a Shwartzmann reaction-like mechanism. Kissmeyer-Nielsen in Copenhagen reported a similar case of what he termed a hyperacute rejction. This experience established the crossmatch test as a major test in histocompatibilty testing. The Panel-Reactive Antibody (PRA) test was first reported by Terasaki in 1971 to identify presensitized patients at higher immunological risk of rejecting their transplant.

Conclusion.

During the fifties and sixties, transplantation immunology began to emerge as a distinct scientific discipline. Six transplantation symposia were held during 1954-1964 under sponsorship of the New York Academy of Sciences and participants at these meetings included biologists, geneticists, immunologists, pathologists, surgeons and serologists. Peter Medawar concluded that *"One of the distinguishing marks of modern science is the disappearance of sectarian loyalties. Isolationism is over; we all depend upon and sustain each other"*. The "Transplantation Society" was established in 1967 and was its membership reflected a diverse group of scientists, physicians and surgeons devoted to make transplantation as an clinically effective therapeutic modality to treat patients with end-stage disease. The diversity was also characterized by the differences in scientific concepts and experimental approaches as illustrated by the statements by two noted experts in the transplantation field ten years later. Roy Calne had the opinion that "*Progress in transplantation would come less from basic immunologic research than from the search for better immunosuppressive drugs"* whereas Leslie Brent stated that *"The immunological solution of rejection might involve a time-scale of progress that is greater than self-interest and our natural urge for human advance demand"*. More than twenty years have gone by and, looking at all the accomplishments in the transplantation field, we must conclude that both men were right.

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Kidney

Diagnostic Categories for Renal Allograft Biopsies ('97)*

1. Normal, see Definitions

2. Antibody mediated rejection-demonstrated to be due, at least in part, to anti-donor antibodies

Type	Histopathological Findings
Immediate (Hyperacute)	Polymorph accumulation in glomerular and peritubular capillaries with subsequent endothelial damage and capillary thrombosis
Delayed (Accelerated Acute)	

3. Borderline Changes:"Suspicious" for acute rejection

Grade	Histopathological Findings
"Suspicious"	This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (1 to 4 mononuclear cells/tubular cross section) and at least i1

4. Acute Rejection

Type (Grade)	Histopathological Findings
IA	Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (> 4 mononuclear cells/tubular cross section or group of 10 tubular cells)
IB	Cases with significant interstitial infiltration (> 25% of parenchyma affected) and foci of severe tubulitis (> 10 mononuclear cells/tubular cross section or group of 10 tubular cells)
IIA	Cases with significant interstitial infiltration and mild to moderate intimal arteritis (v1)
IIB	Cases with severe intimal arteritis comprising > 25% of the lumenal area (v2)
III	Cases with "transmural" arteritis or fibrinoid change and necrosis of medial smooth muscle cells (v3 with lymphocytic inflammation)

5. Chronic/Sclerosing Allograft Nephropathy§

Grade	Histopathological Findings
Grade I (mild)	Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection
Grade II (moderate)	Moderate interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection
Grade III (severe)	Severe interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection

6. Other

Changes not considered to be due to rejection, see Differential Diagnosis

§ Glomerular and vascular lesions help define type of chronic nephropathy; chronic/recurrent rejection can be diagnosed if typical vascular lesions are seen

* The recommended format of report is a descriptive narrative signout followed by numerical codes in parentheses. Categorization should in the first instance be based solely on pathologic changes, then integrated with clinical data as a second step. More than one diagnostic category may be used if appropriate

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2. Solez K, et al. Report of the third Banff conference on allograft pathology (July 20-24, 1995) on classification and lesion scoring in renal allograft pathology. *Trans Proc* 1996;28(1):441-4.
3. Racusen L, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int 1999;55:713-723*

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Kidney

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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The Banff 97 working classification of renal allograft pathology

[Dialysis-Transplantation]

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Abstract

The Banff 97 working classification of renal allograft pathology.

Background. Standardization of renal allograft biopsy interpretation is necessary to guide therapy and to establish an objective end point for clinical trials. This manuscript describes a classification, Banff 97, developed by investigators using the Banff Schema and the Collaborative Clinical Trials in Transplantation (CCTT) modification for diagnosis of renal allograft pathology.

Methods. Banff 97 grew from an international consensus discussion begun at Banff and continued via the Internet. This schema developed from (*a*) analysis of data using the Banff classification, (*b*) publication of and experience with the CCTT modification, (*c*) international conferences, and (*d*) data from recent studies on impact of vasculitis on transplant outcome.

Results. Semiquantitative lesion scoring continues to focus on tubulitis and arteritis but includes a minimum threshold for interstitial inflammation. Banff 97 defines "types" of acute/active rejection. Type I is tubulointerstitial rejection without arteritis. Type II is vascular rejection with intimal arteritis, and type III is severe rejection with transmural arterial changes. Biopsies with only mild inflammation are graded as "borderline/suspicious for rejection." Chronic/sclerosing allograft changes are graded based on severity of tubular atrophy and interstitial fibrosis. Antibody-mediated rejection, hyperacute or accelerated acute in presentation, is also categorized, as are other significant allograft findings.

Conclusions. The Banff 97 working classification refines earlier schemas and represents input from two classifications most widely used in clinical rejection trials and in clinical practice worldwide. Major changes include the following: rejection with vasculitis is separated from tubulointerstitial rejection; severe rejection requires transmural changes in arteries; "borderline" rejection can only be interpreted in a clinical context; antibody-mediated rejection is further defined, and lesion scoring focuses on most severely involved structures. Criteria for specimen adequacy have also been modified. Banff 97 represents a significant refinement of allograft assessment, developed via international consensus discussions.

Standardization of renal allograft biopsy interpretation and reporting is necessary to guide therapy in transplant patients and to establish an objective end point for clinical trials of new antirejection agents. The Banff Working Classification of Renal Allograft Pathology is an international schema recently developed to fill this need. The classification, which originated in a meeting held in Banff, Canada on August 2 to 4, 1991, was published in 1993 [1], has been clinically validated in numerous studies [2-8], and is now widely used by center pathologists and in large international trials of immunosuppressive agents. Subsequent meetings have been held in Banff every two years to refine the classification. For National Institutes of Health clinical trials, a modification of the Banff grading system, the Collaborative Clinical Trials in Transplantation (CCTT) classification was developed; this classification and a clinical validation study were published in late 1997 [9]. This article is the report of the March 7-12, 1997, Fourth Banff Conference on Allograft Pathology, a meeting at which pathologists using the Banff schema and those using the CCTT modification met with clinical investigators to review new clinical and experimental observations on the pathology of the renal allograft, with an emphasis on mechanisms and diagnosis of rejection.

METHODS

Banff 97, the combined classification described here, is a product of an international consensus discussion begun at Banff and continued via the Internet. This modified schema for renal allograft rejection was brought about through several major influences, including (*a*) analysis of data from clinical trials using the Banff classification and observation of actual practice in use of the classification worldwide, (*b*) publication of and experience in the use of the CCTT modification [9], and (*c*) international consensus discussions that took place at the Second, Third [10], and Fourth Banff Conferences and at intervening meetings. In addition, data on prognosis and renal function from the Syntex/Roche mycophenolate mofetil trials [11], data from the CCTT trials [9], and a recent study focused on vascular lesions in rejection [12] have demonstrated that vasculitis of any severity has significant implications for response to therapy, and graft function and outcome, and provide a major rationale for this 1997 revision ("Banff 97"). This combined classification focuses on histologic "types" rather than "grades" of rejection. Since there are significant changes in this revised schema, there is strong incentive in many circumstances to retain the older classifications, but to incorporate Banff 97 when a new study is initiated.

RESULTS

Banff 97, presented in Table 1, represents a significant modification of the grading of acute/active rejection in Banff 93-95. Nonetheless, the new version retains the basic construct of the earlier schema, which includes the range of findings seen in allograft biopsies and also provides for semiquantitative grading of changes of both acute/active rejection and chronic/sclerosing allograft nephropathy. To clarify the changes made, the categorization and grading of acute changes are discussed in the context of the earlier schemas, Banff 93-95 and CCTT.

1. Normal, see Definitions
2. Antibody-mediated rejection
   Rejection demonstrated to be due, at least in part, to anti-donor antibody
   A. Immediate (hyperacute)
   B. Delayed (accelerated acute)
3. Borderline changes: "Suspicious" for acute rejection
   This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (1 to 4 mononuclear cells/tubular cross section) and at least i1
4. Acute/active rejection

Type (Grade)	Histopathological findings
IA	Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells)
IB	Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of severe tubulitis (>10 mononuclear cells/tubular cross section or group of 10 tubular cells)
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 (v3 with accompanying lymphoctic inflammation)

5. Chronic/sclerosing allograft nephropathy[b]

Grade	Histopathological findings
Grade I (mild)	Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific changes suggesting chronic rejection
Grade II (moderate)	Moderate interstitial fibrosis and tubular atrophy (a) or (b)
Grade III (severe)	Severe interstitial fibrosis and tubular atrophy and tubular loss (a) or (b)

6. Other — Changes not considered to be due to rejection, see Table 14.

[a] The recommended format of report is a descriptive narrative signout followed by numerical codes in parentheses. Categorization should in the first instance be based solely on pathologic changes, then integrated with clinical data as a second step. More than one diagnostic category may be used if appropriate.

[b] Glomerular and vascular lesions help define type of chronic nephropathy; chronic/recurrent rejection can be diagnosed if typical vascular lesions are seen.

Table 1. Banff 97 diagnostic categories for renal allograft biopsies[a]

The initial modification of the schema is a change in definition of specimen adequacy. To

diagnose and categorize rejection, adequate cortex must be present in the material examined, and the change has been made to ensure more adequate cortical sampling. With the new emphasis on arteritis, a more generous minimal arterial sampling is also recommended. For Banff 97, an "adequate" specimen is now defined as a biopsy with 10 or more glomeruli and at least two arteries; the threshold for a minimal sample is seven glomeruli and one artery. It is also recommended that at least two separate cores containing cortex be obtained or that there be two separate areas of cortex in the same core. The recommendation for slide preparation is seven slides containing multiple sequential sections, three stained with hematoxylin and eosin (HE) stain, three with periodic acid-Schiff (PAS) stain or silver stains, and one with a trichrome stain. The PAS stain and silver stains enhance the identification of glomerulitis and tubulitis and any destruction of tubular basement membranes. These stains also enhance the recognition of chronic features such as arteriolar hyaline, increased mesangial matrix, double contours in glomerular capillaries, and thickened tubular basement membranes. The trichrome stain is useful in defining interstitial fibrosis. It is recommended that histologic sections should be cut at 3 to 4 microns, as the current definitions of lesion grading are not appropriate either for 1 micron plastic sections or for "routine" thicker sections obtained at some institutions.

Acute/active lesion scoring

Semiquantitative lesion scoring provides the morphologic basis for the rejection classification. While the basic features used to diagnose rejection are tubulitis and arteritis, a minimal threshold for interstitial inflammation must be reached to diagnose rejection of the tubulointerstitial type. Glomerulitis, although not a specific criterion for rejection, may have implications for late graft function, and is also graded. Tubulitis and vasculitis, as the cardinal features of rejection, will be considered first.

The Banff 93-95 schema grades tubulitis ("t" score) based on the greatest number of infiltrating mononuclear cells in the tubular epithelium (that is, having breached the tubular basement membrane and lying beneath or between tubular cells) per tubular cross section; if the tubule is sectioned longitudinally, results are expressed per 10 tubular cells (the average number of cells per cross-section). In the CCTT modification, significant tubulitis is defined by number of tubules with tubulitis in 10 serial high-powered fields from the area with the most inflammatory infiltrate. Banff 97 retains a focus on most severely inflamed tubules to grade tubulitis and requires that the tubulitis be present in more than one focus in the biopsy (Table 2). The most inflamed tubules should be sought in the most inflamed areas in the biopsy. Inflammatory tubular injury and/or breakdown of tubular basement membranes are included as significant histologic findings in Banff 93-95 and the CCTT modification, and are included in Banff 97 in the "t3" grade. Since tubulitis is seen routinely in atrophic tubules in native kidneys and cannot be interpreted as a specific response to alloantigen, tubulitis should not be graded in moderately-to-severely atrophic tubules, that is, tubules reduced in caliber by 50% or more.

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

[a] Applies to tubules no more than mildly atrophic

Table 2. Quantitative criteria for tubulitis ("t") score[a]

Arteritis is likewise a defining feature for rejection diagnosis in the allograft. Both Banff 93-95 and the CCTT formulations distinguish intimal arteritis, carefully defined as lymphocytic infiltration beneath the endothelium, from arteritis with inflammation in the media and/or with fibrinoid necrosis of the vessel wall. Parenchymal necrosis and/or interstitial hemorrhage were recognized as possible manifestations of severe arteritis by both classifications. Banff 93-95 vasculitis ("v") scores focused on intimal arteritis, with v1 defined as mild-to-moderate in at least one artery, v2 as moderate-to-severe in more than one artery, and v3 as severe in many arterial cross-sections and/or with transmural arteritis, fibrinoid change, and necrosis. However, because there is the potential for significant sampling error in defining vasculitis, it was agreed that the focus of grading should be on the most severely involved vessel (analogous to tubulitis scoring). A score of v3, or severe vasculitis (v3), is now reserved for those cases with transmural arteritis and/or arterial fibrinoid change and smooth muscle necrosis with accompanying lymphocytic inflammation in the vessel (Table 3). In reporting vasculitis, the total number of arteries and the total number involved by vasculitis should be recorded. If there is interstitial hemorrhage and/or infarction, an asterisk should be added to the "v" score. Interstitial hemorrhage and/or infarction alone (that is, v0*), while raising the specter of rejection with vascular involvement not sampled by the biopsy, is no longer considered adequate to presumptively score v3.

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 - Transmural arteritis and/or arterial fibrinoid change and medial smooth muscle necrosis with lymphocytic infiltrate in vessel
Note number of arteries present and number affected. Indicate infarction and/or interstitial hemorrhage by an asterisk (with any level v score).

Table 3. Quantitative criteria for intimal arteritis ("v")

While not itself a signal criterion for rejection, a background of interstitial inflammation is required to diagnose rejection of the tubulointerstitial type. Because minimal (and even significant) mononuclear inflammation is present in many protocol biopsies from asymptomatic patients, at least 10% of cortex must be inflamed as a threshold for grading of interstitial inflammation. Severe inflammation (i3) is defined when greater than 50% of the cortex is inflamed (Table 4). Areas that cannot be meaningfully graded for assessment of interstitial infiltrates are fibrotic areas, the immediate subcapsular cortex, and the adventitia around large veins and lymphatics. The infiltrate in classic cellular rejection consists of T lymphocytes and monocyte/macrophages. If there are more than 5 to 10% eosinophils, neutrophils, or plasma cells in the infiltrate, an asterisk is added to the "i" score, and other differential diagnoses should be considered, for example, hypersensitivity reaction or infection, as discussed later here. Moreover, the quality of the infiltrate must be analyzed in the context of clinical information. For example, tapering and withdrawal of immunosuppression may be followed by rejection infiltrates with a substantial component of plasma cells.

i0 - No or trivial interstitial inflammation (<10% of unscarred parenchyma)
i1 - 10 to 25% of parenchyma inflamed
i2 - 26 to 50% of parenchyma inflamed
i3 - more than 50% of parenchyma inflamed
Indicate the presence of remarkable numbers of eosinophils, PMNL, or plasma cells (specify which) with an asterisk (*).

Table 4. Quantitative criteria for mononuclear cell interstitial inflammation ("i") scores

Glomerulitis is graded in both the CCTT and Banff classifications, although it is not used as a criterion for rejection since its significance has been and remains controversial. The Banff schema grades glomerulitis, defined by mononuclear cell infiltrate and endothelial cell enlargement, by the percentage of glomeruli involved and whether the process is segmental or global within involved glomeruli. In the CCTT, glomerulitis may be absent, "focal," or "severe." The grading of glomerulitis in Banff 97 is shown in Table 5, with g1 defined as glomerulitis in less than 25% of glomeruli and g3 as glomerulitis that is mostly global and in more than 75% of glomeruli. Polymorphonuclear leukocytes in glomerular capillaries are not a feature of transplant glomerulitis, but may be seen in antibody-mediated rejection or in early thrombotic microangiopathy.

g0 - No glomerulitis
g1 - Glomerulitis in less than 25% of glomeruli
g2 - Segmental or global glomerulitis in 25 to 75% of glomeruli
g3 - Glomerulitis (mostly global) in more than 75% of glomeruli

Table 5. Quantitative criteria for early allograft glomerulitis ("g") score

The Banff 97 classification: Acute/active rejection

Acute/active rejection in the Banff 93-95 schema was divided into three grades: I, mild, characterized by moderate tubulitis; II, moderate, further divided into IIa with marked tubulitis and no vasculitis and IIb with mild-to-moderate intimal arteritis; and III, severe, characterized by severe intimal arteritis or transmural arteritis or intramural necrosis. In this earlier Banff classification, recent focal infarction and interstitial hemorrhage without obvious cause could be regarded as grade III rejection. In the CCTT modification, acute/active rejection was divided into three types: I, with significant tubulitis; II, with arterial or arteriolar endothelialitis; and III, with arterial fibrinoid necrosis or transmural inflammation.

The Banff 97 classification of acute/active rejection-related changes is shown in Table 6, and is compared with rejection categories from Banff 93-95 and the CCTT modification. In view of the recent studies that provide evidence that vasculitis *per se* has implications for response to therapy and/or graft survival [9, 11, 12], Banff 97 focuses on types of rejection. Type I is tubulointerstitial rejection without arteritis, further divided into type IA with focal moderate tubulitis and IB with severe tubulitis. Type II, vascular rejection, is characterized by intimal arteritis, further divided into IIA if the intimal arteritis is mild-to-moderate, and IIB if severe. Type III, severe rejection, is with transmural arteritis with or without fibrinoid and smooth muscle necrosis. Those cases with only mild tubulitis and/or with only mild focal interstitial inflammation remain in a

"borderline" category.

Banff 97	Banff 93–95	CCTT
Suspicious for acute rejection, borderline	Borderline	Type I[a]
Type IA (tubulointerstitial with t2 and at least i2)	Grade I	Type I[a]
Type IB (tubulointerstitial with t3 and at least i2)	Grade IIA	Type I[a]
Type IIA (vascular with v1)	Grade IIB	Type II
Type IIB (vascular with v2)	Grade III	
Type III v3 - (fibrinoid change/transmural arteritis)	Grade III	Type III

[a] Additionally requires at least i1 and at least 2 of the 3 following features: edema, activated lymphocytes, or tubular injury

Table 6. Overview of acute rejection

As in the previous working classifications, antibody-mediated rejection is also included, but now recognizing two forms, immediate (hyperacute) and delayed (accelerated acute). Except in classic hyperacute rejection occurring immediately post-transplant, antibody-mediated rejection should be confirmed by repeat cross-match, as discussed below. Antibody-mediated rejection can occur as an isolated rejection response or combined with cell-mediated rejection as an antibody-mediated component. The morphology of classic "pure" antibody-mediated rejection may be quite distinctive. In other cases, the antibody-mediated component is superimposed on cell mediated vascular changes (Discussion).

Lesion scoring: Chronic/sclerosing

Chronic/sclerosing changes develop in renal allograft with renal ischemia, hypertension, drug effects, infection, increased ureteral pressure, and nonimmune inflammatory processes, in addition to a subset due to chronic or recurring immune reaction to the graft [13]. Chronic changes may be seen in glomeruli, interstitium, tubules, and vessels, although not necessarily simultaneously or to the same degree. Because sampling error is less of a problem in sampling of tubules and interstitium, these features are the basis of the grading of severity of chronic allograft nephropathy. The grading of chronic interstitial fibrosis and tubular atrophy and/or loss remains unchanged from Banff 93-95, with quantitation based on the percentage of cortical parenchyma involved (Tables 7 and 8).

ci0 - Interstitial fibrosis in up to 5% of cortical area
ci1 - Mild - interstitial fibrosis in 6 to 25% of cortical area
ci2 - Moderate - interstitial fibrosis in 26 to 50% of cortical area
ci3 - Severe - interstitial fibrosis in >50% of cortical area

Table 7. Quantitative criteria for interstitial fibrosis ("ci")

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 in >50% of the area of cortical tubules

Table 8. Quantitative criteria for tubular atrophy ("ct")

The grading of chronic glomerular changes related to rejection, previously defined by mesangial matrix increase and basement membrane thickening, has now been refined. The presence of "double contours" in capillary loops, created by mesangial interposition, is the most specific change of chronic transplant glomerulopathy [14], whereas mesangial matrix increase is a potentially important but less specific finding. Therefore, the two are now graded separately. Severity of chronic glomerulopathy is now graded by the extent of "double contours" in the most severely affected glomerulus. The total number of glomeruli and the total number of non-specifically sclerotic glomeruli must be recorded (Table 9). An increase in mesangial matrix is graded by the percentage of nonsclerotic glomeruli with at least moderate mesangial matrix increase. Moderate mesangial matrix increase, in turn, is defined by expansion of the matrix in the mesangial interspace between adjacent glomerular capillaries to exceed the width of two mesangial cells in at least two lobules. Grading of mesangial matrix increase ("mm" score) is shown in Table 10. Transplant glomerulopathy often also includes mesangiolysis and progressive sclerosing changes; the latter may be difficult to distinguish from membranoproliferative glomerulonephritis or, in some cases, focal segmental glomerulosclerosis.

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 the number of glomeruli and percentage sclerotic.

Table 9. Quantitative criteria for allograft glomerulopathy ("cg")

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

Table 10. Quantitative criteria for mesangial matrix increase ("mm")[a]

Vascular changes potentially enable identification of kidneys with chronic/sclerosing changes due to chronic rejection. Specific chronic vascular changes that suggest that vascular changes are due to "chronic rejection" are disruptions of the elastica, best seen on special stains, and inflammatory cells in the fibrotic intima. Proliferation of myofibroblasts in the expanded intima and formation of a second "neointima" are also useful features [15, 16]. Fibrointimal thickening in vessels without these features is a significant finding, especially if it is of new onset and is graded, but it is not regarded as specific for "chronic rejection." Recognizing that vascular changes may be focal, chronic vascular changes are graded based on the extent of occlusion of the most severely affected vessel (Table 11).

cv0 - No chronic vascular changes
cv1 - Vascular narrowing of up to 25% luminal area by fibrointimal thickening of arteries ± breach of internal elastic lamina or presence of foam cells or occasional mononuclear cells[a]
cv2 - Increased severity of changes described above with 26 to 50% narrowing of vascular luminal area[a]
cv3 - Severe vascular changes with >50% narrowing of vascular luminal area[a]
[a] In the most severely affected vessel. Note if lesions characteristic of chronic rejection (breaks in the elastica, inflammatory cells in fibrosis, formation of neo-intima) are seen.

Table 11. Quantitative criteria for vascular fibrous intimal thickening ("cv")

Finally, arteriolar hyaline change, particularly if nodular and documented to be of new onset, may be an important manifestation of cyclosporine or FK506 toxicity [17], as discussed later here, and has a separate lesion scoring in the schema. The scoring of this lesion remains unchanged from Banff 93-95 (Table 12). Arteriolitis is a lesion that is currently of uncertain significance; if present, it is designated by an asterisk added to the "arteriolar hyalinosis" ("ah") score.

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.

Table 12. Quantitative criteria for arteriolar hyaline thickening ("ah")

Because it is often impossible to define the precise cause or causes of chronic allograft damage, the term "chronic/sclerosing allograft nephropathy" is preferable to "chronic rejection," which implies allogeneic mechanisms of injury, unless there are specific features to incriminate

such a rejection process. However, recognition of those cases that do represent "chronic/recurrent rejection" may be important, as there are preliminary data suggesting that therapy may be efficacious in these cases [18]. In chronic/sclerosing allograft nephropathy, grades 1 (mild), 2 (moderate), and 3 (severe), as mentioned earlier here, may be modified by "a" (no changes strongly suggestive of chronic rejection in glomeruli and/or vessels present) or "b" (changes strongly suggestive of chronic rejection present (Table 1). If convincing diagnostic features are present, a diagnosis of "chronic/recurrent rejection" can be made.

The Banff 97 combined working classification

The Banff 97 combined classification of renal allograft pathology includes acute/active rejection, chronic/sclerosing allograft nephropathy, and other morphologic findings, including *de novo* and recurrent diseases, toxic changes, and infection (Tables 1 and 13). Major changes from the previous Banff schema are summarized in Table 14.

Post-transplant lymphoproliferative disorder
Nonspecific changes
focal intersitial inflammation without tubulitis
reactive vascular changes
venulitis
Acute tubular necrosis
Acute interstitial nephritis
Cyclosporine or FK506-associated changes, acute or chronic
Subcapsular injury
“healing in ”
Pretransplant acute endothelial injury
Papillary necrosis
De novo glomerulonephritis
Recurrent disease
immune complex glomerulonephritis
focal segmental glomerulosclerosis
diabetes
hemolytic-uremic syndrome
other
Pre-existing disease
Viral infection
Obstruction/reflux, urine leak
Other

Table 13. Other non-rejection diagnoses in renal allograft biopsies

Lesion scoring - Acute
severity of vasculitis is based on most severely involved vessel
moderate vasculitis (v2) is now severe intimal arteritis (more than 25% luminal occlusion)
severe vasculitis (v3) now requres inflammatory changes in muscle wall
interstitial hemorrhage and/or necrosis is no longer sufficient to grade v3
threshold for grading interstitial inflammation is more than 10% of non-scarred cortical parenchyma
Lesion scoring - Chronic
transplant glomerulopathy (cg) is now defined by "double contours"
cg now graded by severity in most involved glomerulus
chronic vascular changes (cv) now flagged as due to chronic rejection if characteristic changes seen
mesangial matrix increase (mm) now scored separately
Combined schema
antibody-mediated rejection replaces "Hyperacute rejection" and is further defined
acute rejection now defined as "types": I, tubulointerstitial; II, vascular; and III, severe

[a] Asterisks now are used to denote unusual cell composition of intersitial infiltrates (1*), presence of hemorrhage and/or necrosis (v*), and arteriolitis (ah*).

Table 14. Changes from Banff 93, 95[a]

DISCUSSION [Context Link]

The Banff 97 Working Classification represents input from the two classifications most widely used in large clinical rejection trials and in clinical practice worldwide to diagnose acute rejection. This new international classification follows earlier classifications that took the approach of semiquantitative grading of rejection lesions to provide an acute rejection index. Finkelstein et al published such a classification in 1976, in the pre-cyclosporine era [19]. This classification graded interstitial inflammation, glomerulitis, and arteritis; intimal arteritis and tubulitis were not recognized separately. Mild rejection had interstitial inflammation; moderate and severe rejection were characterized by vasculitis. Banfi et al published a similar classification in the same era [20], recognizing an irreversible form of rejection with large artery changes and infarction. In 1983, Matas et al proposed a schema with eight grades, the first four defined by minimal-to-severe tubulointerstitial nephritis, categories 5 through 7 defined by minimal-to-moderate vasculitis, and category 8 reserved for cases with severe vascular rejection with fibrinoid necrosis [21]. These grades showed a general correlation with survival, although numbers in some of the categories were too small to draw firm conclusions.

Several studies have concluded that the presence of vasculitis in a renal allograft biopsy is associated with poorer response to therapy and/or outcome. For example, Visscher et al found that in cases with steroid-resistant rejection, the response to OKT3 was lower in those with vascular injury (arteritis and/or chronic changes) [22]. Vasculitis (intimal arteritis ± fibrinoid necrosis) has also been reported to impact negatively on allograft survival [23]. In a pediatric series, all of those with vasculitis (mostly severe) lost their allograft [2]. While a deleterious

impact of vasculitis on rejection outcome has not been a uniform finding [19], three very recent studies, summarized briefly later here, reach a conclusion similar to these earlier studies, and have led to the Banff 97 categorization of acute/active rejection changes as "types" (tubulointerstitial or vascular) rather than "grades" of rejection.

The Roche mycophenolate mofetil study [11] included 87 biopsies scored blinded to clinical history or outcome using the Banff criteria. The highest tubulitis and vasculitis scores in the biopsy/biopsies obtained post-transplant from each case, as defined by the Banff 93-95 grading system, were recorded. The finding of vasculitis of any grade was significantly correlated with allograft loss. Outcome, defined by graft survival, was independent of rejection therapy cohort.

In a study of the modified Banff grading system used in the CCTT [9], in which type I rejection is defined by tubulointerstitial inflammation, type II by intimal arteritis, and type III by arterial necrosis or transmural inflammation, there was a significant correlation of these patterns with severity of clinical rejection. Clinically severe rejection was defined in these protocols as a rejection episode that was steroid resistant, treated with ATG, OKT3, or FK506, or was of early onset, occurring within 10 days of transplantation. The odds ratio for severe rejection was 6.2 for Type I and 37.9 for Type II. Since this classification does not provide semiquantitative grading of severity of individual inflammatory changes, no correlations with severity of inflammatory changes were defined, except that extent of tubulitis or interstitial infiltrate did not correlate with severity.

In a more recent study, Nickeleit et al analyzed the prognostic significance of vascular lesions in rejection [12]. They found that rejection with endarteritis was significantly less responsive to steroid therapy than rejection without endarteritis. One-year graft failure was also somewhat higher in the group with arteritis (28%) than without (21%), although the difference was not significant. Conversely, severity of interstitial inflammation and tubulitis (defined by CCTT criteria) did not correlate with response to therapy or outcome.

The threshold for rejection diagnosis is an important component of any diagnostic grading system. It is clear that some inflammatory changes are to be expected in any allograft, but do not necessarily signal rejection. Examination of protocol biopsies in asymptomatic patients has revealed that, in some cases, significant interstitial inflammation may be present [24, 25]. This observation led to a de-emphasis of interstitial inflammation in establishing a diagnosis of rejection in both the Banff and CCTT classifications. Similarly, mild tubulitis, defined in the Banff schema as no more than four inflammatory cells in the most inflamed tubule, has been documented in biopsies from well-functioning allografts as well and is, therefore, not included as a criterion for rejection.

Rush et al established a protocol in which they biopsied asymptomatic patients at intervals post-transplant [26]. Using Banff criteria, they found that approximately one-third of these patients had "subclinical rejection," that is, i2t2 with a less than 10% change in serum creatinine. Patients randomized to early protocol biopsies and treatment of this "subclinical rejection" had a significantly lower creatinine at 24 months than those patients randomized to the control arm [27]. This finding suggests that the threshold of i2t2 for the diagnosis of rejection is likely appropriate, even in those cases with no change in serum creatinine, since untreated chronic graft injury may result.

The significance of "borderline" rejection [mild tubulitis (t1) only, or focal tubulitis with only mild interstitial inflammation (i1)] has been difficult to define. If mild tubulitis, as defined by the Banff criteria, was included as a criterion for rejection in the study by Rush et al, over 50% of

the patients would have subclinical rejection, likely leading to unnecessary increase in immunosuppressive therapy. A few studies have looked at this "borderline" cohort. In some series, patients with borderline rejection usually responded to antirejection therapy; however, the finding of borderline changes with mild tubulitis does not always correlate with clinical rejection as defined by response to therapy [28-30]. In some centers, biopsies are obtained after treatment is initiated so that inflammatory changes may have diminished in individuals that did indeed have a significant rejection episode; in this circumstance, i1t2 lesions may, in fact, have clinical significance as an indicator of rejection. It is clear that these mild inflammatory changes can only be adequately interpreted in a clinical context; borderline changes in biopsies obtained in the context of decreased function may require therapy, whereas borderline changes in protocol biopsies performed on patients with stable graft function may not [30]. Possible diagnoses for this category include the following: suspicious for acute rejection, borderline for acute rejection, borderline inflammatory changes only, possible (early) acute rejection, probable (early) acute rejection. The final designation may depend on center experience, therapy, time after transplant, and other clinical and morphological features, including other signs of inflammatory cell activation or tissue injury.

The criteria for rejection diagnosis in the CCTT modification included tubulitis plus two of the following three criteria: interstitial edema, activated lymphocytes (or blasts), or tubular injury. However, on evaluation of the individual pathologic criteria for rejection, removal of these three additional criteria resulted in reclassification of only two cases, one that responded to antirejection therapy and one that did not [9]. Moreover, in those centers in which biopsy is frequently performed after steroid bolus therapy, edema and activated lymphocytes are much diminished within one to two days. These additional criteria, however, may occasionally be useful when combined with other morphologic findings and in clinical context in those cases with borderline changes [31].

Type I and type II rejection are both thought to be manifestations of cell-mediated rejection. However, type II may be seen in and type III is strongly suggestive of an antibody-mediated component to the rejection process. Other pathologic features suggesting an antibody-mediated component have been identified in cases in which antidonor antibody has been identified [32, 33]. These features include widespread endothelial injury with more severe vasculitis (frequently accompanied by fibrinoid changes in the vessel walls), glomerular and small vessel thromboses, infarctions, glomerulitis, marginating cells, and especially polymorphonuclear leukocytes, in peritubular capillaries. When these features are prominent, the biopsy findings should be graded according to the Banff criteria, and the possibility of an antibody-mediated rejection component should be indicated as well. The presence of antibody-mediated rejection should be confirmed by a repeat donor-specific cross-match.

Banff 97 also includes grading of chronic/sclerosing change in renal allograft biopsies. This remains an important component, as most allografts are now lost to often slowly evolving and clinically indolent sclerosis in the allograft. Recognizing that the tubulointerstitial changes are most accurately sampled and have the strongest correlation with outcome in native as well as allograft kidneys [34], the grading of severity of chronic rejection continues to focus on interstitial fibrosis and tubular atrophy and loss. However, identification of distinctive vascular changes may enable the diagnosis of chronic rejection, which in turn may be amenable to therapy [18]. The other major grading system that focuses on chronic changes is the Chronic Allograft Damage Index (CADI), which provides semiquantitative assessment of a number of chronic and inflammatory features that have been validated as clinically relevant predictors of allograft outcome [35]. The CADI and the Banff schema have been adjusted to provide equivalent information.

It must be emphasized that although rejection-related changes are a focus of the Banff 97 schema, there are a number of other disease processes that may involve the allograft and must be considered in the differential diagnosis (Table 13). Those processes that produce inflammatory changes in the allograft must be differentiated from acute rejection. Polymorphonuclear leukocytes (PMNL) in the interstitium and especially in tubular lumina may signal acute bacterial infection, although they may be seen in cases in which there is significant ischemic injury and infarction (which may in turn be rejection related). If PMNL are confined to peritubular and glomerular capillaries, the possibility of severe acute endothelial injury and possible antibody-mediated rejection must be considered. While numerous eosinophils may be a feature of the inflammatory response to alloantigen, the possibility of a hypersensitivity reaction must be in the differential as well.

Viral infections must always be considered, as inflammatory infiltrates in this setting are typically mononuclear, and significant tubulitis may be seen. The specimen should be examined carefully for evidence of viral cytopathic features such as megalic cells, nuclear smudging, or intranuclear or cytoplasmic inclusions. If the clinical or pathological index of suspicion is high, immunohistology or *in situ* hybridization can be used to enhance identification of viral agents. Cytomegalovirus [36], polyoma (BK) virus [37], and adenovirus [38] may all infect the allograft. Colvin believes that relatively severe tubular cell injury with relatively mild inflammation should suggest the possibility of a viral infection [39]. Infection may, of course, coexist with rejection, making diagnosis and therapy problematic.

Plasma cells may likewise be a component of the rejection response, but may also signal infection. If the plasma cells are part of an aggressive infiltrate, that is expanding and displacing normal structures, and especially if the cells are atypical, post-transplant lymphoproliferative disorder (PTLD) must be ruled out. The separation of renal Epstein-Barr virus (EBV)-associated PTLD from severe acute rejection at biopsy remains very important, as the appropriate treatment is reduction of immunosuppression for PTLD, but aggressive anti-T-cell therapy for severe rejection. Potential differential features have been identified [40]. PTLD typically shows expansile or nodular mononuclear infiltrates with irregular foci of serpiginous necrosis. PTLD lesions may be focal or diffuse, and the latter may result in extensive involvement of the pericalyceal adipose tissue and nerves. The infiltrates in PTLD generally show the entire spectrum of lymphocyte differentiation, including immunoblasts, plasma cells, large cleaved/noncleaved cells, and small round lymphocytes. Cells with marked nuclear atypia are usually present and help in the differential diagnosis from rejection. Some biopsies have a monotonous appearance, and such patients may be histologically and clinically indistinguishable from intermediate-to-high grade lymphomas in nonimmunocompromised patients.

Although they are not as readily found as in severe rejection, PTLD cells can also be associated with tubulitis. Of course, rejection and PTLD can coexist in a biopsy [41], making accurate diagnosis especially difficult. In most cases, and especially with limited biopsy material, the final diagnosis must await the results of immunophenotyping, and EBV *in situ* hybridization. With rare exceptions, PTLD lesions are B-cell preponderant and EBV positive, whereas rejection is associated with a primarily T-cell infiltrate, which is EBV negative. CD20 (B-cell marker) and CD3 (T-cell marker) immunohistochemistry is a reliable way of phenotyping infiltrates in formalin-fixed material. The most sensitive technique for demonstrating EBV in routinely processed tissue is *in situ* hybridization for EBV-encoded small RNA [42]. In lesions with significant numbers of plasma cells, staining for kappa and lambda light chains is a convenient way of identifying lesions that are clearly clonal. If sufficient fresh tissue is available, immunoglobulin gene rearrangement and oncogene studies should also be performed, as molecular findings have also been related to ultimate prognosis [43].

Toxic effects of cyclosporine and of tacrolimus also remain important differential considerations. Toxic effects of cyclosporine have been studied for some time, but tacrolimus is a relatively new agent, and its toxicity profile is still being defined. Cyclosporine and tacrolimus share a closely related mechanism of action, which is paralleled by an overlap in the toxicity profile of these two drugs. The pathology of tacrolimus nephrotoxicity appears to be similar to cyclosporine toxicity [44-47], although it has been much less completely studied. Tubular vacuolization is the most common finding in biopsies performed during clinical episodes of tacrolimus nephrotoxicity; tubular vacuoles may be seen in proximal as well as distal tubules, and although these are often isometric, focal coalescence into larger vacuoles is also present. As with cyclosporine, tacrolimus therapy may also be associated with microvascular toxicity characterized by damage to the glomerular capillaries and renal arterioles. Arteriolar damage mediated by tacrolimus sometimes results in an acute arteriolopathy characterized by endothelial swelling, mucoid intimal thickening, eosinophilic globules in the media, and focal medial necrosis. Scattered thrombi may be seen in capillary loops or afferent arterioles. Prolonged tacrolimus therapy results in arteriolar hyaline eosinophilic deposits comprised of fibrin, IgM, C3, and C1q, which may be difficult to distinguish from those due to aging, hypertension, and diabetes mellitus. As with CsA, arteriolar myocyte vacuolization can be seen; this lesion is a nonspecific manifestation of vessel spasm and should be ascribed to tacrolimus toxicity only after exclusion of other causes of vessel injury. Druginduced vasospasm and the hyalinization of the interlobular arteries and arterioles may lead to ischemic injury accentuated in the medullary rays and probably also the medullary inner stripe [48], leading to striped or diffuse interstitial fibrosis.

Significant tubulointerstitial inflammation or vasculitis may also be components of recurrent or *de novo* renal disease in the allograft. These differential considerations must be considered at any time post-transplant and become more likely as time post-transplant increases. A good pretransplant clinical history can be invaluable in considering differential diagnoses.

Finally, future advances in analysis of renal allograft biopsies can already be predicted, and the classification and grading of acute/active rejection will continue to evolve. The significance of specific morphologic findings-including glomerulitis, arteriolitis, and infiltrates with unusual cellular features-for acute and chronic allograft function and outcome will continue to be investigated. Emphasis in biopsy assessment will shift from diagnosis to prediction of later allograft function and outcome, potentially enabling early intervention. Indeed, two recent studies have shown that chronic histologic changes detected in early protocol biopsies and graded using the 93-95 Banff schema were predictive of long-term outcome [49, 50]. Clinical utility of renal allograft biopsies, for both diagnosis and prediction of outcome, will be enhanced by application of immunostaining and molecular studies. Identification of effector cells such as NK cells and cytotoxic T-lymphocytes and of monocyte/macrophages may enhance diagnosis and/or predict later dysfunction. Molecular studies show promise in refining the diagnosis of acute/active rejection [51], although much more needs to be done to establish validity in cases with borderline features and to disseminate the technology. It will be important to establish which molecular markers correlate with interstitial infiltrates and which correlate with invasive inflammation (tubulitis, intimal arteritis). Also, more precise definition of histologic and molecular features of indolent graft injury and sclerosis should enable better understanding of pathogenesis of progressive damage and enable appropriate therapy. There is clearly much work to be done in optimizing the assessment of the renal allograft by the pathologist as we move into the 21st century. Many of these issues will receive focused attention at the Fifth Banff Conference on Allograft Pathology in 1999 and in other international forums, which have become logical venues for such consensus in the global medical community.

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OVERVIEW

ROLE OF DONOR KIDNEY BIOPSIES IN RENAL TRANSPLANTATION

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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

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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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Polyomavirus Allograft Nephropathy: Sequential Assessment of Histologic Viral Load, Tubulitis, and Graft Function Following Changes in Immunosuppression

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Our initial cases of polyoma virus allograft nephropathy (PVAN) received pulse steroids due to anxiety about concomitant acute rejection triggered by the presence of tubulitis. However, our current policy is to reduce immunosuppression in all cases. The aim of this study was to determine whether clinical follow-up in these patient categories shows any differences in: (a) histologic viral load, (b) grade of tubulitis, and (c) graft function. Reduced viral load assessed within 8 weeks was seen in 4/20 (20.0%) biopsies treated initially by increased immunosuppression, compared to 15/19 (83.3%) biopsies treated with reduced immunosuppression ($p = 0.001$, Fisher's exact test). Yet, >70% reversal of the rise in serum creatinine occurred in only 3/19 (15.8%) and 1/19 (5.3%) patients, respectively, in these two groups. Improved tubulitis was seen in 11/20 (55%) of biopsies treated with steroids, despite the lack of beneficial effect on serum creatinine in 12/19 (63.1%) instances. In biopsies not treated with any change in immunosuppression, the serum creatinine remained stable in 1/5 (20%) and worsened in 4/5 (80%) biopsies. These data demonstrate that in biopsies with PVAN and tubulitis, reduced immunosuppression is more effective in lowering viral load than steroid therapy. Lack of parallelism between viral load, tubulitis grade, and serum creatinine illustrates a complex interplay of viral and alloimmune factors leading to graft injury.

Key words: BK virus, histology, immunosuppression, interstitial nephritis, kidney, nephropathy, pathology, transplantation

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Introduction

Polyomavirus nephropathy (PVAN) is an increasingly recognized complication in kidney transplant recipients (1–5). The histology is characterized by viral inclusions, interstitial inflammation, and tubulitis. The significance of tubulitis in these cases has been a subject of some debate. Most clinicians accept it as a part of viral-induced parenchymal injury, and reduce immunosuppression after a diagnosis of PVAN. However, others contend that tubulitis is a manifestation of concurrent acute cellular rejection, and advocate increased immunosuppression (6). In an attempt to clarify this controversy, we performed a study to determine the effect of changes in immunosuppression on: (a) histologic viral load, (b) grade of tubulitis, and (c) serum creatinine as a marker of graft function. The series presented includes our initial cases, who were briefly treated with steroids because of certainty about concomitant acute rejection, and our subsequent cases treated by reduced immunosuppression from the outset, despite the presence of concomitant tubulitis.

Materials and Methods

The diagnosis of PVAN was provisionally made by histologic documentation of polyomavirus inclusions in the tubular epithelium accompanied by varying degrees of interstitial inflammation. The diagnosis was confirmed in all cases by immunohistochemistry or *in-situ* hybridization, as previously reported (2). Viral infection localized to the nuclei in the tubular epithelium. Both proximal and distal tubules were affected. The number of infected cells varied from 1 to 10 per tubular cross-section, with 1–70% of the sampled renal parenchyma showing evidence of viral cytopathic effect. All biopsies were reviewed in detail by a pathologist and graded for tubulitis and interstitial inflammation using Banff 1997 criteria (7). Changes in these histologic parameters were assessed both as Banff scores, and, when the score was numerically unchanged, by a 'gestalt approach'. The viral load, assessed by light microscopy, immunohistochemistry, and *in-situ* hybridization, was graded semi-quantitatively as grade 0, 1 + (less than 5 infected cells), 2 + (5–15 infected cells), or 3 + (>15 infected cells). Follow-up biopsies were recorded as complete viral clearance, improved, unchanged, or worse compared to the index specimen. In 12 biopsies the tissue viral load was also determined using a published real-time quantitative PCR assay (8). Clinical data were abstracted from the medical records. For each biopsy studied, we obtained a baseline creatinine (4 weeks prior to biopsy), a peak serum creatinine, a post-therapy creatinine (2 weeks after therapy), and the most

recent serum creatinine, to assess the current graft status. Changes in immunosuppression in relation to each biopsy were individually analyzed. If these changes led to >70% reversal of the rise in serum creatinine, the biopsy was classified as 'complete response'. Graft dysfunction episodes with 30–70% reversal qualified for 'partial response', <30% reversal as 'stable creatinine', and rise in creatinine >30% as 'worse'. A Banff score of t_0 was designated as 'complete response' with regard to tubulitis. This study was approved by the University of Pittsburgh Institutional Review Board (IRB protocol # 000622).

Results

The 66 biopsies studied here were obtained from 31 patients with polyoma virus allograft nephropathy. There were 12 females and 19 males, with a mean age of 47 ± 13 years (range 21–72, median 50) years. The native kidney diseases that led to transplantation were diabetes mellitus (9/31, 29%), chronic glomerulonephritis (5/31, 16.1%), hypertension (6/31, 19.1%), focal segmental glomerulosclerosis (1/31, 3.2%), Alport's syndrome (2/31, 6.5%), Henoch Schonlein purpura (1/31, 3.2%), dysplastic kidney (2/31, 6.5%), polycystic kidney (1/31, 3.2%), reflux (2/31, 6.5%), and systemic lupus erythematosus (2/31, 6.5%). The time of onset of viral nephropathy was 11 ± 6.8 months (range 2–32, median 11) months after transplantation. The mean and median baseline serum creatinine prior to the diagnosis of PVAN was 2.0 ± 1 mg/dL and 2.6 mg/dL, respectively. At the time viral nephropathy was diagnosed, the mean and median serum creatinine were 2.6 ± 1 mg/dL and 2.6 mg/dL, respectively. Eleven patients (35.5%) lost their grafts, five underwent allograft nephrectomy, and one was re-transplanted. Chronic allograft nephropathy was the primary cause of graft loss in all cases. However, persistent viral DNA and antigens indicative of ongoing viral nephropathy were also demonstrable in five patients. One patient showed no viral DNA or antigens in the tissue, but viruria could be detected by PCR. The probability of graft survival 1 year after diagnosis was 53.8%. Excluding patients whose grafts had failed, the most recent serum creatinine, measured 95.4 ± 102 weeks after the diagnosis of PVAN, was 3.6 ± 1.5 mg/dL.

Tables 1 and 2 summarize data on clinico-pathologic correlations presented in 3 categories, depending on whether the diagnosis of PVAN led to an initial increase, decrease, or no change in overall immunosuppression. Table 1 focuses on 45 biopsies, each of which was followed by a repeat biopsy obtained within 8 weeks. In this group, 20 biopsies were treated with increased immunosuppression, 19 biopsies with decreased immunosuppression, and 6 biopsies with no change in immunosuppression. Table 2 presents an analysis of 21 biopsies with regard to long-term changes in viral load, tubulitis, and serum creatinine. In this group, 11 biopsies were treated with increased immunosuppression, and 10 biopsies with decreased immunosuppression. The smaller number of data points in this table reflects the

Table 1: Changes in immunosuppression correlated with short-term effects on viral load, tubulitis, and serum creatinine

	Initial increase				Decrease				No change			
	CR	PR	NR		CR	PR	NR		CR	PR	NR	
			Stable	Worse			Stable	Worse			Stable	Worse
Viral load	3/20 [15]*	1/20 [5]*	7/20 [35]	9/20 [45]	7/19 [41.2]	8/19 [42.1]	0/19 [0]	4/19 [21.1]	1/6 [16.7]	2/6 [33.3]	0	3/6 [50]
Tubulitis	2/20 [10]	9/20 [45]	5/20 [25]	4/20 [20]	0	5/19 [26.3]	4/19 [21.1]	10/19 [52.6]	0	1/6 [16.7]	0	5/6 [83.3]
Creatinine	3/19 [15.8]	4/19 [21.1]	1/19 [5.3]	11/19 [57.9]	1/19 [5.3]	3/19 [5.8]	2/19 [10.5]	13/19 [68.4]	0	0	1/5 [20]	4/5 [80]

*p = 0.001 CR + PR Increased vs. Decreased Immunosuppression (Fisher's Exact Test).
Abbreviations: CR-complete response; PR-partial response; NR-no response. Parentheses indicate per cent values.

Table 2: Long-term effects on viral load, tubulitis, and serum creatinine

	Initial increase in immunosuppression				Decrease in immunosuppression			
	CR	PR	NR		CR	PR	NR	
			Stable	Worse			Stable	Worse
Viral load	8/11 [72.8]	2/11 [18.2]	0/11 [0]	1/11 [9.1]	7/10 [70]	2/10 [20]	0/10 [0]	1/10 [10]
Tubulitis	3/11 [27.3]	6/11 [54.5]	1/11 [9.1]	1/11 [9.1]	0/10 [0]	5/10 [50]	2/10 [20]	3/10 [30]
Creatinine	3/10 [30]	2/10 [20]	0/10 [0]	5/10 [50]	0/9 [0]	2/9 [22.2]	1/9 [11.1]	6/9 [66.6]

No statistically differences were found amongst different biopsy categories.
Abbreviations: CR-complete response; PR-partial response; NR-non response. Parentheses indicate per cent values.

lack of availability of a follow-up sample for histologic examination.

PVAN biopsies treated with initial transient increase in immunosuppression

The increased immunosuppression in these cases was temporary, and was prompted by the presence of tubulitis, which is conventionally regarded as a marker of acute cellular rejection in the allograft kidney. In an attempt to separate the short-term consequences of steroid therapy and the subsequent long-term effects of reduced immunosuppression (after steroid therapy had failed), data were separately analyzed for follow-up biopsies performed <8 weeks (Table 1, n = 20) and >8 weeks (Table 2, n = 11) after the first diagnosis of PVAN.

In biopsies performed within 8 weeks of diagnosis (Table 1), serum creatinine showed a complete therapeutic response in 3/19 (15.8%) episodes of renal dysfunction, a partial response in 4/19 (21.1%) episodes, and no response in 12/19 (63.2%) patients. The 'no response' category included one patient in whom creatinine remained stable, and 11 patients in whom the serum creatinine worsened after initial therapy. Changes in viral load generally paralleled the trends in serum creatinine. Thus, a decreased viral load (partial response) was seen in 1/20 (5%), and clearance of virus (complete response) in an additional group of 3/20 (15%) samples. The percentage of biopsies showing viral clearance or decreased viral load was significantly lower in patients treated with an initial increase in immunosuppression, compared with patients whose immunosuppression was reduced at the outset (20% vs. 83.3%, p = 0.004, Fisher's exact test). Partial or complete resolution of tubulitis was observed in a higher proportion of cases treated with an initial increase in immunosuppression, compared to biopsies where immunosuppression was reduced at the outset (55% vs. 26.3%). However, an associated improvement in serum creatinine (complete or partial response) was seen in only 7/19 (36.8%) biopsies, and it was of a transient nature.

In biopsies performed beyond 8 weeks of initial diagnosis, after reduction of immunosuppression (Table 2), serum creatinine showed a complete therapeutic response in 3/10 (30.0%) episodes of renal dysfunction, partial response in 2/10 (20%) episodes, and deterioration in the remaining 5/10 (50.0%) episodes. As a rule, the viral load was substantially improved. Thus, decreased viral load occurred in 2/11 (18.2%), and complete clearance of virus in 8/11 (72.8%) of samples. The latter group included all five biopsies with complete or partial creatinine response. Tubulitis showed complete resolution in 3/11 (27.3%) and partial improvement in 6/11 (54.5%).

PVAN biopsies treated with no change in immunosuppression (Table 1, n = 6)

All of these biopsies were performed within 8 weeks of the initial diagnosis of PVAN to assess the progression of viral nephropathy (Table 1). Serum creatinine was stable in 1/5 (20.0%), and worse in 4/5 (80%) cases. No data were available for one biopsy. The grade of tubulitis improved in 1/6 (16.7%), and worsened in 5/6 (83.3%) biopsies. Decreased viral load was demonstrable in 2/6 (33.3%) of follow-up biopsies, while 1/6 (16.7%) biopsies showed complete viral clearance. The remaining 3/6 (50%) samples showed increased viral cytopathic effect in the tubular epithelium.

PVAN biopsies treated with decreased immunosuppression from the outset

Serum creatinine measured within 8 weeks of biopsy showed: (a) complete therapeutic response in 1/19 (5.3%) episodes of renal dysfunction, (b) partial response in 3/19 (15.8%) episodes, (c) stable values in 2/19 (10.5%), and (d) progressive rise in the remaining 13/19 (68.4%) episodes (Table 1, n = 19). There was decreased viral load in 8/19 (42.1%) and complete clearance of virus in 7/19 (41.2%) samples.

In biopsies performed >8 weeks after the index specimen (Table 2, n = 10), serum creatinine showed: (a) complete therapeutic response in 0/9 (0%) episodes of renal dysfunction, (b) partial response in 2/9 (22.2%) episodes, (c) stable values in 1/9 (11.1%), and (d) progressive rise in the remaining 6/9 (66.6%) episodes. Decreased viral load was demonstrable in 2/10 (20.0%) and complete clearance of virus in 7/10 (70.0%) of samples. Tubulitis showed improvement in 5/10 (50.0%) of follow-up biopsies. The lack of improvement or worsening of tubulitis in half of the

patients, despite reduction in viral load, suggests that reduction of immunosuppression was complicated by the development of irreversible viral or alloimmune injury to the graft.

In 12 biopsies, we correlated changes in immunosuppression with intra-tissue viral concentrations measured by real-time quantitative PCR. Four biopsies were treated by increased immunosuppression, and in 2 instances, this led to increased viral load in a follow-up biopsy. The remaining 2 follow-up biopsies showed no significant change in viral load, within the limits of assay precision. Seven biopsies were treated with decreased immunosuppression, and 6 of these showed decreased viral load, while the seventh biopsy showed no significant change in tissue viral concentration. One biopsy was not treated by any change in immunosuppression, and in this case, a follow-up biopsy showed increased viral load.

Discussion

PVAN is a complication of excessive immunosuppression in kidney transplant patients. Most clinicians reduce the dose of immunosuppressive drugs after the diagnosis is made, but some have advocated a brief course of steroids if tubulitis is present. The purpose of this study was to determine the effect of changes in immunosuppression on: (a) histologic viral load, (b) grade of tubulitis, and (c) serum creatinine as a marker of graft function. Clinical response as assessed by serum creatinine did not show statistically significant differences between the patients treated with an initial increase or decrease in immunosuppression. Thus, complete reversal of serum creatinine within 8 weeks of biopsy was seen in 3/19 (15.8%) of biopsies treated by increased immunosuppression, and in 1/19 (5.3%) of biopsies treated with decrease in immunosuppression (Table 1). However, the majority of biopsies treated with steroids showed either no change (1/19 = 5.3%), or actual worsening (11/19 = 57.9%) of serum creatinine. Even when improvement in serum creatinine occurred following steroid therapy, it was of a transient nature.

Biopsies treated with no change in immunosuppression showed a persistently high viral load in 3/6 (50.0%) of the cases (Table 1). Increased immunosuppression led to persistent or worsened viral cytopathic effect in 16/20 (80%) of biopsies within 8 weeks. In contrast, reduction of immunosuppression led to viral clearance in 7/10 (70%) of biopsies after long-term follow-up (Table 2). These observations form the basis of our current policy to reduce the immunosuppression, whenever a diagnosis of PVAN is made. Nonetheless, the clinical course of individual patients is variable, and reduction of viral load did not always translate into improved graft function, probably due to irreversible chronic allograft nephropathy (9), and perhaps the confounding effect of alloimmune injury. Long-term follow-up did not show any statistically significant differences between patients who did or did not receive a brief initial course of steroid therapy (Table 2). It is also worth mentioning that viral clearance was observed even in a few patients getting no therapy or steroid therapy for presumed concomitant rejection.

A somewhat paradoxical observation made during this study is that partial or complete resolution of tubulitis was observed in 11/20 biopsies treated with initial increase in immunosuppression (Table 1). This suggests that tubulitis in these biopsies was, at least partially, related to an associated component of alloimmune injury. In this context, it is pertinent to recall that Hirsch et al. have recently reported 4 cases of PVAN with concurrent acute cellular rejection, which responded to steroid therapy (10). These patients had been diagnosed relatively early during the course of a study which mandated prospective monitoring of urine and blood for BKV infection. In contrast, Limaye and colleagues have documented a patient with acute cellular rejection and BKV viremia, where the administration of steroids led to a fall in serum creatinine, but a progressive increase in viral load, culminating in viral nephropathy (11). This variable response of PVAN to steroid therapy highlights our lack of complete understanding of all the clinical variables that affect viral replication in the allograft kidney. We postulate that host cellular and humoral immunity play an important role in determining ultimate clinical outcome in individual patients. Further exploration of this notion would require the development of immunologic assays directed against specific viral antigens.

Acknowledgments

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Nephrology
Dialysis
Transplantation

Special Feature

Fernando Valderrabano Memorial Lecture

Renal transplantation 2004: where do we stand today?

Claudio Ponticelli

Ospedale San Luca, Istituto Scientific Auxologica

On September 6, 2001, Professor Fernando Valderrabano (Hospital Gregorio Marañon, Madrid) died at the age of 59 years. He was a leading figure in Spanish nephrology, a full professor of Medicine/Nephrology at the University Complutense of Madrid, and an outstanding scientist who published more than 300 articles in medical journals. He was a very intelligent and cultured person, and a man of great style who enjoyed a wide range of hobbies and interests in addition to his medical work. All his colleagues and friends mourn his passing.

Professor Fernando Valderrabano 29.12.1941–6.9.2001

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Abstract

In spite of considerable progress in immunosuppressive and supportive treatment, numerous problems persist which interfere with the success of renal transplantation. Before transplantation has been performed, factors impacting on outcome include the donor (living *vs* cadaver, age and HLA system) as well as the recipient (age, immunological reactivity, potential sensitization and duration of dialysis). These are the main factors that affect the outcome of the transplant, particularly in the long-term. After transplantation a number of events may put graft function at risk: potential recurrence of the primary renal disease in the allograft; '*de novo*' renal disease triggered by infections, drugs or autoimmunity; and non-specific progression promoters, such as diabetes, hypertension, proteinuria, nephrotoxic agents and/or viral infections. The two most frequent causes of chronic allograft dysfunction are (i) chronic rejection (often triggered by preceding acute rejection, delayed graft function or poor compliance) and (ii) calcineurin-inhibitor nephrotoxicity (more likely to develop in kidneys of older donors or in marginal kidneys). The differential diagnosis between these two entities is generally difficult, but some histological clues (reduplication of glomerular basement membrane, obliterating vasculopathy and C4d deposits) as well as the demonstration of humoral antibodies are pointers suggesting rejection. Treatment of chronic graft dysfunction is difficult, whatever the cause, particularly in cases with advanced renal lesions. Therefore, early diagnosis is of paramount importance. In this regard, graft biopsy can be of great help. In spite of many problems and complications, not only short-term but also long-term results of renal transplantation are improving progressively, as documented by CTS data showing that in Europe for transplants performed between 1982 and 1984 the mean graft half-life was 7 years, while for transplants performed between 1997 and 1999 it was 20 years.

Keywords: calcineurin inhibitors; chronic allograft nephropathy; chronic rejection; recurrent disease; renal transplantation

Introduction

Today, renal transplantation is the treatment of choice for most patients with end-stage renal failure. Yet, in spite of the continuous progress in immunosuppressive and supportive therapy, a number of factors still interfere with the complete success of renal transplantation. Some factors, present at the time of transplantation, concern the donor as well as the recipient, while other complications originate after transplantation. In this review, particular attention will be paid to the main factors and events that impair graft function in the long-term.

Factors at the time of transplantation

The donor

The *source* of the donor can strongly affect the results of renal transplantation. The renal graft half-life is by far longer for living-donor than for cadaver transplants [1]. This finding cannot be attributed only to a better histocompatibility. In fact, the graft half-life of transplants between spouses who are obviously HLA-mismatched is more than one-third better than that of cadaver donor grafts [2]. The difference may be accounted for by a number of factors. First of all, the quality of the kidney of a living donor can be carefully assessed, while that of a cadaver donor must be evaluated in a hurry and under difficult conditions. Second, brain death causes a hypertensive crisis and also an autonomic storm leading to profound ischaemia and endothelial damage of peripheral organs, even when blood pressure is normal [3]. Third, ischaemia-reperfusion injury is obviously less severe with living donation. Finally, brain death is associated with an upregulation of cytokines and chemokines that favour overexpression of HLA antigens by endothelial and tubular epithelial cells, thus, increasing the risk of acute [4] and chronic rejection [5].

The *age* of the donor is also important. While in the recent past most donors were younger than 50 years, today the age of the donor is increasing progressively. The UNOS registry documented that the higher the age of the donor, the worse the long-term outcome of the graft [1]. Some investigators feel that the poorer results of kidneys of elderly donors are mainly caused by the age-dependent progressive reduction of glomerular filtration rate and renal reserve. To overcome this problem it has been proposed to transplant both kidneys of borderline cadaver donors into one single recipient. Alfrey *et al.* [6] reported good results with dual transplantation in 287 patients, i.e. a 5 year graft survival rate of 69%. Surprisingly, however, in this series the mean age of the donor (58 years) was by no means very advanced and the mean creatinine clearance was borderline, at best (mean: 77 ml/min). It is well justified to ask whether a similar result would not also have been seen by transplanting the two kidneys into two recipients. As a matter of fact, Bunnapradist *et al.* [7] reviewed the data of the US Renal Data System and reported that the 3 year graft survival of kidney grafts coming from donors above age 55 years was 70% for single transplants and 65% for dual transplants. On the other hand, Halloran *et al.* [8] did not find a relation between the initial creatinine clearance of an old donor and subsequent graft survival. He hypothesized that the main problem of old kidneys is replicative senescence rather than decreased renal function. Actually, a strong association has been found between specific markers of replicative senescence and the presence of chronic allograft nephropathy in biopsies of kidney transplants from older donors [9,10]. If so, the best way of utilizing old kidneys could be to transplant them to old recipients. In this regard, the group of La Charité in Berlin reported that in old recipients graft survival was similar for those transplanted with old kidneys and for those who were given kidney grafts based on HLA match, waiting time and cold ischaemia time, irrespective of the age of the donor [11].

The role of *HLA* typing with modern immunosuppression has been a matter of controversy. While there is evidence that long-term survival is better for transplants with no antigen mismatch than for mismatched transplants [12], lesser degrees of mismatch are of minor clinical relevance [13]. The analysis of more than 50 000 renal transplant recipients showed that the effect of donor age on patient survival was greater than that of HLA match [14].

The recipient

Not only the age of the donor, but also the *age* of the recipient is increasing in recent years. The UNOS data show that the results are worse for recipients above age 50 years. The main cause of graft failure is death with a functioning graft. As expected, the older the age, the higher the risk of death. On the other hand, the risk of graft failure caused by acute or chronic rejection tends to decrease with age [1]. Since death is due mainly to cardiovascular disease and since malignancy is more frequent at advanced age, intensified cardiovascular investigation and search for malignancies are indicated and appropriate therapeutic measures should be taken before an elderly patient is considered suitable for transplantation. It is also important to assess the patient's nutritional status and rehabilitation, since frail elderly patients are at particular risk of infectious complications. On the other hand, however, since the risk of rejection is less in elderly recipients [1], immunosuppressive therapy can be less aggressive. Particularly, steroid-free

immunosuppression is indicated in elderly recipients to reduce the risk of cardiovascular complications [15], infection and diabetes.

It would be of great utility to know the *immune reactivity* of the recipient in order to adjust immunosuppression accordingly. Unfortunately, we still have no valid pre-transplant markers, but recent data have shed some light on this problem. Susal *et al.* [16] proposed to measure the immunological reactivity of the patient before transplantation by measuring serum CD30, which is expressed on CD4+ and CD8+ T cells that secrete TH2-type cytokines. Patients with CD30 levels <100 U/ml had a significantly better 5 year graft survival than patients with higher serum levels. Rotondi *et al.* [17] measured the serum chemokine CXCL10/IP-10 and found significantly higher pre-transplant blood levels in patients who had graft failure than in patients with good graft function. Uboldi de Capei *et al.* [18] reported that high interleukin (IL)-10 producers mismatched for class I, but matched for class II HLA antigens and low IL-4 producers (independent of HLA match) are protected from chronic rejection. Although it is still too early to tailor immunosuppression according to these parameters, there is hope that in the near future good markers of immune reactivity will permit us to find the immunosuppressive regimen that is most appropriate for the individual patient. In the past, patients who lost their first transplant because of rejection were considered at high immunological risk. More recently, the UNOS data [1] showed that the graft half-life was similar for first (10.6 years) and second transplants (9.4 years). Patients who lost their first graft because of an accelerated rejection may still be considered 'strong responders', however.

Patients who have developed high titres of panel-reactive *anti-HLA antibodies* (PRA) following pregnancies, blood transfusions or transplants are at increased risk of graft failure [19]. Moreover, most patients with very high titre PRA cannot be transplanted because the crossmatch with a potential donor is likely to be positive. In the past, attempts to remove preformed anti-HLA antibodies with immunoadsorption had some success [20]. More recently, Glotz *et al.* [21] reported good results by pre-treating 15 hypersensitized patients with a 3 month course of intravenous high-dose immunoglobulin before transplantation. Thirteen patients were actually desensitized and were transplanted immediately. One patient lost the graft because of thrombosis and another because of rejection. All the other patients were alive with a functioning kidney graft after >1 year. Another potential approach is pre-treatment with the anti-CD20 monoclonal antibody rituximab, which may reduce the PRA titres dose-dependently [22].

The duration of dialysis treatment is a problem that has been neglected so far. Strong evidence suggests that the results of pre-emptive transplantation, before dialysis is started, are far better [23,24]. Using a paired donor kidney analysis, Meier-Kriesche and Kaplan [23] demonstrated that the longer the time on dialysis, the worse the long-term outcome of renal transplantation. This was true both for living and cadaver allografts. The authors concluded that the time waiting on dialysis is the strongest modifiable factor influencing transplant outcome.

What to do before transplantation?

From a theoretical point of view, transplantation ideally should be performed between HLA-identical subjects, the kidney should preferably come from a living young donor and the recipient should also be young, have no preformed anti-HLA antibodies and low immunoreactivity and should receive the transplant before starting dialysis treatment. The real world is quite different: only a small minority of patients receive a well-matched kidney; in Europe, as of 2001, only 15% of patients receive a living donor transplant; donor and recipient age increases progressively; and the duration of dialysis treatment while the patient is on the waiting list gets longer and longer. On the other hand, it would be unethical to refuse transplantation to a patient only because of his/her old age, long duration of dialysis or hypersensitization, since even in elderly patients renal transplantation offers higher life expectancy [25] and better quality of life [26] than does dialysis. It is also not advisable to discard marginal donors because of the persistent shortage of kidney grafts.

In order not to penalize patients at risk and not to compromise the success of transplantation, it is advisable to take some practical measures. Hypersensitized patients should be treated with intravenous immunoglobulins or rituximab and should be transplanted immediately after their PRA titres have decreased substantially. Patients with high immunological reactivity and those who lost a previous graft because of an early rejection should receive aggressive immunosuppression. In contrast, frail patients, such as older recipients, those with long exposure to dialysis as well as HCV- and HBV-positive patients should receive less-aggressive immunosuppression, possibly altogether avoiding the use of corticosteroids. Finally, in patients who receive a kidney from elderly or marginal donors the use of calcineurin inhibitors should be avoided or at least minimized, since these kidneys are particularly vulnerable to the nephrotoxic effects of these agents.

Post-transplant events

Specific diseases

Recurrence of primary disease may lead to graft failure, particularly in the long-term. It is difficult to assess the risk of recurrence for the individual renal diseases, because duration of follow-up and indication for biopsy are so heterogeneous in the available reports. If one reviews the most recent large series [27–32], it appears that some diseases, such as immuno-

Table 1. Risk of recurrence and relative risk of graft failure of primary renal disease

Disease	Recurrence	Relative risk
IgA GN	30%	1.2
MN	6–30%	1.2
SLE	3–30%	1.1
FSGS	20–40%	2.3
MPGN	3–48%	2.5
HS purpura	20–40%	2.6
HUS	6–56%	5.6

IgA GN, immunoglobulin-A glomerulonephritis; MN, membranous nephropathy; SLE, systemic lupus erythematosis; FSGS, focal segmental glomerulosclerosis; MPGN, membranoproliferative glomerulonephritis; HS purpura, Henoch–Schoenlein purpura; HUS, haemolytic uraemic syndrome.

globulin-A nephritis, membranous nephropathy and lupus nephritis, do not affect the 10 year graft survival even when they have recurred in the graft (Table 1), although over even longer periods recurrence of these renal diseases may eventually contribute to graft failure. More dangerous is the recurrence of focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, Henoch–Schoenlein purpura and, particularly, haemolytic uraemic syndrome (Figure 1). In many cases the recurrence of these diseases leads to the loss of graft, although sporadic cases of response to plasmapheresis or immunoadsorption have been reported [33–35].

'*De novo*' *thrombotic microangiopathy* may occur in patients on cyclosporin, tacrolimus, anti-mTOR agents or OKT3. Only half of cases show the typical picture of haemolytic uraemic syndrome. In the remaining patients, systemic signs and symptoms may be absent and there is only a progressive decline of graft function [36]. In these patients, renal biopsy is indispensable for early diagnosis of thrombotic microangiopathy. Prompt withdrawal of the offending drug leads to recovery in some patients. Plasmapheresis may also be helpful. In a large series [37], graft function recovered in 23 of 29 patients with post-transplant thrombotic microangiopathy. In all patients, calcineurin inhibitors were stopped and plasmapheresis was administered for a mean of 8.5 days.

Aggressive immunosuppression may reactivate *polyoma BK virus*, which is usually latent in the urinary tract. As a consequence, ~5–6% of transplant patients develop interstitial nephritis, which causes graft failure in about half the cases. There are not specific symptoms or signs. The diagnosis should be suspected in any patient with progressive graft dysfunction, particularly if treated with a combination of tacrolimus and mycophenolate mofetil [38]. Cells in the urine with viral inclusions, so-called 'decoy cells', may be used to monitor the patient, although the presence of decoy cells is sensitive but not very specific. Detection of virus DNA in plasma by polymerase chain reaction is more specific, but expensive. Once again, renal biopsy is of paramount importance. It shows interstitial nephritis with cytopathic changes and inclusion bodies (Figure 2). Staining with monoclonal antibodies against the simian virus can confirm the diagnosis. Reduction of immunosuppression or replacement of tacrolimus and mycophenolate mofetil with leflunomide, an immunomodulator agent

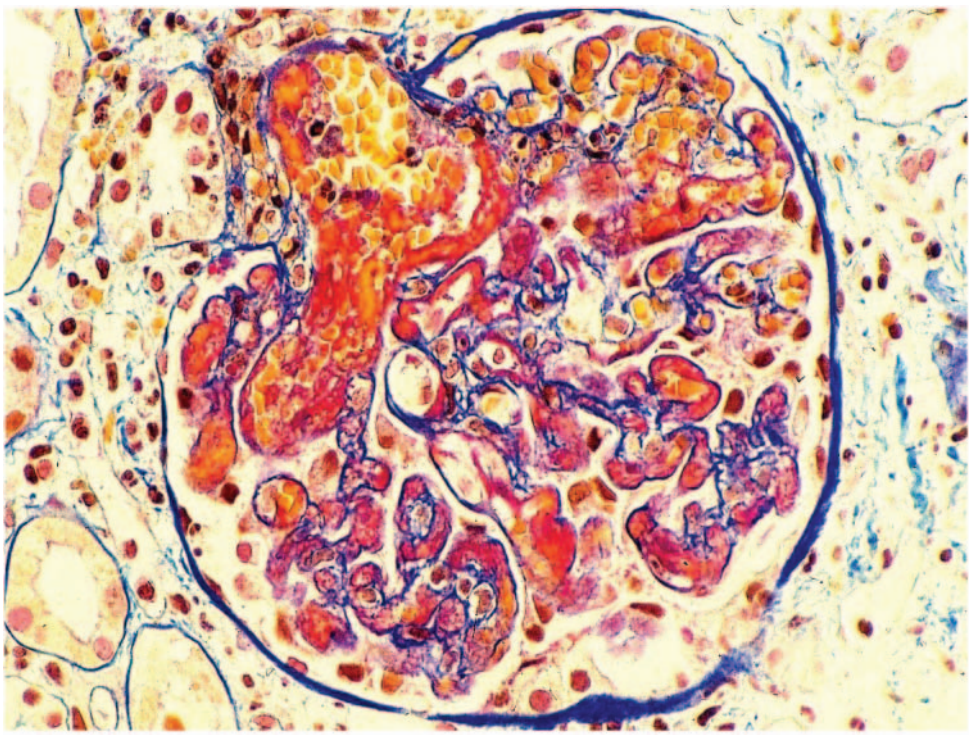

Fig. 1. Recurrence of haemolytic uraemic syndrome and severe thrombotic microangiopathy. (Courtesy of Dr G. Banfi, Nephrology, IRCCS Ospedale Maggiore, Milan, Italy.)

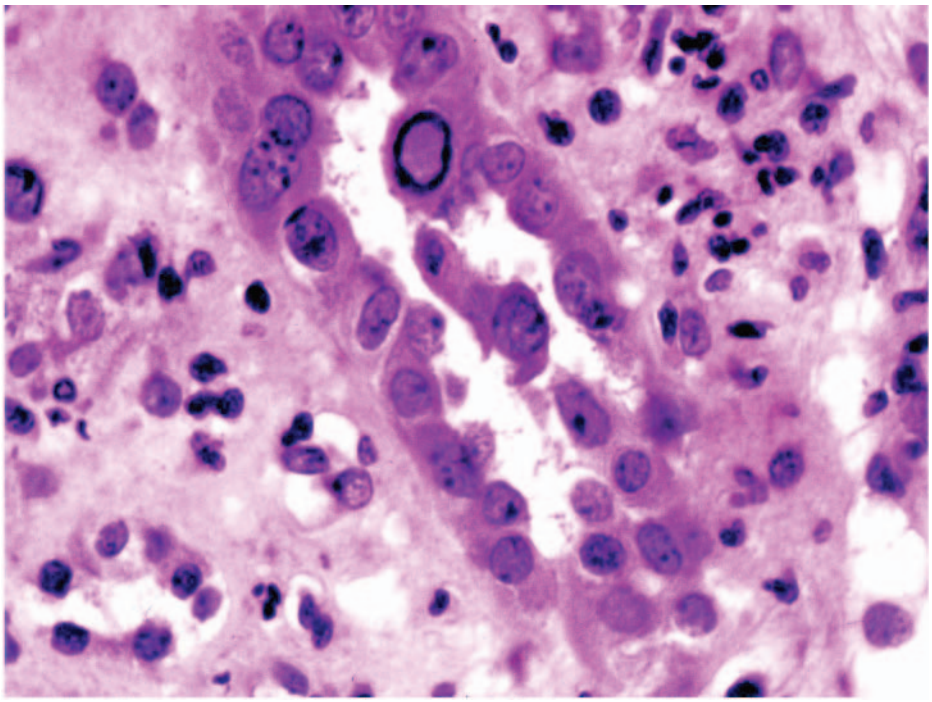

Fig. 2. Polyoma BK virus nephritis. Interstitial nephritis with severe tubular damage. The nuclei of epithelial cells are enlarged with chromatin irregularly distributed and vesicular changes (decoy cells). Note in a tubular cell the large nucleus with chromatin circumferentially distributed around a central halo (howl eye). (Courtesy of Dr G. Banfi, Nephrology, IRCCS Ospedale Maggiore, Milan, Italy.)

with antiviral properties, may rescue the kidney in a number of cases. Cidofovir has also been used with success in anecdotic observations [38].

Besides BK virus, cytomegalovirus (CMV), herpes viruses 1 and 2 and adenovirus may cause *interstitial nephritis* as well. Moreover, a number of drugs that are often used in renal transplant recipients, such as antibiotics, sulphonamides, allopurinol, diuretics and non-steroidal anti-inflammatory drugs, may also cause interstitial nephritis. The diagnosis is difficult, since eosinophilia, fever and rash are generally absent because of the administration of corticosteroids and immunosuppressive drugs. The diagnosis rests on renal biopsy.

The transplanted kidney may also develop *'de novo' glomerulonephritis*. The most frequent forms are membranous nephropathy usually related to HBV infection [39] and membranoproliferative glomerulonephritis in HCV carriers [40]. However, cases of '*de novo*' idiopathic membranous nephropathy [41], acute glomerulonephritis [42], collapsing focal glomerulosclerosis [43] and minimal change nephropathy [44] have been described as well. Although the pathogenesis of these cases is still obscure, one may speculate that the proinflammatory alloimmune response in a transplanted subject modifies anti-inflammatory mechanisms that protect from autoimmunity. Consequently, the immune responses to autoantigens may be subverted by alloimmunity, resulting in an autoimmune response.

The potential development of chronic graft dysfunction from *calcineurin-inhibitor toxicity* is well known. These drugs may cause persistent vasoconstriction and endothelial lesions (Figure 3) that eventually lead to interstitial fibrosis and tubular atrophy. Important contributors are activation of the renin–angiotensin system, increased synthesis of osteopontin and chemokines as well as diminished production of nitric oxide. All these factors may trigger excessive production of profibrogenic transforming growth factor-β1) and/or directly cause tubulointerstitial damage [45]. Factors increasing the risk of severe nephrotoxicity are the dose of the calcineurin inhibitor, the age of the patient and his or her renal function. 'Marginal' kidneys are more vulnerable to the nephrotoxicity of calcineurin inhibitors. To prevent the development of severe renal toxicity, the blood levels of cyclosporin and tacrolimus should be monitored regularly; the doses should be adjusted accordingly; particularly, the possibility of pharmacokinetic interferences should be taken into account between calcineurin inhibitors and drugs that increase (macrolides, triazolic antifungal, calcium-channel blockers, etc.) or decrease (antiepileptic agents, rifampin and derivates, etc.) the bioavailability of calcineurin inhibitors; and the simultaneous use of nephrotoxic agents should be avoided whenever possible. In patients with graft dysfunction, a renal biopsy should be performed to exclude or confirm a diagnosis of nephrotoxicity. It should be kept in mind that the lesions caused by calcineurin inhibitors can be halted or even improved by reducing or stopping the drug in the due time [46].

In summary, specific diseases represent a frequent cause of graft failure. In a number of cases, an early

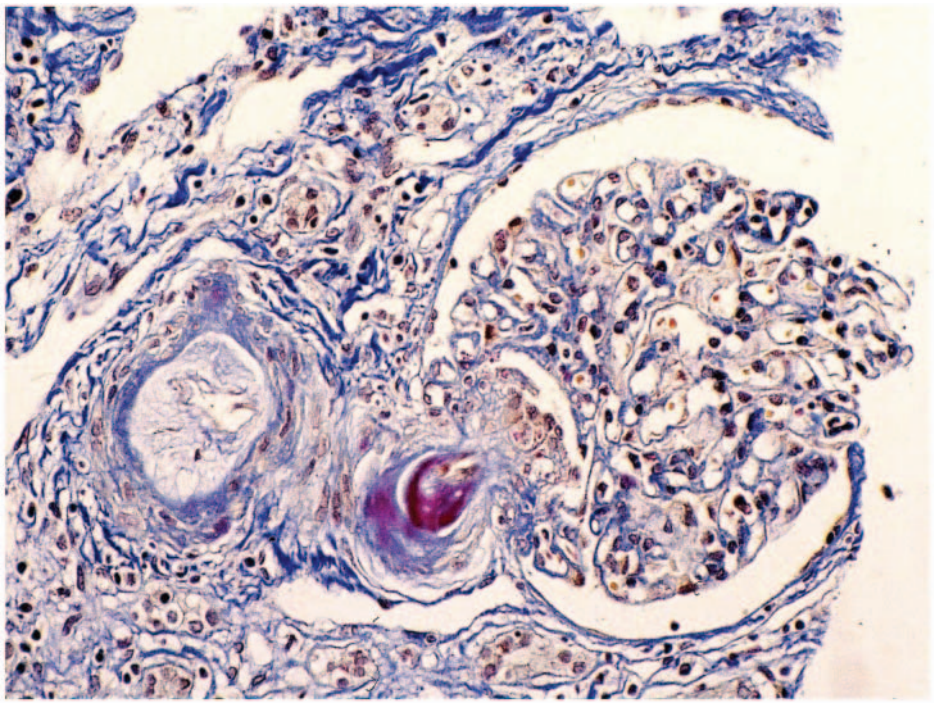

Fig. 3. Cyclosporin-related arteriolopathy. Mucinoid thickening of the intima with intraluminal thrombosis. (Courtesy of Dr G. Banfi, Nephrology, IRCCS Ospedale Maggiore, Milan, Italy.)

diagnosis and an appropriate treatment may allow the reversal of graft dysfunction. The diagnosis is often difficult. Renal biopsy is the most important tool to establish the diagnosis and should be performed in any case of graft deterioration of uncertain origin.

Non-specific causes of graft dysfunction

Up to 20–25% of renal transplant recipients develop overt *'de novo' diabetes* [47,48]. These patients have an increased risk of cardiac, cerebrovascular and peripheral vascular disease [49]. Moreover, patients with post-transplant diabetes may develop a diabetic nephropathy and graft dysfunction in the long-term [47,50].

Arterial hypertension is frequent in renal transplant patients. Opelz *et al.* [51] showed a strong association between the values of blood pressure and the risk of chronic graft dysfunction.

The inappropriate use of *nephrotoxic agents* may also expose to progressive graft dysfunction. Aminoglycosides, fluoroquinolones, cidofovir, foscarnet, sulphonamides, non-steroidal anti-inflammatory drugs, analgesics, contrast media, etc. may cause renal toxicity, which is usually dose-dependent. Patients showing an increase in serum creatinine should be always asked about the use of potentially nephrotoxic drugs.

The role of *proteinuria* in the progression of renal disease has been the subject of numerous experimental and clinical studies. Roodnat *et al.* [52] showed that both patient survival and graft survival (censored by death) were significantly lower in renal transplant recipients with proteinuria than in non-proteinuric patients.

CMV infection is a frequent complication in renal transplantation. More than 50% of seronegative [53] and ~10% of seropositive transplant patients [54] may develop symptomatic CMV disease. Apart from the well-known consequences of CMV disease, the infection can increase the risk of acute [55] and chronic rejection [56] through overproduction of mediators, cytokines, chemokines and growth factors.

To prevent the deleterious impact of these factors on progression, fasting and postprandial glucose should be checked frequently and glucose intolerance should be treated as early as possible; arterial hypertension should be treated aggressively, trying to keep blood pressure levels within the normal range; nephrotoxic agents should not be used unless strictly necessary; angiotensin-converting enzyme inhibitors and/or angiotensin-receptor blockers should be considered in patients with proteinuria; and CMV infection should be prevented or treated with specific antiviral agents.

Transplant glomerulopathy

This still mysterious entity is characterized clinically by proteinuria and progressive graft dysfunction. Graft biopsy shows enlarged glomeruli, mesangiolysis and glomerular capillary enlargement with microaneurysm formation. In advanced stages, reduplication of glomerular basement membranes is seen. Electron microscopy shows widening of the subendothelial

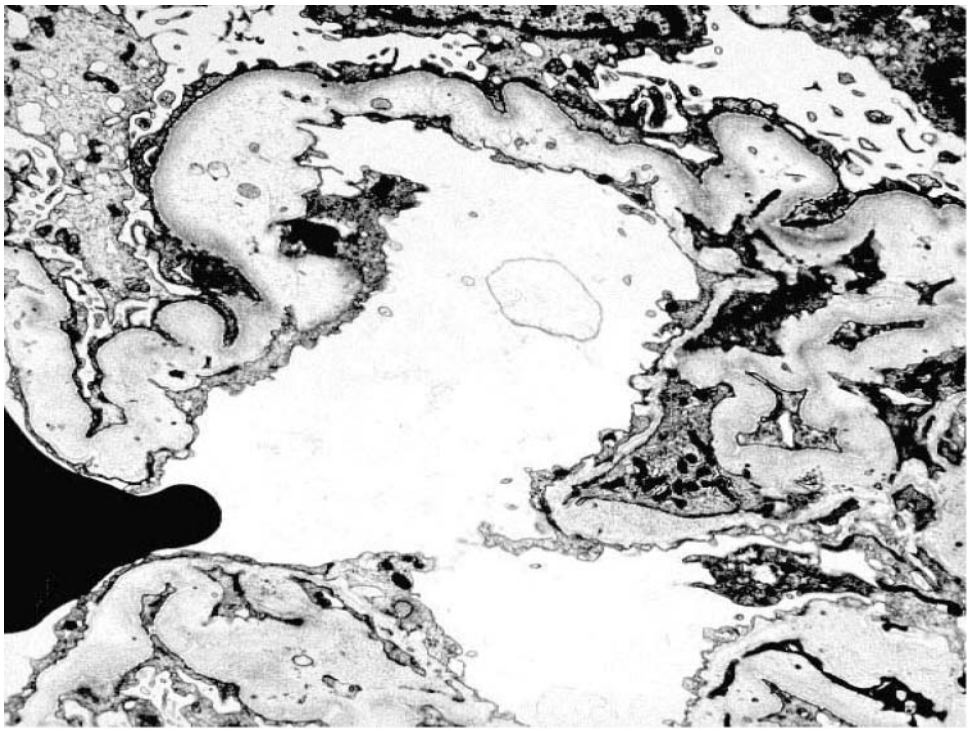

Fig. 4. Transplant glomerulopathy. Reduplication of glomerular basement membrane with large subendothelial space (electron microscopy). (Courtesy of Prof. M.J. Mihatsch, University of Basel, Switzerland.)

space (Figure 4). Transplant glomerulopathy is often classified as an expression of chronic rejection, but other investigators prefer to consider it as a separate entity. The prognosis is poor [57]. However, a few patients may maintain some degree of renal function even for years. There is no effective therapy.

Post-transplant predictors of chronic rejection

There is evidence that *acute rejection* can influence the long-term outcome of renal transplantation. Graft half-life is longer in patients who never experienced acute rejection [58]. However, the long-term impact of rejection on graft function is related more to its characteristics than to its occurrence. Long-term graft survival is better in patients who had only a single episode than in patients with two or more episodes of rejection [59,60]. Opelz [61] showed that when rejection is completely reversible, it does not affect 5 year graft survival. Sijpkens *et al.* [62] pointed out that the prognosis is worse for patients who had late rejection than for those who had early rejection: 10 year graft survival censored by death was 86% for patients who developed rejection by the third post-transplant month and 45% for patients who had rejection after the third month. Long-term graft survival is usually excellent in patients with borderline or grade I rejection, according to the Banff '97 classification [63], while the prognosis is worse for patients with grade II and very poor for patients with grade III rejection [64,65]. More recently, the Banff classification has been revised by adding the category 'humoral rejection', defined by either the presence of deposits of C4d (a split product of the C4 component of complement) in peritubular capillaries and/or the presence of circulating donor-specific antibodies [66]. The histological equivalents are the presence of neutrophils in the peritubular capillaries and glomeruli and fibrinoid necrosis of arteries. Thus, the impact of an acute rejection on the long-term outcome depends on the number of rejections, on the reversibility (complete or partial), on the time of onset (early or late), on the histological outlook according to the Banff criteria and on the development of humoral antibodies.

The occurrence of *delayed graft function* (DGF) may require dialysis, may prolong hospitalization and may expose to an increased risk of infection. Whether DGF *per se* affects long-term graft survival is still controversial. However, there is agreement that the combination of DGF with rejection has a deleterious effect on graft survival [67,68]. As a matter of fact, it is very difficult to identify acute rejection in an oliguric patient. Moreover, the endothelial damage caused by reperfusion injury and by acute rejection may eventually result in the development of a chronic obliterative vasculopathy (Figure 5). Thus, efforts should be made to prevent or attenuate the damage caused by ischaemia-reperfusion injury. Reduction of the cold ischaemia time has been advocated by some investigators. However, two large studies [69,70] showed that, at least up to 30–36 h, cold ischaemia time does not significantly affect graft survival. Intracellular perfusion solutions are now extensively used after it has been demonstrated that they reduce the risk of DGF. Antioxidant and antiapoptotic agents proved to be effective in experi-

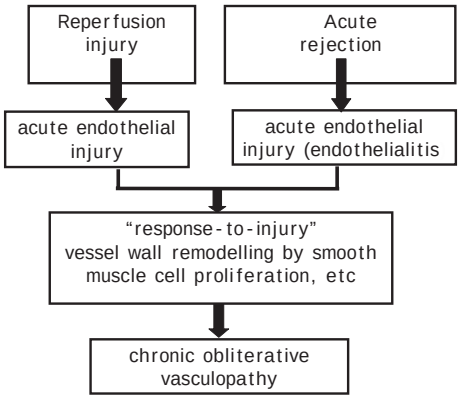

Fig. 5. The combination rejection–delayed graft function can lead to chronic obliterative vasculopathy.

mental models, but they are still not used widely in clinical practice. As the problem of ischaemia-reperfusion injury is becoming more and more important with the more frequent use of marginal donors, further studies are needed to overcome this potential consequence.

An important cause of late graft failure is poor patient *compliance*. Its frequency is poorly known, as many patients are reluctant to admit non-adherence to therapy. A recent paper [71] reviewed the studies devoted to the problem of compliance. Cross-sectional studies based on a self-report questionnaire suggested poor compliance in 22% of transplant recipients. Cohort studies indicated that 36% of the cases of graft loss were preceded by episodes of non-adherence. Meta-analysis of these studies showed that the odds of graft failure increased seven-fold in non-adherent patients. Poor compliance is often related to the complexity of and disfiguration from treatment as well as to the social isolation of the patient. To improve the compliance of the patient, the treatment should be simplified; the patient should be informed about the effects of the drugs and the consequences of a poor adherence; and the clinician should have a firm partnership with the patient and should pay attention to their problems, by modifying therapy in case of disturbing side effects. Significant improvements in graft survival might be obtained by improving the compliance of our patients.

Chronic allograft nephropathy

This term encompasses most causes of late dysfunction and has been adopted to indicate a progressive and irreversible histological and functional deterioration of the transplanted kidney. However, for the clinician it is of great importance to know the main cause of chronic allograft nephropathy (CAN). In this regard there is much confusion, because in many cases a late renal biopsy shows non-specific features rendering a correct diagnosis almost impossible. The transplant community seems to be divided into two parties: those (including this writer) who feel that the main cause of late graft failure is chronic rejection and those who feel that it is the chronic toxicity of calcineurin inhibitors.

The term *chronic rejection* should be applied only to cases of CAN caused by a cellular or humoral alloimmune response. Unfortunately, it is not easy to recognize whether a CAN is caused by rejection or by non-immunological causes, as in both cases the graft biopsy shows interstitial fibrosis, tubular atrophy and glomerular sclerosis. Some cases of late rejection, characterized by major infiltration by mononuclear cells, are probably sustained by T-cell activation, favoured by inadequate immunosuppression or poor compliance. If promptly recognized, unfortunately unusual, such late rejections may benefit from standard anti-rejection therapy plus reinforcement of maintenance therapy. However, most cases of chronic rejection are caused by humoral antibodies, either directed against HLA or minor antigens [72]. Besides the presence of *de novo* humoral antibodies, some histological features are considered to be specific for chronic humoral rejection, such as multilayering lamination of the basement membrane of peritubular capillaries on electron microscopy [73], arterial intimal fibrosis with intimal mononuclear cells (Figure 6) and a bright linear staining of CD4 along over half of peritubular capillaries [66]. Theoretically, plasmapher-

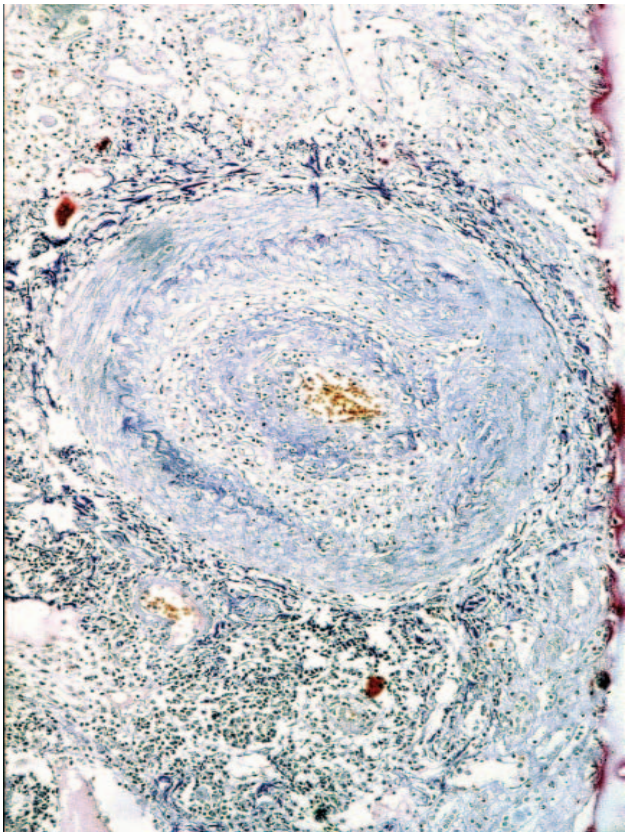

Fig. 6. Severe obliterative transplant arteriopathy in an interlobular artery. (Courtesy of Dr G. Banfi, Nephrology, IRCCS Ospedale Maggiore, Milan, Italy.)

esis, intravenous immunoglobulins and/or rituximab might obtain some reduction of circulating antibodies, but it remains unclear whether these measures may actually benefit the clinical outcome in patients with chronic humoral rejection. The problem is further complicated by the fact that the nephron loss, caused by T cell-mediated or humoral rejection, can trigger a vicious circle perpetuating the progression through non-immunological factors, such as glomerular hyperfiltration, hypertension, proteinuria, hyperlipidaemia and atherosclerosis. Moreover, anti-graft antibodies can stimulate cell proliferation that may result ultimately in the development of transplant arteriosclerosis [74]. Finally, release of novel self-antigens caused by rejection might trigger an indirect recognition of alloantigens by antigen-presenting cells of the recipient as demonstrated in lung transplantation [75]. This synergistic interplay of immunological and non-immunological events might explain why it is so difficult to manage chronic rejection.

Also, in cases of CAN caused by *calcineurin-inhibitor toxicity* or by *accelerated senescence*, graft injury can trigger non-specific accelerating factors that contribute to progressive graft dysfunction. In a number of patients, some improvement of renal function has been achieved by replacing calcineurin inhibitors with mycophenolate mofetil [76,77] or with sirolimus [78]. However, even with graft biopsy, it is not easy to exclude immunological activation in these cases of CAN. A number of patients are, therefore, exposed to the risk of late irreversible rejection after stopping the calcineurin inhibitor. It is also possible to speculate that the overexpression of chemokines and cytokines and a release of antigens from the damaged kidney can favour an indirect recognition and T-cell sensitization that may trigger a late rejection even in cases of CAN originally triggered by non-immunological factors.

Conclusions

Many factors and events can complicate the outcome of renal transplantation and can eventually lead to progressive renal dysfunction and graft failure. Some of these factors are unmodifiable a priori and for some other complications we do not have any effective therapy. A recent review of the American data concluded that, in spite of a marked decrease in acute rejection, there is a lack of improvement in long-term graft survival [79]. Should we conclude that progress in renal transplantation is limited, i.e. that we have achieved better graft survival in the short-term without having achieved any significant impact in the long-term? This is not the impression of this writer. In Milan we reviewed our own results in patients treated with kidney transplantation. The review included patients transplanted between 1983 and 2000. Consequently, a number of patients were treated with too high doses of cyclosporin and others could not profit from the use of newer immunosuppressive and supportive therapy. In spite of these drawbacks, the cumulative graft half-life was 20 years. If the data were censored by death, the pure graft half-life would have been 31 years [80]. At any rate, not only single-centre results, but also the cumulative European data clearly show that there has been a progressive improvement of the graft half-life in spite of the older age of donors and recipients. The data of CTS reported a graft half-life of 7 years for cadaver grafts transplanted between 1982 and 1984 *vs* a graft half-life of 19.5 years for graft transplanted between 1997 and 1998 [81].

In summary, many different factors and events may lead to chronic graft dysfunction. In the case of specific renal diseases or drug-related nephrotoxicity, prompt recognition and treatment of the underlying cause may slow progression. Thus, an early diagnosis is of paramount importance and the use of renal biopsy in doubtful cases should be encouraged. Whatever the cause of graft dysfunction, non-specific accelerating factors, such as hypertension, CMV infection, glucose intolerance, proteinuria etc., should be treated early and aggressively. The differential diagnosis between chronic rejection and chronic drug toxicity is difficult, but some clues may help to identify the immunological nature of a CAN. In many cases an early biopsy is helpful, while a late biopsy is generally of no use. Today, although many unresolved problems persist, long-term graft survival is possible for many transplant recipients, if they are monitored regularly by experienced clinicians. It is likely that in the near future the results will even be improved further by the introduction of newer immunosuppressive agents with a better therapeutic index.

Conflict of interest statement. The author is an external consultant of Novartis, Italy.

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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.		

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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

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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.

Grading of Disease Activity in Nonalcoholic Steatohepatitis

Grading Terminology		Criteria			
Grade	**Descriptive**	**Steatosis**	**Hepatocyte Ballooning**	**Lobular Inflammation**	**Portal Inflammation**
1	Mild	Mainly macrovesicular, may involve up to 66% of the lobules	Occasional, Zone 3	Scattered neutrophils, occasional mononuclear cells	None to mild
2	Moderate	Any extent, usually mixed macro- and microvesicular	"Obvious", Zone 3	Neutrophils may be noted associated with ballooned hepatocytes, pericellular fibrosis; mild chronic inflammation may be seen	Mild to moderate
3	Severe	Typically >66% (panacinar); commonly mixed steatosis	Marked, predominantly Zone 3	Scattered acute and chronic inflammation; neutrophils may concentrate in Zone 3 areas of ballooning and perisinusoidal fibrosis	Mild to moderate

Staging of Nonalcoholic Steatohepatitis

Staging Terminology

Stage	Descriptive	Comments
1	Zone 3 perivenular, perisinusoidal (pericellular) fibrosis	Fibrosis at these sites may be focal or extensive
2	Stage 1 changes + periportal fibrosis	Periportal fibrosis may be focal or extensive
3	Bridging fibrosis	May be focal or extensive
4	Cirrhosis	Cirrhosis

Reference

- Brunt, EM: Nonalcoholic steatohepatitis: Definition and pathology. *Semin Liv Dis* 21(1):3-16, 2001.

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

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.

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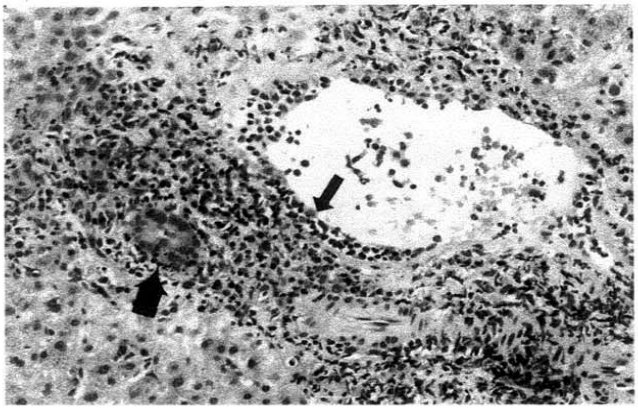

FIG. 1. Grading of acute rejection assumes the diagnosis has already been established: this portal tract shows all three of the typical histopathological features, two of which are required to make the diagnosis. There is: 1) a portal inflammatory infiltrate containing blastic lymphocytes and eosinophils; 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.

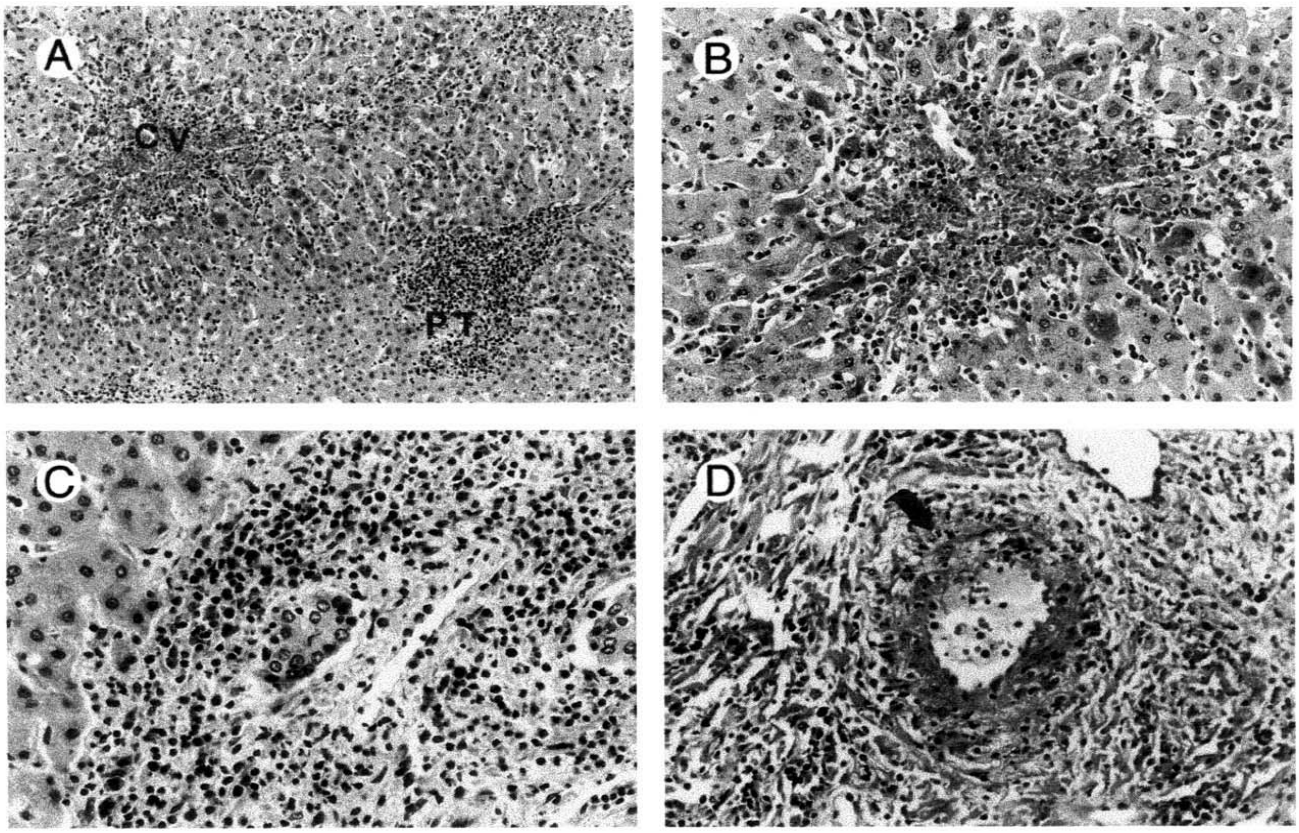

FIG. 2. (A) Low power photomicrograph of a failed liver allograft with severe acute rejection. Note the prominent portal tract (PT) and central vein (CV) inflammation, associated with confluent perivenular necrosis, which is shown at a higher magnification in (B). These findings would elicit a diagnosis 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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Pathophysiology of Chronic Allograft Rejection CME

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Pathophysiology of Chronic Allograft Rejection

Introduction

Advances in immunosuppression, operative techniques, and patient management have greatly reduced allograft failures that occur within the first year after solid organ transplantation. When they occur, these failures are usually attributable to technical complications, preservation injury, and acute rejection (AR). However, life-long immunosuppression is required to prevent the development of chronic rejection (CR), a complication that occurs in a majority (except for liver allograft recipients) of those who survive for more than 5 years. Thus, either CR or the multiple and sometimes severe adverse side effects of chronic immunosuppression, such as infection, malignancy, hypertension, hyperlipidemia, diabetes mellitus, irreversible kidney damage, bone loss, and depression, limit morbidity-free long-term survival.

Onset and Magnitude of the Problem

Chronic rejection is defined as an indolent but progressive form of primarily immunologic injury to the allograft, which more slowly compromises organ function than AR. Although the clinical symptoms of CR are dependent on the function of the specific organ allograft, the most common histologic manifestation is a progressive narrowing of the muscular arteries. This is referred to as "graft vascular disease" or "obliterative arteriopathy" (OA)", which can be likened to an accelerated form of atherosclerosis.

Obliterative arteriopathy damages the allograft primarily by compromising the arterial blood flow, predisposing to chronic ischemic damage and infarction. Other common characteristics of CR include patchy interstitial inflammation, fibrosis and associated parenchymal atrophy, destruction of epithelial-lined conduits such as bronchioles in lung allografts and bile ducts in the liver, and depletion of organ-associated lymphoid tissue.

Chronic rejection usually begins within weeks to months after transplantation, often during severe and/or persistent AR episodes (see Risk Factors in next section). Chronic rejection can also insidiously develop years after transplantation, particularly in inadequately immunosuppressed patients with late-onset AR. In general, the incidence of CR increases with time after

transplantation and eventually affects a majority of solid organ allografts, except for liver allografts. By 5 years after transplantation, CR affects only about 5% of liver recipients, but up to 80% of lung allograft recipients (Table 1) and 30%-40% of heart, kidney, and pancreas allograft recipients.

Table 1. Estimates of the Incidence of Chronic Rejection in Various Solid Organ Allografts

Allograft	Approximate Incidence of OA at 5 Years after Transplantation	References
Heart	25%-60%	(116, 281, 523-526)
Heart-Lung	8%-20% (obliterative arteriopathy in heart) 50%-60% (obliterative bronchiolitis in lung)	(47, 113, 114, 116, 524, 527)
Kidney	40%-50% (chronic allograft nephropathy)	(450, 528)
Isolated lung	28/45% (single/double lung) to 80%	(35, 113, 226, 304, 305, 529)
Liver	4%-6% in adults 8%-12% in children	(41, 46, 530)
Pancreas alone	30%-70%*(late graft loss),	(531, 532)
Pancreas and kidney	20%-40%*	(337, 339, 531)

* Estimates based on graft survival rates after 1 year, with the assumption that most late graft failures are due to CR.

Some interesting curiosities emerge when more than 1 organ from the same donor is transplanted into a single recipient. For example, the incidence of CR is about 40% in isolated cardiac allografts, but falls to 10%-20% when a heart is combined with the lungs in a composite graft. This "composite effect" is most predictable and significant when other organ(s) are combined with a liver allograft.[1-3]

Etiology and Risk Factors

Alloimmune immunologic injury was suspected as the major reason for the chronic deterioration of kidney allografts when it was first observed nearly 50 years ago[4-5] -- hence the designation CR. The premise that CR is primarily immunologically mediated is best supported by the observation that isografts rarely suffer this complication, and if they do, it is much delayed and less severe in comparison to allografts.[6-9] The effect of major histocompatibility complex (MHC) or human leukocyte antigen (HLA)-matching on long-term heart, lung, and kidney allograft survival is also cited as proof of an immune etiology for CR.[10-22] With complete 6-antigen matches, immunologic causes of allograft failure are minimized.[23,24)] Any degree of mismatching usually results in a progressive attrition of graft function over time,[10-17,25] which is in large part related to CR. However, some studies have shown that the actual impact of imperfect matching on long-term allograft survival has been less than expected.[10,11,13,17,26-30]

The importance of immunologic injury in the development of CR is also indirectly supported by many clinical studies, in which severe or persistent AR[25,31-46] or inadequate immunosuppression[43,46-48] increases the incidence of CR. Chronic rejection is also more common after transplantation across a variety of donor-recipient matching characteristics, such as racial

barrier (eg, Caucasian donors into non-Caucasian recipients)[11,12,14,17,49]; sex mismatching (male donor to female recipient and vice versa)[37,50]; and prior viral infections (CMV-positive donor to CMV-negative recipient).[51-58] Chronic rejection is also more common after the use of immune-activating drugs, such as alpha-IFN.[59-61]

Although the immunologic factors listed above are generally considered to be most important, nonimmunologic factors -- such as magnification of immunologic reactivity, various aspects of the repair response, and disruption of structural integrity -- also contribute to the development of CR. Included under nonimmunologic factors are older donor age,[25,46,62] inadequate functional capacity of the donor organ to meet the metabolic demands of the recipient,[63] prolonged cold ischemia,[41,64,65] donor atherosclerosis,[66-68] and conventional risk factors for atherosclerosis in the recipient.[62,69-71]

Immune Mechanisms in Chronic Allograft Rejection

Since CR often begins during severe or persistent AR episodes, it is important to briefly review those aspects of AR that predispose to the development of CR. Subsequently, immunologic mechanisms involved in CR are discussed.

Setting the Stage for Chronic Rejection: Acute Rejection and Direct Allorecognition

Implantation of any solid organ allograft results in a characteristic cycle of heightened immune activation,[27,72-74] followed by evolution toward a more stable relationship when immunosuppression can be considerably lowered. This prototypic series of events is attributable to the donor hematolymphoid cells[75] that emigrate from the allograft into recipient lymphoid tissues, simultaneously with an influx of recipient cells into the allograft.[76-79] Direct interaction between donor and recipient cells at these sites causes bidirectional stimulation of both donor and recipient T cells. At these sites, dendritic cells[75,79] engage and stimulate allogeneic T-cell blastogenesis, cytokine secretion, and mitogenesis, a process known as "direct allorecognition" (ie, donor dendritic cellsdirectly stimulating recipient T cells).[80-82]

Several lines of evidence support the contention that AR is dominated by direct allorecognition[80,83] (reviewed in Shirwan[84]). These include: (1) the high precursor frequency of T cells recognizing allogeneic major histocompatibility complex (MHC) molecules directly; (2) marked amelioration or absence of AR, but persistence of CR in allografts depleted of donor antigen-presenting cells (APC) prior to transplantation; (3) enhancement of AR by pretreatment of donors with agents that increase the number of donor APCs; and (4) ability of T-cell lines specific for direct recognition of allogeneic MHC molecules to induce AR in immunocompromised hosts.[84] The high precursor frequency and strength of the reaction explain the brisk polyclonal activation of T cells, secretion of cytokines and chemokines, and subsequent upregulation of various costimulatory and adhesion molecules on surrounding tissues. This is followed by maturation of cytotoxic T cells; expansion and maturation of B cells; and recruitment of macrophages, eosinophils, neutrophils and other effector cells, all of which have the potential to damage the organ.[85-88] The robust nature of the direct presentation pathway explains the frequency of clinical symptoms and the potential for rapid allograft failure with AR.

Direct allostimulation has been associated with a predominance of TH1 activation, reflected in the type of cytokines (IL-2, gamma-IFN, TNF-alpha, GM-CSF, IL-3) and chemokines[89-92] involved. Monitoring and controlling the severity of this reaction in the allograft is the mainstay of patient management during the first several months after transplantation. Fortunately, the direct allostimulation pathway is highly sensitive to increased immunosuppression, and AR is controllable in a majority of patients.[80-82]

The severity of AR is influenced by many factors, including but not limited to age, sex, race, and general overall health of the recipient. In general, younger, healthier, female, and black recipients show higher responsiveness to donor organs. Responsiveness is also influenced by prior exposure to the same or similar alloantigens via blood transfusions, previous transplants, or pregnancy. Clinically, the severity of AR is gauged by a combination of symptoms and histopathologic findings in an allograft biopsy. The hallmark histopathologic feature of severe AR, which is predictive of graft failure and a higher incidence of CR, is arterial inflammation or necrosis. Some histopathologic grading systems, which are less reliable, also include indirect evidence of rejection-related ischemia, such as interstitial hemorrhage or ischemic necrosis. Ischemic infarction is the final common path of organ damage in AR.

Ongoing Immunologic Injury: The Transition to Chronic Rejection and Indirect Allorecognition

Once the donor hematolymphoid cells are destroyed or die out, there is an evolution of the donor-recipient immunologic interface to one that is less violent, but not necessarily less capable of immunologic injury. This coincides with a transition from direct to indirect alloantigen presentation. The latter refers to the uptake of donor allopeptides by recipient APCs, followed by MHC-restricted presentation of peptides to recipient T cells.[82,83,93-95]

Increased transcripts for granzyme B, the presence of TH1 cytokines such as TNF-alpha, GM-CSF, IFN-gamma,[96] and chemokines, suggest that strong TH1 activation during AR might explain the evolution to CR, because activated recipient macrophages play an important role. Activated macrophages have been strongly implicated in virtually all aspects of CR: indirect allopresentation, tissue injury, upregulation of adhesion molecules, alterations in blood flow, and release of fibrogenic growth factors.[9,97-108]

Evidence supporting the shift to the indirect pathway during CR is less compelling than that supporting the role of direct presentation in AR, but strong enough to seriously consider as an important immunologic mechanism,[82,83,93-95] as reviewed in Shirwan.[84] Factors in support of the indirect pathway include: (1) ongoing immunologic injury in the allograft, despite disappearance of donor APCs[3]; (2) influx of activated recipient macrophages[3]; (3) c) the important role of alloantibodies in CR, mediated by B cells serving as APCs for CD4+ T cells generating these antibodies; (4) susceptibility of allografts to CR that have been depleted of donor APCs prior to transplantation; and (5) the high incidence of CD4+ T-cell responses to donor MHC allopeptides via indirect recognition in patients with CR.[93,94] Indeed, allografts that manifest persistent AR[83,94] and those that evolve toward CR[83,93,94] show evidence of increased indirect alloantigen presentation and diminished direct presentation.[80]

The indirect alloresponse is oligoclonal and initially involves only a few dominant antigen peptides on donor MHC class II DR determinants.[82,83,93,94] However, indirect presentation can be associated with "epitope spreading" to new determinants on donor MHC and tissue-specific antigens or "autoantigens."[82,83,93,94,109-111] More importantly, the indirect pathway is less sensitive to immunosuppressive blockade by cyclosporin A, which explains the comparatively poor response of CR to increased immunosuppression (see section on Treatment of Chronic Rejection).

Shortly after the discovery of TH1 (inflammatory CD4+ T cells) and TH2 (helper CD4+ T cells), it was thought that TH1 predominant responses were associated with AR, whereas TH2 profiles were more typical of allograft acceptance or tolerance. However, more recent evidence suggests that this explanation was overly simplistic.[89-92] TH2 predominance has also been associated with the indirect pathway of allopeptide presentation and production of alloantibodies,[89-92] cytokines and growth factors typically seen in CR.[84] Evidence supporting this contention includes the presence of CD4+ TH2 cells in chronically rejecting organs, the critical role of TH2-type cytokines in the regulation of effector mechanisms of CR, and the inability of immunosuppressive drugs to prevent AR, but not CR.[84]

How Immunologic Injury Leads to the Common Manifestations of Chronic Rejection in Different Organs

The immunologic injury discussed above disrupts normal allograft structure and function. It also triggers repair responses that lead to the common histopathologic features of CR in all allografts: OA; patchy interstitial inflammation; fibrosis and atrophy of parenchymal cells; destruction of epithelial conduits; and atrophy and destruction of organ-associated lymphoid tissues and lymphatic vessels.Although all of these changes occur to some extent in most allografts with CR, 1 or more of these features usually predominate in particular organs (Table 2). For example, the primary manifestation of CR in heart and kidney allografts is OA (see sections on Manifestations of Chronic Rejection). In lung allografts, destruction of the small bronchioles, termed "obliterative bronchiolitis" (OB), is the major manifestation of CR,[35,112-116] and vascular disease is generally considered to be of secondary importance.[35,112-114,116] In liver allografts, both bile duct loss and OA (see sections on Manifestations of Chronic Rejection) contribute to allograft failure.[117-121]

Table 2. Principal Manifestation of Chronic Rejection in the VariousVascularized Allografts

	Common Features of Chronic Rejection			
Allograft	**Vascular disease**	**Interstitial inflammation, fibrosis, and parenchymal atrophy**	**Destruction of epithelial conduits**	**Atrophy/destruction of organ-associated lymphoid tissues and lymphatic vessels**
Heart	+++ OA	+	Not applicable	+
Kidney	+++ OA Glomerular sclerosis	++	++ tubules	+
Pancreas	+++ OA	++	++ ducts and acini	Not well studied
Lung	+ OA	++	+++ obliterative bronchiolitis	+
Liver	++ OA Venous sclerosis	++	+++ bile ducts	+
Intestine	++	++	++ crypts	++ can affect mucosal immune

				system

Paul[111] recently summarized the various hypotheses used to explain the pathogenic mechanisms leading from immunologic tissue injury to the development of CR in kidney allografts. These hypotheses can be extrapolated to extra-kidney allografts and are useful in conceptualizing potential therapeutic interventions. Fundamental to all of these hypotheses is acceptance of the premise that CR develops as a pathogenic "response to injury," similar to atherosclerosis.[97,122,123] The basic algorithm is as follows:

Severe or persistent immunologic injury -> disruption of normal structure -> repair response-> parenchymal fibrosis, OA and other manifestations of CR

Immunological tissue injury in the nontransplant setting is a common event. However, the injury is usually mild and transient, and organ structure and function return to normal after the insult is eliminated. In allografts, rejection-related immunologic injury is often severe and/or persistent and eventually results in progressive arterial narrowing and interstitial fibrosis in those allografts that develop CR. Any hypothesis used to completely explain the pathogenesis of CR must therefore account for both the severity and/or persistence of the immunologic injury and the propensity to develop fibrosis and vascular disease.

The Immunolymphatic Theory of Chronic Rejection

The *immunolymphatic theory* of CR contends that freedom from CR requires robust tolerance; and since tolerance is a biologic function of the immune system, the presence of the donor immune system (hematopoietic chimerism) is required.[3] An important component of the donor immune system is the population of APCs that reside within the allograft and recipient lymphoid tissues.[3,124] These cells help maintain tolerance to the organ[3] and enable the organ to respond to environmental challenges.[3]

Destruction of these cells by severe and/or persistent immunologic injury has several consequences. This not only eliminates the possibility for robust tolerance, but also disrupts the egress of lymphatic cells and fluid that normally accumulate in the interstitium of the allograft. This compromises the normal response to environmental challenges, such as infection. It also predisposes to the development of OA and interstitial fibrosis, because cytokine and growth factor-rich fluid stagnates in the allograft. Thus, normal physiology (ie, production and reabsorption of lymph), is transformed into a driving force of tissue pathology.

The Cytokine Excess Theory of Chronic Rejection. The *cytokine excess theory* suggests that CR develops because of excessive scar formation in the allograft in response to the tissue injury.[111] Evidence supporting this contention includes the correlation between high intragraft TGF-beta production and increased risk of late graft failure.[96,102,125-131] Although most studies have focused on TGF-beta because of its role in fibrogenesis, more mediators will become the focus of study as their role in organ repair reactions becomes clarified.

The Loss of Supporting Architecture Theory of Chronic Rejection. The *loss of supporting architecture theory*[111] is based on the concept that repair processes such as tubular regeneration after injury are dependent on the 3-dimensional stromal framework, including the basement membrane. Destruction of the supporting architecture by severe injury compromises repair responses. Parenchymal cells that would normally migrate and divide to repair a defect might undergo apoptosis. The net effect is a tendency of the allograft to develop excessive scarring in response to injury instead of regeneration to the normal architectural baseline.

The Premature Senescence Theory of Chronic Rejection. The *premature senescence theory*[111,132] suggests that the excessive fibrosis of CR may be the result of accelerated aging of allograft parenchymal and endothelial cells. This accelerated aging may occur as a consequence of exposure to multiple stressors, such as organ harvesting, implantation, and rejection episodes, which subject these cell populations to ischemia, oxidative damage, and increased demand for cell division. Over time, the parenchymal and endothelial cells reach their Hayflick limit[133,134] and, similar to normal aged organs, the allograft displays a propensity to heal by fibrosis after injury.

All of the above hypotheses suggest valid mechanisms for linking tissue injury and abnormalities of the repair response to the development of CR. Common to all however, is a central theme of tissue injury and repair. Thus, whatever mechanisms are ultimately responsible for the development of CR, it seems clear that limiting the severity and/or persistence of immunologic injury will be the most effective way of diminishing the impact of CR on long-term allograft survival.[111]

Common Features of Chronic Rejection

Graft Vascular Disease or Obliterative Arteriopathy

Obliterative arteriopathy, also referred to as graft vascular disease, is the most widely recognized manifestation of CR. It is a progressive narrowing of muscular allograft arteries due to fibrointimal hyperplasia and mural remodeling.[3,135-138] In some respects, it is similar to atherosclerosis (AS), which is endemic in the general population, but most evidence suggests that OA and AS are distinct disorders. Identifying distinguishing features and avoiding confusion between these conditions necessitate precise terminology. "Obliterative arteriopathy" will be used throughout this review to refer to the artery disease associated with CR in all allografts. "Atherosclerosis" will be used to refer to the common endemic disease.

In some allografts, overlap between OA and AS is considerable, so distinguishing between the 2 disorders using various clinical, radiologic, and even histopathologic techniques can be quite difficult.[70,139,140] This is particularly true for heart allografts, which are normally prone to AS. It is less of a problem in atheroresistant organs such as the liver. A further complicating issue is the increased susceptibility of allograft recipients to AS compared with the general population, because of a higher incidence of hypertension, hyperlipidemia, and diabetes mellitus, which are side effects of immunosuppressive medications.

In general, the distribution of OA lesions in allografts is one of the key features used to distinguish it from AS. AS preferentially affects the large extracorporeal arteries, such as the epicardial portions of the coronary arteries.[116,141-143] OA more often involves both the extraorgan (eg, epicardial, hepatic hilar) and medium-size intraorgan muscular arteries.[116,141-145] It has been our experience, however, that OA lesions are not as diffuse as one might expect from a review of the literature.[116,141-145] Even though the arterial tree is more diffusely involved, OA lesions in both large and smaller arteries are often patchy in distribution, and it has also been our impression that they often begin and evolve more quickly near branch points.[142] Involvement of the allograft by either OA or AS is usually established by angiographic and various other imaging studies (see sections on Manifestations of Chronic Rejection).[44,136,146-148]

Models Used to Study Obliterative Arteriopathy. Except for kidney allografts, the association between immunologic injury and the development of arterial disease is difficult to study in human tissue biopsies. Therefore, animal models are extensively used to isolate and study the various aspects of OA. One popular model exploits the weak immunologic barrier between LEW and F-344 rats, where only minor histocompatibility barriers are crossed.[98,149-154] Another model employs

transient treatment with various forms of immunosuppression, the most popular being a short course of cyclosporine or anti-CD4 monoclonal antibodies.[155,156] The general approach in these models is to allow a nonlethal level of AR to occur, which subsequently evolves into CR.

In these models and in human tissue samples, it is relatively clear that the allogeneic arterial endothelium is injured during AR, primarily by antidonor antibodies and/or T cells and macrophages, which can directly invade the arterial intima.[100,149,151-153,155-157] This injury disrupts intimal homeostasis and triggers a stereotypic repair response [122,123,158] that eventually narrows the lumen of affected arteries.[64,139,142,145]

Because OA is so strongly correlated with arterial intimal inflammation, it is tempting to conclude that this is the predominant or only pathway leading from immunologic injury to OA.[97,142,155,159-161] However, arterial inflammation is not always observed,[3,162] and fibrointimal hyperplasia can progress without it.[162-166] When arterial inflammation is not seen, it is thought that antibody alone initiates the damage or that the stereotypic vascular repair response can proceed without continued immunologic injury.[163,166]

Even though OA can occur without intimal inflammation, most investigators would agree that cellular immunity importantly contributes to its development. The preferential localization of leukocytes in the adventitia and intima suggests that these are the most important antigenic targets or sites of damage.[97,142,145,155,159,160,167] The adventitia is rich in lymphatics and donor dendritic cells, making it a site of both peripheral sensitization and a conduit for emigrating leukocytes in AR.[3,97,142,155,167,168] When rejection is mild, the inflammation is usually limited to the adventitia.[3] However, adventitial injury alone can also trigger an intimal repair response.[3,137,169] When rejection is severe, or when the recipient harbors antidonor antibodies, mononuclear and/or neutrophilic endotheliitis is usually also present.[97,98,139,143,149-152,170,171]

Immunophenotypic analyses have shown that the arterial inflammation consists primarily of an admixture of T cells and macrophages.[70,97,142,159,161,172] In some studies, CD8+ T cells are the most common,[159,172] a subset of which show perforin positivity, thus identifying a cytolytic effector pathway.[159,161] $CD4^-$ and $CD8^-$ double negative and gamma-delta-positive T cells have been cultured in vitro from affected vessels.[173] The presence of occasional dendritic cells signals ongoing antigenic presentation.[97] Macrophages, however, often become the predominant inflammatory cell population, which may be related to the increased deposition of ground substance and lipid trapping, both of which stimulate phagocytosis.[70,97,142,161,172,174-176] The macrophages permeate the adventitia, media, and intima, and proliferate within the artery.[142] Foam cell transformation is more common in early lesions and most often seen in liver allografts.[70,120,142,143,145,174-177]

Substitution of immune deficient and/or knockout mice as recipients into CR models has further clarified the nature of the immunologic injury by isolating the contribution of humoral and cellular components.[162,165] The development of OA in combined immune-deficient mice treated with repeated injections of exogenous antidonor antibodies[162] nicely illustrates the importance of alloantibodies. Other models also clearly show a role for antibody-mediated damage.[178,179] However, OA also develops in B-cell knockout mice that presumably are incapable of an antibody response,[162,165] showing an important contribution by cellular immunity. Thus, both cellular and humoral immunity contribute to the arterial injury.

Histopathologic Studies of Acute Arterial Injury. The evolution from acute arterial injury to OA can be precisely followed by detailed histopathologic studies.[123,142,143,180] Common early intimal findings include endothelial activation, which histologically manifests as hypertrophy or a "hobnail" appearance with eosinophilic transformation of the cytoplasm.[3,142,155,157] This correlates with functional activation,[156,157] including upregulation of class II MHC and adhesion molecules.[3,99,142,181-184] Endothelial damage can occur via a variety of mechanisms, including direct antibody and complement-mediated or CTL Fas-mediated apoptosis,[161] the result of which is a

loss of barrier function and the influx of clotting proteins (including fibrin), platelets, blood cells, and lipids. Edema and increased deposition of intercellular matrix and lipid[174] and a local turnover of cells[3,161] are seen in all 3 layers but are especially common in the intima. Taken all together, these processes result in disruption of intimal homeostasis and trigger the stereotypic repair response, typically seen in the arteries of all types of allografts. The response includes recruitment of medial myocytes, infiltration of foamy macrophages,[97,98,139,143,149-152,170] and proliferation of intimal myofibroblasts that eventually cause lumenal narrowing and predispose to thrombosis.

In the media, early changes include edema and degeneration or frank necrosis of individual myocytes.[3,167] Later, the media can become thinned,[142,185] presumably as a result of intimal migration of medial myocytes in response to injury. More importantly, the arteries affected by intimal thickening begin to dilate in an attempt to compensate for the lumenal narrowing. Eventually, even this compensatory mechanism fails. Compensatory failure could be related to replacement of the media by foam cells and/or fibroblasts or adventitial fibrosis, so that the artery is no longer a flexible muscular conduit, but a rigid tube formed by a mixture of fibroblasts and foam cells.

Changes in the adventitia of arteries have received far less attention than they deserve. Early after transplantation, the adventitia is a primary site of sensitization, which results in inflammation and edema.[3,168] The adventitial inflammation often persists during the transition to CR,[3,97,142] which is followed by the development of adventitial fibrosis[3,186] in fully developed lesions. These adventitial abnormalities likely contribute to the ultimate failure of the artery to dilate in compensation for the intimal thickening.

The Arterial Repair Response. At some stage in its development, the stereotypic arterial repair response proceeds independently of the arterial injury,[163,164,166] and it is the repair response that is the final common pathway for the development of OA and organ dysfunction. This has led to a natural preoccupation with understanding the molecular mechanisms of myofibroblast proliferation,[187] with the aim of finding key points of possible therapeutic intervention. A number of investigators have utilized the isolated aortic allograft model, introduced by Hayry.[32] Although this model does not address the organ parenchyma,[166,168,188-191] it offers a quick way of screening agents that might be of potential therapeutic benefit.

Multiple and redundant signaling molecules initiate and/or promote smooth muscle cell proliferation and fibrogenesis,[32,98,130,187,192-195] similar to the situation observed with AS in the general population.[196-199] Included are growth factors, cytokines, and chemokines.[9,32,98-101,130,187,192-195,200-203] For example, it has been shown that platelet-derived growth factor A and B,[192,193,204] fibroblast growth factor,[192,194,205] insulin-like growth factor-1[187] and interleukin (IL)-1[187] can be released by myofibroblasts, endothelial cells, and inflammatory cells into the arterial intima, stimulating smooth muscle cells to migrate to the site of injury and proliferate.

Macrophages are also an important source of molecules causing damage and initiating the repair response and fibrogenesis.[9,96,98-101,153,154,200,206] This helps explain the inhibition of OA by an essential fatty acid-deficient diet, which interferes with leukocyte chemotaxis.[98] Transforming growth factor beta-1 might also importantly contribute to fibrosis.[102,130,192,195,202] As well, IL-6 has been linked to the glomerular proliferative lesions in chronic kidney allograft rejection.[99] The reader is referred to several excellent reviews for a more detailed account of this complex area.[32,34,187,192,196-198,206-208]

Patchy Inflammation, Fibrosis, and Parenchymal Atrophy

The inflammation associated with CR consists predominantly of lymphocytes, macrophages, and plasma cells. The infiltrates are often organized into nodular aggregates, replete with recipient

APCs and germinal centers.[3,97,124,209] Most studies of CR have shown primarily CD4+ with fewer CD8+ T cells[3,96,209-212] and compared with AR, in CR there are usually more recipient macrophages and B cells.[181,212,213] Donor interstitial hematolymphoid cells are invariably destroyed in chronically rejecting allografts.[3] Anatomic arrangement and immunohistochemical studies are supportive of ongoing indirect alloantigen presentation.

The development of patchy fibrosis and associated parenchymal atrophy is often the first manifestation of CR,[142] an observation that has been used to predict the later development of CR in renal[214] and lung allografts.[147,215] The fibrosis usually develops in areas of ongoing immunologic damage and is associated with the deposition of tenascin and other matrix components,[102,216] endothelin,[217] and activation of interstitial myofibroblasts.[102] Larger scars representing healed infarcts and fibrosis in watershed regions supplied by the terminal circulation suggest that ischemia also contributes to fibrogenesis.[142]

Destruction of Epithelial-lined Conduits

Epithelial cell-lined conduits used for exchange of substances with the environment (eg, bronchioles, bile ducts, pancreatic ducts, renal tubular epithelium) are particularly prone to damage during CR. There are several possible nonexclusionary explanations for this observation: (1) the presence of a basement membrane, which could potentially play a role in migration, positioning, and costimulation of T cells[218]; (2) an immunologically active antigenic profile that is significantly different from other parenchymal cells, including expression of class I and II MHC and various adhesion and costimulatory molecules[218,219]; (3) the presence of nearby APCs and lymphatics that facilitate the functional role of these conduits in processing environmental antigen for local presentation and traffic to the regional lymph nodes[218]; (4) the ability of the epithelial cells to produce proinflammatory and fibrogenic cytokines; and (5) a deficient arterial blood supply.

Basement membranes contain matrix components important to the migration, positioning, and costimulation of T cells.[218] In lung allografts, staining for type IV collagen revealed early focal bronchiolar basement membrane damage, manifested by thickening and subsequent splitting and duplication, which later led to OB.[215] Epithelial cell "immunogenicity" is also enhanced by unique antigenic profiles[218] and their ability to upregulate immunologically active adhesion and costimulatory molecules. When activated, biliary epithelial and bronchiolar epithelial cells can also produce a variety of proinflammatory and fibrogenic cytokines and chemokines, such as IL-1-beta, IL-6, IL-8, TNF-alpha, and TGF-beta.[128,218-225] This enables the epithelial cells to dynamically participate in immune reactions, including rejection and response to foreign antigens, and to repair reactions. For example, El-Gamel and associates[128] showed that increased expression of TGF-beta during injury is a risk factor for the development of OB. Kallio and colleagues[107] showed that nitric oxide production by bronchial epithelial cells has at least a partial protective role in OB by either directly or indirectly inhibiting smooth muscle cell proliferation and modulation of the immune response towards TH-2 cytokines.

The epithelial-lined conduits also transport environmental antigens, are intermixed with APCs, and are surrounded by a rich lymphatic plexus which drains to the regional lymph nodes. Exposure to environmental or microbial antigens can cause local immune activation. In an allograft, this triggers upregulation of foreign MHC, adhesion, and various other immunologically active molecules, thereby potentiating rejection reactions. The inflammatory response to either the environmental or alloantigen(s) can compromise the structural integrity of the conduit and the surrounding microvasculature and lymphatic drainage. This, in turn, can inhibit efficient antigen clearing and cause ischemic damage, leading to a vicious cycle alternating between a persistent and inadequate immune response to environmental antigens and allogeneic injury. This concept is illustrated by the rapid decline of lung transplant recipientswithout OB who acquire a pulmonary infection.[226]

A separate arterial circulation primarily supplies allograft bile ducts and bronchioles.[117,118,227] Obliteration of these arteries by CR, or failure to reanastomose the vessels at the time of transplantation, can cause ischemic injury to the conduits.[117,118,227] Thus, in lung and liver allografts, both direct immunologic injury and ischemia contribute to the destruction of epithelial conduits[57,219] and bile ducts[40,48,117,119,121,228-232] during CR.

Disruption of Lymphatics and Organ-Associated Lymphoid Tissues

Hematolymphoid cells constantly travel into, transiently occupy, and then leave the interstitium of all vascularized organs. These cells are primarily derived from progenitors that migrate hematogenously from the bone marrow and consist of mature T and B cells, macrophages, hematopoietic stem cells, and dendritic cells. Maturation of local precursors can also contribute to this pool. In concert, they monitor the microenvironment and communicate with regional lymph nodes via the circulation and lymphatics. Organs such as the lung and intestine have a large specialized compartment of organ or mucosal-associated lymphoid tissue (MALT), commensurate with their task of directly dealing with antigens from the external environment. By contrast, the liver is richly endowed with a large macrophage population, consistent with its role as a filter of various opsonized material and other physiologic debris. Although not as extensive or well known, the kidney,[233] heart,[234,235] and pancreas also have considerable intraorgan immune networks.

Complete revascularization of an allograft results in the reestablishment of connections between the intraorgan immune network of the donor and the immune system of the recipient. Consequently, recipient immune cells circulate through donor organ-associated lymphoid tissues (GALT, BALT, portal lymphoid tissue)[1,236-239] and regional donor lymph nodes.[240] Conversely, donor immune cells migrate into recipient spleen and lymph nodes.[76,78,79] Early after transplantation, this results in an "in vivo mixed cell response," which typically manifests as AR.[236-240]

Transplantation disrupts the efferent lymphatics, which results in organ edema and contributes to the reimplantation response. In the absence of additional insults, the lymphatic channels reconnect within 2 to 3 weeks.[241,242] However, damage from AR again disrupts the lymphatic microvasculature and causes increased production of lymph fluid. These cause the graft to swell,[241,243-246] and cytokine and growth factor-rich fluid stagnates within the allograft and lymph nodes, predisposing to the development of fibrosis and exaggerated repair responses.

Repeated rejection-related damage eventually results in atrophy, fibrosis, and depletion of the lymphatic microvasculature and organ-associated lymphoid tissue,[3,247] such as BALT[246,248] or GALT.[239] This undoubtedly impairs adequate processing of infectious and other environmental antigens from chronically rejecting allografts and contributes to their functional decline.[47,54,57,248-251]

The Contribution of Nonimmune Injury to the Development of Chronic Rejection

A variety of nonimmunologic factors, such as older donor age, long cold ischemic time, preservation injury, delayed graft function, recipient hypertension, and hyperlipidemia, can potentially contribute to the development of CR. However, these factors are generally considered to be of secondary importance. The prevalence of AS in elderly donors and the propensity of older organs to heal by fibrosis are likely explainations for the association between older donor age and CR. Factors such as hypertension, hyperlipidemia, and diabetes mellitus, all of which are side effects of immunosuppressive therapy, contribute to the development of vascular disease, and therefore have the potential to accelerate OA. The stress of donor organ harvesting,

including donor brain death,[252] long cold ischemia times with subsequent preservation injury, and delayed graft function can stress the allograft. All together these manipulations increase allograft immunogenicity by stimulating cytokine and chemokine production and activating dendritic cells,[253,254] resulting in magnification of the immune response and damage.

Another important contributor to CR in kidney allografts is "functional overload," or an insufficient number of glomeruli or renal mass to satisfy the metabolic demands of the recipient (eg, small or diseased donor into a large recipient). This leads to progressive glomerular injury in the remnant glomeruli characterized by glomerular hyperfiltration, hypertrophy, and subsequent systemic hypertension. Hyperfiltration-mediated renal damage in allografts appears to become a problem when creatinine clearance is less than 60 mL/min.[255] In addition, ischemia and rejection combine to decrease the number of nephrons leading to hypertension and glomerulosclerosis and contribute to the downward spiral of injury and inadequate functional capacity.[63]

Manifestations of Chronic Rejection in the Heart Allograft

OA is the major manifestation of CR in cardiac allografts. In some studies, OA has been seen more frequently in patients with AR[256,257] and in those who develop antidonor HLA antibodies.[257] Some investigators suggest that early-onset (< 2 years) OA has a stronger association with AR than late-onset (> 3 years) OA,[256] in which there are multiple contributing factors. Cytomegalovirus (CMV) also has the potential to contribute to OA in heart allografts[51,258-260] by augmenting vascular growth factor production, altering the alloimmune response directly, or changing the expression of cytokines and cell adhesion molecules.[260] OA is also associated with direct arterial inflammation and/or necrotizing arteritis in tissue sections,[139,159,261] but the correlation between the development of OA and prior AR episodes is not as clear-cut in heart allografts as it is in other vascularized allografts. One source of the problem is distinguishing OA from AS in heart allograft recipients, who have many risk factors for coronary artery disease.

Since AS is endemic in the general adult population, it is frequently transmitted from the donor to the recipient.[66-68] This explains the higher incidence of vascular abnormalities seen early after transplantation with use of older donors. [67,135,262,263] Thus, both OA and AS contribute to the arterial disease seen in allografts after cardiac transplantation.[68,263-266] In an individual patient or lesion, however, it is often difficult to determine the relative contribution of OA or AS, since the 2 disease processes can show overlapping features. In addition, atheromas are frequently inflamed with donor hematolymphoid cells, providing a strong immunogenic stimulus at this site that could trigger vascular rejection. Conversely, McManus and colleagues [174,176,267] showed that OA lesions can imbibe lipids, suggesting that an "atherogenic" environment may accelerate OA.

Despite the overlap between OA and AS, preexisting donor AS does not necessarily place the recipient at increased risk for the development of OA.[67,268] This suggests that at least some of the pathogenic mechanisms responsible for OA are different from those involved in AS.[135] The influence of OA on AS and vice versa may explain some of the more recent apparent inconsistencies in the distribution of OA noted by investigators using intravascular ultrasound.[269] Clearly, more detailed investigation into the association between OA and AS is needed.

Coronary angiography is the standard method for surveillance of heart allograft vasculopathy (both OA and AS), although it underestimates the incidence and severity of disease.[50,136,265,270-272] Gao and associates[273] originally classified the angiographic abnormalities in heart allograft recipients into 3 categories: (1) type A, characterized by discrete or tubular stenoses; (2) type B, characterized by diffuse concentric narrowing; and (3) type C, characterized by narrowed irregular vessels with occluded branches. This classification has proved useful for distinguishing the relative contribution of OA and AS to arterial pathology in cardiac allografts.[135]

Type A lesions of the primary epicardial coronary vessels with less frequent secondary branch involvement are the most common type of lesion(s) encountered in AS in the general population. Thus, Type A lesions can be detected in heart allografts shortly after transplantation, which is consistent with transmission of donor disease. However, evolution of type A lesions to include narrowing of secondary and tertiary branches develops more frequently in heart allografts than in native hearts. Type B and C lesions involving primary, secondary, and tertiary branches are much more common in allograft coronary arteries than in native hearts.[273] Total vessel occlusion is seen predominantly in the proximal or middle vessel segments of arteries with AS, whereas distal involvement can be seen in up to one half of patients with OA. Failure to develop collateral vessels is also more common in allograft recipients with OA.

Based on the above angiographic observations, Gao and associates[273] concluded that coronary artery disease in heart transplant recipients represents a mixture of typical AS and unique transplant-related progressive distal OA that occurs without collateral vessel development. Several other investigators have drawn the same conclusion using the more recently developed intravascular ultrasound.[68,136,263-266,270] This technique offers some advantages over angiography in terms of sensitivity[136,262,270,272,274] and the ability to more precisely characterize changes in coronary vessel shape and wall thickness[275] and vascular remodeling.[136,270] This technique also enables one to follow the changes over time and study the reaction of the vessel wall to injury. Nevertheless, the conclusions are the same. Lesions frequently detected early after transplantation are segmental and eccentric intimal thickening and lumenal narrowing, primarily involving the epicardial coronary arteries and their primary branches, and reflect donor AS in the general population. Over time, more distal, diffuse, noncalcific and concentric intimal hyperplasia develops,[68,265,266,272,276] which likely represents OA.

Serial intracoronary acetylcholine infusions and quantitative angiography can be used to study endothelial cell dysfunction, which is frequently detected in allograft coronary arteries beginning early after transplantation.[277,278] A close association between endothelial dysfunction and intima abnormalities can be documented in some[277] but not all studies, presumably because of the episodic nature of the immune injury.[264] Another abnormality is impaired responsiveness of epicardial arteries to increased flow.[278] Resting 2D echocardiography and dobutamine stress echocardiography (DSE)[279] offer noninvasive methods of monitoring heart allograft vasculopathy with reasonably comparable but less sensitive results when compared to angiography and IVUS.[270,279]

Cardiac dysfunction associated with CR is largely due to ischemic damage to the heart, which can manifest as ventricular dysrhythmias, congestive heart failure or myocardial infarction and cardiogenic shock.[135,148,280] Although the classic symptoms of myocardial ischemia, such as angina pectoris, are thought not to be reliable indicators in the denervated allograft heart,[281] symptoms of myocardial infarction in 25 heart transplant recipients[280] included chest pain (n=2), arm pain (n=3), weakness (n=16), dyspnea (n=11) and palpitations (n=8). Three episodes were clinically silent, detected only as new electrocardiographic changes during routine follow-up, and 2 patients had old Q-waves. Of the 8 patients hospitalized with symptoms, only 7 had typical Q-wave changes on electrocardiography; 5 had nonspecific ST-segment changes and 2 had no documented changes. Serial creatine phosphokinase levels were obtained in 13 patients, and values were elevated in 8. Other manifestations of CR and myocardial ischemia include cardiac enlargement with ventricular dilatation, loss of papillary muscle function and mitral regurgitation, ventricular wall dysfunction,[281] and constrictive pericarditis.[282]

Endomyocardial biopsy is not a reliable method of establishing the diagnosis of chronic cardiac allograft rejection, because the biopsies do not show characteristic changes that can be reliably associated with CR alone,[135,141,283-288] except in rare cases.[289] Intimal thickening typical of OA usually does not affect the small penetrating intracardiac arterioles present in endomyocardial biopsies.[186] In contrast to kidney allografts,[214] collagen density in biopsy specimens does not

appear to predictably increase with time in patients who develop CR.[290] This is because fibrosis can develop from repeated biopsy sampling of the right ventricle, even in the absence of CR.

There is, however, some evidence that nodular subendocardial lymphocytic aggregates, or Quilty lesions,[283] may be associated with CR. Quilty lesions are present in 4%-25% of human endomyocardial allograft biopsies and are detected on at least 1 occasion in 58%-78% of patients.[286,287,291,292] Although Quilty lesions are not the equivalent of therapy-requiring AR, some clinical studies show an association with AR in the underlying myocardium[287,293-296] or with OA.[288] Patients in the Stanford series with Quilty lesions who underwent transplantation in the early 1980s had the highest incidence of graft vasculopathy,[297] but more recently the incidence of OA has significantly decreased in these patients.[297] This perplexing observation might be explained by the fact that these patients had the most potential for improvement, particularly with the addition of new immunosuppressive regimens. For example, tacrolimus has significantly lessened the incidence of Quilty lesions in our heart transplant recipients[298] and significantly increased the half-life of kidney allografts, [299] presumably by lessening CR.

Even though a causal association of Quilty lesions with OA is unlikely, Quilty lesions might not be innocuous histopathologic curiosities. Their organized lymphoid structure[3,135] and presence in animal models with persistent alloactivation and developing OA[3] suggest that they may represent indolent ongoing immunologic damage via indirect allorecognition. Kemnitz and associates[295] speculated that Quilty lesions may even represent a form of vascular rejection, since the heart develops embryologically from primitive vessels.

As in native hearts with AS, coronary artery bypass grafting,[300] angioplasty,[301] laser revascularization and/or stenting[302] have been used in an attempt to restore distal arterial flow in heart allografts affected either with OA or AS, or both. The lesions most amenable to treatment with these procedures are those preferentially involving the proximal portions of the artery with a segmental distribution and sparing of the distal portions. Procedural success rates are similar to those in native hearts with similar lesions.

Manifestations of Chronic Rejection in the Lung Allograft

The term "bronchiolitis obliterans syndrome" (BOS) is used to connote lung allograft deterioration secondary to progressive airway disease for which there is no other cause.[303] It is widely presumed, but unproved, that this is the principal manifestation of CR in lung allografts.[303] BOS appears to equally affect all lung transplant recipients, including single, bilateral, and heart-lung transplantation patients.[304,305]

Although specific symptoms are lacking, patients typically experience worsening respiratory debilitation. The characteristic physiologic hallmark of BOS is airflow limitation, as evidenced by progressive decline in the forced expiratory volume in 1 second, to values less than 80% of posttransplantation baseline.[303,306,307] The baseline value is defined as the average of the 2 previous highest consecutive measurements being obtained 3 to 6 weeks apart. In some patients, the baseline value will rise over time, especially in the first 6 to 9 months.[303] The highest baseline value achieved is used for all subsequent measurements.

The term obliterative bronchiolitis (OB) is the principal histopathologic manifestation of CR in lung allografts, which refers to the findings of inflammation and fibrosis occurring predominantly in the walls and contiguous tissues of membranous and respiratory bronchioles, with resultant narrowing of their lumens.[308]It is generally assumed that OB and BOS are synonymous. However, BOS does not necessarily require histologic confirmation[303];the clinical and functional aspects of this syndrome are not always consistent with the typical pathology, often because of sampling problems related to small transbronchial biopsies. Therefore, a staging and

classification system was devised that allows for diagnosis of BOS even in the absence of biopsy confirmation of OB.[303]

Compared to CR in other vascularized allografts, CR is a particularly pervasive problem in lung transplantation, developing in about 15% of survivors per year,[305] and is the most significant obstacle to long-term, morbidity-free survival.[307] Once discovered, BOS tends to progress over time.[307] Risk factors for the development of OBand CR include severe AR, [226] late-onset AR,[42] inadequate immunosuppression,[42] HLA mismatching,[18,19,226] development of anti-HLA antibodies,[19,306] CMV mismatching (seropositive donor in seronegative recipient),[226,306] lung infection,[226] and organizing pneumonia.[226]In one study, p-glycoprotein and metallothionein expression by inflammatory cells was suggested as a marker of aggressive or persistent cases of AR leading to OB.[309] Donor and recipient characteristics such as sex, age, underlying disease, type of transplant, and graft ischemic time does not appear to affect BOS onset, progression, or prognosis.[226] Once developed, the prognosis of BOS is degraded in the face of superimposed AR and lung infection.[226]

Although serial pulmonary function tests are the standard method used to screen lung allograft recipients for the development of BOS, repeated high-resolution computed tomographic examination is another relatively sensitive and specific, noninvasive method of monitoring the development of OB and BOS.[308,310] Chest radiographs usually show nonspecific parenchymal abnormalities consisting of linear nodular, nodular, confluent nodular, or diffuse alveolar opacities, which are also present in other infectious and noninfectious complications. The presence of central bronchiectasis may also be a distinctive radiographic finding in this group of patients.[311]

The use of transbronchial biopsies is encouraged to establish the diagnosis of OB and CR in support of a clinical diagnosis of BOS. In one study, 38% of transbronchial biopsies from BOS patients were diagnostic of OB; another 29% showed chronic inflammation and fibrosis, suspicious for CR.[115] Inadequate sampling by the bronchoscopist was the major reason for a negative biopsy.[115] In another study, the sensitivity and specificity of one transbronchial biopsy procedure with an average procurement of 7.6 tissue fragments was 17.1% and 94.5%, respectively.[312] The predictive value of a positive procedure for the presence of disease was 65.5% and that of a negative procedure for the absence of disease was 65.2%.[312] Although false-positive diagnoses of OB on transbronchial biopsies are uncommon, OB can be associated with bronchiolitis obliterans organizing pneumoniasyndrome[313] accompanying viral pneumonitis, and chronic inflammation and fibrosis with AR and infection.[115] Thus, although the sensitivity of transbronchial biopsy and the predictive value are low, attempts should be made to support a clinical diagnosis of BOS through biopsy.[312]

Usual treatment for BOS has been to increase the maintenance immunosuppression regimen or augment immunosuppression by adding other agents or by switching to entirely new ones.[304] Most of these strategies have been effective in achieving some reduction in the rate of decline in graft function, although true cure of BOS has been rare. Retransplantation is the most aggressive therapeutic strategy for OB, but for a number of logistic reasons, such as significant adhesions between the allograft and parietal pleura, it is applicable only to a tiny minority of lung transplant recipients with OB.[304]

Manifestations of Chronic Rejection in the Kidney Allograft

Chronic rejection in kidney allografts is characterized by an indolent and variable loss of function, which mostly occurs in combination with proteinuria and hypertension.[111] Since many of the pathophysiologic and immunologic features of CR were first recognized in kidney allografts,[5,185,314,315] the study of CR is probably most comprehensive in this organ. In kidney allografts, CR is also known as chronic allograft nephropathy (CAN), a term which acknowledges the important contribution of nonimmunologic factors such as hypertension, hyperfiltration injury and other insults, to the progressive decline of renal structure and function that plagues many

long-surviving kidney allografts with CR. Paul[111] has recently written a comprehensive and authoritative review of CAN.[111]

As in other organs, risk factors for CAN are divided into immunologic and nonimmunologic, although the latter assumes a greater importance in kidney allografts because they are frequent causes of disease in native kidneys. Immunologic factors are repeated AR episodes,[25,45,316,317] vascular rejection episodes,[45,316] rejections that occur late after transplantation,[45] MHC mismatching,[25,317] donor and recipient gender differences,[65] and recipient race.[12] Nonimmunologic factors that augment the immunologic injury or contribute to the architectural or vascular pathology of CR include advanced donor age,[25,316,317] delayed graft function,[316] donor source (living-related and living-unrelated vs cadaveric),[317] cold ischemia time,[317] delayed graft function,[255,317] size mismatching or insufficient nephron mass,[43,63,255,318] hyperlipidemia,[65,319,320] and hypertension.[65,111,319] The presence of the angiotensin-1-converting enzyme gene-deleted allele has been associated, when in homozygosity, with increased risk of cardiovascular diseases and with an accelerated progression of organ damage in a variety of kidney diseases. The DD genotype is associated in pediatric kidney graft recipients with a shorter long-term kidney graft survival and suggests a possible role of this genotype as a cofactor in the progression of nonimmunologic injuries leading to chronic kidney allograft failure.[321]

The histopathology of CR in the kidney is not thought to be specific,[322] because similar histopathologic changes can be seen in other disorders such as hypertension, diabetes, and hyperlipidemia. However, transplant glomerulopathy and multilayering of the peritubular capillaries are highly characteristic features of CAN.[111] Even though the histopathologic findings are not specific, biopsy findings can provide important information for patient management. Besides establishing the cause of dysfunction, early protocol biopsies contribute important prognostic information.[323] For example, poorer long-term function can be predicted using 6-month protocol biopsy specimens immunostained for collagen III: increased percent areas occupied by type III collagen correlates with decreased GFR.[214] In addition, early-protocol biopsies of stable, well-functioning kidney allografts reveal a high prevalence of clinically unsuspected acute and chronic pathology, including rejection,[324] which is predictive of long-term allograft outcome.[325,326] Larger glomeruli at baseline, measured by a simple point-counting technique, are an early predictor of risk for late allograft dysfunction and may help to identify a subpopulation of patients in whom treatment to prevent or ameliorate glomerular enlargement and/or hypertension may be efficacious.[318]

Currently, the most effective option to prevent chronic allograft nephropathy is to avoid graft injury from both immune and nonimmune mechanisms.[111] Once the functional decline is linked to irreversible structural damage, such as fibrosis and/or glomerulosclerosis, little can be done to reverse the trend.

Manifestations of Chronic Rejection in the Small Bowel Allograft

Transplantation of the immunologically difficult small bowel has only recently been clinically achieved to any significant extent,[327-329] largely because of ineffective control of AR, which was made routinely possible by the use of tacrolimus immunosuppression.[330] Thus, study of CR changes in small bowel allografts has heavily relied on experimental animal models.[1,239,331-333] However, a recent study of CR in small bowel allografts at the University of Pittsburgh showed that CR was associated with an increased number and severity of AR episodes, as seen in other types of allografts. The composite effect is also seen when the small bowel is simultaneously transplanted with the liver, in which case the incidence of CR decreases substantially.

Typical clinical findings that support the diagnosis of CR include persistent diarrhea with nonhealing ulcers, repeated bouts of AR, and either noncompliance with immunosuppressive medications or deliberate withdrawal of immunosuppression because of life-threatening infection.[38] Endoscopic and radiographic studies used to support the diagnosis of CR include

focal loss of mucosal folds, focal ulcers and mural thickening, and pruning of the mesenteric arterial arcades.[334] Histopathologic features of CR include OA, hyperplasia of the muscularis propria,[335] ongoing crypt epithelial cell apoptosis with crypt loss, villous blunting, focal mucosal ulceration, fibrosis of the lamina propria and submucosa, and destruction of donor mesenteric lymph nodes and Peyer's patches.[1,38,239,331-333,335,336]

As with other types of allografts, the biopsy diagnosis of CR in small-bowel allograft recipients is difficult. The vessels affected by OA, primarily the distal branches of the mesenteric arteries and the larger arteries of the serosa and muscularis propria, are not routinely sampled. Therefore, one is forced to rely on surrogate findings, including focal, nonhealing ulcers; a distinctive combination of loss of crypts, and epithelial regeneration and crypt epithelial apoptosis; and a disordered mucosal and villous architecture with fibrosis.[38]

Manifestations of Chronic Rejection in the Pancreas Allograft

Pancreas transplantation is used to treat type I diabetes mellitus, but the operation is largely restricted to diabetic patients who also suffer from renal failure and who are in need of simultaneous kidney transplantation.[337-340] This is because of an unfavorable risk:benefit ratio associated with isolated pancreas transplantation and long-term immunosuppression vs the ability to control blood glucose with exogenous insulin therapy.[337,338] Potential benefits to the patient increase substantially for those in need of both kidney and pancreas transplantation.[340] The most obvious advantage is an improved quality of life, but there is emerging evidence of a slow beneficial effect on diabetic complications, which reduces long-term morbidity and mortality compared with kidney transplantation alone in diabetics.[337,338,340] Currently, insulin independence rates for combined kidney-pancreas transplantation is 60 % at 5 years; and late graft failures are largely attributable to CR.[337-339]

As in other organs, CR in pancreas allografts often begins during AR. The simultaneous kidney allograft from the same donor is thought to provide a reliable surrogate marker of immune activity in the pancreas.[341] Therefore, acute pancreas allograft rejection is often indirectly monitored via kidney allograft function and kidney allograft biopsies. Other parameters used for the diagnosis of pancreas allograft rejection include: decrease in urinary insulin and C peptide; and increase in serum amylase, lipase, anodal trypsinogen, and pancreas specific protein. Cytologic evaluation of pancreatic juice and urine have also been used in the diagnosis of pancreas allograft rejection. In bladder-drained pancreas allografts, urinary amylase has been used as a measure of pancreas exocrine function.[341] Lastly, blood glucose monitoring is only a crude indicator of pancreatic function, and because rejection does not primarily target the islets, elevated blood glucose is a late finding. Therefore, loss of insulin function is a late finding and not generally useful for early recognition of injury.

In our experience, pancreas allografts are the least well monitored of all solid organ allografts. Consequently, subclinical rejection-related pancreatic allograft injury is not uncommon and, therefore, CR is a significant problem. Recently, however, direct biopsy monitoring of the pancreas allograft has received greater acceptance and can be used to diagnose AR, CR, recurrent type I diabetes mellitus, and a host of other pancreatic complications that occur after pancreas transplantation.[143,342-345] As in other organs, chronic pancreas allograft rejection primarily manifests as mild patchy inflammation, progressive fibrosis and loss of acinar parenchyma, obliterative arteriopathy, and focal ductal destruction.[143,342-345] Further research is needed to determine the rate and impact of recurrent autoimmune diabetes on islet destruction.

Fortunately, islet injury is a late finding in chronic pancreas allograft rejection and occurs mostly as a result of dense fibrosis of the surrounding tissue and islet atrophy, rather than direct immunologic injury to the islets. Isolated inflammation of islets, or so-called isletitis, is rarely, if ever, seen independently of pancreas allograft rejection. When present, isletitis is usually seen in association with inflammation of the surrounding acinar structures typical of higher grades of

AR.[342] In other studies, isletitis occurs in up to 25% of grafts,[344] and 36% of grafts with isletitis also had selective loss of beta cells from the islets, indicative of recurrent type I diabetes mellitus.[344]

Loss of islet function can also occur as a reversible side effect of both tacrolimus and cyclosporine and with recurrent diabetes mellitus.[346] Reversible drug-induced structural damage includes cytoplasmic swelling, vacuolization, apoptosis, abnormal immunostaining for insulin, and marked decrease or absence of dense-core secretory granules in beta cells, which can result in dysfunction of beta cells.[346] A ratio of insulin:glucagon cells of less than 1.0 appears to be specific for recurrent diabetes. In the absence of isletitis, this is a reasonable method for detecting recurrent disease at an early stage.[347] The obvious treatment for end-stage CR of the pancreas is a return to exogenous insulin therapy.

The Special Case of Liver Allografts

Chronic liver allograft rejection is uncommon. Over the past 2 decades, the incidence at 5 years after transplantation has steadily declined from about 15%-20% to current projections of 3%-5%.[135,348] This decline has been attributed to the unique immunologic properties of the liver allograft, better recognition and control of acute and the early phases of CR, and the remarkable regenerative capabilities of the liver.[27,119,330,349-356] Nevertheless, CR is still an important cause of late liver allograft dysfunction and failure,[348,357-361] and from a practical perspective, proper recognition of CR is essential for long-term patient management because clinicians frequently lower or discontinue immunosuppression in long-term survivors.[362-365]

Chronic liver allograft rejection usually begins within several months after transplantation and allograft failure typically occurs within the first year after transplantation.[40,41,46,119,348,366-368] In contrast to other vascularized allografts, the incidence of CR in the liver usually decreases with time after transplantation,[40,46,367] except for in a small group of patients with late-onset CR.[46]

As in all other organs, "immunologic" factors are the most important contributors to the development of CR in the liver allograft. Among these, the number and severity of AR episodes are the most significant,[41,46] regardless of the immunosuppressive regimen. In cyclosporine-treated cohorts, late-onset AR episodes,[37,119,369,370] younger recipient age,[119,356] male-to-female sex mismatch, a primary diagnosis of autoimmune hepatitis or biliary disease,[356] baseline immunosuppression,[37,353,354] and non-caucasian recipient race[49,119] have all been associated with an increased risk of developing CR.

The association between liver allograft survival and MHC/HLA matching is controversial.[26,371-373] Some studies show that mismatching at the MHC class II locus increases the incidence of AR[26,372,374] and allograft failure from AR.[26] Other studies show no effect of DR mismatching on graft survival,[371,373] while still another study showed a beneficial effect of class II mismatching on nonrejection-related causes of graft failure.[372] It has been hypothesized that MHC matching appears to play a dualistic role in liver transplantation: mismatching increases the incidence of AR, but MHC matching might contribute to a greater susceptibility to recurrent inflammatory liver disease.[372,373] The effect of CMV infection on CR is also controversial.[37,54,119,367,374,375] In a large tacrolimus-treated cohort, many of the matching factors described above were not significant risk factors for CR, but the influence of the number and severity of AR episodes remained.[376]

A nonalloantigen-dependent or nonimmunologic risk factor that contributes to the development of CR in the liver is donor age > 40 years,[376] which is also a well-described risk factor in kidney and heart allografts.[132,377,378] Older donor organs have also been found to have a higher incidence of AR.[374]A clinical diagnosis of CR should be suspected in a patient with a history of AR who develops progressive cholestasis and an increase in canalicular enzymes that is unresponsive to antirejection treatment.[379] This usually occurs as the end-stage of unresolved AR or after multiple

episodes of AR, particularly within the first year after transplantation.[41,46,117,119,121,228-231,352,366,379-382] CR can also appear indolently without preceding clinically recognized episodes of AR,[41,46,117,119,121,228-231,352,366,379-382] but this presentation is relatively uncommon and may simply reflect inadequate monitoring.[348] In fact, late-onset CR (more than 1 year after transplantation) is typically seen in inadequately immunosuppressed patients, either as a result of noncompliance or because of infectious or neoplastic toxicity of overimmunosuppression.[46]

Unresolved or indolent rejection may become apparent only as a persistent elevation of liver injury tests. If clinical symptoms are present, they usually resemble those of AR until allograft dysfunction becomes severe enough to cause jaundice. Biliary sludging or appearance of biliary strictures, hepatic infarcts, and finally loss of hepatic synthetic function, which can manifest as coagulopathy and malnutrition, are other late findings presaging allograft failure.[379]

Standard liver injury test abnormalities in a patient with CR in general show a progressive cholestatic pattern that manifests as preferential elevation of gamma-glutamyl transpeptidase and alkaline phosphatase.[359,379,383] The early transition from AR to CR may be marked by persistent elevation of aspartate aminotransferase,[380] which, along with total bilirubin, is associated with graft failure from CR.[41,46,384] A similar pattern of liver injury test elevations and histopathologic changes can be seen with obstructive cholangiopathy and arterial narrowing or thrombosis.[359] Cholangiography and/or angiography may be required in some cases to distinguish between CR and biliary obstruction.[384] Selective hepatic angiography showing pruning of the intrahepatic arteries with poor peripheral filling and segmental narrowing can also be used to support the diagnosis of CR.[40,146,379,385]

The final diagnosis of CR should be based on a combination of the clinical, radiologic, laboratory, and histopathologic findings.[348] In a biopsy specimen, the minimal diagnostic criteria for CR are: (1) the presence of bile duct atrophy or pyknosis, affecting a majority of the bile ducts, with or without bile duct loss; (2) convincing foam cell OA; or (3) bile duct loss affecting greater than 50% of the portal tracts.[348,383] Cases with either isolated bile duct loss or foam cell arteriopathy alone may occur, but usually both features are found together.[117,120,121,380] Unfortunately, arteries with pathognomonic changes are rarely present in needle biopsy specimens, and therefore, what has been observed about OA in the liver has come from examination of failed allografts removed at the time of retransplantation. Therefore, in biopsy specimens, considerable significance is placed on damage and loss of small bile ducts. However, a similar pattern of duct injury and ductopenia can be seen as a result of nonrejection-related complications, such as obstructive cholangiopathy, hepatic artery stricturing or thrombosis, adverse drug reactions, and CMV infection.[383,384] In cases of CR identified by bile duct injury or loss alone, other nonrejection-related causes of ductal injury and loss (which histopathologically appear similar to CR), such as adverse drug reaction, hepatic artery or biliary tract obstruction or stricturing, should be reasonably excluded.

Since the early state of CR in the liver is reversible, much emphasis has been placed on precisely defining its histopathologic features.[348] Within the portal tracts, CR manifests as damage and loss of small bile ducts and small branches of the hepatic artery. Bile duct damage is due to a combination of direct immunologic injury and indirect ischemic damage resulting from OA, small artery and arteriolar loss and destruction of the peribiliary capillary plexus.[117,386] Early histopathologic changes in the bile ducts that presage their disappearance include eosinophilic transformation of the biliary epithelial cytoplasm, uneven nuclear spacing, syncytia formation, nuclear enlargement, and hyperchromasia resembling cytologic dysplasia, and ducts only partially lined by biliary epithelial cells.[349,352,384,387] Late bile duct and arterial damage is gauged by the extent of loss of these structures, which is based on quantitative morphometry.

Inflammation during CR in liver allografts is usually mild and consists of lymphocytes, plasma cells, and macrophages.[96,181,388] Despite a paucity of portal inflammation, intraepithelial lymphocytes are located adjacent to degenerating biliary epithelial cells,[40,48,117,119,121,228-232] which

show uneven spacing of individual epithelial cells, eosinophilic transformation of the cytoplasm, and ducts only partially lined by bile duct cells.[135] The portal infiltrate is T-cell predominant in CR and contains both CD4 and CD8 subsets,[96,181,388] with the latter predominating in and around the biliary epithelium.[388] Increased gamma-IFN, IL-2, and granzyme B transcripts[96] have also been detected, the last of which strongly suggests that a cytolytic effector pathway of injury is involved. The damaged ducts can also show inappropriate expression of the major ABO blood group antigens.[389]

In a normal liver, Crawford and colleagues[390] defined a portal tract as "a focus within the parenchyma containing connective tissue (by Masson's trichrome stain) and at least 2 lumenal structures embedded in the connective tissue mesenchyme, each with a continuous connective tissue circumference." Using this definition, 93 +/- 6% and 91 +/- 7 % of portal tracts in needle biopsies contain bile ducts and hepatic artery branches, respectively.[380,390] Bile duct loss is considered present when < 80% of the portal tracts contain bile ducts; arterial loss is considered present when < 77 % of the portal tracts contain hepatic artery branches.[348]

Since CR can result in loss of both the bile duct and hepatic artery branches,[117,380] strictly applying the above definition, which requires 2 of 3 normal portal profiles (bile duct, hepatic artery and portal vein), will not be possible in some CR cases.[348] When both bile duct and arterial loss are seen, recognition and counting of the portal tracts ultimately depends on the subjective interpretation of the pathologist.[348] Recognition of portal tracts in such cases should be based primarily on the location (cholestasis in CR is centrilobular), shape, and internal structure of the connective tissue mesenchyme. If both bile ducts and arteries are destroyed, determination of bile duct and arterial loss should be based on a count of the total number of portal tracts with and without bile ducts and arteries, and a comparison with expected values from normal livers.[348]

As in other organs, OA is a manifestation of CR in liver allografts, but it is rarely seen in needle biopsies because medium-sized perihilar muscular arteries are most commonly and severely affected, and these arteries are not routinely sampled in a peripheral needle core biopsy. Instead, examination of failed allograft hepatectomy specimens obtained at various times after transplantation have shown that early CR is marked by the accumulation of subintimal, medial, and adventitial foamy macrophages, which accompany the lymphocytic inflammation typical of AR-related arteritis. The foamy macrophages accumulate over time and can be accompanied by the proliferation of donor-derived subintimal myofibroblasts, similar to OA in other solid organ allografts.

The early phase of CR in the terminal hepatic venules and surrounding perivenular parenchyma is characterized by subendothelial and perivenular mononuclear inflammation accompanied by perivenular hepatocyte dropout, pigment-laden macrophages, and mild perivenular fibrosis.[348,356,376,380,382] Spotty acidophilic necrosis of hepatocytes, or so-called transitional hepatitis, may occur during evolution to the late stages of CR.[380,391] At this stage, it may be difficult to distinguish between hepatitis and rejection. Late CR is characterized by severe (bridging) perivenular fibrosis with at least focal central-to-central or central-to-portal bridging and occasional obliteration of terminal hepatic venules.[356,376,380,382] Other common findings in late CR include perivenular hepatocyte ballooning and dropout, hepatocanalicular cholestasis, regenerative hyperplasia, and intrasinusoidal foam cell clusters.Bile duct loss in more than 50% of the portal tracts, severe (bridging) perivenular fibrosis, and small arterial loss have all been associated with a greater incidence of eventual graft failure from CR.[119,352,356,376] However, it is important to remember that bile duct loss is potentially reversible. This was first recognized when patients with clinical and histopathologic features of established CR markedly improved, either with or without augmented immunosuppression.[119,349,352,356,384,387] In several of these cases, serial biopsies chronicled an apparent regrowth of bile ducts even when initial bile duct loss exceeded 50%, and serum bilirubin levels were greater than 10 mg/dL.[119,349,352,356,384,387] Sampling errors and/or functional adaptation to ductopenia could theoretically account for these observations, but the more likely explanation is that the liver undergoes architecturally intact repair. The important

practical implication is that the biopsy findings do not absolutely define a point of no return; they provide information and the likelihood of reversal, which should be combined with a complete clinicopathologic evaluation prior to the decision to continue medical therapy or proceed with retransplantation.[348]The quantitative analysis of both bile duct and arterial loss raises issues of sampling problems.[348] It has been suggested that examination of a minimum of 20 portal tracts is needed to ascertain the diagnosis of CR in liver allografts,[351,367] but recent studies showed the median number of portal tracts in a standard liver allograft biopsy was 9 - 11[356,380,383] and CR could be correctly and reliably rendered on these samples. Smaller biopsies with fewer than 10 portal tracts should be interpreted with greater caution.[348] The most significant potential error is a false-positive diagnosis of CR or categorization of a case in the advanced stages when there is still potential for recovery. Potential sampling errors can be minimized by obtaining 1 large or multiple biopsies. The more portal tracts sampled, the more likely the specimen is to be representative of the entire organ. A point that cannot be overemphasized is establishing a close correlation between the biopsy findings and the clinical profile.[348]An unresolved question is whether there is a form of late-onset CR that resembles chronic viral or autoimmune hepatitis (without evidence of infection with known hepatitis viruses or the presence of autoantibodies) that leads to cirrhosis.[348] This concern was initially raised by Kemnitz and colleagues.[392,393] Recent recognition of this syndrome at several large centers has brought the issue to the forefront for discussion.Hubscher,[394] who coined the term idiopathic post-transplant hepatitis (IPTH), showed that more than 40% of 12-month protocol liver biopsies from recipients at Birmingham, United Kingdom, showed an unexplained chronic hepatitis. By definition, the histopathologic changes of typical AR or CR were not present. The patients with IPTH were more likely to have autoimmune disorders (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis) or seronegative fulminant hepatic failure than they were to have their original diseases. Fortunately, only a small proportion of cases progressed to cirrhosis, but the long-term outlook for the others is uncertain.[394] Whether IPTH represents a form of recurrent autoimmune disease, infection by an unknown virus, an adverse drug reaction, an unrecognized form of rejection, or a combination of 1 or more of these factors, is uncertain.[348] Further study is needed in this area.There are 2 fascinating aspects of chronic liver allograft rejection: its reversibility,[119,352] which has already been discussed; and an appreciably lower incidence compared with other types of allografts (Table 1,[30]). Experimental animal studies have also shown that a liver allograft is spontaneously accepted without immunosuppression in some small animal species[395,396] and is the best composite organ in that it can completely protect other organs from the same donor from CR.[3]

Resistance of liver allografts to AR and CR and the ability to induce tolerance are well known. Hypotheses used to explain this observation can be broadly separated into 2 general categories, based on whether emphasis is placed on the parenchyma or nonparenchymal cells in the liver.[135,397] One argument in favor of parenchymal cells is the release of soluble donor MHC class I antigen from hepatocytes.[398] This hypothesis, however, is not tenable because recent studies show that murine liver allografts are routinely accepted between strains of mice that show no difference between the class I loci, but are mismatched for class II loci.[399] In addition, fully allogeneic liver allografts from class I or II MHC-deficient mice, which do not shed soluble MHC antigens,[399,400] are also accepted. Additional evidence refuting this hypothesis is that other organs also secrete soluble MHC antigens,[401] but they are routinely rejected and infusion of exogenous soluble MHC usually leads to only slight prolongation of allograft survival.

Another potential explanation for the importance of the parenchyma relies on the concept that allogeneic hepatocytes provide only 1 0f 2 signals needed for allogeneic lymphocyte activation.[402] Lack of important costimulatory molecules is thought to result in the induction of anergy in the responding lymphocyte populations.[402] However, when transgenic mice that aberrantly express the allogeneic MHC class I molecule H-2Kb (Kb) in the liver (using a metallothionein promoter[403]), are crossed with a strain that develops CD8+ T cells specific for the Kb molecule, lymphocyte-mediated autoimmune liver damage can be induced under certain conditions. Interestingly, most of the CD8+ cells responsive to Kb were eliminated by the intense intrahepatic activation, but some of the liver continued to show chronic low-grade inflammation.[403] However, great care had

to be taken to assure that alloantigen expression was limited to the liver.[403] Interpretation of these complicated experiments is difficult, but it appears that the liver may be able to effect an activation-induced partial clonal deletion of allogeneic lymphocytes,[403] as originally proposed by Kamada.[396] Deletion of activated cytolytic T cells may be a special property of the liver,[403-405] because alloantigen expression on other nonhematolymphoid cells such as pancreatic islets does not lead to anergy,[406] but to immunologic ignorance,[407] which is an important difference.

It has been our contention that so called "hepatic tolerogenecity" is, in large part, a result of hematolymphoid cells within the liver and subsequent microchimerism.[27,74,408] The donor hematolymphoid cells are thought to provide 2 important functions. First, they stimulate intense (IL-2 and gamma-IFN-associated) activation-induced apoptosis of donor-responsive recipient lymphocytes and sustain chimerism via engraftment and/or survival of donor hematopoietic stem cells contained within the liver.[409-411] The possibility of activation-induced death of donor-reactive lymphocytes has been recognized for quite some time.[72,412] Recent studies show that it occurs both within the allograft and in recipient lymphoid tissues.[413-422] Irradiation of the donor liver[416] or a short course of methylprednisolone breaks spontaneous acceptance of the liver allograft and reduces survival of subsequent donor strain skin grafts.[416] Similar observations have been made in the miniature swine model, in which corticosteroid therapy was able to block tolerance induction (see section on Nonchimeric Tolerance).

Unfortunately, in humans, activation-induced apoptosis or deletion is apparently not able to induce tolerance in the majority of liver allograft or other solid organ allograft recipients. Even if partial clonal deletion and graft adaptation enable reduced immunosuppression during the first several months after transplantation, the recipient immune system still recognizes the allograft as foreign in most long-term survivors; consequently, the majority still require life-long immunosuppression to prevent CR.[362]

The second function of the donor hematolymphoid cells is to sustain the presence of multilineage donor hematolymphoid cells. Several groups have shown that the continued presence of donor hematolymphoid cells in the recipient[27,408,423-428] is associated with improved long-term allograft survival, although the existence of a cause-and-effect relationship is controversial.[429-433]

Treatment and Prevention of Chronic Rejection

Treatment of established or "end-stage" CR is generally considered to be futile, because the major structural manifestations are irreversible. Therefore, attempts are made to prevent the disease entirely, or to identify patients in the early stages of CR before irreversible damage has occurred. Since CR evolves directly from damage during AR in many cases, the first point of interdiction is controlling the severity and duration of AR episodes. This is usually accomplished by adjusting baseline immunosuppression and augmentation with additional corticosteroids and monoclonal antibodies, if needed. The next point of potential therapeutic intervention is at the early stages of CR, when ongoing immunologic damage to the allograft is obvious, but before irreversible damage has occurred. This strategy is most effective in liver allografts because of their marked regenerative capacity. The most popular approach at this time is a switch in baseline immunosuppression, or the addition of another nonsteroidal immunosuppressive drug (Table 3). Once irreversible damage has occurred, continued attempts at treatment with increased immunosuppression may have deleterious consequences, but attempts to restore arterial blood flow past localized arterial narrowing have been made in the heart allograft with OA (see section on Manifestations of Chronic Rejection in the Heart Allograft).

Table 3. Clinical Treatment of Chronic Rejection in Various Organs

Treatment	Reference(s)	Rationale	Allograft	Results

Change of baseline immunosuppression				
Cyclosporine to Tacrolimus	(31, 330, 384, 446, 452, 457, 463, 533)	Limit damage from AR, early CR	Liver	Effectively limits damage from AR. Reverses early phase of CR.
Cyclosporine to Tacrolimus	(441, 455, 456)	Limit damage from AR, early CR	Lung	Effectively limits damage from AR. Slows the functional decline with CR, but does not reverse defects.
Cyclosporine to Tacrolimus	(446, 534, 535)	Limit damage from AR, early CR	Kidney	Effectively controls refractory acute rejection; increases grafts survival
Cyclosporine to Tacrolimus	(536)	Limit damage from AR, early CR	Heart	Effectively controls refractory acute rejection; increases grafts survival
Addition of secondary immunosuppressive agent				
Mycophenolic acid to CyA or Tacrolimus	(461), (537)	Limit damage from AR, early CR	Kidney	Either minimally effective or not effective
Azathioprine to Mycophenolate in CyA Patients	(458)	Limit damage from AR, early CR	Liver	Effectively limits damage from AR. Reverses early phase of CR
Cyclophosphamide	(538)	Limit immunologic damage in CR	Lung	Slowed or stopped decline of pulmonary function in 6/7 OB patients.
Pharmacologic Control of Repair Response or "Nonimmunologic factors"				
Pravastatin (3-hydroxy-3-methyl-glutaryl co-	(539, 540)	Limit damage from AR, early CR.	Heart	Decreases incidence of

enzyme A (HMG-CoA) reductase inhibitors (HRIs))		AR, early CR. Limits pathologic repair response (lipid lowering).		acute and severity of CR; lowers lipid levels
Pravastatin	(541)	Limit damage from AR, early CR. Limits pathologic repair response (lipid lowering)	Kidney	Decreases incidence of AR; lowers lipid levels
Diltiazem (calcium channel blockers)	(542, 543)	Block early proliferative vascular response to injury and lipid accumulation in arteries	Heart	Prevented arterial lumenal narrowing seen in untreated controls.
Losartan	(126)	Limits fibrogenic repair response	Kidney	Decreases plasma TGF-beta levels in plasma
Recombinant human superoxide dismutase	(544)	Limits AR, limits damage from free radicles	Kidney	Decreased incidence of AR; increased half-life of kidneys from 5 to 15 years
Carvedilol (antihypertensive)	(545)	Limit damage from "nonimmunologic" co-factors	Kidney	Lowered lipid levels and controlled blood pressure, but no beneficial effect on CAN
L-arginine (precursor of nitric oxide)	(546)	Limits arterial wall response to injury by balancing vasodilatation with vasconstriction	Heart	Ameliorates endothelial dysfunction commply seen in cardiac allografts
Angiopeptin (somatostatin analogue)	(547)	Limits arterial repair response by inhibiting smooth muscle cell proliferation	Heart	Safe; marginal effect on incidence of AR; long-term follow-up needed to assess impact on CR.
Angiotensin enzyme converting agents	(548, 549)	Limits repair response; antihypertensive	Kidney	Beneficial effect on CAN, stabilizes glomerular size. Selectivitv in

				proteinuric kidney allograft recipients

Prevention of CR is being attempted with the induction of tolerance or by pharmacologic intervention that either lessens the immunologic damage or limits the repair response. Currently, experimental tolerance-inducing clinical trials are being attempted at several sites,[434-437] but for the vast majority of nonhepatic vascularized allograft recipients, attempts to prevent CR are limited to controlling AR and the early stages of CR by baseline immunosuppression and drugs that limit repair responses.

Limiting Graft Damage From Acute Rejection

As discussed above, implantation of any solid organ allograft results in a characteristic cycle of heightened immune activation, brought about by the initial engagement of the donor and recipient lymphoid systems[27,72-74] and followed by evolution toward a less violent interface. This appears to coincide with a transition from the direct to indirect pathway of alloantigen presentation. In most allograft recipients this occurs within 6-12 months after transplantation and consequently, at this time, clinicians can begin to slowly lower the immunosuppression on a trial-and-error basis with close monitoring of organ function. Usually, an attempt is made to lower and then discontinue corticosteroid therapy, followed by further reduction in baseline immunosuppressive agent(s). By 5 years or more after transplantation, immunosuppression can be completely withdrawn in about 15%-20% of liver allograft recipients.[438,439] Unfortunately, in other types of allograft recipients, immunosuppression cannot be lowered and AR evolves directly into CR. It is uncertain why some allograft recipients evolve toward CR while others maintain a more stable relationship with the allograft.

Many clinical studies show that a reduction in the incidence and severity of AR is associated with a lower frequency of CR.[31,45,64,307,324,338,339,369,440-445] This is usually achieved when a more potent baseline immunosuppressive drug such as tacrolimusis used, when 2 or more drugs are used for baseline immunosuppression, and/or when induction therapy with antilymphocytic monoclonal antibodies is used.[450] Tacrolimus has been one of the most effective drugs in achieving this goal in all organs.[31,329,330,354,444-449] The effectiveness of limiting the number and severity of AR episodes supports the contention that prevention of tissue injury during AR limits the subsequent repair responses that lead to CR.[111]

Interestingly, the clinical approach to reducing CR (eg, reducing AR by more powerful immunosuppressive agents and closer monitoring),[325,451] is conceptually at odds with recent studies showing that activation-induced clonal deletion via apoptosis may be required for tolerance induction.[418-420] The activation-induced clonal deletion or stripping could be thought of as a donor-specific biologic equivalent of the nonspecific T cell-depleting antibodies used in many tolerance-inducing protocols (see section on Induction of Allograft Tolerance). It has even been suggested that the mainstay immunosuppressive agents, cyclosporine and tacrolimus, both of which are calcineurin inhibitors, actually prevent tolerance induction by blocking this process.[418-420] Regardless, the incongruity of current clinical and experimental approaches used to lessen the impact of CR on long-term survival needs to be aired and addressed.

Pharmacologic Treatment of the Early Stages of CR

Refractory or severe AR has become less common in some allografts (ie, liver and heart allografts) because of a larger arsenal of more effective immunosuppressive agents, but it still occurs, and when it does, many of these patients are at high risk for the development of CR. The transition from severe and/or persistent AR to CR is marked by development of structural and/or functional decline that presages irreversible damage, which has been referred to as early CR.

The most popular therapeutic approach in patients with early CR is to increase or change baseline immunosuppression.[339,441,452-458] The goal is to limit or stop the ongoing immunologic injury and allow the graft to recover before completely irreversible damage has occurred. An additional advantage can be realized if the new immunosuppressive drug does not contribute to the development of allograft fibrosis.[459,460] Generally, patients with early CR are converted from cyclosporine to tacrolimus, or baseline immunosuppression (cyclosporine or tacrolimus) is lowered and another agent, such as mycophenolate mofetil or azathioprine, is added.[339,441,452-458]

In general, most but not all[461] treatment studies of early CR carried out in kidney, lung, heart, and pancreas allograft recipients show that the rate of functional and structural decline can be slowed, but significant functional and structural improvement is uncommon.[339,441,453-458] Liver allografts, however, are the exception; the structural and functional deterioration associated with CR can be completely reversed with a change in immunosuppression.[46,452,462,463] And occasionally, structural improvement has been reported to spontaneously occur.[41,119,352] The ability to reverse CR in liver allografts has been attributed to its unique immunologic properties and to its almost limitless regenerative capacity, which enables the organ to more easily recover from injury without fibrosis.[41,46]

Induction of Allograft Tolerance

Achieving tolerance to the allograft without the need for continuous immunosuppression is the "holy grail" of transplantation immunobiology and medicine. It eliminates the threat of both CR and toxicity from long-term immunosuppression. This goal has already been practically realized for a small percentage of fortunate liver allograft recipients,[27,408] some of whom have been completely withdrawn from immunosuppression for years without adverse consequences.[27,408,439] Unfortunately, this ideal outcome does not occur in the majority of liver allograft recipients.

Immunosuppression can rarely be completely withdrawn from nonhepatic allograft recipients, although it can be significantly lowered,[438,439,464-466] but with the threat of CR. Consequently, CR could be viewed either pessimistically as an indolent rejection reaction, or optimistically as incomplete tolerance. We prefer the latter viewpoint, since tolerance may not be an all-or-none phenomenon, but rather representative of various levels of unresponsiveness.[467]

Chimeric Tolerance. Since the advent of transplantation immunobiology, tolerance achieved by hematopoietic chimerism has been widely recognized as the ideal and most robust form of tolerance (for recent comprehensive reviews see references 468-471). Owen[472] was the first to show that nonidentical twin cattle sharing a placental circulation develop chimeric immune systems, each composed of hematopoietic cells from both individuals.[472] Shortly thereafter, Medawar showed that these chimeric twins were tolerant to skin grafts from their twin siblings.[473,474]

In the fetus or in immunodeficient recipients, engraftment of allogeneic hematopoietic cells can be achieved without immunosuppression,[472,475-477] because the recipient immune system is immature or defective and unable to reject the donor cells. In mature immunocompetent individuals, some type of recipient conditioning is required, typically irradiation and/or cytoreductive chemotherapy combined with T cell-depleting antibodies and/or conventional pharmacologic immunosuppression. The intent is to provide a "clean slate"[469] for the newly developing T-cell repertoire, to prevent rejection of the donor cells by mature recipient T cells and facilitate long-term engraftment of the donor hematopoietic cells.[469] This is followed by infusion of donor bone marrow alone, or a combination infusion of donor and recipient bone marrow.[478-486]

Two types of chimerism are achieved with these protocols[469]: full chimerism, in which the entire recipient hematolymphoid system is replaced by that of the donor; and mixed chimerism, in which a functionally integrated immune system is composed of various proportions of hematolymphoid

cells from the donor and recipient. A third type called microchimerism is a recently described phenomenon, similar to mixed chimerism.[27,408,424] Microchimerism refers to trace populations of multilineage donor hematopoietic cells that persist in a fraction of stable, unconditioned, long-term vascularized allograft recipients treated only with conventional immunosuppressive regimens.[27,408,424] Although some investigators are quick to draw a clear distinction between microchimerism and mixed chimerism,[429,430,469] in our opinion, the only difference is the proportion of donor cells.

The robust tolerance associated with mixed chimerism is strictly dependent on the persistence of donor cells (chimerism).[487,488] It is primarily mediated by central deletion,[481,482,485] although peripheral mechanisms are also likely to contribute to the process,[489,490] similar to mechanisms of self tolerance.[491-494] Specifically, successful mixed chimeras show clonal deletion of self-reactive T cells[485,488,495] and consequently, lack donor responsiveness in a mixed lymphocyte reaction (MLR), accept donor-specific allograft(s) without immunosuppression, and are resistant to CR.[496,497]

Unfortunately, the mixed chimera approach to tolerance induction, which is so successful in small experimental animals, has not gained widespread clinical acceptance. The major drawback is the significant morbidity and mortality associated with the recipient conditioning[485,498] needed to ensure engraftment of the donor hematopoietic cells. The subsequent threat of graft-versus-host disease is another serious consideration, particularly when major MHC barriers are crossed, as is currently the practice in solid organ transplantation.[499,500] Efforts are being made, therefore, to modify the protocols in an effort to lessen the associated morbidity without compromising donor stem-cell engraftment. These modifications include lower doses of irradiation, the use of facilitator cells to enhance engraftment of donor stem cells,[501,502] higher doses or repeated infusions of donor bone marrow,[486,498,503] and costimulatory molecule blockade.[469]

The acid test for tolerance-inducing regimens is their efficacy in subhuman primates and clinical trials. Tolerance was induced in monkeys using a combination of total body irradiation, thymic irradiation, antithymocyte globulin, donor bone marrow transplantation, and a 4-week course of cyclosporine.[504] However, these studies showed that splenectomy may also be required in the initial conditioning regimen to induce B-cell tolerance and prevent the production of alloantibodies,[504] which are important in the development of CR.

A multiple myeloma patient with renal failure was recently treated with simultaneous kidney-bone marrow transplantation from an HLA-identical sibling.[505] The conditioning regimen consisted of cyclophosphamide, antithymocyte globulin, thymic irradiation, and, after transplantation, cyclosporine (given for 73 days). Mixed chimerism was easily detectable until 105 days after transplantation, but thereafter was either nondetectable or fell into the microchimeric range. Nevertheless, the patient was able to be weaned from all immunosuppression with normal kidney allograft function 14 weeks after discontinuance of cyclosporine.[505] The long-term outcome in this patient is yet to be determined.

Several centers are currently evaluating clinical protocols whereby vascularized organ allograft recipients are given an infusion of unfractionated donor bone marrow at the time of transplantation.[434-437] In some protocols, additional donor bone marrow infusions are given several weks later.[434] In general, no specific conditioning therapy is used and the patients are treated with conventional immunosuppressive agents. The intent is to enhance donor hematopoietic stem-cell engraftment and possibly the ability of allostimulatory cells to deplete donor-reactive mature T cells via activation-induced apoptosis. It is hoped that the bone marrow infusion will enhance engraftment of donor hematopoietic stem cells, thereby increasing the number of donor hematolymphoid cells persisting long term without harsh conditioning therapy. Although it is too early to critically evaluate the results, the treatment protocol seems relatively safe and indeed enhances the number of patients becoming chimeric and the number of donor cells present, albeit modestly (~ 1% at 2 years).[434-437,506] A trend toward decreased donor-specific

hypo- or unresponsiveness[434-437] and perhaps a lower incidence of CR[435,437] has also been observed. However, the long-term efficacy of this approach is yet to be determined.

Nonchimeric Tolerance. There are numerous protocols to achieve tolerance in small experimental animals which apparently do not depend on hematopoietic chimerism.[507] In fact, almost every new immunosuppressive agent has been treated as tolerance-inducing, based on long-term allograft survival following preconditioning or a short course of treatment. Many of these studies suggest that the "tolerogenic" signal resides in the allograft parenchyma and stroma,[402,508,509] soluble donor MHC antigens,[510,511] or donor peptides,[510-513] and not in the donor hematopoietic cells. However, in these nonchimeric models, tolerance is usually nondeletional, more difficult to define, and less robust. It is therefore less likely to withstand the rigors of environmental and infectious exposures that are likely to occur in human allograft recipients.[469] Unfortunately, many investigators have spent years mistakenly studying tolerance models that actually were models of CR.[3]

A series of recent investigations in in-bred miniature swine[514-520] showed that long-term kidney allograft acceptance (tolerance induction) can be achieved across class I MHC barriers with a short course of cyclosporine. However, if a heart is transplanted instead of the kidney, the heart allograft develops CR.[521] If a simultaneous kidney-heart allograft is conducted (composite effect), the tolerance returns and the heart allograft is resistant to CR.[521] In these studies, the induction of tolerance was dependent on at least 1 matched MHC locus between the donor and recipient, with complete class II matching appearing to be the most successful way of assuring robust long-term graft survival without spontaneous rejection episodes.[522] Tolerance was associated with a permanent change in the recipient immune system. If the accepted allograft was removed, the tolerant recipients accepted a second renal transplant MHC matched to the original donor without additional immunosuppression. This observation was not dependent on graft adaptation, because retransplantation of the accepted allograft into na?ve recipients resulted in prompt rejection in most of the animals.[520] Methylprednisone, old age, and thymectomy[515] or thymic injury[516] interfered with the induction of stable tolerance in this model.[517] A series of studies to determine whether microchimerism was associated with tolerance in this model yielded inconclusive results.[518]

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Recurrent Hepatitis C in Liver Allografts

Prospective Assessment of Diagnostic Accuracy, Identification of Pitfalls, and Observations About Pathogenesis

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Rationale and Design: The accuracy of a prospective histopathologic diagnosis of rejection and recurrent hepatitis C (HCV) was determined in 48 HCV RNA-positive liver allograft recipients enrolled in an "immunosuppression minimization protocol" between July 29, 2001 and January 24, 2003. Prospective entry of all pertinent treatment, laboratory, and histopathology results into an electronic database enabled a retrospective analysis of the accuracy of histopathologic diagnoses and the pathophysiologic relationship between recurrent HCV and rejection.

Results: Time to first onset of acute rejection (AR) (mean, 107 days; median, 83 days; range, 7–329 days) overlapped with the time to first onset of recurrent HCV (mean, 115 days; median, 123 days; range, 22–315 days), making distinction between the two difficult. AR and chronic rejection (CR) with and without co-existent HCV showed overlapping but significantly different liver injury test profiles. One major and two minor errors occurred (positive predictive values for AR = 91%; recurrent HCV = 100%); all involved an overdiagnosis of AR in the context of recurrent HCV. Retrospective analysis of the mistakes showed that major errors can be avoided altogether and the impact of unavoidable minor errors can be minimized by strict adherence to specific histopathologic criteria, close clinicopathologic correlation including examination of HCV RNA levels, and a conservative approach to the use of additional immunosuppression. In addition, histopathologic diagnoses of moderate and severe AR and CR were associated with relatively low HCV RNA levels, whereas relatively high HCV RNA levels were associated with a histopathologic diagnosis of hepatitis alone, particularly the cholestatic variant of HCV.

Conclusions: Liver allograft biopsy interpretation can rapidly and accurately distinguish between recurrent HCV and AR/CR. In addition, the histopathologic observations suggest that the immune mechanism responsible for HCV clearance overlap with those leading to significant rejection.

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Hepatitis C virus (HCV)-induced cirrhosis is the leading indication for liver transplantation throughout the world.[32] Unfortunately, reinfection is nearly universal and recurrent disease occurs in a majority of recipients. HCV replication can be detected in the RNA-positive recipients within days after transplantation.[27] Allograft dysfunction can occur as early as 1 week following transplantation in patients with high viral loads before transplantation,[6] but the majority of HCV-positive recipients usually first show signs of recurrent disease between 2 and 3 months.[11,31]

Distinguishing among recurrent viral hepatitis and AR and CR and other causes of allograft dysfunction is based primarily on liver biopsy evaluation. Guidelines to recognize individual syndromes were proposed more than a decade ago,[13,16] but experience has shown the distinction to be problematic.[1,19,25]

Steatosis is an early nonspecific finding in recurrent HCV[5]; spotty hepatocyte necrosis, lobular disarray, and Kupffer's cell hypertrophy are more reliable features that specifically point toward recurrent HCV as the cause of allograft injury.[31,33] Later in the course of recurrent HCV, predominantly mononuclear portal inflammation with variable interface activity and low-grade bile duct damage signal the transition from acute to chronic hepatitis. Chronic HCV is characterized by mononuclear portal inflammation that is frequently arranged into nodular aggregates and mild bile duct damage that is neither as severe nor as widespread as is seen in acute rejection (AR) or chronic rejection (CR).[31] Interface activity, including a type II ductular reaction, is also more common in hepatitis than in rejection. Retrospective analysis of case material to determine whether specific histopathologic criteria are useful in distinguishing among recurrent HCV and other causes of allograft dysfunction is fraught with pitfalls.

The Electronic Data Interface for Transplantation (EDIT)[15] software simultaneously creates an information portal for patient management and populates a research database. Real-time entry of all pertinent treatment, laboratory, and histopathology data into this software has enabled us to accomplish our primary goal. This objective was to prospectively and rigorously test our ability to distinguish among recurrent HCV, rejection, and other various causes of liver allograft dysfunction in HCV RNA-positive recipients enrolled in a recent "immunosuppression minimization protocol."[36] A secondary goal was to retrospectively analyze errors and determine how they might be avoided.

The treatment protocol used in this patient population is based on the concept that alloantigen migration to the central lymphoid tissues of the recipient early after transplantation is an important event that simultaneously triggers rejection and tolerogenic immune responses.[35–37] Pretransplant immunodepletion with anti-leukocyte antibodies is used to rein in the expected early alloresponse into a manageable range. This is combined with minimal posttransplantation monotherapy immunosuppression in an attempt to facilitate activation-induced apoptosis of donor-reactive lymphocytes.[36] A final goal of this study was to determine whether histopathologic observations in this unique patient population shed any insight into our conceptual understanding of HCV pathogenesis in the allografted liver.

MATERIALS AND METHODS

Patient Population and Pathology Workflow

All primary liver allograft recipients with documented HCV infection (RNA positive by PCR) enrolled in the protocol between July 29, 2001 and January 24, 2003 were initially included in this study (n = 53). Five patients either died within 1 week of transplantation or did not undergo any posttransplantation biopsies and were removed from the study. This left a total of 48 patients for analyses. The remaining patients were followed until March 31, 2003. Rationale for the treatment protocol is reported by Starzl et al.[36]

Briefly, all liver allograft recipients were treated immediately before transplantation with either broadly reactive rabbit anti-thymocyte globulin (Thymoglobulin; Sangstat, Menlo Park, CA; n = 25) or Alemtuzumab (Campath 1H; n = 22) and simultaneously with 1 to 2 g methylprednisolone to concomitantly prevent cytokine reactions.[36] One patient inadvertently missed pretreatment. After transplantation patients were treated with tacrolimus monotherapy with the goal of achieving target trough levels of 10 ng/mL. Any additional immunosuppression, such as steroids or other agents, was added only temporarily to control biopsy-proven rejection. Beginning at 4 months after transplantation, patients that had been on tacrolimus monotherapy for the preceding 60 days were considered for weaning.[36] After obtaining a protocol biopsy that was rejection-free, twice daily tacrolimus doses were consolidated to once daily doses for a few weeks. In the continued absence of rejection, baseline immunosuppressive therapy was further weaned by spacing doses to every other day and subsequently to longer intervals.[36] During immunosuppression weaning, unacceptable elevations of liver injury tests were investigated by liver allograft biopsies and other tests when appropriate. Mostly all biopsies were processed on a "STAT" basis with interpretation occurring on the same day the biopsy was obtained.

Experienced transplant pathologists (A.J.D., M.A.N., P.R., or T.W.) initially reviewed all liver allograft biopsies. Each biopsy was evaluated according to a protocol[15] that listed 31 histologic findings for scoring (http://tpis.upmc.edu/tpis/schema/AlloLiver.html). Consultation among the pathologists in difficult cases was routine at the time of signout. All biopsies were reviewed a second time immediately before a weekly clinicopathologic conference, when the free text diagnosis assigned by the primary pathologist was converted into "coded" diagnosis(es) by a single pathologist (A.J.D.) who re-reviewed the slides.[15] The diagnoses were also ranked in perceived order of importance with the most important listed first and discussed during the conference. Since all difficult cases were also discussed among the pathologists at the time of signout, there was only one instance of a significant disagreement between the signout diagnosis and coded diagnosis, which did not impact the results of this study.

Histopathologic Criteria for the Distinction Between Recurrent HCV and AR and CR and Assessment of Follow-up

The criteria used to distinguish between AR and recurrent HCV were based on those originally developed for HBV.[13,16] Specifically, mild AR was diagnosed when either of the following conditions was met: 1) portal inflammation with inflammatory bile duct damage involving $\geq$50% of the bile ducts; or 2) mononuclear perivenular inflammation involving $\geq$50% of the terminal hepatic venules, associated with hepatocyte necrosis and/or dropout. These criteria for mild AR require more extensive tissue injury than listed for the Banff criteria[3] in allografts not otherwise affected by a coexistent disease. Slightly more extensive tissue injury than usually seen in allografts without coexistent disease was also required for a diagnosis of moderate and severe AR, but the Banff criteria did not have to be adjusted.

In general, a biopsy was considered adequate when it contained six or more portal tracts and four or more terminal hepatic venules. Early and late CR was diagnosed using the Banff criteria.[12] Recurrent HCV was diagnosed when lobular or interface necro-inflammatory activity was more prevalent and prominent than bile duct inflammation and damage.

Patient outcome was used to gauge the accuracy of the prospectively entered histopathologic diagnoses. AR treat-

ment consisted of bolus steroid therapy. If unsuccessful, daily tacrolimus therapy was re-instituted and other agents were added. For the purpose of this study, the diagnosis of AR was considered correct if peak liver injury test abnormalities at the time of diagnosis showed a sustained improvement of at least 50% during the first week after additional immunosuppressive therapy. Liver injury tests eventually normalized with increased immunosuppression in all of the patients with AR alone.

Return to daily tacrolimus therapy until whole blood levels registered at least 10 ng/mL was used to treat CR; this was supplemented in some cases by simultaneous administration of other agents. A diagnosis of early CR was considered correct if there was a sustained decrease of 50% or more in total serum bilirubin therapy during the 2 months following treatment. Liver injury test eventually returned to normal or near-normal levels in all of the patients with early CR alone.

Recurrent hepatitis C was treated either by no change in immunosuppression therapy or weaning of immunosuppression in patients more than 4 months after transplantation. This was supplemented by interferon-α (INTRON A or PEG-INTRON; Schering, Kenilworth, NJ) and/or ribavirin (REBETROL; Schering) in patients that agreed to treatment and were able to tolerate the side effects. A diagnosis of recurrent HCV was considered correct if there was either no worsening of liver injury tests for at least 2 weeks following the decision not to augment immunosuppression and/or introduce anti-viral therapy. However, most patients specifically treated for HCV with decreased immunosuppression and anti-viral therapy showed noticeable improvement.

EDIT

The EDIT software used to collect data for this study was designed and developed specifically for the Thomas E. Starzl Transplantation Institute at UPMC and described earlier in greater detail.[15]

Data Handling and Statistical Analysis

Pertinent data from EDIT were first rendered anonymous by stripping it of unique patient identifiers, according to the exempt institutional review board-approved protocol (IRB#020177). The cohort was described using estimates of central tendency (means, medians) and spread (standard deviation, range) for continuous data and frequencies and percentages for categorical data. Groups were compared using the χ^2 test for differences in proportions (categorical data) and the Wilcoxon Rank Sum test (continuous data). To identify potential predictors of AR and CR, Cox regression models were constructed. Time-dependent covariates were used when appropriate. For comparison of liver injury tests, only those laboratory values that were obtained within −2 to 0 days prior to biopsy were eligible. However, the time range for eligible laboratory results was increased from −14 to 0 days for biopsies showing CR because of the slower changes in liver injury tests associated with this diagnosis. All analyses were performed using Statistical Analysis System (version 8.2).

RESULTS

Patient Characteristics and Graft and Patient Survival

Donor and recipient age, sex, race, and viral genotype (if available) are shown in Table 1. Coexistent diseases complicating HCV-induced cirrhosis are shown in Table 2. The mean and median follow-up for this group of patients was 292 and

TABLE 1. Donor and Recipient Characteristics, Follow-up Period, and HCV Genotypes in Study Population

	Study Group (N = 48)	
Variable	**N**	**[Column %]**
Gender		
Male	35	72.9%
Female	13	27.1%
Donor gender		
Male	29	60.4%
Female	19	39.6%
Race		
White	45	93.8%
Black	0	0.0%
Other	3	6.3%
Donor race		
White	42	87.5%
Black	5	10.4%
Other	1	2.1%
Age (yr)		
Mean (SD)		52.3 (8.3)
Median		50.8
Range		36.1–70.6
Donor age (yr)		
Mean (SD)		47.6 (14.6)
Median		50.7
Range		13.7–78.4
Follow-up (days)		
Mean (SD)		292.0 (172.7)
Median		240.0
Range		10–650
Genotype		
1a	16	33.3%
1b	8	16.7%
3a	2	4.2%
Other	7	14.6%
Missing	15	31.3%

TABLE 2. Coexistent Diseases in Patients Who Underwent Liver Transplantation Primarily for HCV-Induced Cirrhosis

Coexistent Disease 1	Coexistent Disease 2	No. of Recipients
None		28
Hepatocellular carcinoma		11
Alcoholic liver disease		5
Alcoholic liver disease	Hepatocellular carcinoma	1
Chronic HBV		1
Metabolic disease		2
Total		48

240 days, respectively, with a range of 10 to 650 days. Patient survivals at 1, 3, and 6 months and 1 year after transplantation were 98%, 94%, 85%, and 80%, respectively; graft survivals for the same intervals were 94%, 90%, 83%, and 78%. There were eight deaths and nine graft failures. The causes of death included liver allograft failure from primary dysfunction (n = 3) complicated by myocardial infarction (n = 1), cerebral anoxia (n = 1), or multiorgan failure (n = 1). Three patients died with functioning allografts from an intracranial bleed (n = 1), sepsis (n = 1), and a motor vehicle accident (n = 1). Two other patients died because of liver allograft failure secondary to recurrent cholestatic HCV (n = 1) and a combination of hepatic artery thrombosis and recurrent HCV (n = 1). The causes of graft failure included patient death (with functioning graft; n = 3), primary dysfunction without (n = 1) or with patient death (n = 3), and liver allograft failure from cholestatic hepatitis (n = 1) or a combination of hepatic artery thrombosis and recurrent HCV (n = 1). None of the allografts failed primarily from either AR or CR.

Biopsy Timing and Diagnoses

There were a total of 179 biopsies included in this study. The timing of the biopsies and the number of biopsies per patient are shown in Table 3. In total, grade mild AR or greater was diagnosed on 45 of 179 (25%) biopsies from 23 of 48 (48%) patients (Table 4). The mean and median time to first onset of AR was 107 and 83 days, respectively, with a range of 7 to 329 days. Early CR was diagnosed on 17 of 179 (9.5%) biopsies from 6 of 48 (12.5%) patients. The mean and median times until the first onset of early CR were 302 and 300 days, respectively, with a range of 170 to 413 days. None of the patients developed late CR.[8,12] Acute and/or chronic hepatitis was diagnosed on 86 of 179 (48%) biopsies from 31 of 48 (65%) patients. The mean and median times until the first onset of recurrent HCV were 115 and 123 days, respectively, with a range of 22 to 315 days.

TABLE 3. Number and Timing of Liver Allograft Biopsies Obtained to Determine the Cause of Allograft Dysfunction

	No.	%
Biopsies/person		
1	11	22.9
2	8	16.7
3	10	20.8
4	6	12.5
5–12	13	27.1
Biopsies/time period		
0–7 days	12	6.7
8–30 days	40	22.3
31–60 days	16	8.9
61–90 days	11	6.1
90–180 days	50	27.9
181–365 days	37	20.7
>365 days	13	7.3

Correlation of Histopathologic Diagnoses With Liver Injury Test Profile

Correlation of the histopathologic diagnosis with the liver injury test profile is shown in Table 5. Patients with a primary diagnosis of AR alone showed significantly lower serum aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGTP) levels than patients with a primary diagnosis of AR and a secondary diagnosis of recurrent HCV. This is likely attributable to the more restrictive criteria used for a primary diagnosis of AR in the context of recurrent HCV. Conversely, however, there was no significant difference in the liver injury test profile in patients with a primary diagnosis of recurrent HCV alone versus those with a primary diagnosis of recurrent HCV and a secondary diagnosis of AR (Table 5).

TABLE 4. Timing of First Onset of AR and CR and Recurrent HCV in the Study Population

Time to first AR (days)	
Mean (SD)	106.6 (109.8)
Median	83.0
Range	7–329
Time to first CR (days)	
Mean (SD)	302.3 (80.8)
Median	300
Range	170–413
Time to recurrent HCV (days)	
Mean (SD)	114.6 (58.5)
Median	123.0
Range	22–315

AR, acute rejection; CR, chronic rejection; SD, standard deviation.

TABLE 5. Correlation of Liver Injury Test With Histopathologic Diagnoses

Primary Diagnosis	Other Diagnoses		AST	GGTP	TB
AR (n = 11)	HCV −	Mean	222.1	232.5	7.0
		SD	203.0	137.9	6.0
		Median	110.0	181.5	4.7
AR (n = 19)	HCV +	Mean	497.7	738.1	5.0
		SD	464.2	520.0	4.3
		Median	270.0	589.0	5.0
		P value	0.049	0.001	0.39
HCV (n = 42)	AR −	Mean	184.1	519.4	4.5
		SD	143.5	578.6	5.4
		Median	163.0	395.0	2.0
HCV (n = 7)	AR +	Mean	178.2	632.0	2.6
		SD	116.8	374.2	2.4
		Median	149.0	774.5	1.0
		P value	0.9	0.4	0.4
HCV (n = 39)	AR−/CR−	Mean	186.2	416.7	4.2
		SD	145.7	453.1	5.3
		Median	154.5	287.0	1.7
CR (any position) (n = 17)	HCV +/−	Mean	335.4	558.4	11.2
		SD	324.5	253.6	5.0
		Median	154.0	513.5	12.8
		P value	0.4	0.04	0.0004

AR, acute rejection; CR, chronic rejection; HCV, hepatitis C virus hepatitis; TB, total bilirubin; +, present; −, absent.

A diagnosis of CR, regardless of ranking, was associated with significantly higher GGTP and total bilirubin levels compared with patients with HCV alone. This difference is attributable to the more diffuse bile duct damage and senescence seen in CR,[26] which is not part of the histopathologic spectrum of HCV alone.

Examples of Correct Identification and Treatment of Rejection and Recurrent Hepatitis

Two predominant histopathologic patterns comprised an "AR profile" in the context of recurrent HCV: 1) portal inflammation with bile duct damage involving a majority of the portal tracts; and/or 2) perivenular mononuclear inflammation involving a majority of central veins. The latter finding was associated with perivenular hepatocyte necrosis and dropout with or without portal inflammation. Early CR was recognized by senescence changes involving a majority of the bile ducts.[26] A representative example of each AR profile and one for recurrent HCV are described below.

The clinical course of the first patient, a 54-year-old man, is represented graphically in Figure 1. A protocol preweaning biopsy obtained 109 days after transplantation showed recurrent HCV alone, characterized by mild portal inflammation that was focally arranged into nodular aggregates, mild focal interface activity, and mild steatosis. No bile duct damage or perivenular inflammation was seen and liver injury tests were minimally abnormal, so weaning from immunosuppression began (Fig. 2A). Fifty days later (postoperative day 206), elevated liver injury tests prompted a repeat biopsy that showed moderate to severe AR (Figs. 2B, C), characterized by moderate portal inflammation with prominent bile duct damage involving virtually all of the bile ducts. The patient was initially treated with a pulse of corticosteroids. A follow-up biopsy obtained 11 days later (day 217) showed partial resolution of the portal inflammation, but senescence changes appeared in a majority of the bile ducts signaling the onset of

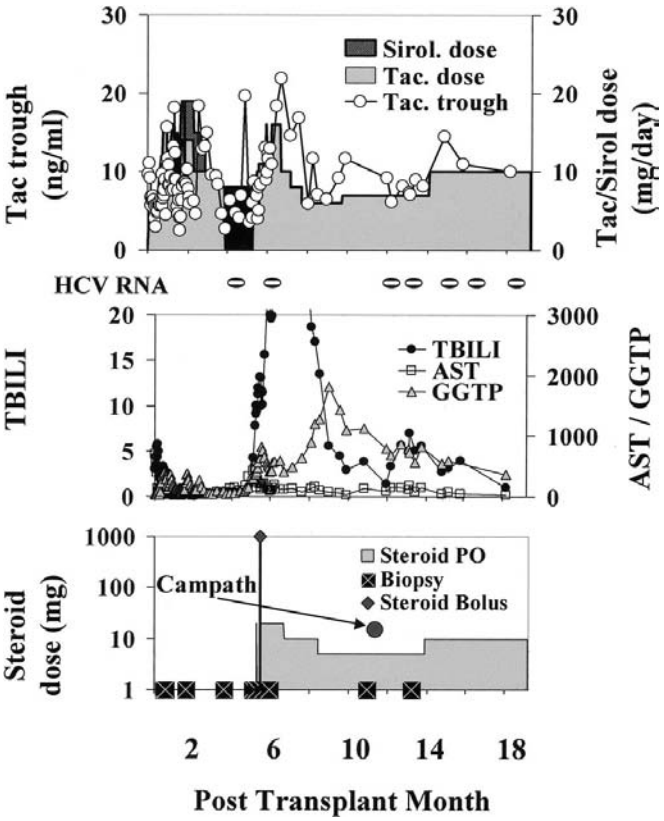

FIGURE 1. Dose and blood levels of baseline immunosuppression (top panel), HCV RNA levels and anti-viral therapy (second panel from top), liver injury test (third panel from top), and timing of biopsies and augmentation of immunosuppression (bottom panel) in a patient correctly diagnosed as developing AR after weaning of immunosuppression. Note the dramatic increase in total bilirubin (Tbili), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGTP) after weaning of immunosuppression. A correct histopathologic diagnosis (see Fig. 2) of rejection prompted return to daily tacrolimus therapy and treatment with corticosteroids, which eventually resulted in resolution of liver injury tests abnormalities. Despite the low levels of HCV RNA, the PCR for HCV was positive.

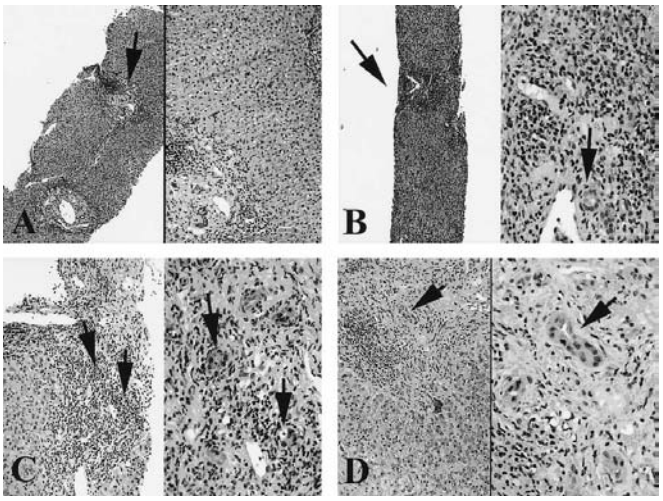

FIGURE 2. Appearance of liver allograft biopsies from the patient whose clinical course is shown in Figure 1. A: A protocol preweaning liver biopsy obtained on day 109 showed mild chronic portal and spotty hepatocyte necrosis but without bile duct damage or portal or central venulitis. These findings prompted a diagnosis of recurrent HCV alone. B and C: Another biopsy was obtained after weaning of immunosuppression on day 206 because of markedly increased liver injury test (see Fig. 1). Note the prominent mononuclear portal inflammation and prominent bile duct damage (arrows), which involved the majority of the ducts. Attention is drawn to the bile ducts (C; arrows), which are shown at higher magnification on the right side of (C). D: A follow-up biopsy obtained 11 days later (day 217) after treatment with increased immunosuppression showed partial resolution of the portal inflammation, but a majority of the bile duct showed senescence-related changes, prompting a diagnosis of early CR (arrows). An example of an affected bile duct (arrow) is shown at higher magnification in the right panel of D. A return to daily tacrolimus and maintenance corticosteroids eventually resulted in near normalization of the liver injury tests.

early CR (Fig. 2D). Re-institution of daily tacrolimus and maintenance corticosteroid therapy eventually lowered the markedly elevated liver injury test (total serum bilirubin peaked >50 mg/dL) to near-normal levels without a concomitant increase in HCV RNA levels.

The clinical course of the second patient, a 52-year-old woman, is represented graphically in Figure 3. A preweaning biopsy obtained about 3.5 months after transplantation showed low-grade perivenular inflammation, which was not treated with increased immunosuppression because of normal liver injury tests. There was minimal histopathologic evidence of recurrent HCV at this time. Approximately 3.5 months after the start of weaning, a sharp rise of AST and GGTP prompted the liver biopsy shown in Figure 4. Mild portal inflammation with minimal interface activity and mild focal bile duct damage combined with prominent perivenular inflammation and hepatocyte dropout resulted in focal central-to-central bridging necrosis. A primary histopathologic diagnosis of mild acute cellular rejection prompted treatment with 2 pulses of corticosteroids, followed by a single injection of Alemtuzumab. This led to a prompt return of liver injury tests to normal levels, without a significant rise in the HCV RNA levels.

The clinical course of a 45-year-old man successfully recognized and treated as suffering from recurrent HCV alone after withdrawal from immunosuppression is shown in Figure 5. This patient first had evidence of recurrent HCV in a biopsy obtained on day 150 after transplantation, manifest as primarily as spotty acidophilic necrosis of hepatocytes with minimal portal inflammation. This was followed by biopsies on days 232, 296, and 388, all of which showed changes characteristic of recurrent chronic HCV including variable mononuclear portal inflammation, interface activity, lobular disarray, and spotty hepatocyte necrosis (Fig. 6). No significant bile duct

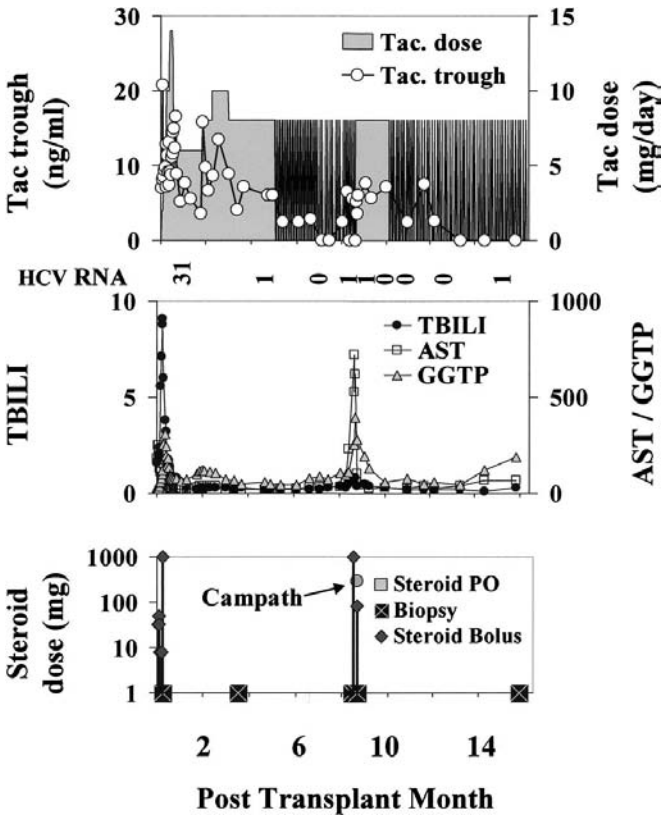

FIGURE 3. Clinical course of a patient who developed AR after being weaned from immunosuppression. Approximately 5 months after transplantation (top panel) and several weeks after a protocol biopsy that showed minimal focal perivenular inflammation, the immunosuppression was lowered (top panel). Approximately 3 months after spaced dosing of tacrolimus and low whole blood levels of tacrolimus <5 ng/mL (top panel), the patient developed elevations of the AST and GGTP to levels >600 IU/L (third panel from top). This prompted a repeat liver biopsy shown in Figure 4. Note the low levels of HCV RNA during the course.

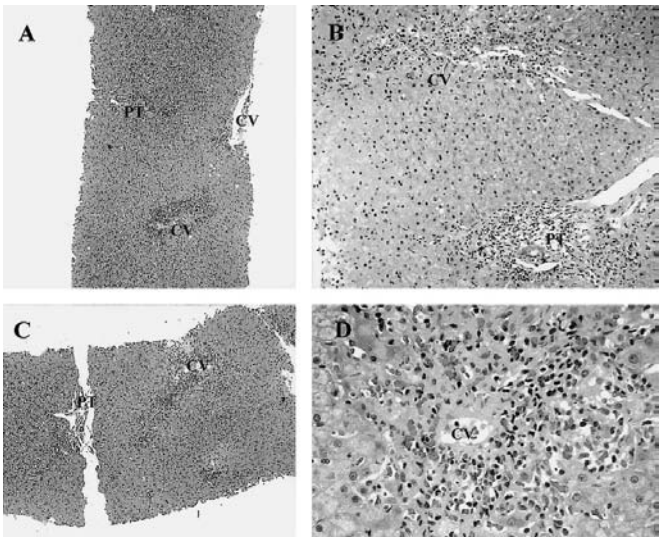

FIGURE 4. Liver biopsy from the patient whose clinical course is shown in Figure 3. It was obtained 8.5 months after transplantation and 3 months after weaning of immunosuppression. The most striking changes were perivenular inflammation, centrilobular hepatocyte dropout, and early perivenular fibrosis, which prompted a diagnosis of mild AR. A: Note that the inflammation is concentrated around the central veins (CV). B and C: Note the mild portal tract (PT) inflammation with focal mild bile duct damage but prominent perivenular inflammation. D: Higher magnification of an involved central vein showing the prominent perivenular inflammation consisting of lymphocytes and plasma cells. There is also red blood cell congestion, hepatocyte dropout, and early perivenular fibrosis.

damage or perivenular inflammation was seen in any of the biopsies. While being maintained on low levels of baseline immunosuppression (Fig. 5), the patient experienced fluctuating liver injury tests and HCV RNA levels over 6.5 months until treatment with a combination of interferon and ribavirin caused a dramatic lowering of the liver injury tests.

Identification and Explanation of Errors

We identified one major error and two minor errors in the pathology diagnoses. The major error occurred in a 36-year-old man who was subjected to an initial biopsy on day 39 because of elevated AST levels. The biopsy showed changes consistent with recurrent HCV alone, manifest as very mild chronic portal inflammation and spotty acidophilic necrosis of hepatocytes without bile duct damage or venulitis. Weaning of immunosuppression resulted in an increase in liver injury tests (Fig. 7), which in turn, prompted a repeat biopsy on day 82. It showed markedly increased portal inflammation. Bile duct damage was present but involved a minority of the portal tracts. There was also prominent lobular disarray, a type II ductular reaction, and hepatocyte necrosis. A mistaken primary diagnosis of mild AR with a secondary diagnosis of recurrent HCV (Fig. 8) prompted re-institution of daily tacrolimus therapy and a short cycle of steroids.

Treatment with more immunosuppression caused an immediate worsening of liver injury tests. Another follow-up biopsy obtained almost a week later (day 88) showed noticeably less portal inflammation but centrilobular hepatocyte swelling and hepatocanalicular cholestasis appeared, clear indicators of the development of cholestatic hepatitis (Fig. 9). Recognition of the mistake in this follow-up biopsy prompted a lowering of immunosuppression; the patient was also started on pegylated interferon and ribavirin. The anti-viral therapy resulted in dramatic lowering of the liver injury tests and lower viral loads (Fig. 7). An additional follow-up biopsy obtained on day 100 showed changes of recurrent HCV alone.

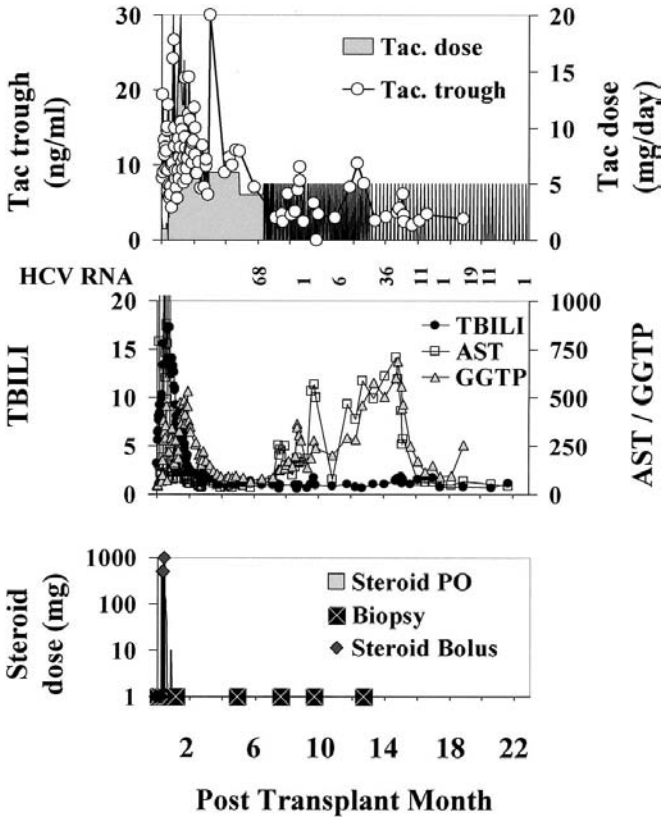

FIGURE 5. Clinical course of a patient correctly recognized and treated as recurrent HCV alone. Note that weaning from immunosuppression began approximately 4 months after transplantation (top panel) after a protocol biopsy (bottom panel) showed changes of recurrent HCV alone. Marked fluctuation of liver injury tests after weaning from immunosuppression prompted several liver allograft biopsies, all of which showed changes of recurrent HCV alone (see Fig. 6). Consequently, the patient was treated with a combination of interferon and ribavirin (panel second from top), which eventually resulted in a normalization of liver injury test (middle panel).

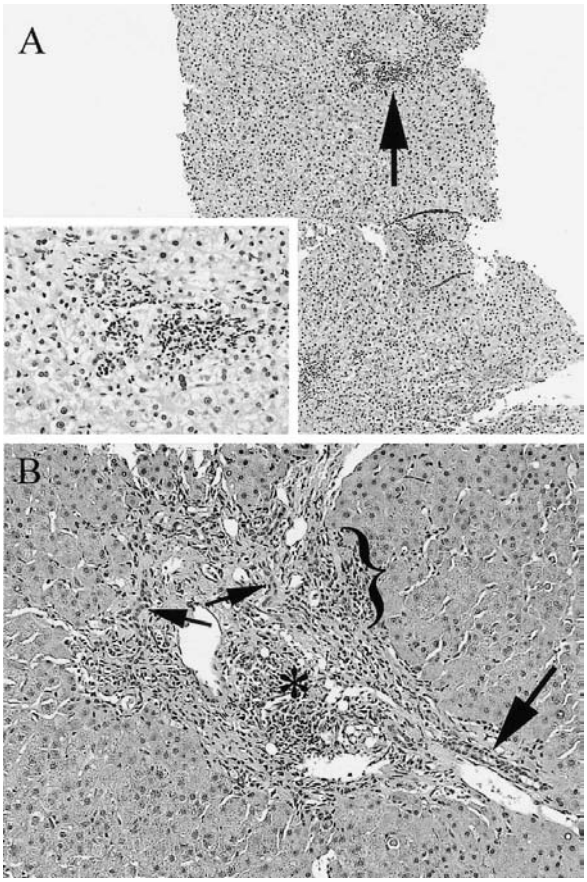

FIGURE 6. Liver allograft biopsies obtained from the patient whose clinical course is shown in Figure 5. A: The protocol preweaning biopsy obtained approximately 4 months after transplantation showed mild mononuclear portal inflammation, mild interface activity, and spotty acidophilic necrosis of hepatocytes. There was no evidence of bile duct damage or perivenular inflammation. The inset in the lower left corner shows the portal tract marked by the arrow at higher magnification. B: A repeat biopsy obtained about 6 months after weaning, during the peak of liver injury test abnormalities also showed changes of recurrent HCV alone. Note the mild mononuclear portal inflammation arranged into a small nodular aggregates (*), intact bile ducts (arrows), and type II ductular reaction at the interface zone (brace). None of the biopsies from the patient showed any changes of AR or CR.

A minor diagnostic error occurred approximately 10 months after transplantation and 6 months after weaning of immunosuppression. A secondary diagnosis of mild AR resulted in treatment with a single bolus of steroids, which in turn resulted in a slight worsening of liver injury tests. Follow-up biopsies 1, 4, and 6 months later showed predominantly or only recurrent HCV. Rejection activity, if present at all in any of these biopsies, was of indeterminate severity.

The final minor error occurred during the interpretation of a protocol biopsy obtained 4 months after transplantation, just before beginning weaning of immunosuppression. A secondary diagnosis of mild AR was ignored by the clinicians and weaning proceeded without any worsening of the near-normal liver injury tests.

A total of 105 of the 179 (59%) biopsies were obtained more than 30 days after transplantation and therefore were considered "at risk" for confusing AR with recurrent HCV. All errors were similar: AR was overdiagnosed in the context of recurrent HCV. The positive predictive values of rejection and hepatitis diagnoses were 91% and 100%, respectively.

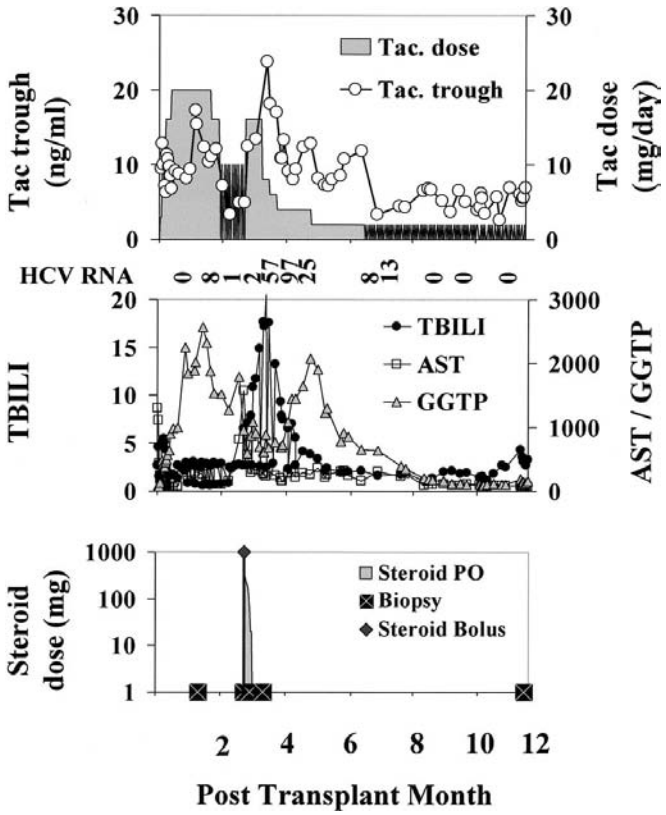

FIGURE 7. Clinical course of the patient whose liver allograft biopsy was misinterpreted as primarily AR, when in retrospect, the changes represented aggressive recurrent HCV. Weaning from immunosuppression (top panel) began several months after transplantation because a preweaning biopsy obtained on day 39 showed changes of recurrent HCV alone. By day 82, the liver injury tests as well as total serum bilirubin increased dramatically (third panel from top). This prompted a repeat liver biopsy, shown in Figure 8, which was misinterpreted as showing primarily AR with a secondary diagnosis of recurrent HCV. Return to daily tacrolimus therapy (top panel) and treatment with a pulse of corticosteroids (bottom panel) resulted in a further worsening of liver injury tests (third panel from top). A repeat biopsy 6 days later (Fig. 9; day 88) showed features of cholestatic hepatitis, a diagnosis that led to a decrease in immunosuppression and treatment with interferon and ribavirin. Eventually the anti-viral therapy led to a marked improvement in liver injury tests and a dramatic fall in HCV RNA levels (second panel from top).

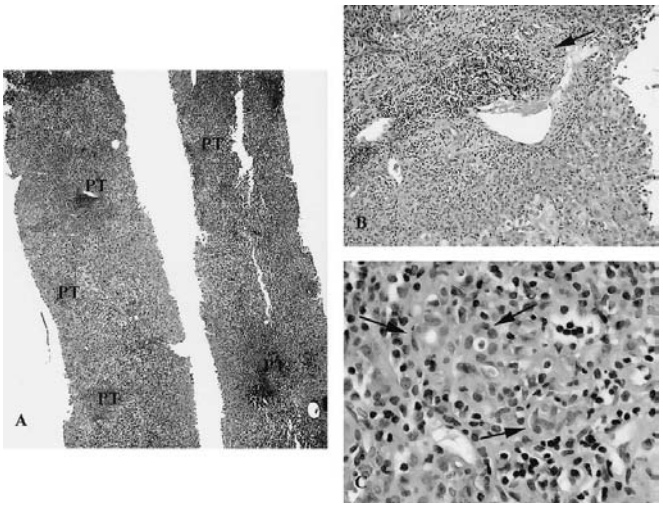

FIGURE 8. Liver allograft biopsy misinterpreted as showing primarily AR with a secondary diagnosis of recurrent HCV. The clinical course of this patient is shown in Figure 7. A: Note the prominent portal tract (PT) inflammation. B: In this portal tract, there is mild to moderate mononuclear portal inflammation and a ductular reaction at the interface zone, but minimal inflammatory bile duct damage (arrow). C: In contrast, other portal tracts from the same biopsy showed easily identifiable lymphocytic infiltration and damage of the small bile ducts (arrows). The prevalence of inflammatory bile duct damage was greater than is usually seen with HCV alone, but in retrospect, did not involve a majority of the bile ducts.

Retrospective Analysis of Errors and How They Might Be Avoided

In our opinion, the most serious error occurred because of anxiety over the uncertainty about the impact of conflicting influences of the treatment protocol on the biologic and histopathologic manifestations of recurrent HCV and rejection. It was reasoned that depleting antibody pretreatment might raise viral levels[11] early after transplantation. Subsequent weaning of immunosuppression had the potential to "re-arm" the immune system that could cause either severe hepatitis or severe rejection.[13,16] In retrospect, the major mistake would have probably not occurred if we had strictly adhered to our original histopathologic criteria and ignored the low blood levels of immunosuppression. In addition, retrospective analysis of the clinical course of this patient showed that HCV RNA levels were >50 million IU/mL at the time of the misinterpreted biopsy. Thereafter, an attempt was made to include routine monitoring of HCV RNA levels in patient management, but values were routinely not available until recently during preparation of this manuscript.

Correlation of Histopathology Diagnosis With HCV RNA Levels

Serial plasma HCV RNA levels according to the histopathologic diagnosis were plotted versus time after transplantation (Fig. 10). In general, HCV RNA levels were greatest during the first 6 months after transplantation, although the results varied among patients and values fluctuated significantly over time in individual patients.

Quantitative HCV RNA levels near the time of biopsy were available for 10 of 14 patients with histopathologic diagnoses of moderate or severe AR or early CR (Fig. 10). All of the episodes in these 10 recipients occurred more than 100 days after transplantation, and all but one of these patients, who had titers of 11.8 million IU/mL, showed HCV RNA levels of <10 million IU/mL at the time of the rejection diagnosis. HCV RNA levels were not available near the time of the biopsies in the 4 remaining patients, all of whom experienced moderate or severe AR or early CR less than 30 days after transplantation. The two patients who developed cholestatic HCV showed HCV RNA levels of >50 million IU/mL at the time of diagnosis. There was a wide range of HCV levels (0–30 million IU/mL) in recipients with a primary histopathologic diagnosis of recurrent HCV (Fig. 10).

Analysis of Risk Factors for the Development of Acute and Chronic Rejection

Six of the 11 HCV-positive recipients simultaneously maintained on low immunosuppression and treated with a combination of interferon and ribavirin developed significant rejection, defined as moderate or severe AR or CR. The remaining 5 of 11 patients treated with a combination of interferon and ribavirin, 2 other recipients treated with interferon alone, and a third patient treated with ribavirin alone did not develop significant rejection.

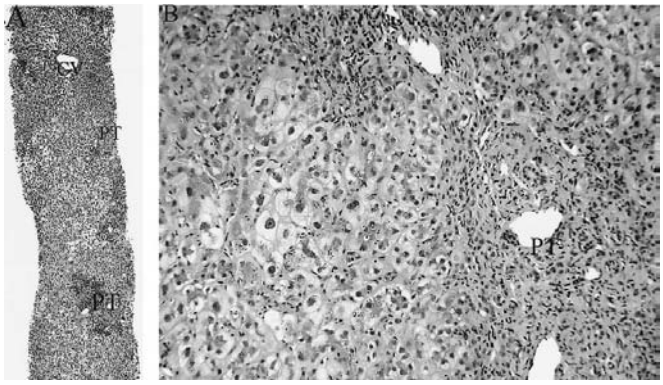

FIGURE 9. Follow-up biopsy from the patient whose clinical course and previous liver allograft biopsy are shown in Figures 7 and 8, respectively. A and B: Treatment with increased immunosuppression caused a dramatic decrease of the portal inflammation compared with the biopsy shown in Figure 8; it also caused marked hepatocyte swelling, hepatocanalicular cholestasis (left side of B), and a prominent ductular reaction at the interface zone (right side of B), all of which are characteristic features of cholestatic hepatitis. HCV RNA levels measured retrospectively from a sample obtained near the time of this biopsy were >50 million IU/mL (PT, portal tract).

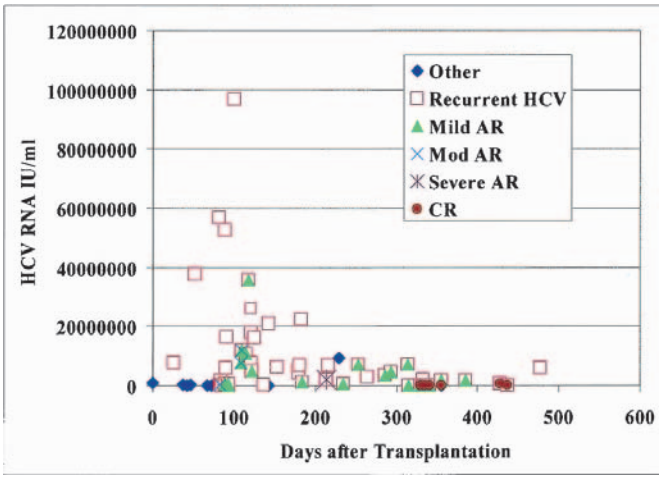

FIGURE 10. Correlation between quantitative HCV RNA levels and primary histopathologic diagnosis plotted against time after transplantation. The HCV RNA levels were obtained within a window from 14 days before until 3 days after the corresponding biopsy. Note that the highest levels of HCV RNA are detected during the first 6 months after transplantation, and thereafter levels generally decrease. In addition, HCV RNA levels >10 million IU/mL are almost invariably associated with a histopathologic diagnosis of recurrent HCV alone, and very high levels are seen in association with cholestatic hepatitis. Conversely, moderate and severe AR, and especially early CR, almost invariably shows relatively low HCV RNA levels. The point corresponding to the major mistake was not included in this graph.

Univariate models were first examined for predictors of AR and/or CR. Those parameters that reached a significance level of 0.10 were then used in multivariable models. Parameters that were considered for these models included basic donor and recipient characteristics such as age, race, and gender. Other variables included were cold ischemia time, pretransplantation crossmatch, antibody pretreatment, and posttransplantation anti-viral therapy. Only moderate or severe AR was used in the analysis for AR.

Predictors of AR in this patient population include female recipients ($P = 0.04$; hazard ratio [HR] = 4.0) and pretreatment with Thymoglobulin versus CamPath pretreatment ($P = 0.05$ HR = 8.0). Since older donor livers tended to develop CR sooner than younger donors, the CR model and the AR or CR model was adjusted for donor age. Treatment with interferon and ribavirin appeared to be a predictor of CR ($P = 0.03$; hazard ratio = 12.6); AR was weakly predictive of CR ($P = 0.11$; HR = 2.8), probably because of the small number of patients that experienced CR. Predictors of either AR or CR included female recipients ($P = 0.02$; HR = 4.7), pretreatment with Thymoglobulin ($P = 0.08$; HR =6.6), and treatment with interferon and ribavirin ($P = 0.09$; HR 4.0). Indeed, 13 of 14 patients who developed moderate or severe AR or early CR received Thymoglobulin pretreatment and of these, 4 of 6 who went on to develop early CR were also treated with anti-viral therapy for recurrent HCV.

DISCUSSION

In conventionally treated liver allograft recipients, AR usually occurs during the first month after transplantation; recurrent HCV usually first appears more than 1 month after transplantation. Thus, timing alone can be used to determine the cause of new onset liver allograft dysfunction. For patients enrolled in this protocol, the mean time to first onset of AR was 107 days, which significantly overlapped with first onset of recurrent HCV at 115 days. Consequently, distinguishing between rejection and recurrent HCV could not be based solely on time since transplantation and was especially troubling and particularly reliant on biopsy evaluation. The unusually long interval until the first onset of AR[15,40] is likely attributable to the protocol: pretransplantation immunodepletion followed by weaning of immunosuppression. Regardless, this study prospectively documents that interpretation of liver allograft biopsies can be used to quickly (6–7 hours) and accurately distinguish recurrent HCV from AR and CR, even under challenging conditions.

We found that the most problematic biopsies are a subset of those showing changes primarily of recurrent HCV. The troubling subset also shows bile duct damage or perivenular inflammation that is more prevalent than usually encountered with HCV alone, but still involving ≤50% of bile ducts or central veins, respectively. In such cases, it is our opinion that AR should be considered mild at most, and as a secondary diagnosis. These patients should not be treated with additional immunosuppression. Instead, they should be closely followed and subjected to re-biopsy if liver injury tests continue to rise.

Increased immunosuppression should be considered as a treatment option only when rejection is judged to be the primary insult. This condition is met when obvious bile duct damage or perivenular inflammation and hepatocyte dropout clearly involves most of the bile ducts or central veins, respectively. In our experience, AR-related findings should be obvious for biopsies in which AR is the primary insult. Such biopsies are usually graded as moderate or severe AR according to the Banff schema (1997) and usually associated with higher liver tests than HCV alone or mild rejection alone,[15] as confirmed in this study.

The above algorithm is recommended because liver allografts are very "forgiving" compared with other allografts: they can recover from most rejection-related insults and repair without fibrosis.[15] Conversely, unnecessary additional immunosuppression can significantly worsen hepatitis or even trigger fibrosing cholestatic hepatitis, and such patients usually suffer significant and often permanent liver damage from severe recurrent HCV. Furthermore, all of the mistakes in this series and in most other reports[1,19,25] were in the same direc-

tion; too great an emphasis was placed on mild AR findings in the context of recurrent HCV.

Although this study was carried out under a specific immunosuppressive protocol, it is our opinion that histopathologic findings and recommendations are generically applicable to other liver allograft recipients subjected to different approaches to immunosuppression. This contention is supported by the following observations: 1) the algorithm used in this study to distinguish between viral hepatitis and rejection was developed long ago in patients under a different immunosuppression protocol[13,16,31]; 2) even with low immunosuppression and the increased risk of rejection, we still overestimated the risk of AR; and 3) the same tendency to overdiagnose AR in the context of recurrent HCV occurs in liver allograft recipients treated with different approaches to immunosuppression. This statement is particularly true if the histopathologic findings are not clear-cut.[1,19,25]

Correlation of the biopsy findings with the clinical course, including an examination of serum HCV titers, serial liver injury tests in relation to immunosuppression, weekly meetings to discuss and collate all of this information, and importantly, insistence by the clinicians that there be unequivocal histopathologic evidence of significant rejection before giving any additional immunosuppression offered the best approach to optimal management. Real-time availability of graphical representations of the clinical course (ie, pertinent clinical, biochemical, and treatment data) made possible by the EDIT software greatly facilitated the entire process and reporting of the results.

Serial HCV RNA levels provided information useful for the histopathologic interpretation, but caution is urged against placing too much emphasis on HCV levels alone. There is a wide variation of HCV levels among patients, the values fluctuate significantly over time in individual patients, and there is considerable overlap in patients with different histopathologic diagnoses, particularly early after transplantation. Nevertheless, similar to other studies, the highest HCV RNA values were observed early after transplantation and during episodes of cholestatic HCV.[6,28,42] In contrast, all but one of the patients with late onset (>60 days) moderate or severe AR or CR showed HCV RNA levels <10 million IU/mL and most showed levels <2 million IU/mL. Thus, a diagnosis of moderate or severe AR or CR occurring more than 60 days after transplantation should be made with extreme caution in a patient with HCV RNA levels of >10 million IU/mL. Similar results were obtained by Gottschlich et al[19] who showed that higher HCV RNA levels were more frequently associated with an unequivocal diagnosis of recurrent HCV.

Relatively high HCV RNA levels during the first 6 months followed by a return to relatively low levels thereafter is most probably related to antibody pretreatment, disruption by transplantation of the previously established equilibrium between the host and virus, and later weaning of immunosuppression.[21,22,24,41] The unusual responsiveness of HCV levels and liver injury tests to anti-viral therapy in the weaning patients (unpublished observation) is likely attributable to lower overall immunosuppression, and in particular, the infrequent and sparing use of pulse or recycle corticosteroid therapy.[6,9,18,30,42] Our hope is that HCV might be more easily controlled or eliminated after transplantation, but using this protocol in HCV-positive recipients requires very close patient monitoring. We have already observed an increased incidence of early CR,[15] but there have been no graft failures from either AR or CR. In addition, most of the cases of early CR occurred early in the protocol and have recovered to near-normal liver injury tests with appropriate treatment. We are also aware that assessment of efficacy for both rejection and HCV will require longer-term follow-up because "re-arming" the immune system after immunodepletion has the potential to accelerate liver damage from recurrent HCV.[7,32]

The relatively low (<10 million IU/mL) HCV RNA levels and a paucity of hepatitis histopathologic findings during moderate or severe AR and CR versus high HCV RNA levels in cholestatic hepatitis and a complete absence of rejection-related histopathologic findings are interesting observations. Significant AR and CR in liver allografts have been associated with a strong T_H1-type hepatic microenvironment and cytotoxic T-lymphocyte response.[20,34,38,42] These effector mechanisms also are crucial determinants of HCV clearance and HCV-induced liver damage.[10,21,22,41] In contrast, cholestatic HCV has been associated with high viral levels and a strong T_H2-type intrahepatic microenvironment[42] and hepatic tolerogenesis.[29] Consequently, rejection and HCV clearance appear to be closely linked because all of the risk factors for significant rejection in this study (pretreatment with Thymoglobulin vs. CamPath, low-immunosuppression and anti-viral therapy including interferon) simultaneously enhanced viral clearance.

It is tempting to speculate about the role that hepatic dendritic cells might play in the outcome because of their ability to initiate and perpetuate immune responses. HCV interacts with DC-SIGN,[17,39] a receptor on dendritic cells that has the capacity to regulate their maturation and promote T_H2-type microenvironment.[2,39] Since dendritic cells are classically associated with rejection[14] and HCV appears to diminish their allostimulatory capacity,[4,23] HCV may be particularly suited for co-survival of the virus as well as the liver recipient. Early immunosuppression needed to prevent rejection enhances HCV replication, which in turn helps to subvert the alloresponse. Thus, the virus protects itself from clearance and the allograft from rejection. Conversely, while weaning of immunosuppression with addition of interferon may re-arm the immune system and promote HCV clearance, it also could increase the risk of rejection, and both rejection and the immune response leading to HCV clearance can significantly damage the liver. It seems, therefore, that the optimal approach for the treatment of HCV-positive liver allograft recipients would be

similar to hepatitis B. Agents that directly interfere with viral replication are needed.

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Standardized Cardiac Biopsy Grading

Grade	Histopathological Findings
0	No rejection
1	**A** = Focal (perivascular or interstitial) infiltrate without necrosis
	B = Diffuse but sparse infiltrate without necrosis
2	One focus only with aggressive infiltration and/or focal myocyte damage
3	**A** = Multifocal aggressive infiltrates and/or myocyte damage
	B = Diffuse inflammatory process with necrosis
4	Diffuse aggressive polymorphous ± infiltrate ± edema, ± hemorrhage, ± vasculitis, with necrosis

Additional Required Information*

■ **Biopsy less than 4 pieces**	
■ **Humoral rejection (positive IF, vasculitis, or severe edema in absence of cellular infiltrate**	
■ **"Quilty" effect**	**A** = No myocyte encroachment
	B = With myocyte encroachment
■ **Ischemia**	**A** = Up to 3 weeks posttransplant
	B = Late ischemia
■ **Infection present - biopsy therefore uninterpretable**	
■ **Lymphoproliferative disorder**	
■ **Other (specify)**	

* Must be added to biopsy report if present

Reference Billingham ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group. *J Heart Trans* 1990;9(6):587-93.

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

[FRAMES] [NO FRAMES]

INTERNATIONAL SOCIETY FOR HEART TRANSPLANTATION

With the emergence of many different grading systems for heart and lung biopsy interpretation, the International Society for Heart Transplantation realized the necessity to establish a standardized grading system for the purpose of multicenter trials and for publication so that results from different centers could be compared effectively. Our purpose is not necessarily to change the grading systems in individual centers but to find one to which most grading systems may be extrapolated for the purpose of publications and multicenter drug trials. The charge to the heart and the lung study groups was to provide simple grading systems so that there could be conformity of interpretation of heart and of lung rejection among pathologists. Basic grading systems are presented in the following articles that will now be required for all manuscripts accepted by THE JOURNAL OF HEART AND LUNG TRANSPLANTATION. In taking this step to require standardization of grading systems, the International Society for Heart Transplantation has taken a leadership role and has made a significant contribution to more meaningful scientific results in the field of thoracic organ transplantation.

Margaret E. Billingham, MD
Stanford, California

A Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection: Heart Rejection Study Group

The International Society for Heart Transplantation:
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14/1/25335

At the Society meeting in Munich in 1989 and again at the Colorado Springs Symposium in June 1989, Bruno Reichart, MD, then president of the Society, cited the many grading systems of rejection in heart and lung transplantation,[1-7] and he made urgent appeals to pathologists to come together for the purpose of establishing a universal grading system for biopsy interpretation. For this purpose, an international meeting of heart and lung pathologists from large transplantation centers from which previous publications on grading

TABLE I Standardized cardiac biopsy grading

Grade	"New" nomenclature	"Old" nomenclature
0	No rejection	No rejection
1	A = Focal (perivascular or interstitial) infiltrate without necrosis B = Diffuse but sparse infiltrate without necrosis	Mild rejection
2	One focus only with aggressive infiltration and/or focal myocyte damage	"Focal" moderate rejection
3	A = Multifocal aggressive infiltrates and/or myocyte damage	"Low" moderate rejection
	B = Diffuse inflammatory process with necrosis	"Borderline/severe"
4	Diffuse aggressive polymorphous ± infiltrate ± edema, ± hemorrhage, ± vasculitis, with necrosis	"Severe acute" rejection

"Resolving" rejection denoted by a lesser grade.
"Resolved" rejection denoted by grade 0.

systems had emerged convened at Stanford University Medical Center in August 1990. The object of the meeting was to find a simple and easily understood grading system that could be used by most pathologists. Furthermore, it was recognized that the grading system should have a high degree of reproducibility among all pathologists. Standardization of grading systems will result in more accurate comparison of results and will allow the establishment of multicenter immunosuppressive trials.

TECHNICAL CONSIDERATIONS

Because acute rejection is often focal, the standardized cardiac biopsy grading requires four to six undivided pieces of myocardium, depending on the size of bioptome used, to reduce sampling error to 2%. The tissue should be fixed in 10% buffered formalin and be paraffin embedded. At least one piece of evaluable myocardium should be frozen, fixed in OCT freezing compound, and saved for all biopsies in the first 6 weeks after transplant. No tissue needs to be routinely fixed for electron microscopic evaluation.

Adequacy of Sample

As to adequacy of sample, four pieces of myocardium are required, 50% of which must be evaluable myocardium and not biopsy site or scar. If a 7F bioptome or smaller is used, then six pieces of myocardium are required, 50% of which should also be evaluable. Biopsy specimens should not be divided.

Histologic Requirement

For histologic examination, a minimum of "three step levels" must be made through the paraffin block with at least three sections of each level. Slides should be stained routinely with hematoxylin and eosin, and one additional slide should be stained with a connective tissue stain such as Masson's trichrome.

GRADING OF CELLULAR REJECTION BY ENDOMYOCARDIAL BIOPSY

A new standardized cardiac biopsy grading is tabulated in Table I.

Grade 0 (No Acute Rejection)

Grade 0 should be used when there is no evidence of acute rejection or myocyte damage on the biopsy specimens. Equivocal findings of rejection should also be graded zero.

Grade 1A (Focal, Mild Acute Rejection)

Grade 1A represents focal, perivascular, or interstitial infiltrates of large lymphocytes that cause no myocyte damage, as seen in Figure 1. One or more pieces of the biopsy tissue may be involved.

Grade 1B (Diffuse, Mild Acute Rejection)

The grade 1B represents a more diffuse, perivascular or interstitial (or both) infiltrate of large lymphocytes with no myocyte damage, as seen in Figure 2. One or more pieces of biopsy tissue may be involved.

Grade 2 (Focal, Moderate Acute Rejection)

Grade 2 should be used when there is only one focus of inflammatory infiltrate (large aggressive lymphocytes with or without eosinophils), which is sharply circumscribed. Architectural distortion with myocyte damage should be present within the solitary focus, as seen in Figure 3.

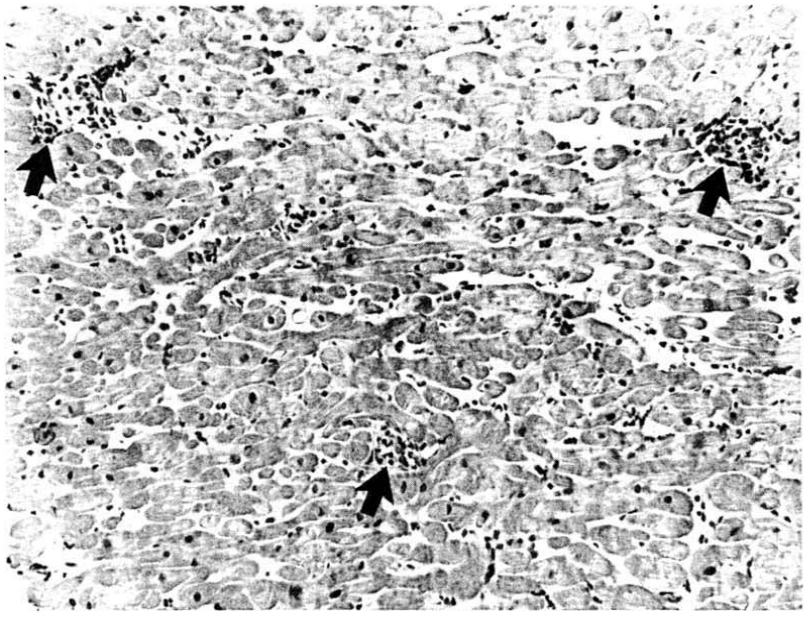

FIGURE 1 Grade 1A (ISHT Standardized Grading System). Section from a cardiac biopsy specimen shows focal, perivascular *(arrows)*, sparse infiltrate of lymphocytes, with no myocyte damage. (Hematoxylin and eosin. Magnification ×250.)

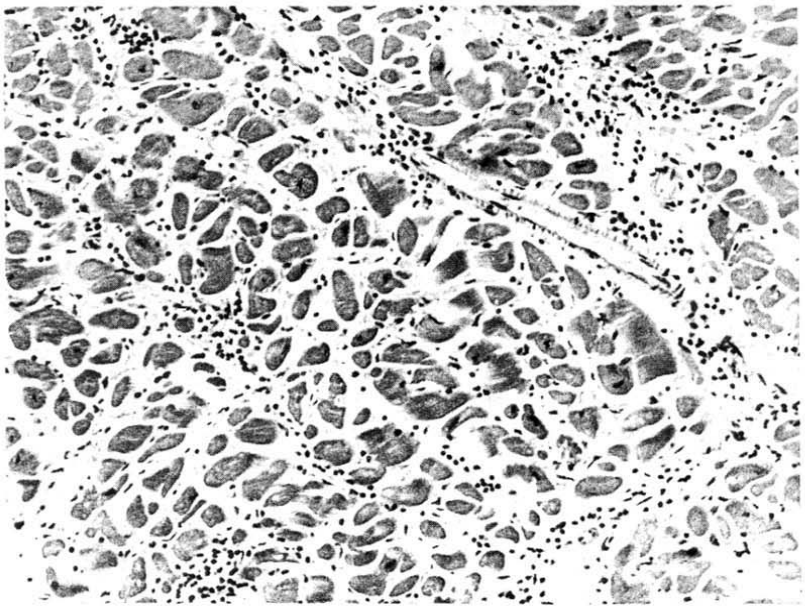

FIGURE 2 Grade 1B (ISHT Standardized Grading System). Section from a cardiac biopsy specimen shows a sparse, diffuse interstitial lymphocytic infiltrate without myocyte damage. (Hematoxylin and eosin. Magnification ×500.)

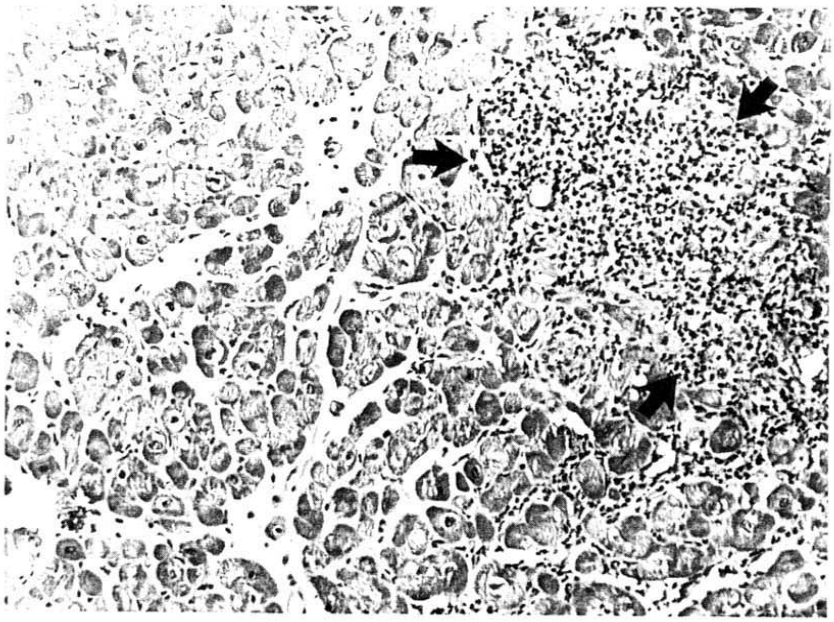

FIGURE 3 Grade 2 (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows a solitary, circumscribed focus of inflammatory cells *(arrows)*, with myocyte damage. Other pieces from the biopsy were clear of any infiltrate. (Hematoxylin and eosin. Magnification ×500.)

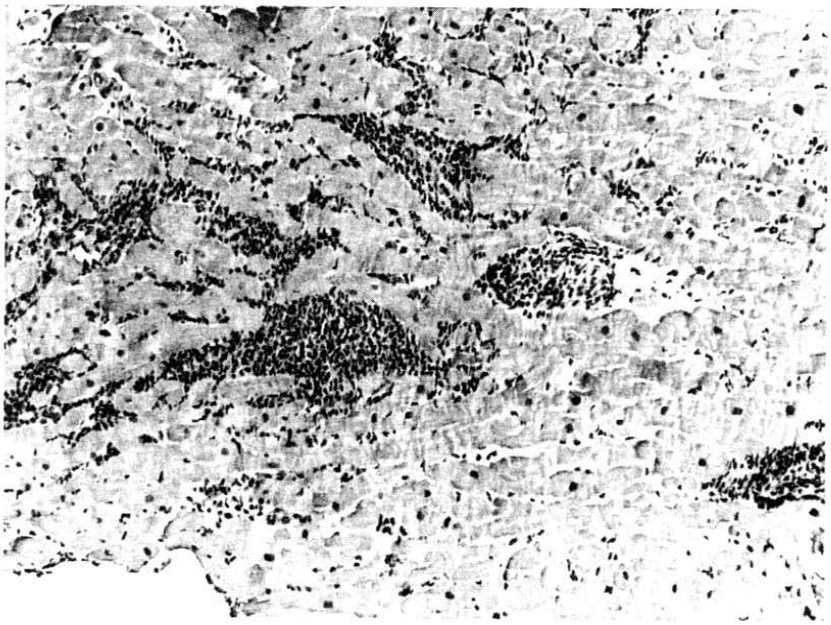

FIGURE 4 Grade 3A (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows an obvious multifocal pattern of inflammatory infiltrate, some with myocyte encroachment and damage. (Hematoxylin and eosin. Magnification ×250.)

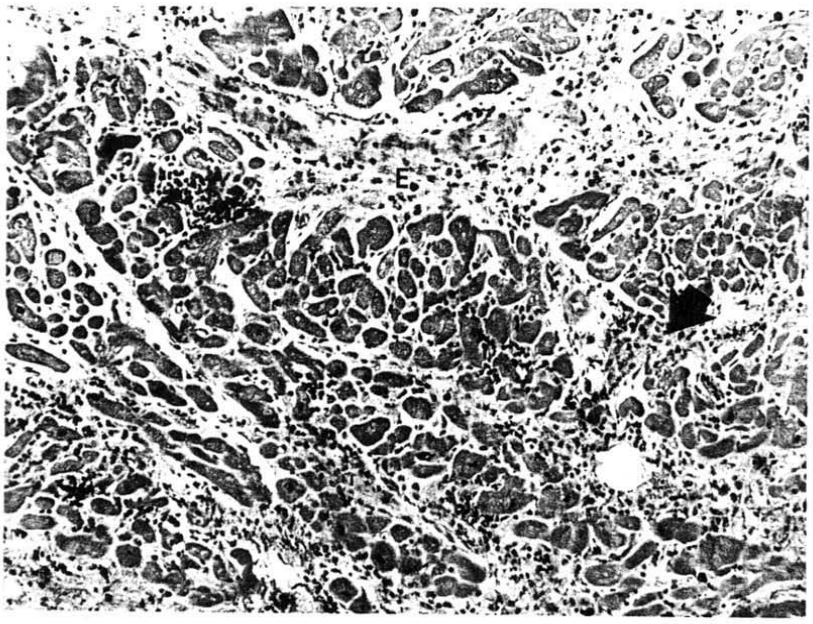

FIGURE 5 Grade 3B (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows a diffuse inflammatory process with focal myocyte damage *(arrow)* and edema *(E)*. All of the pieces were involved similarly. (Hematoxylin and eosin. Magnification ×250.)

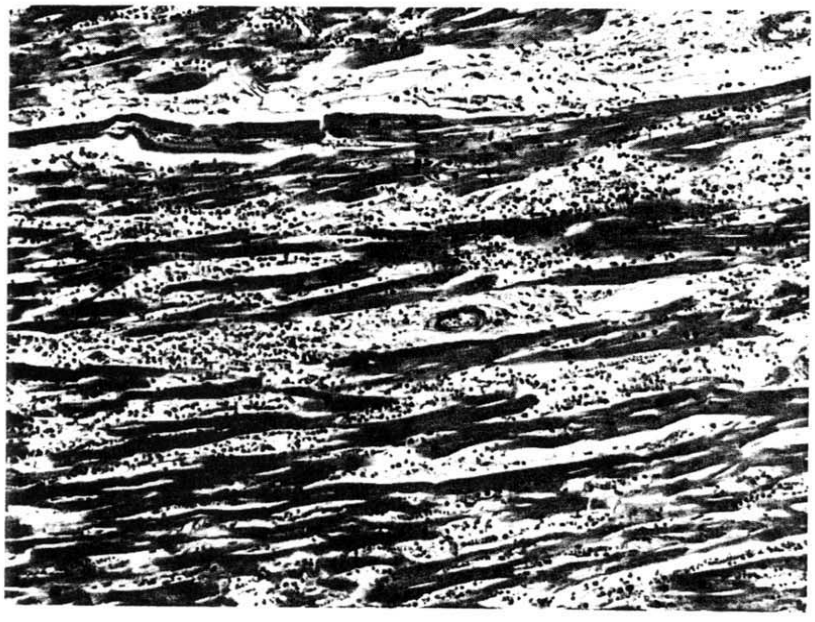

FIGURE 6 Grade 4 (ISHT Standardized Grading System). Section of cardiac biopsy specimen shows edema, hemorrhage, and an aggressive polymorphous inflammatory infiltrate. (Hematoxylin and eosin. Magnification ×250.)

TABLE II Standardized cardiac biopsy grading

Additional required information Check and specify "A" or "B"	
☐	Biopsy less than 4 pieces
☐	Humoral rejection (positive IF*, vasculitis, or severe edema in absence of cellular infiltrate
☐	"Quilty" effect A = No myocyte encroachment B = With myocyte encroachment
☐	Ischemia A = Up to 3 weeks posttransplant B = Late ischemia
☐	Infection present—biopsy therefore uninterpretable
☐	Lymphoproliferative disorder
☐	Other (specify)

*Immunofluorescence.

Grade 3A (Multifocal Moderate Rejection)

Grade 3A represents multifocal inflammatory infiltrates consisting of large aggressive lymphocytes with or without eosinophils. These infiltrates may involve one or more of the pieces of biopsy tissue, as seen in Figure 4.

Grade 3B (Diffuse, Borderline Severe Acute Rejection)

Grade 3B represents a diffuse inflammatory process within several of the pieces of biopsy tissue. Myocyte damage is present, as well as an aggressive inflammatory infiltrate of large lymphocytes and eosinophils with an occasional neutrophil. Hemorrhage is not usually seen in this grade (Figure 5).

Grade 4 (Severe Acute Rejection)

Grade 4 represents a diffuse aggressive, polymorphous inflammatory infiltrate that includes aggressive lymphocytes, eosinophils, and neutrophils (Figure 6). Myocyte necrosis and damage is always seen. Edema, hemorrhage and vasculitis are usually present. In some cases in which the patient has been treated aggressively with immunosuppression for some time, the edema and hemorrhage may be more prominent than the cellular infiltrate.

"Resolving" acute rejection in the old nomenclature will be replaced by denoting a lesser grade than that given in a recent biopsy. In addition, the term "resolving" may be used in parenthesis after the grade number. Likewise, "resolved" rejection should be denoted by grade 0; however, in this case also, "resolved" rejection may be included after the numerical grade in parenthesis.

Table II shows the additional required information that should be added to any biopsy report using the standardized grading system.

—Inadequate biopsy: Check box for biopsies of less than four pieces. The number of pieces should be written in the box.

—Immunofluorescence: If immunofluorescence is performed, and humoral rejection is suspected, the box on humoral rejection should be checked. It is suggested that in those centers where it can be easily done immunoflourescence be performed on all biopsy specimens in the first 6 weeks after heart transplantation, as previously described.[8]

—"Quilty" effect: The Quilty effect has been described many times and is an endocardial infiltrate that may not be associated with acute rejection.[9] Whenever a Quilty effect is present, the appropriate box should be checked. If the Quilty effect encroaches into the myocardium from the endocardium, that should also be designated with the letter "B."

—Ischemia: Evidence of myocyte damage from ischemia at the time of heart transplantation or reperfusion injury is often seen in the first early biopsies after transplantation. If ischemia is present, it should be checked in the appropriate box; the letter "B" should be used for late ischemia occurring 3 or more months after heart transplantation.

—Infection: Whenever infection is found to be present in the endomyocardial biopsy, as evidenced by a mixed inflammatory infiltrate that is not characteristic of acute rejection or organisms (viral,

fungal, or protozoal) are found, this should be indicated in the appropriate box.

—Lymphoproliferative disorder: If the endomyocardial biopsy provides evidence of a lymphoproliferative disorder, as previously described,[10] the appropriate box should be checked.

In conclusion, the above is a proposal for use in interpreting endomyocardial biopsies in cardiac or in combined heart-lung allografts. By using this scheme, different institutions can construct their data to support or argue against certain propositions espoused by other investigators. The International Society for Heart Transplantation will require that this grading system be used in all Society-sponsored publications in the future. It is expected that the study group will meet again in the future to evaluate the adequacies or deficiencies of this proposal.

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A Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection: Lung Rejection Study Group

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The grading of rejection in combined heart-lung, double lung, and unilateral lung allografts has created several nomenclatural problems in the transplant community. Establishing a uniform grading system, which could be used as a working formulation for comparing data from independent institutions, promoting improved patient care, and developing alternative therapies, is essential. Although it is recognized that all grading systems currently in use have individual merits, there is a need for a single comprehensive scheme that

The Pathology of Heart Transplant Biopsy Specimens: Revisiting the 1990 ISHLT Working Formulation

E. Rene Rodriguez, MD[a]

The ultimate goal of a heart transplant team is successful long-term outcome for the patient. One of the many critical duties necessary for success is accurate and expeditious study of heart pathology after transplantation. Correctly assessing the endomyocardial biopsy specimen to monitor for rejection requires intimate knowledge of the pathophysiology of the rejection process as it affects the heart. A detailed discussion of the biologic aspects of rejection is beyond the scope of this article; therefore, this review will focus on diagnostic use of the 1990 International Society for Heart and Lung Transplantation Working Formulation of Cardiac Allograft Pathology (ISHLT-WF90).[1]

Two recent meetings that addressed the pathology of transplantation prompted this review. The first meeting was the Sixth Banff Conference on Allograft Pathology, held in Banff, Canada, April 22–23, 2001.[2] The first 5 meetings at Banff used an interdisciplinary approach to discussion and to improving the pathology schemas used to evaluate pathology of kidney[3–6] and liver transplants.[7] Given the success of their work in other solid organ transplantations, the organizers of the Banff conferences invited a group of pathologists, cardiologists, cardiac surgeons, and other scientists to address the pathology of the human cardiac allograft at Banff. The goal of this work group was to exchange ideas and to share vast experience using the ISHLT-WF90. The second meeting that influenced this review was the "Endpoints" conference sponsored by the American Society of Transplantation and the American Society of Transplant Surgeons, held at the Natcher Center of the National Institutes of Health in Bethesda, November 5–6, 2001. The goal at this conference was consensus on objective endpoints that could be used as surrogate markers in clinical trials related to solid organ transplants. For the heart group at the conference, it was clear that endomyocardial biopsy remains the gold standard for diagnosis. However, the ISHLT-WF90 has some limitations because it has not been revised critically in more than a decade. This article reviews the issues discussed in detail at the Banff meeting that support the wisdom of refining the ISHLT-WF90, in light of the knowledge acquired during the decade since its implementation.

I will refer to the combination of speakers and audience at the Banff meeting as "the working group" (list of names and institutions provided as an appendix). After the first session, a consensus was reached that the ISHLT-WF has withstood the test of time and the intent of the clarification paper from 1998 was understood.[8] However, several topics required further clarification or modification and thus were chosen for further discussion as described below.

HUMORAL REJECTION

In this review, the term *humoral rejection* (Figure 1) refers to the immunopathologic process of antibody- and complement-mediated graft dysfunction (Table I).[9–12] A recent clinico-pathologic study expands the definition of humoral rejection by adding hemor-

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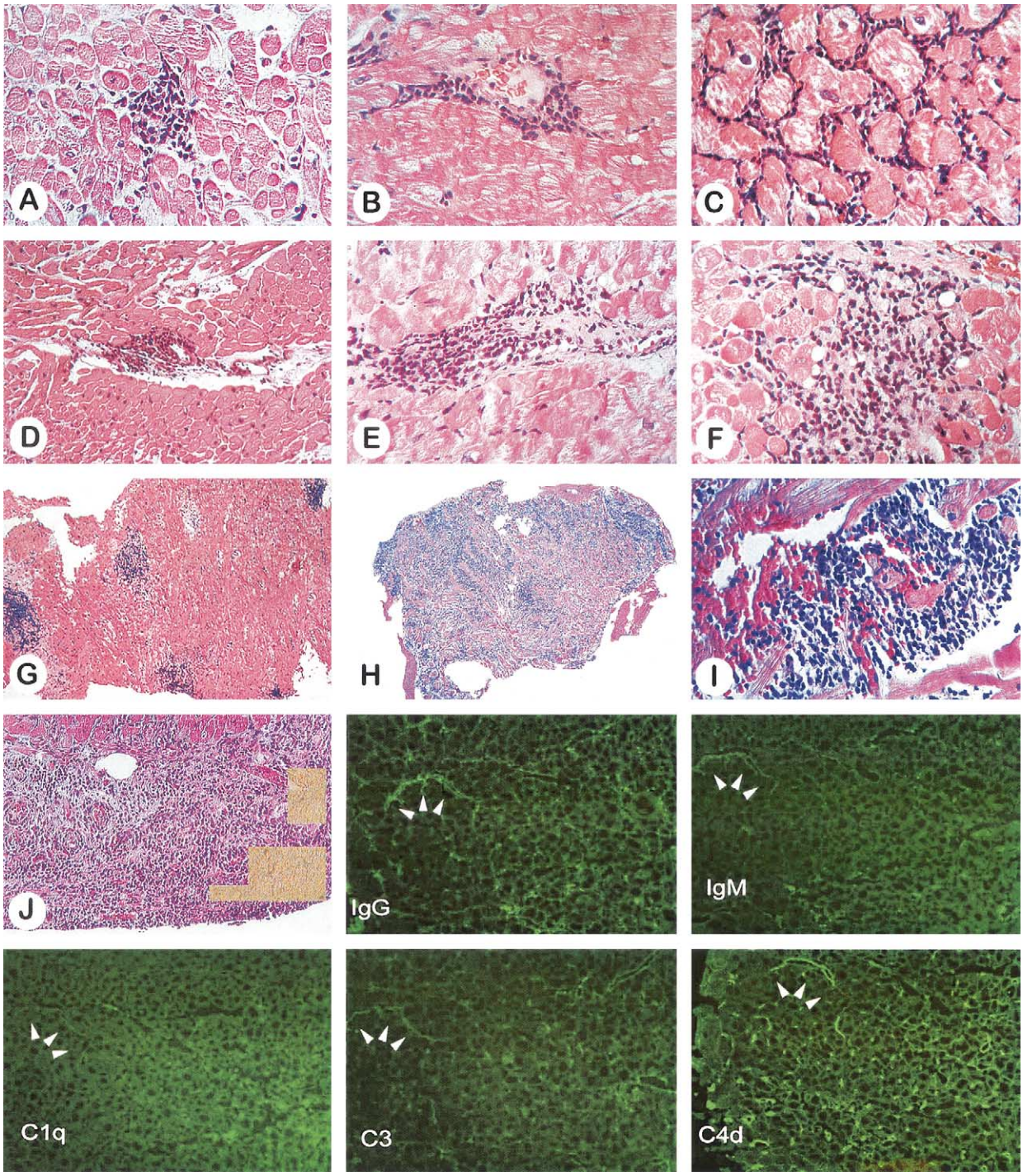

FIGURE 1 Light microscopy of cellular and humoral rejection. A. Endomyocardial biopsy specimen showing a focus of lymphocytic infiltrate around a small arteriole with a few red blood cells in the lumen of the vessel. The infiltrate is beginning to extend into the interstitial space between the myocytes. There is no evidence of myocyte damage. (hematoxylin and eosin [H&E], ×40). B. Perivascular infiltrate around a venule with open lumen and intravascular red cells. Early expansion of the interstitial space without evidence of myocyte damage. (H&E, ×40). C. Grade 1B rejection consisting of a conspicuous mononuclear inflammatory infiltrate that forms a network-like pattern of interstitial dark

TABLE I Definition of humoral rejection at Banff

Light microscopic findings:
A. Intravascular polymorphonuclear leukocytes and macrophages with or without endothelial swelling
B. Vasculocentric, lymphocyte-poor inflammatory infiltrate
C. Myocyte injury including necrosis in areas adjacent to (affected?) vessels with infiltrates
Immunofluorescence microscopy: Document the presence of immunoglobulins, complement, and, potentially, fibrin and HLA-DR positivity in the biopsy. At least 2 patterns exist:
A. The most common shows deposits in capillaries, arterioles, and small arteries.
B. The second pattern shows deposits around myocytes.
Immunohistochemistry: It is common to distinguish between elongated macrophages and swollen endothelial cells by staining with CD68 and CD31 in adjacent sections.
Clinical pathology studies: Identification of donor specific antibody in serum

rhage and neutrophilic infiltrates to the spectrum.[13] The authors of this study state that their minimal criteria to diagnose humoral rejection included capillary endothelial cell swelling and any immunoglobulin and complement staining in the capillaries. However, immunohistologic markers demonstrated that most of the swollen cells in capillaries were macrophages.[13,14]

A unified and consistent definition of diagnostic criteria for humoral rejection in biopsy specimens or

blue cells surrounding individual myocytes. Lymphocytes form 1 to 3 rows of "Indian files" between myocytes without evidence of myocyte injury. (H&E, ×40). D. Vasculocentric lymphocytic infiltrate. Small arterioles cut in a cross section showing aggregates of lymphocytes forming an asymmetric "cuff" of mononuclear cells around the arteriole. (H&E, ×40). E. Similar lesion in which the inflammatory infiltrate is also asymmetrically distributed around the vessel, giving an impression of widening the interstitial space at the left end of the vessel. (H&E, ×40). F. Grade 2 rejection. Interstitial inflammatory infiltrate consisting mostly of lymphocytes and some macrophages. The infiltrate covers an area of about 300 × 500 μm, thus representing a large area of myocyte dropout. Vacuoles are present mostly within myocytes, but others are difficult to assign to a specific cell type. (H&E, ×40). G. Grade 3A rejection. Several foci of mononuclear inflammatory infiltrate are present. Each focus measures >300 μm, representing myocyte dropout. (H&E, ×4) H. Grade 3B rejection. Diffuse extensive infiltrate of the myocardium in Grade 3B rejection. Instead of making small nests of lymphocytes, the inflammatory infiltrate extends through the interstitial space, separating the myocytes and in some areas destroying the myocytes. (H&E, ×4). I. Severe rejection showing lesions consisting of lymphocytes and macrophages that disrupt the architecture and are associated with neutrophils, eosinophils, and necrotic myocytes. (H&E, ×40 X). J. Endocardial lymphocytic infiltrates (ELI) (Quilty effect), showing layers of lymphocytes within the endocardium. No extension into subjacent myocardium. Small networks of open capillaries are a common feature in larger ELIs. Exuberant ELI can produce marked thickening of the endocardium, but no infiltration into the myocardium (Quilty type A). IgG. Immunofluorescence microscopy of endomyocardial biopsy specimen showing abundant (++ to +++) linear deposits of immunoglobulin (Ig) G in peri-arteriolar (arrowheads) and peri-capillary locations. The intensity of the deposits around myocytes is less than that of capillaries and arterioles. (×40). IgM. Minute focal granular deposits in the same arteriole (arrowheads) shown in the IgG stain. (×40). C1q. No evidence of complement fragment C1q deposition. C3. Linear deposits of C3 in peri-arteriolar (arrowheads) and peri-capillary locations. (×40). C4d. Compared with C3, the linear deposits of C4d are more intense in arteriolar (arrowheads) and peri-capillary locations. Also note the presence of complement around the myocytes in a pattern following that of IgG deposition.

in explanted hearts has not been well established.[10–18] The reported frequency of humoral rejection ranges from 44% to 59%.[13,15,16] Despite these high percentages, the ISHLT-WF90 mentions only briefly that a piece of tissue should be frozen for immunofluorescence and an entry should be made to indicate whether there is humoral rejection, recording the following: "positive immunofluorescence, vasculitis or severe edema in the absence of cellular infiltrate."[1] However, the ISHLT-WF90 does not indicate how many pieces of endomyocardia should be examined in this manner, whether both light microscopic and immunofluorescence findings must be present, how to interpret the pattern or intensity of humoral rejection, or how to interpret the combined findings of cell-mediated and humoral-mediated rejection.

Humoral rejection has been referred to as "vascular humoral rejection"[15] or more recently "cardiac vascular (microvascular) rejection."[16,17] It usually occurs within the first month after transplantation and affects most notably the components of the cardiac capillary network.[15] However, combined cellular inflammatory infiltration of the larger vessel walls and intimal proliferation, which results in severe luminal narrowing and eventually loss of the graft, of the epicardial coronary arteries may not show a prominent humoral rejection component.[18] Furthermore, when the humoral response is intense, there are subtle light microscopic findings in the biopsy specimen. One is "finding the combination of prominent endothelial cell swelling and/or vasculitis on light microscopy and the deposition of immunoglobulin and complement by immunofluorescence."[15] Interstitial edema occurs, but even under the best circumstances, freezing can create artifacts that mimic edema, making accurate interpretation difficult. Thus, evaluation of edema in small biopsy pieces is unreliable.

Other methods for evaluating humoral rejection that the literature recommends include identification of fibrinogen, immunoglobulin (Ig)G, IgM, and complement components C1q and C3 in frozen sections.[15] Deposits of IgM, IgG, or complement in the microvasculature[13] or myocytes[19,20] indicate humoral rejection. Since publication of the ISHLT-WF90, additional reagents that detect complement have been reported.[21,22] Recently, it was shown that the presence of fibrin may be part of the humoral rejection process.[23] On the technical side, immunohistochemistry of paraffin-embedded tissue to detect humoral rejection markers (i.e., IgG, IgM, etc.) is not reliable, because the fixation process may precipitate many serum proteins in the tissue and give a false-positive rate >90%.[24]

Humoral rejection in the pediatric population can present with or without concomitant cellular rejection, and the humoral component may persist after treatment.[25] Such episodes of humoral rejection also are seen in the adult population, particularly when humoral rejection develops many months or even years after transplantation. Patients who undergo second cardiac transplantations may experience a similar phenomenon.

In some studies, humoral rejection correlates well with the presence of anti-HLA antibodies in serum,[26,27] perhaps suggesting that combined use of immunofluorescence and serologic measurement of anti-HLA antibodies may be a more reliable way to diagnose humoral rejection and that diagnosis may not be possible with 1 test alone. In one study, serum specimens were obtained, on average, within 1.8 days of biopsy and were analyzed for anti-HLA antibodies.[26] The authors found good correlation between the presence of these antibodies and linear deposits of immunoglobulins or complement components in the biopsy specimens. Nonetheless, evidence of antibodies in the circulation with specificity for non-HLA antigens on the graft is a possibility and should support the diagnosis of humoral rejection. At our institution, humoral rejection is usually suspected clinically when there is evidence of suboptimal graft function and the endomyocardial biopsy specimen shows either no evidence of cellular rejection (i.e., Grade 0) or mild rejection (Grade 1). This combination of findings should prompt the cardiologist to obtain tissue for immunofluorescence. Patients with prolonged warm or cold ischemia experience poor survival of cardiac transplants. When biopsies obtained 1 to 3 weeks after transplantation are evaluated with immunofluorescence, a diffuse capillary and pericapillary deposition of complement components C4d or C3d can be detected in the absence of IgM, IgG, and IgA. Approximately 80% of the biopsy specimens with complement deposition also had light microscopy evidence of peritransplant ischemic injury. However, only 45% of the biopsy specimens without complement C4d or C3d deposition showed ischemic injury. Thus, ischemic changes are associated with complement activation.[22] Therefore, predominant complement activation should not be the only criterion used to diagnose true humoral rejection, particularly in the early post-transplant period.

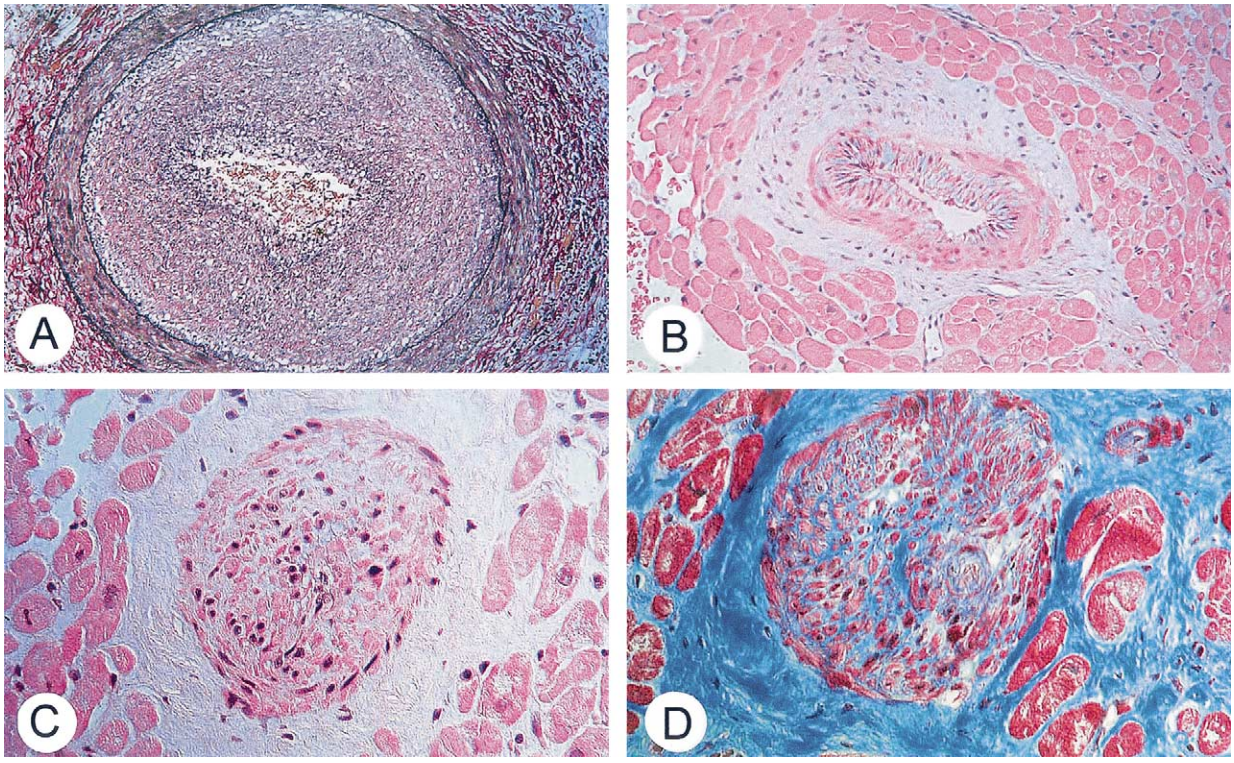

FIGURE 2 Cardiac allograft vasculopathy, light microscopy. A. Epicardial coronary artery with severe luminal narrowing by intimal proliferation of cells and deposition of connective tissue (darker red media and light pink-red intima). The intima shows marked proliferation of cells that almost completely narrows its lumen. Conspicuous vacuolation of the cells indicates intracellular lipid accumulation. Note the intact elastic laminae (Verhoeff-van Gieson elastic stain, ×1.25) B. Small intracardiac coronary artery shows early proliferative disease in the intima and abundant proteoglycan accumulation (light blue discoloration subjacent to the endothelial cells). Note the abundant fibrosis of the adventitia (hematoxylin and eosin [H&E], ×20). C. Occlusive proliferation of smooth muscle cells in a small intramural coronary artery. (H&E, ×40) D. This stain proves that most of the luminal narrowing is secondary to smooth muscle proliferation (red-staining cells) and little accumulation of collagen. (Masson's trichrome) (×40).

Discussion of Humoral Rejection at Banff

The working group agreed that humoral rejection is a real process that clinically affects a significant number of cardiac transplant patients (although no percentage was agreed upon). The working group also agreed that there was no consensus (in the working group or in the literature) about the incidence of clinically significant humoral rejection in the heart. The working group concurred that the ISHLT-WF must strive to establish clear pathologic criteria for humoral rejection in the heart. The literature on this topic is sparse, and no reproducibility criteria exist among heart transplant centers as to what constitutes humoral rejection. Consensus was reached that humoral rejection must be recognized early and unambiguously. The working group also agreed on the necessity of clear criteria, because isolated markers are not fully reliable or specific for humoral rejection. The pathologic markers of humoral rejection identifiable in endomyocardial biopsy tissue suggested by the working group were IgG, IgM, IgA, C1q, C3d, C4d, CD68/KP1 (monocytes/macrophages), and CD34 (endothelium). There was no consensus on the usefulness of fibrinogen, fibrin, and HLA-DR. During the discussion about establishing these criteria, the working group agreed on a need to show reproducible results with these markers in diagnosing humoral rejection. While discussing this issue, the working group agreed that humoral rejection must be better defined because no standardized pathologic criteria exist. Table I summarizes suggested features.

Clinical pathology studies may be useful (for cases in which ischemia–reperfusion injury is present concomitantly with rejection). Identifying donor-specific antibody should be another criterion used to support the diagnosis of humoral rejection.

The working group also recognized that a sub-set of patients may predominantly experience vascular acute cell-mediated rejection, which affects small arteries and arterioles. Mononuclear sub-endothelial or transmural inflammatory infiltrates present within arterial and arteriolar walls (not venules) are the likely hallmark of these lesions. The incidence and significance of these lesions is not known.

The working group agreed that it is premature to speculate about the association between the vascular humoral component and/or the predominantly vascular acute cell mediated rejection seen in some biopsies and the development of epicardial coronary artery vasculopathy (Figure 2) in the cardiac allograft.

ACUTE CELLULAR REJECTION

Morphologically, acute cellular rejection is a mononuclear inflammatory response, predominantly lymphocytic, directed against the cardiac allograft (Figure 1). In severe cases, the granulocytes also participate in the rejection process. Characterizing sub-types of lymphocytes in cardiac biopsy tissue has shown no reproducible correlation between the type of cell that infiltrates the graft and the severity of rejection or the presence of humoral rejection. Although B cells also are found in these allografts, they do not correlate with the degree of rejection.[28] However, some studies report good correlation between the presence of CD8+ T-cells and rejection grade.[29] The discrepancy in these studies may relate to the fact that the immune response to the allograft is a continuous process in flux, which is usually dissected in small "time-lapsed" views for pathologic study. Furthermore, if lymphocyte phenotypes are further sub-classified by the presence of naive cells (CD45RA) and memory cells (CD45RO), naive cells are more abundant in biopsy tissue during mild as opposed to moderate rejection.[30] Immunohistochemical studies indicate that macrophages are the predominant cell infiltrating some allografts.[31] Although HLA molecules are a major anti-genic target of lymphocytes in cellular rejection,[32] HLA Class I and Class II antigens express readily in any inflammatory condition, ranging from myocarditis to rejection. Unlike the good correlation found in serologic studies of HLA mismatches between donor and recipient and subsequent development of rejection,[32] detection of HLA molecules in tissue sections does not have a predictive value per se.

Detecting cytokine expression in tissue shows that interleukin (IL)-2 is prominent in cases of severe rejection, whereas IL-6 or interferon-gamma are expressed only mildly.[33] Endothelial antigens such as vascular endothelial growth factor are expressed in the microcirculation of the graft; expression is confined to areas where there is fibrin deposition, macrophages and neutrophils.[34] In a recent study, several cytokines were localized by combining the detection of mRNA using in situ hybridization and the detection of the protein product using immunohistochemistry in heart biopsies.[35] In biopsies with rejection Grade 3A or 3B, expression of mRNA for IL-6, IL-8, IL-9, and IL-10 was strong, whereas expression of IL-2, IL-4, IL-5, and tumor necrosis factor α (TNF-α) was weak and was detected in lymphocytes. In biopsy specimens with lower grades of rejection, mRNA for IL-6 and IL-9 was present, and occasionally mRNA for IL-1–β, TNF-β, and interferon-α (IFN-α) were detected. When detected with antibodies, IL-2, IL-3, and IL-10 were detected in biopsy specimens with greater rejection grades, whereas few cells expressed IL-6, IL-8, and IFN-α. In biopsy specimens with lesser grades of rejection, weaker expression of these cytokines was observed. The level of IL-12 expression was equal in all biopsies, and IL-4 was barely detected in any biopsy. Interleukin-3, IL-6, IL-10, and IL-12 were detected in lymphocytes and in macrophages. Thus the authors concluded that T-helper cell cytokine production and other intragraft elements regulate cardiac allograft rejection.[35] Among the adhesion molecules, expression of intercellular adhesion molecule 1[33,36–40] and E-selectin[39,41,42] seems to correlate with upcoming rejection, whereas vascular adhesion molecule 1[38,42] is a somewhat better marker in evaluating the effect of therapy in an acute episode of rejection.[38]

Grading Acute Cellular Rejection

Through the years, several methods have been proposed to assess the histologic grade of rejection. These now have historic interest and will not be reviewed here. The grades proposed in the ISHLT-WF90[1] are based primarily on the amount of inflammatory infiltrate and the presence of myocyte damage; the pattern of inflammatory infiltration plays a minor role.

Discussion of Acute Cellular Rejection at Banff

The working group agreed that in light of 10 years of cumulative experience since its publication, revisit-

TABLE II Definition of acute cellular rejection at Banff

Use the term *injury* to include 1 or more of the following: myocyte encroachment, architectural distortion and dropout, as well as reversible and irreversible cell injury. Substituting the term *aggressive infiltrates* with *dense infiltrates* may be more appropriate. In this context, the wording for the grades of cellular rejection would read
Grade 0—no evidence of cellular rejection
Grade 1A—focal perivascular or interstititial infiltrate *without myocyte injury*
Grade 1B—multifocal or diffuse sparse infiltrate *without myocyte injury*
Grade 2—single focus of *dense* infiltrate *with myocyte injury*
Grade 3A—multifocal *dense* infiltrates *with myocyte injury*
Grade 3B—diffuse, *dense* infiltrates *with myocyte injury*
Grade 4—diffuse and extensive polymorphous infiltrate *with myocyte injury*; may have hemorrhage, edema, and microvascular injury

ing the ISHLT-WF was a necessary and worthy effort. However, there was strong consensus that any changes in the formulation should reflect current pathologic practice and should not affect the grading of historical samples. In other words, the issue was not one of changing current ISHLT grading scales, but of more clearly defining how expert cardiac pathologists currently interpret the ISHLT-WF (Table II).

Confusion has arisen from the use of the term *myocyte damage* and *myocyte necrosis* as a required feature in several grades of cellular rejection. The most difficult issue in interpreting endomyocardial biopsy specimens from human allografts has been the inability to obtain consensus about the definition of *myocyte damage*, even among experienced pathologists trained in and dedicated to cardiovascular pathology. The morphologic spectrum of myocyte injury seen in the myocardium is wide. With light microscopic examination, subtle changes are difficult to see by the occasional observer. Simple changes in the myocytes, such as vacuolization, hydropic change, or perinuclear halos (or retrogressive changes),[43,44] indicate milder injury, whereas coagulation necrosis, myocytolysis, and nuclear pyknosis indicate more severe injury. Thus, the term *damage*is ambiguous. Without clear coagulative necrosis or fragmentation of the sarcoplasm or typical nuclear changes such as pyknosis in the myocytes, identifying damaged cells in paraffin sections stained with hematoxylin and eosin is a subjective matter. Ultrastructural studies show clearly that subtle myocyte damage is present.[45] Myocyte degeneration is easily distinguished from necrosis. Damage to endothelial cells, basal lamina, or other components also is recognized easily.[46] Myocyte necrosis as defined by ultrastructural criteria is common in humoral rejection, whereas myocyte degeneration (with the potential for recovery) is more common in cellular rejection.[47] Some of the myocyte damage seen during rejection may actually be reversible;[45,48] however, with light microscopy, some of the myocyte changes that represent sublethal damage are indistinguishable from actual early necrosis.

The working group suggested replacing terms *damage* and *necrosis* with the encompassing term *injury* and that this later term should be clearly defined in the text and illustrations of the ISHLT-WF, if it is revised. The term *injury* would encompass several types of myocardial alteration including 1 or more of the following: myocyte encroachment, architectural distortion, and dropout, as well as reversible and irreversible cell injury. In addition, the working group confirmed that actual necrosis was rare and that architectural changes were the hallmarks used to define Grades 2 and greater. Myocyte necrosis vs myocyte injury was particularly significant in pediatric biopsy specimens.

The pathologic terms used on the grades listed in Table II should be clearly defined in the text of any attempted modification of the ISHLT-WF90. By implication, it is worthwhile to point out that Grade 2 cellular rejection should be retained. The working group agreed to preserve Grade 2 because 1) it is still used in transplant centers to make therapeutic decisions; 2) it is part of many databases of prospective information on the outcomes of transplanted patients; 3) it is still used as a standard in some clinical trials. (This particular issue also was relevant at the "Endpoints" conference in November 2001, because Grade 2, or the transition from Grade 2 to Grade 3A, is an endpoint frequently used in clinical trials). In some transplant centers, Grade 2 is no longer used as a discrete defining point to make therapeutic decisions. The fact that observers can

confuse Grade 2 rejection with Quilty type B lesions is a problem that the working group recognizes; however, practical solutions include attention to histologic detail (vascularity, fibrosis); using many levels of sectioning; and using immunohistochemical stains that can, in most instances, allow the pathologist to distinguish between these two lesions.

ADDITIONAL INFORMATION FOR THE BIOPSY REPORT

The ISHLT-WF90 suggests additional items be recorded during interpretation of endomyocardial biopsy specimens.

Inadequate Biopsy

This term should be used when the specimen consists of $\leq$4 pieces[1], and when myocardia present in 1 or more of these pieces is $<$50% of the piece. However, in some instances, valuable information (such as rejection) is present in the other (adequate) biopsy pieces, and although a diagnosis of rejection cannot be rendered, the findings can be documented in the report as part of the histologic description or as a note.

Ischemic Damage

The presence of ischemic damage should be documented. Such foci may persist for several weeks, perhaps because of delayed inflammatory response secondary to immunosuppression.[49]

Quilty Effect or Endocardial Lymphocytic Infiltrates

The ISHLT-WF90 describes this phenomenon and recommends that its presence or absence be recorded. But the ISHLT-WF90 does not address its pathologic significance. Endocardial lymphocytic infiltrates,[50] also known as Quilty effect[51] or lymphoma-like lesions,[43] are collections of T and B cells with histiocytes[52,53] seen in the endocardium of transplanted hearts. Plasma cells are present in about 50% of these infiltrates. Occasional eosinophils and neutrophils may be seen.[53] Capillaries with red blood cells and sometimes prominent endothelial cells are seen within the infiltrate. They vary in size from 0.007 to 1.89 mm^2.[43] Several hypotheses have been proposed to explain the pathogenesis of these infiltrates, including the use of cyclosporine,[52] concomitant infection with Epstein-Barr virus,[54] low local levels of cyclosporine in the areas of endocardium where Quilty effect infiltrates develop,[55] and idiosyncratic responses to cyclosporine,[50] but none has been proven. However, Quilty effect does not occur in the hearts of patients receiving cyclosporine therapy for other organ transplants (i.e., liver, kidney).[56] The Quilty lesion seems to be a phenomenon that occurs only in the endocardium of cardiac allografts.

Quilty effect infiltrates may or may not be found concomitantly with cellular rejection in the myocardial interstitium. The size of the Quilty infiltrates varies greatly. Two morphologic patterns are recognized, Type A and Type B. Type A is confined to the endocardium. Type B, also known as invasive Quilty, is present "if the Quilty effect encroaches into the myocardium from the endocardium."[1]

Quilty Effect vs Moderate Rejection

Some articles discuss the possible confusion of Quilty Type B infiltrates with Grade 2 (focal moderate) rejection.[35] This is a problem for pathologists because of the obvious implications for therapy. Quilty Type B infiltrates can extend deep into the subjacent myocardium. One may imagine how a tangential section through the deeper (myocardial) end of a biopsy may show inflammatory infiltrates that look like rejection if only a few levels of section are examined. However if deeper sections are examined, or better yet, if all the biopsy tissue is examined, it would become obvious that the inflammatory infiltrate in the myocardium is connected to a Quilty type lesion in the endocardium, thus, representing Quilty Type B infiltrate. This type of artifact has prompted some observers to question whether Grade 2 cellular rejection exists.[57] Furthermore, moderate rejection evolves from mild rejection associated with graft dysfunction.[58] Thus, the fact that another pathologic lesion can mimic Grade 2 should never become the reason to deny the existence of Grade 2 itself. A possible solution to this problem is to section and examine all the tissue available. Thus, a possible focus of Grade 2 rejection can easily be tracked down through all the subsequent levels of that particular biopsy piece. In almost every instance, we can clearly establish whether the mononuclear inflammation in question is part of an endocardial Quilty Type B infiltrate that has penetrated deep into the myocardium or is Grade 2 rejection. Very rarely do we have inconclusive results with this approach. Another solution to this problem is to stain the biopsy with antibodies to RANTES (regulated upon activation, normal T cell expressed and secreted). This is helpful in differentiating cellular rejection from Quilty B lesions because the RANTES-positive cells are more abundant in rejection.[59]

Quilty Effect vs Lymphoid Neoplasms

Lymphoid neoplasms occur in 4% to 13% of all transplanted patients. A role for Epstein-Barr virus in the pathogenesis of post-transplant lymphoproliferative disorders has been suggested[60–63] but not proven.[64] In most instances, these neoplasms are monoclonal B-cell type,[65] and their clinical presentation involves the lymph nodes, the central nervous system, the systemic organs, or the transplanted organ itself.[60] T-cell lymphomas also occur,[65,66] but primary cardiac presentation of these neoplasms is not common.[60] Development of multiple myeloma is rare.[67]

Early Peri-operative Necrosis vs Ischemic Changes

Early peri-operative necrosis may be caused by events that affect the donor, such as catecholamine discharge, pressor therapy given during acute care, severe donor trauma, re-implantation damage, or early post-operative damage.[49] A biopsy specimen of the septum taken at the time of implantation is informative, although changes can be subtle. The ISHLT-WF90 makes a distinction in allograft monitoring between ischemia, commonly seen up to 3 weeks after transplantation and representing peri-operative injury, and late ischemia, which occurs after 3 months. Morphologic evidence shows myocyte necrosis, usually coagulative type, with hypereosinophilic myocytes, pyknotic nuclei, and even some karyorrhexis. Myocyte necrosis is usually out of proportion to the inflammatory infiltrate; myocyte vacuolization may be seen. Immunosuppression may delay healing.[49] Although this type of necrosis can be silent clinically, if severe, these changes can compromise the function of the graft in various degrees. Another possibility in hearts damaged during the peritransplant period is the development of interstitial fibrosis.[68] The size of the actual myocardial piece also may influence perception of the amount of fibrosis present.[69]

Allograft Vasculopathy and Biopsy Specimens

Although small arteries or arterioles with vasculopathy may be seen in endomyocardial biopsy specimens, no consistent correlation exists with the anatomic or functional status of epicardial coronary arteries.[70] Despite this lack of correlation, the presence of vasculopathy in the biopsy specimen should be recorded in the final report. Furthermore, the significance of "vasculocentric" inflammatory infiltrates seen in biopsy specimens and their possible correlation with the development of vasculopathy must be studied.

PERSPECTIVES FOR THE FUTURE

New Technologies

Modern pathology practice requires the use and application of a number of technological advances for everyday diagnostic work. Clearly, molecular diagnostics are common practice in other sub-specialties of pathology. The application of such technology to diagnostic evaluation of endomyocardial biopsy specimens should not be overlooked. The pace of knowledge transfer from the research environment to the diagnostic environment has increased. Any classification of allograft rejection should set guidelines for the use of such technological advances. For example, careful understanding of the polymorphisms that modulate the interaction of chemokines and cytokines during rejection will be useful to optimize individual patient immunosuppressive therapy. However, discussion of specific molecular markers is beyond the scope of this review. If necessary, a revised working formulation should take this into account, and we should plan ahead to schedule updates at intervals of 2 to 5 years.

With regard to changes in the epicardial coronary arteries, molecular markers may be detected in the smaller intramural arterioles that are commonly sampled in endomyocardial biopsies. Understanding and interpreting such changes should be performed in a uniform manner. We are in the midst of rapid advances in genomics and proteomics, and these advances are being applied to patient care in research centers. We must strive to identify pathologic markers that predict the development of vasculopathy, whether or not they are present in the endomyocardial biopsy specimen. Any modified or new working formulation to evaluate cardiac allograft pathology should address these issues in a way that allows pathologists to provide useful information to clinicians.

Databases of Pathologic Findings

At Banff, the working group made a very important suggestion: reminding pathologists that the information a pathologist generates can seldom be retrieved from a report that contains only the ISHLT-WF rejection grade in the diagnosis. Because pathology practice varies from place to place, it would be useful if the Working Formulation provided guidelines on how to generate a report easily translated into database terms. Otherwise, subtle pathologic

features eventually found to correlate with outcome may not be retrievable. The ISHLT should not dictate local practices, but should encourage pathologists to make their important observations "retrievable" by electronic queries.

In summary, this review presents a synopsis of cardiac transplantation pathology, mainly from the standpoint of clinical assessment and patient follow-up with endomyocardial biopsy specimen monitoring. The basic lesions characterized 2 decades ago are still the gold standard for defining mild, moderate, and severe rejection. Revisiting the ISHLT-WF from 1990 at Banff 2001 showed that knowledge has evolved from this fundamental base in an organized manner. However, this working formulation has not been formally revised in more than 10 years and does not incorporate the experience accumulated by pathologists around the world. Furthermore, the technology for studying pathologic specimens has advanced tremendously in the past decade. Many of these advances can be applied to clinical specimens, and most others can and should be used in research protocols. Using some of these technologies can establish more accurate diagnosis and can guide therapy. Moreover, pathologic findings are used as surrogate end-points in assessing the effectiveness of therapy. Correlation and interpretation of genomic information from donors and recipients can bring to light interactions between the donor and recipient that could not be addressed before. Individualized therapy can be improved.[71,72] Targeting therapy to the cellular or humoral response may reduce or prevent progression of vasculopathy.[73] The great potential in applying the best of our current technology depends on the accuracy and the thoroughness of our pathologic evaluation of these biopsy specimens.

Finally, the conclusions from this Banff cardiac working group represent observations of more than 100,000 heart transplant biopsy specimens and thus are not anecdotal. The consensus conclusions described above are on target. Should the ISHLT council call for a formal update of the 1990 working formulation, the vision of the organizers of the Sixth Banff Conference on Allograft Pathology to sponsor a session on cardiac allograft pathology at the 2001 and the upcoming Seventh Banff Conference in 2003 should be acknowledged.

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Revised Working Formulation for Classification and Grading of Lung Allograft Rejection - 1995

Acute Rejection*

Grade	Histopathological Findings
A0 (None)	No mononuclear inflammation, hemorrhage or necrosis
A1 (Minimal)	Scattered infrequent perivascular mononuclear infiltrates not obvious at low magnification (40X). 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.
A2 (Mild)	Frequent perivascular mononuclear infiltrates surrounding venules and arterioles readily recognizable at low magnification and usually consist 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 (endotheliitis); 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. A solitary perivascular mononuclear infiltrate of significant intensity to be noted at low magnification still warrants a diagnosis of grade A2 (or greater) rejection
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. Collections of alveolar macrophages are common 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 usually associated with intra-alveolar necrotic cells, macrophages, hyaline membranes, hemorrhage, and neutrophils; there may be associated parenchymal necrosis, infarction, or necrotizing vasculitis. The obvious presence of numerous perivascular and interstitial mononuclear cells seen with grade A4 rejection permits distinction from peri-operative (reperfusion/ischemic) lung injury.

* Pathologists should mention airway inflammation and may choose to grade B lesions (see below).

Chronic Airway Rejection (Bronchiolitis Obliterans)

Classification	Histopathological Findings
Active	In addition to the fibrosis, there are intra and/or peribronchiolar submucosal and peribronchiolar mononuclear cell infiltrates usually associated with ongoing epithelial damage
Inactive	Dense fibrous scarring without cellular infiltrates; this represents old cicatricial change in the small airways with a lack of significant submucosal and peribronchiolar inflammatory infiltrates

Chronic Vascular Rejection

Refers to the vaso-obliterative process affecting arteries and veins, that affects most solid organ transplants, and reflects accelerated atherosclerosis with fibrointimal thickening of the subendothelial area by loose myxomatous connective tissue. A mononuclear cell and foamy cell infiltrate is common

Airway Inflammation§

Grade	Histopathological Findings
B0 (None)	No airway inflammation
B1 (Minimal)	Rare scattered mononuclear cells within the submucosa of the bronchi and/or bronchioles
B2 (Mild)	Circumferential band of mononuclear cells and occasional eosinophils within the submucosa of bronchi and/or bronchioles unassociated with epithelial cell necrosis (apoptosis) or significant transepidermal migration by lymphocytes
B3 (Moderate)	Dense band-like infiltrate of activated mononuclear cells in the lamina propria of bronchi and/or bronchioles including activated lymphocytes and eosinophils, accompanied by evidence of satellitosis of lymphocytes, epithelial cell necrosis (apoptosis) and marked lymphocyte transmigration through epithelium
B4 (Severe)	Dense band-like infiltrate of activated mononuclear cells in the lamina propria of bronchi and/or bronchioles, associated with dissociation of epithelium from the basement membrane, epithelial ulceration, fibrinopurulent exudates containing neutrophils, and epithelial cell necrosis
BX	Ungradeable because of sampling problems, infection, tangential cutting, etc

§ All cases of acute rejection should have a designation indicating whether coexistent airway inflammation is present and may choose to grade the intensity.

Reference Yousem SA, et al. A revision of the 1990 Working Formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Groug (LRSG) *J Heart Lung Transplantation* 1996;15:1-15.

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

[FRAMES] [NO FRAMES]

Revision of the 1990 Working Formulation for the Classification of Pulmonary Allograft Rejection: Lung Rejection Study Group

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In 1990, an international grading scheme for the grading of pulmonary allograft rejection was instituted. The use of this classification has resulted in a uniformity of grading which has allowed inter-institutional collaborations and communication unique in allograft monitoring. In 1995 an expanded group of international pathologists convened and revised the original proposal. This article summarizes the updated classification for pulmonary allograft rejection. In brief, acute rejection is based on perivascular and interstitial mononuclear infiltrates. Each grade of acute rejection should mention the presence of coexistent airway inflammation, the intensity of which may also be graded. Chronic rejection is divided into bronchiolitis obliterans—active or inactive—and vascular atherosclerosis—accelerated arterial or venous sclerosis. J HEART LUNG TRANSPLANT 1996;15:1-15.

In 1990, the International Society for Heart and Lung Transplantation sponsored a workshop to develop guidelines for the standardization of nomenclature in the histologic diagnosis of lung rejection.[1] At this meeting, a core group of pathologists developed a grading scheme for pulmonary allograft rejection which could be used to compare data from independent institutions. The intention was to develop a simple, easily taught, and readily reproducible grading system that incorporated the advantages and benefits of the multiple grading systems used at that time. The working formulation was readily adopted at most institutions performing lung transplantation in 1990, and, over the ensuing 5 years, it was accepted de novo by most new lung transplant programs. For the most part, it has been perceived as an excellent classification and grading formula. To respond to new developments in the field and an increasing wealth of experience by a greater number of pathologists, the Lung Rejection Study Group (LRSG) held a second meeting at the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, in March 1995. The purpose of this meeting was to critically assess the merits of the initial working formulation and, on the basis of published data and, to a lesser extent, experience collected at multiple transplantation centers, improve the initial working formulation. The goal was

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TABLE I Working Formulation for classification and grading of pulmonary allograft rejection

A. Acute rejection		B.* Airway inflammation – lymphocytic bronchitis/bronchiolitis
Grade 0 – None	With/without	
Grade 1 – Minimal		
Grade 2 – Mild		
Grade 3 – Moderate		
Grade 4 – Severe		

C. Chronic airway rejection – bronchiolitis obliterans
  a. Active
  b. Inactive

D. Chronic vascular rejection – accelerated graft vascular sclerosis

*Pathologists may choose to grade B lesions (see text).

to maintain scientific and biologic accuracy while striving to streamline the classification into one that would be pathologically, clinically, and therapeutically relevant. The revised classification was designed so that it was based on histologic findings of acute and chronic lung rejection primarily using transbronchial biopsy as the means of allograft monitoring in both adults and children.[2-8] Although the importance of a morphology-based system for the interpretation of pathologic abnormalities was emphasized, it was noted that all biopsy data needed to be interpreted in an integrated clinical context to allow optimum patient management and clinical decisions.[9] It was also noted that infection and rejection often occur together and can be confused histologically.[10,11] For this reason, exclusion of infection was believed to be essential for accurate and reproducible interpretation of allograft biopsies.

HISTOLOGIC GRADING OF PULMONARY ALLOGRAFT REJECTION

The LRSG recognized that alloreactive injury to the donor lung can affect the vasculature and the airways in both acute and chronic rejection. Acute rejection is characterized by perivascular and subendothelial mononuclear infiltrates and by lymphocytic bronchitis and bronchiolitis. Chronic rejection, by definition, manifests as bronchiolitis obliterans – dense eosinophilic fibrous scarring of the bronchioles – and accelerated vascular sclerosis affecting pulmonary arteries and veins.[2,3,12] Although it is probable that a continuum of vascular and airway histopathologic changes exists in the pulmonary allograft, these changes have been divided into histologic grades based on the intensity of the cellular infiltrate and the presence of dense eosinophilic hyaline fibrosis (Table I). It should be emphasized that the presence of such irreversible hyaline eosinophilic scarring of the airways and vessels represents the key histologic discriminator between acute and chronic rejection.

A. Acute Rejection

The diagnosis of acute rejection is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates. In grading acute rejection, attention should be directed at the intensity of the perivascular mononuclear cell cuffs which surround the blood vessels and at whether the mononuclear cells extend beyond the vascular adventitia and percolate into the adjacent alveolar septa. This latter feature denotes a higher grade of rejection. Although rejection processes usually affect more than one vessel, solitary perivascular infiltrates should be evaluated with criteria that are identical to those which are applied to multiple infiltrates, as outlined later. Infiltrates surrounding small vessels in the submucosa of airways are interpreted as part of the spectrum of airway inflammation and are not diagnostic of acute rejection.

Grade 0 (no acute rejection)

In grade A0, normal pulmonary parenchyma is seen without evidence of mononuclear infiltration, hemorrhage, or necrosis.

Grade A1 (minimal acute rejection)

In grade A1, there are scattered infrequent perivascular mononuclear infiltrates in alveolated lung parenchyma that are not obvious at low magnification (40× magnification); blood vessels, particularly venules, are cuffed by small round, plasmacytoid, and transformed lymphocytes forming a ring of two to three cells in thickness in the perivascular adventitia (Figure 1).

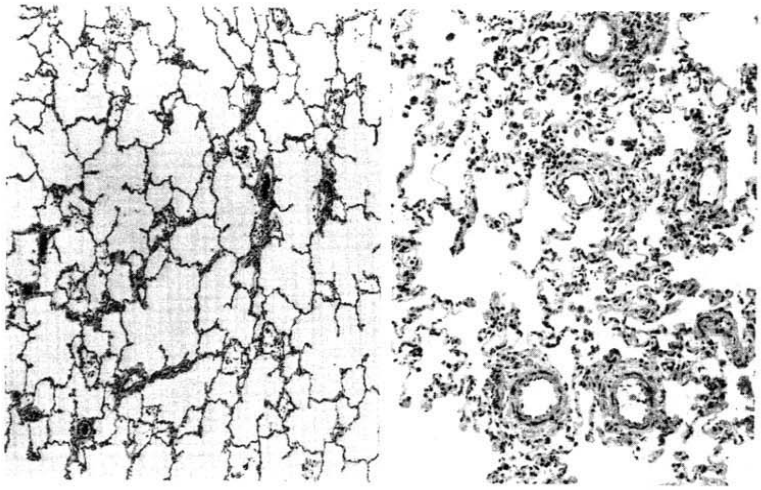


FIGURE 1 Minimal acute cellular rejection (A1): At *left,* no obvious perivascular infiltrates are seen at low magnification. At higher power *(right),* rare isolated mononuclear cells cuffed small pulmonary vessels, primarily venules.

Grade A2 (mild acute rejection)

In grade A2, frequent perivascular mononuclear infiltrates surrounding venules and arterioles are readily recognizable at low magnification (Figure 2); they usually consist of activated lymphocytes, small round lymphocytes, plasmacytoid lymphocytes, macrophages, and eosinophils. There is frequently 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.

Mild acute rejection is distinguished from minimal acute rejection by the presence of unequivocal mononuclear infiltrates which are identified at scanning magnification. Additional helpful features which suggest mild rejection are the presence of subendothelial mononuclear infiltrates, eosinophils, and coexistent airway inflammation. It is also important to note that a solitary perivascular mononuclear infiltrate of significant intensity to be noted at low magnification still warrants a diagnosis of grade A2 (or greater) rejection.

Grade A3 (moderate acute rejection)

Grade A3 shows readily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are usually associated with endothelialitis (Figures 3 and 4); 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 (Figure 5). Collections of alveolar macrophages are common in the airspaces in the zones of septal infiltration.

Grade A4 (severe acute rejection)

In grade A4, there are diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells and prominent alveolar pneumocyte damage usually associated with intraalveolar necrotic cells, macrophages, hyaline membranes, hemorrhage, and neutrophils (Figures 6 and 7); there may be associated parenchymal necrosis, infarction, or necrotizing vasculitis.

Grade A4 acute rejection may be separated from posttransplantation acute lung injury (diffuse alveolar damage) by the obvious presence of numerous perivascular and interstitial mononuclear cells, which are not present in perioperative (reperfusion/ischemic) lung injury.

B. Airway Inflammation – Lymphocytic Bronchitis/Bronchiolitis

In the 1990 classification, acute rejection was divided into four grades based on the presence and intensity of perivascular and interstitial mononuclear infiltrates. Under each grade, four suffixes were offered to reflect coexistent airway inflamma-

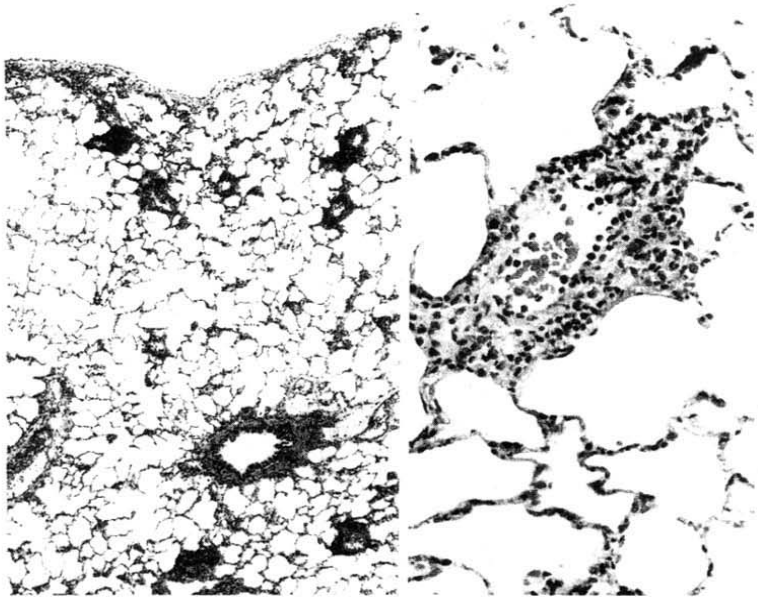


FIGURE 2 Mild acute cellular rejection (A2): At *left,* obvious perivascular infiltrates are present as one scans the lung parenchyma, and they are restricted to the perivascular adventitia. At *right,* the perivascular mononuclear infiltrate is five to eight cells in thickness. No alveolar septal infiltration is seen.

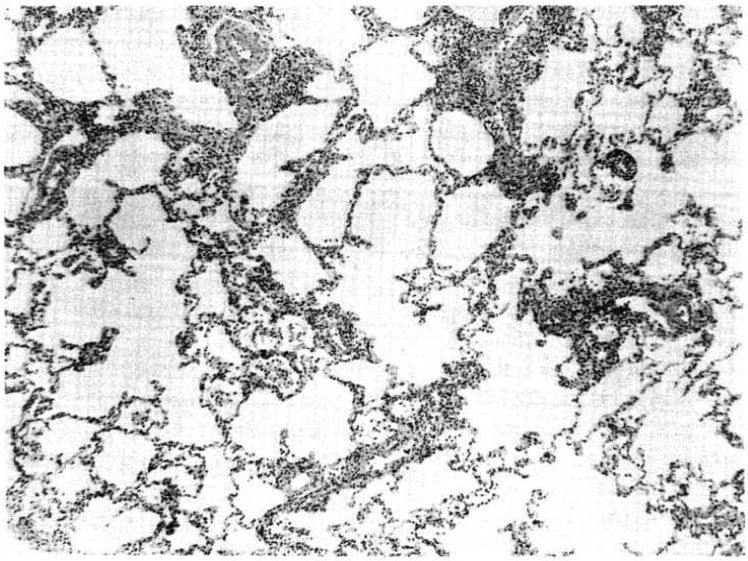


FIGURE 3 Moderate acute rejection (A3): In moderate acute rejection, the mononuclear infiltrates extend beyond the perivascular zones and trickle into adjacent alveolar septa.

tion. These designations did not reflect the intensity of the inflammatory infiltrate and were believed to be cumbersome by the 1995 LRSG. In the 1995 modification of the working formulation of pulmonary allograft rejection, it is recommended that perivascular infiltrates remain the determining factor on which to grade acute rejection. It is also recommended that the presence and intensity

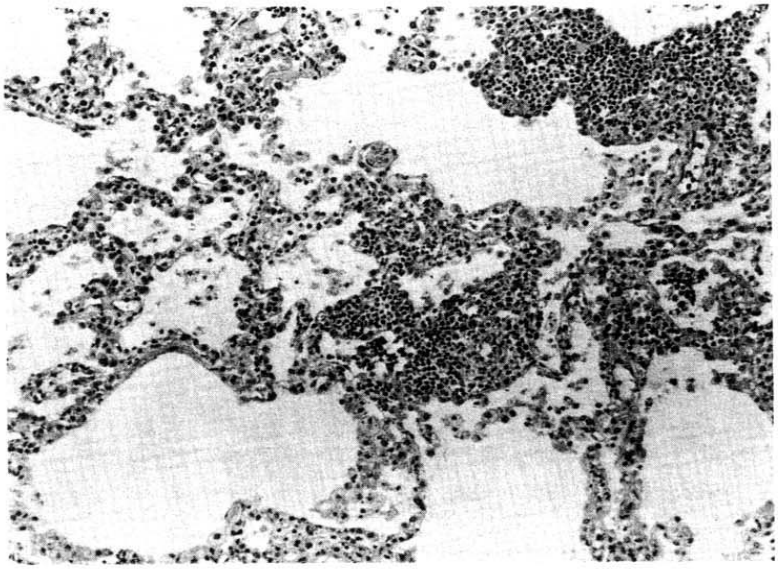


FIGURE 4 Moderate acute rejection (A3): The perivascular and septal mononuclear infiltrates are frequently accompanied by a lymphocytic bronchitis and bronchiolitis. The thickened septa define alveolar sacs which contain numerous macrophages.

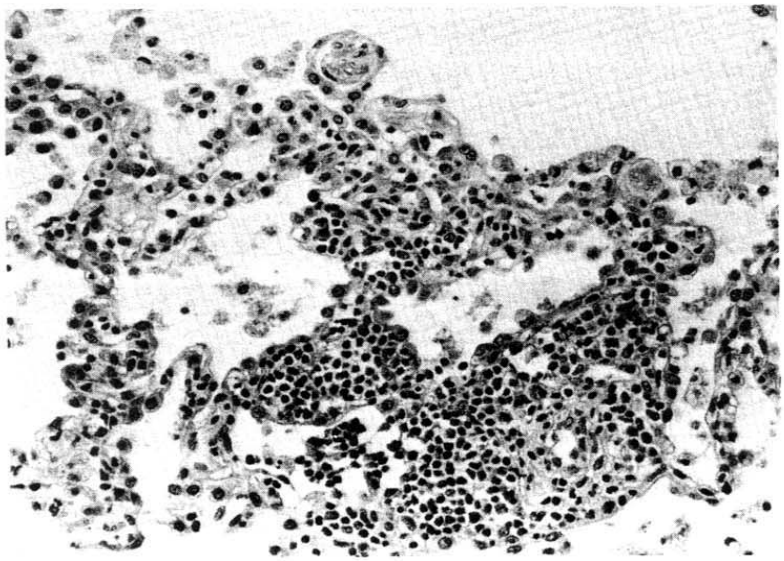
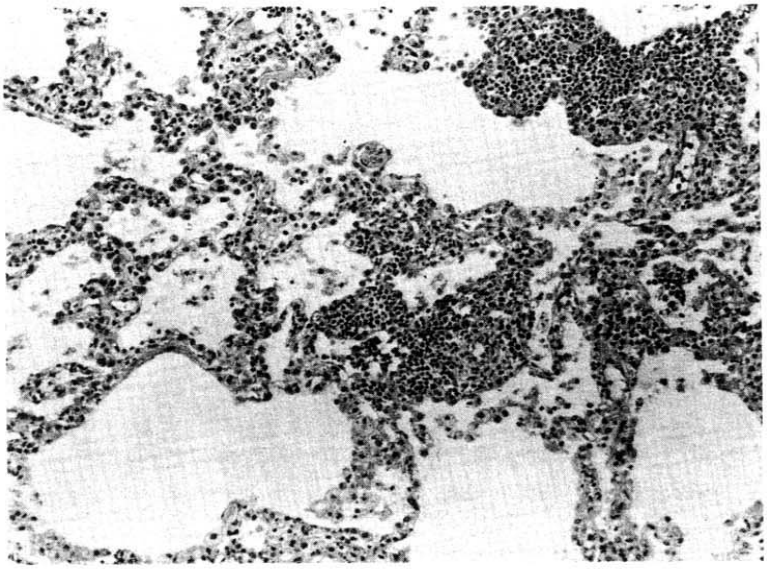


FIGURE 5 Moderate acute rejection (A3): In A3 rejection, endothelialitis and eosinophilic infiltrates are usual. Alveolar pneumocytes may have a hyperplastic or hobnail configuration.

of combined large and small airway inflammation should be noted histologically and recognized as a possible harbinger of bronchiolitis obliterans.[13] The airway inflammation should be listed as a "B" category and can be divided into five grades or simply designated as present or absent, at the discretion of each institution.

B0: No airway inflammation
B1: Minimal airway inflammation—rare scattered mononuclear cells within the submucosa of the bronchi or bronchioles (Figure 8)
B2: Mild airway inflammation—a circumferential band of mononuclear cells and occasional eosinophils within the submucosa of bronchi



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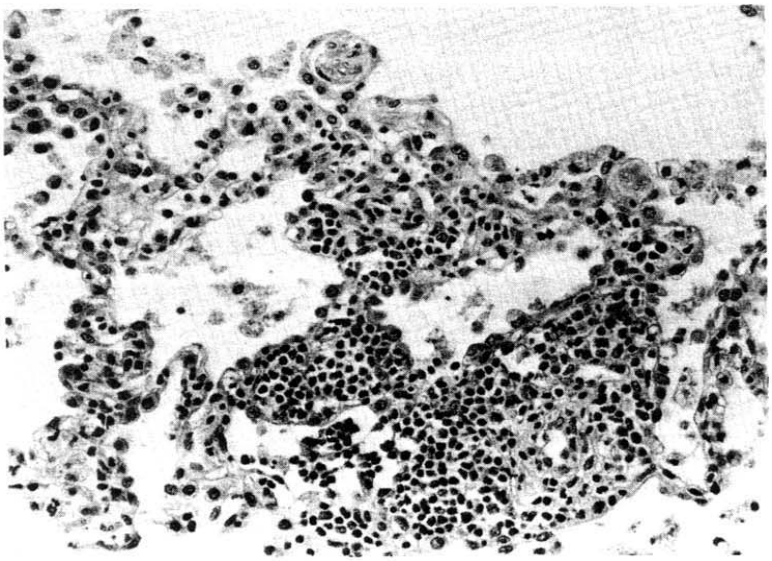

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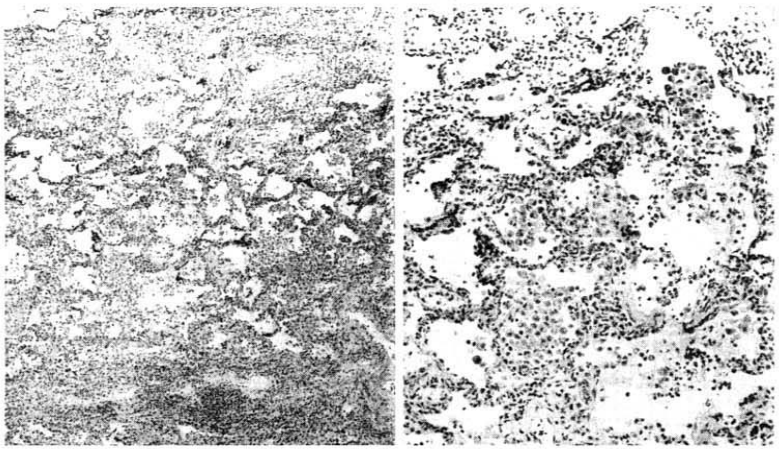

FIGURE 6 Severe acute cellular rejection (A4): Severe acute cellular rejection shows obvious perivascular, peribronchiolar, and alveolar septal mononuclear infiltrates at low magnification *(left)*. At more intermediate powers *(right)*, prominent alveolar macrophages with airspace fibrin, hemorrhage, and hyaline membranes are seen. Neutrophils are prominent and alveolar septa appear thickened and edematous.

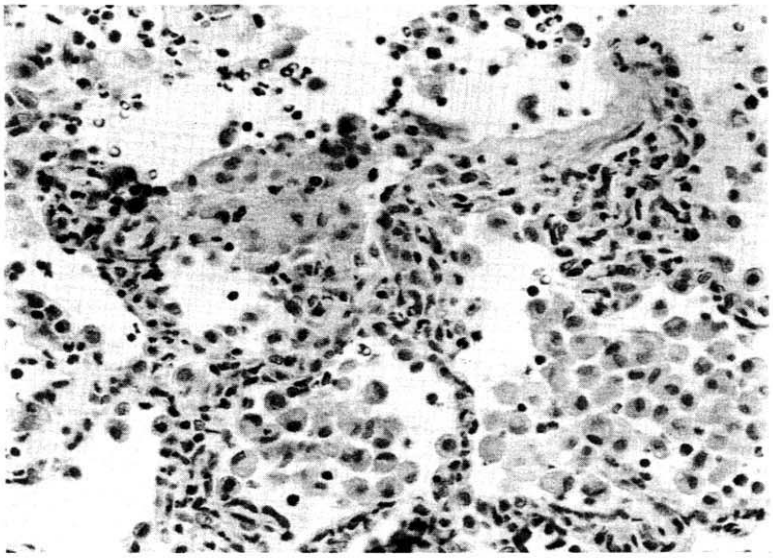

FIGURE 7 Severe acute cellular rejection (A4): Alveolar septa contain a mixed mononuclear and neutrophilic infiltrate that spills into air sacs. These alveoli contain macrophages, blood, fibrin, and hyaline membranes in severe rejection. The mononuclear infiltrate helps discriminate severe rejection from diffuse alveolar damage of other causes.

and/or bronchioles unassociated with epithelial cell necrosis (apoptosis) or significant transepidermal migration by lymphocytes (Figure 9)

B3: Moderate airway inflammation—a dense band-like infiltrate of mononuclear cells in the submucosa of bronchi and/or bronchioles including activated lymphocytes and eosinophils, accompanied by evidence of satellitosis of lymphocytes, epithelial cell necrosis (apoptosis), and marked lymphocyte transmigration through epithelium (Figures 10 and 11).

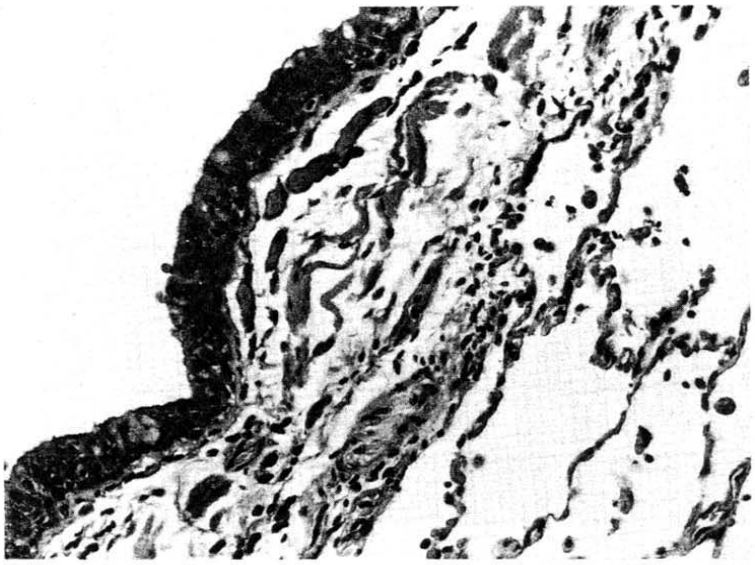

FIGURE 8 Airway inflammation—lymphocytic bronchitis/bronchiolitis, mininal (B1): A scant infiltrate of mononuclear cells is seen in the lamina propria of the airways.

B4: Severe airway inflammation—a dense band-like infiltrate of activated mononuclear cells in bronchi and/or bronchioles, associated with dissociation of epithelium from the basement membrane, epithelial ulceration, fibrinopurulent exudates containing neutrophils, and epithelial cell necrosis (Figures 12 and 13).

BX: Ungradable because of sampling problems, infection, tangential cutting, etc.

Although some members of the LRSG believed that grading the presence and intensity of airway inflammation in acute rejection was important because of the increased risk of developing bronchiolitis obliterans, many other members did not.[13-15] These latter individuals believed that clinicopathologic evidence did not convincingly prove that airway inflammation solely could be used to grade rejection because of its frequent coexistence with airway infection and problems with biopsy adequacy. For these reasons, some institutions may choose only to note the presence of airway inflammation and decline to grade its intensity. Still other centers may opt to focus on the separation of large and small airway inflammation. It should also be highlighted that dense scarring of the bronchioles is not accepted under the "B" designation.

The LRSG proposes to designate the diagnosis of acute rejection with coexistent airway inflammation as follows: Acute rejection grade—, with airway

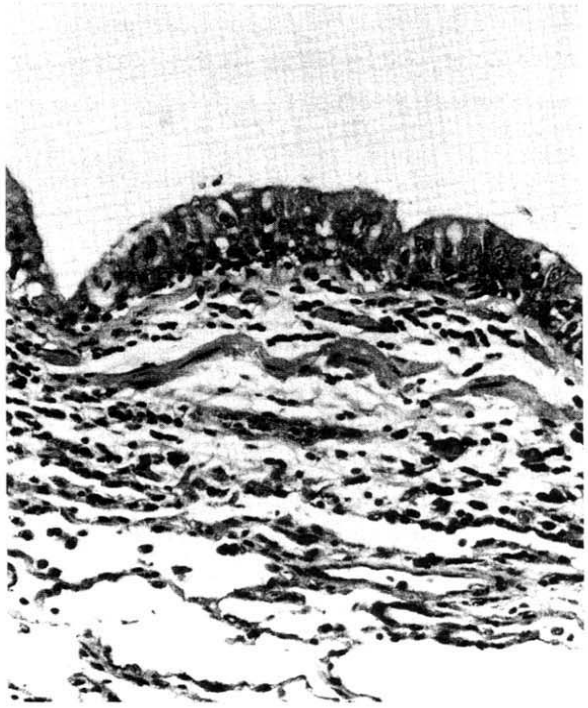

FIGURE 9 Airway inflammation—lymphocytic bronchitis/bronchiolitis, mild (B2): At low magnification, an obvious mononuclear infiltrate is seen in the lamina propria. Rare intraepithelial cells are seen but are unassociated with epithelial cell necrosis (apoptosis).

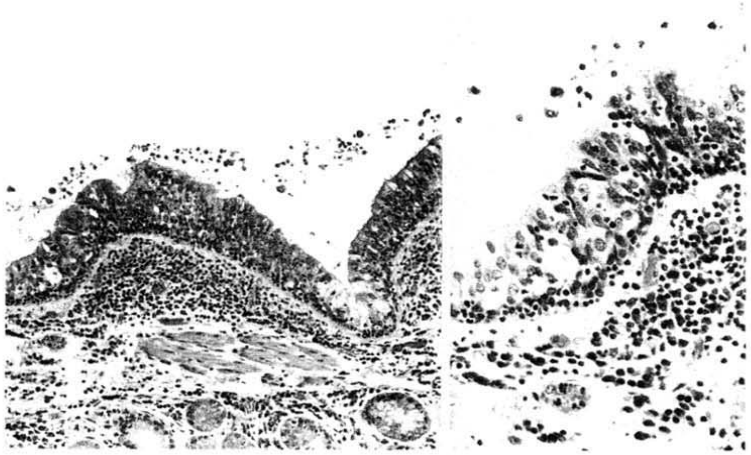

FIGURE 10 Airway inflammation—lymphocytic bronchitis/bronchiolitis, moderate (B3): At scanning power, an obvious band of mononuclear cells fills the lamina propria (left) and infiltrates the overlying epithelium (right). Within the epithelium individual cell necrosis and dropout is seen and a mild luminal exudate is present.

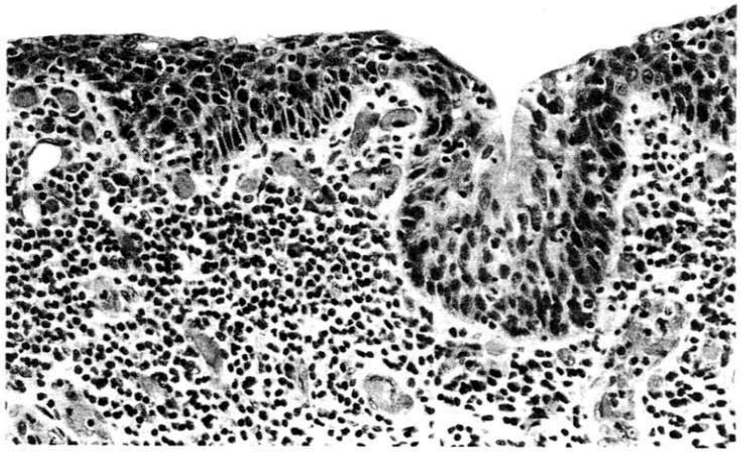

FIGURE 11 Airway inflammation—lymphocytic bronchitis/bronchiolitis, moderate (B3): In some cases, squamous metaplasia with lymphocyte emperipoiesis and epithelial injury may be seen, especially in the large cartilaginous airways.

inflammation, grade—. For example, mild acute rejection in which there is an intense airway infiltrate with epithelial cell necrosis would be diagnosed as "Mild acute rejection, grade A2, with airway inflammation, grade B3". Similarly, the 1990 category of "B lymphocytic bronchitis/bronchiolitis" would now be graded as a "A0 B—" in the 1995 classification. This format emphasizes the need to retain perivascular infiltrates as the primary focus in the histologic classification of acute lung rejection.

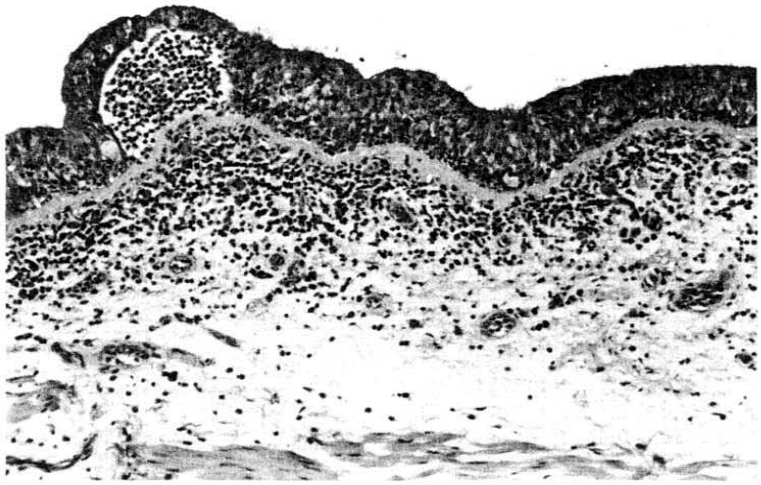

FIGURE 12 Airway inflammation—lymphocytic bronchitis/bronchiolitis, severe (B4): In the early phase of severe airway injury, the epithelium becomes dissociated from the basement membrane. At the site of fracture, a mixed infiltrate of mononuclear and polymorphonuclear cells is present in the subepithelial "blister".

C. Chronic Airway Rejection—Bronchiolitis Obliterans

Bronchiolitis obliterans is a term restricted to membranous and respiratory bronchioles, and, in the context of a pulmonary allograft, refers to dense eosinophilic hyaline fibrous plaques in the submucosa of the small airways which results in partial or complete luminal compromise (Figure 14).[2,3] This scar tissue may be concentric or eccentric, may be associated with fragmentation and destruction of the smooth muscle wall, and may extend into the peribronchiolar interstitium. Mucostasis or foamy histiocytes in the distal airspaces (endogenous lipidosis) are common. Bronchiolitis obliterans rarely develops within the first 3 months after transplantation, usually developing at the end of or after the first postoperative year.

In contrast to the 1990 scheme, the current LRSG believed that a distinction between "subtotal" and "total" forms of bronchiolitis obliterans was not worthwhile in the evaluation of the pulmonary allograft by transbronchial biopsy; however, it was believed that estimation of the relative activity of the inflammatory infiltrate was worthwhile. In those instances of coexistent acute and chronic rejection, the pathology report should reflect these processes in the following manner: "acute rejection, grade—, with active/inactive bronchiolitis obliterans, grade C a/b."

- **a. Active:** In addition to the fibrosis, there are intrabronchiolar and/or peribronchiolar submucosal and peribronchiolar mononuclear cell infiltrates usually associated with ongoing epithelial damage (Figure 15)
- **b. Inactive:** Dense fibrous scarring without cellular infiltrates; this represents old cicatricial change in the small airways with a lack of significant submucosal and peribronchiolar inflammatory infiltrates (Figure 16)

At present the significance of large airway fibrosis is uncertain.[16] In the opinion of the LRSG, this finding is nonspecific and does not warrant a diagnosis of chronic rejection. Inflammation and scarring of the bronchioles is believed to be a more significant reflection of chronic allograft injury.

D. Chronic Vascular Rejection—Accelerated Graft Vascular Sclerosis

In chronic vascular rejection, there is fibrointimal thickening of arteries and veins (Figures 17 and 18). The significance of this change is uncertain but seems to correlate with the presence of coronary artery disease in allograft hearts in combined heart-lung procedures and bronchiolitis obliterans

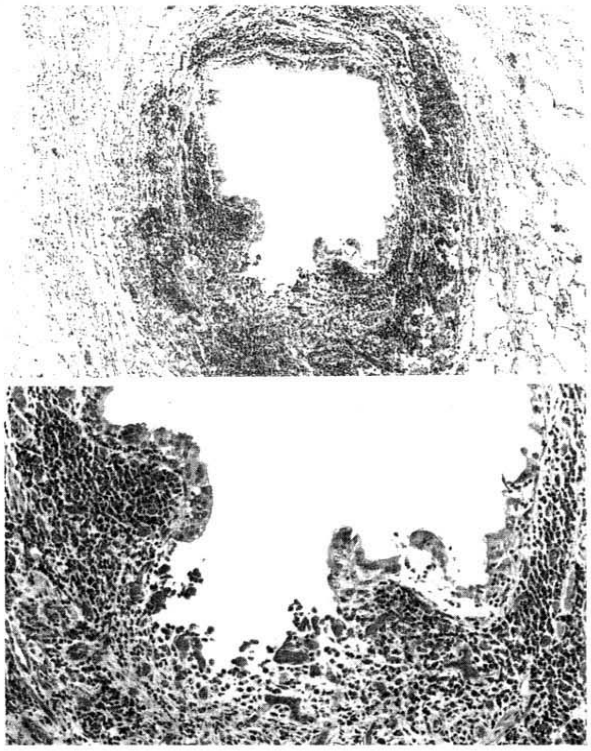

FIGURE 13 Airway inflammation—lymphocytic bronchitis/bronchiolitis, severe (B4): Bronchioles show an intense mural mononuclear infiltrate *(top)* which progresses to ulceration and is accompanied by a neutrophilic infiltrate and luminal exudate *(bottom)*.

in isolated pulmonary allografts.[12] There may also be an "active" component consisting of subendothelial, intimal, and/or medial mononuclear cell infiltrates.

The 1995 LRSG believed that the 1990 category of "vasculitis" was probably not worth retaining, as most cases of vascular injury reflect either severe acute rejection reactions or active graft vascular sclerosis.

RECOMMENDATIONS

Adequacy of Specimens

Transbronchial biopsy remains the mainstay of allograft evaluation.[17,18] It was the uniform opinion of the LRSG that at least five pieces of alveolated lung parenchyma each containing bronchioles and greater than 100 air sacs are necessary to confidently grade acute and chronic rejection. In our experience, it may be necessary for the bronchoscopist to obtain more than five biopsies to provide this minimum number of adequate alveolated pieces. Furthermore, if bronchiolitis obliterans is a consideration, more than five pieces are frequently needed to sample the small bronchioles adequately. Specimens should be gently agitated in formalin to inflate the biopsy fragments and tenderly handled by histotechnicians to avoid crush artifacts when embedding tissue in paraffin.

When appropriate sampling has not occurred, it is essential to note in the pathology report that the biopsy findings may not be fully representative of the changes in the pulmonary allograft.

Histology

Histologic evaluation should include a minimum of (1) sections from three levels of the paraffin block containing the specimens for hematoxylin and eosin stains, (2) connective tissue stains to evaluate the submucosal fibrosis essential for the diagnosis of arteriosclerosis/bronchiolitis obliterans, and (3) silver stains for fungi/pneumocystis. Beyond this minimum workup, individual investigators may augment their evaluation with a wide spectrum of histochemical, immunohistochemical, and in situ hybridization studies.

Review of Clinical History and Previous Biopsies

All biopsies should be studied by the pathologist with full knowledge of the native recipient disease and the results of the last biopsy and current bronchoalveolar lavage. In many instances this requires simultaneous review of prior slides with the current specimen. The biopsy should be reported in this context. Individual institutions may choose to use the term *ongoing rejection* for biopsies which remain unchanged from previous ones; *resolving rejection* for those biopsies where the mononuclear infiltrate is reduced in intensity and the number of activated cells has subsided as compared with a previous biopsy; or *resolved rejection* if the infiltrates have completely disappeared. In these contexts, it is recommended that a rejection score still be included.

Differential Diagnosis of Perivascular and Interstitial Infiltrates

Perivascular and interstitial mononuclear infiltrates are not specific for acute rejection, and other conditions may simulate or mimic alloreactive injury.[11,19] Differential considerations include the following:

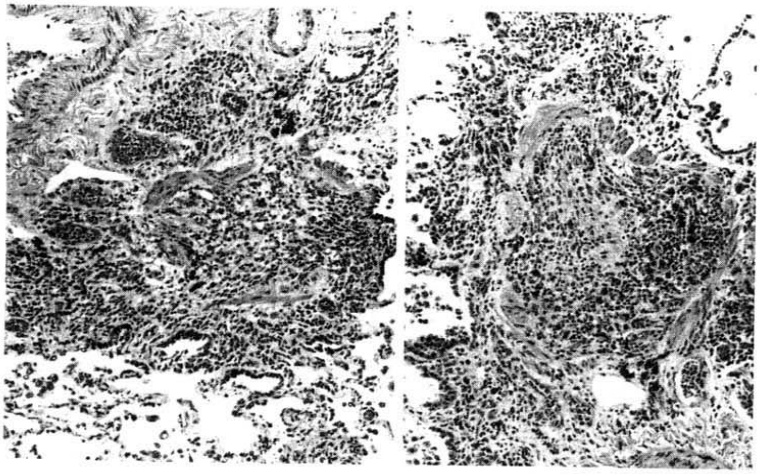

FIGURE 14 Bronchiolitis obliterans, active (Ca): At *left* the bronchovascular bundle is engulfed in an inflammatory process. The bronchiole is completely obliterated, and, at *right,* the lumen contains dense eosinophilic collagen and abundant mononuclear cells. The presence of an airway is confirmed by its position adjacent to a pulmonary artery and its curvilinear interrupted bands of smooth muscle which define the outer circumference of the airway lumen. The mononuclear infiltrate should precipitate an "active" designation to this case of bronchiolitis obliterans.

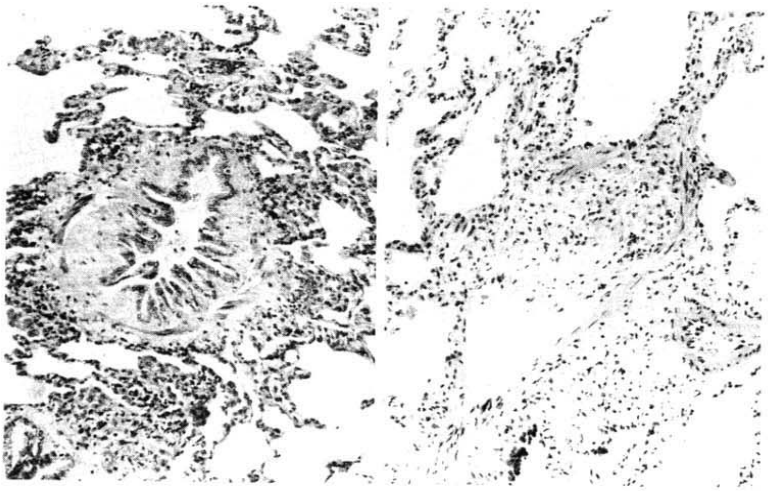

FIGURE 15 Bronchiolitis obliterans, active (Ca): At *left*, the airway contains a subtle diffuse increase in the amount of hyaline collagen throughout the lamina propria, and the airway lumen is partially compromised. At *right*, a bronchiole contains an eccentric deposit of dense scar tissue and its epithelium is flattened and attenuated. Residual smooth muscle defines the airway circumference.

Cytomegalovirus pneumonitis
Pneumocystis carinii pneumonia
Posttransplantation lymphoproliferative disease ranging from pneumonitis to active lymphoproliferative disorders[20]

Bronchial-associated lymphoid tissue, seen particularly in the submucosa and at the bifurcations of large airways, where it is well circumscribed, unassociated with epithelial injury or eosinophils, and frequently accompanied

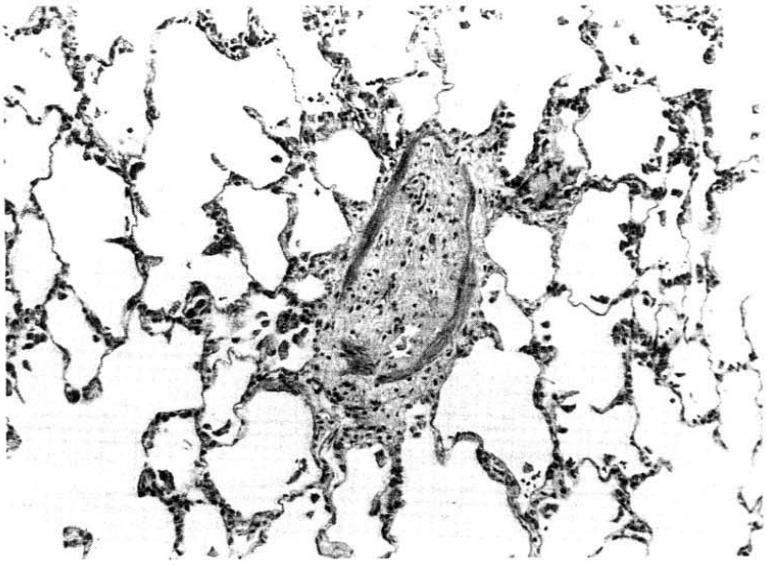

FIGURE 16 Bronchiolitis obliterans, inactive (Cb): In this instance, the lumen of the bronchiole is totally occluded by dense eosinophilic collagen. The luminal scar is ringed by residual smooth muscle.

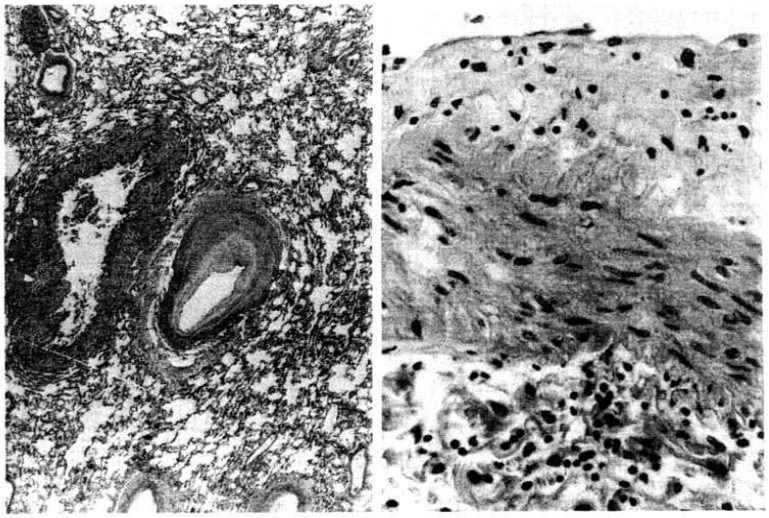

FIGURE 17 Accelerated graft vascular sclerosis (D): At *left*, a small bronchus is dilated while the adjacent pulmonary artery shows a subendothelial fibroelastotic plaque. At *right* the intima is expanded by a mixture of lymphocytes, plasma cells, histiocytes and foamy macrophages in the myointimal connective tissue.

by histiocytes containing particulate matter
Previous biopsy sites
Recurrent primary disease (e.g., sarcoidosis)
Ischemia (preservation injury)

Differential Diagnosis of Airway Inflammation

There are many causes of airway inflammation and scarring of the airways, which may result in complete obliteration in some instances. The following con-

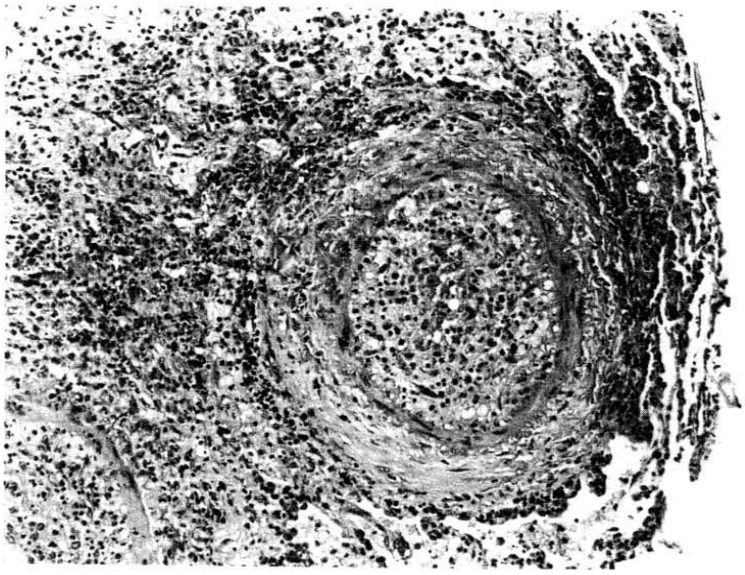

FIGURE 18 Accelerated graft vascular sclerosis (D): At times, the arteriosclerotic process may be highly cellular and be associated with an active mural and adventitial infiltrate. Complete occlusion of pulmonary arteries and veins is uncommon.

ditions represent some nonrejection causes of lymphocytic bronchiolitis/bronchitis and bronchiolitis obliterans.[2,3,21-24]

Infection: Perhaps the most frequent cause of acute and chronic inflammation of the airways is low-grade infection of the respiratory tract, particularly viral, bacterial, mycoplasmal, fungal, and chlamydial in nature.

Aspiration: Because of the loss of cough reflex and tracheal sensitivity after the transplant procedure, patients with pulmonary allografts are predisposed to recurrent aspiration; helpful features in diagnosing aspiration include the identification of exogenous material with an associated foreign body giant cell reaction within the airways, distal organizing pneumonia, and supportive radiologic studies.

Granulation tissue reactions are common in the pulmonary allograft and are manifested as intraairway and intraalveolar plugs of fibromyxoid connective tissue.[20,25] These loose edematous polyps should be distinguished from the dense eosinophilic scarring of bronchiolitis obliterans. Particular settings in which these reactions occur are resolving infection (organizing pneumonia), aspiration, the proliferative phase of diffuse alveolar damage, active or resolving acute rejection reactions, and miscellaneous other conditions. Rare reports of idiopathic organizing pneumonia in pulmonary allografts are another potential cause.[21] A variety of nonspecific reactions may occur in the pulmonary allograft. Although they usually do not cause diagnostic confusion with rejection, awareness of these injury patterns may be helpful in the evaluation of potential causes of graft dysfunction. Such reactions are listed in Table II.

Hyperacute Rejection

The LRSG believed that a morphologic definition of hyperacute lung rejection was not possible at this point in time and that it would require an integrated evaluation of clinical findings, histology, serologic studies, and immunofluorescence evaluation of allograft tissue.

Concomitant Acute Rejection and Infection

The LRSG recognized that the histologic characteristics of acute rejection and infection overlap, especially in cytomegalovirus and *Pneumocystis carinii* pneumonitis.[11,18] In some instances there may also be coexistent infection and rejection. In these cases, we continue to recommend that the pathologist indicate that he or she favors infection or rejection as the predominant process and that a follow-up biopsy be obtained after appropriate antimicrobial therapy to adequately

TABLE II Pathologic alterations in the pulmonary allograft—Nonrejection related

1. Diffuse alveolar damage/patchy acute alveolar injury—usually a manifestation of ischemic/reperfusion injury or other perioperative insults
2. Acute alveolar hemorrhage
3. Alveolar hemosiderosis—usually seen in a previous biopsy site, in resolved high grade acute rejection reactions where endothelial damage was significant, sites of prior infection, or in individuals with coagulation abnormalities
4. Post transplantation lymphoproliferative disorder
5. Recurrent native disease (e.g., sarcoidosis, lymphangioleiomyomatosis, giant cell interstitial pneumonia)
6. Smoker's macrophages in air spaces (if donor was a smoker or recipient continues/begins smoking).

assess the presence of simultaneous rejection.

Some histologic features are so distinctive that they strongly suggest infection in the context of perivascular or interstitial mononuclear infiltrates.[19] Granulomatous inflammation and punctate zones of necrosis are unusual in rejection and should raise the possibility of mycobacterial, fungal, or pneumocystis infection in the former and herpes simplex or cytomegalovirus in the latter context. Pneumocystis and fungal disease can readily be diagnosed with silver stains, as can mycobacterial infection by acid fast stains and culture. Viral infection may be confirmed by immunohistological studies or culture. Clues to cytomegalovirus infection include perivascular edema that outstrips the degree of mononuclear infiltration; disproportionate alveolar septal cellular infiltrates in comparison with perivascular cuffing; the presence of abundant neutrophils with the formation of microabscesses; marked atypia of alveolar pneumocytes; and acute airway inflammation. Finally, airspace consolidation or heavy interstitial infiltrates of eosinophils may suggest fungal infection.[26]

This revised working formulation for the classification of pulmonary allograft rejection is a proposal that is intended to simplify the original classification scheme based on clinicopathologic study and empiric observations. What has been achieved is that the formulation has been stripped of some of its complexity, in terms of the numerous suffixes under acute rejection, the subtotal/total designations of bronchiolitis obliterans, and the presence of "vasculitis." The significance of airway inflammation without fibrosis, in the context of acute rejection, is unclear in the literature. In the 1995 proposal we encourage continued investigation of its significance. As part of this study, it may also be worthwhile to grade the intensity of the latter process, if desired by the institutional pathologist and the clinical care team. This flexibility was emphasized in the original proposal and should be noted once more: the working formulation scheme should not dissuade individuals from constructing their own local classifications but should be used as a means of facilitating communication among clinicians, pathologists, disparate departments, and institutions. Furthermore, the system should always be used in the context of the individual patient, with the histopathology representing one piece of a therapeutic puzzle that includes symptomatology, physical and radiographic findings, pulmonary function studies, and microbiological culture results.

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Bronchiolitis Obliterans Syndrome 2001: An Update of the Diagnostic Criteria

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Bronchiolitis obliterans (BO) is a major cause of allograft dysfunction in lung and heart lung transplant recipients.[1,2] Clinically, progressive airflow limitation develops because of small airway obstruction. The disease has a variable course. Some patients experience rapid loss of lung function and respiratory failure. Others experience either slow progression or intermittent loss of function with long plateaus during which pulmonary function is stable. Histologic confirmation is difficult because transbronchial biopsy specimens often are not sufficiently sensitive for diagnosis. Because BO is difficult to document histologically, in 1993 a committee sponsored by the International Society for Heart and Lung Transplantation (ISHLT) proposed a clinical description of BO, termed *bronchiolitis obliterans syndrome* (BOS) and defined by pulmonary function changes rather than histology. Although this system does not require histologic diagnosis, it does recognize it.[3]

Transplant centers worldwide have adopted the BOS system as a descriptor of lung allograft dysfunction. This allows centers to use a common language to compare program results. In the years since publication of the BOS system, transplant scientists have studied basic and clinical aspects of lung transplant BO. In this document, we update and summarize new information obtained from this research and incorporate, where appropriate, the results into the BOS criteria.

The document will include the following topics: (1) criteria for BOS, (2) BOS considerations in pediatric patients, (3) risk factors for BOS, (4) pathology of BO, (5) surrogate markers for BOS, (6) confounding factors in making a BOS diagnosis, and (7) assessment of response to treatment of BOS.

CRITERIA FOR BOS

Background

When the original definition of BOS was formulated in 1993, the working group had several goals. The group aimed to provide a classification system for airway disease after lung transplantation that did not rely on histopathologic findings, was sensitive and specific, relied on diagnostic techniques available to all lung transplant physicians, and was relatively simple to understand and apply. The resulting classification system defined post-transplant

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pulmonary function using the forced expiratory volume in 1 second (FEV_1) as the primary parameter. For each lung transplant recipient, a stable post-transplant baseline FEV_1 is defined as BOS Stage 0. In patients who experience a decrease in FEV_1, progressive stages of BOS, from 1 to 3, are defined according to the magnitude of the decrease. An additional notation can reflect histologic findings: "a" designates that no BO has been identified, or that no biopsy has been done; and "b" designates that BO has been identified.[3]

Although the ISHLT classification system for BOS has gained universal acceptance, several limitations have been identified. First, the current grading system—which defines BOS 1 as a >20% decrease in FEV_1 from baseline—was not sensitive enough to pick up early, small, but potentially important changes in pulmonary function.[4–6] In addition, the mid-expiratory flow rate ($FEF_{25–75}$) was not used for defining airflow obstruction because the wider intrasubject variability of this index, in particular in recipients of unilateral transplants,[7] and the very high values observed in some patients early after surgery were considered as potential limitations. Yet several reports in recipients of bilateral and heart–lung grafts have shown that $FEF_{25–75}$ is more sensitive than FEV_1 for early detection of airflow obstruction in BOS[4–6] (one study also included recipients of single lung transplants but results in these patients were not reported specifically[8]). These observations have led to a critical re-examination of the BOS criteria, and formulation of the revised classification system as detailed in this document.

Recommendations

1. Definition of BOS: We use the term *bronchiolitis obliterans syndrome* to connote graft deterioration secondary to persistent airflow obstruction (however, note that not all patients in whom airflow obstruction develops have BOS—see confounding conditions discussed below). It is widely presumed, but unproved, that chronic rejection often contributes to functional deterioration. BOS does not necessarily require histologic confirmation; in contrast, the term *bronchiolitis obliterans* is used for a histologically proven diagnosis.
2. Definition of equipment: Spirometric measurements must be made with equipment that conforms to the American Thoracic Society standards for spirometric testing.[9]
3. Definition of baseline: The *baseline value*, to which subsequent measures are referred, is defined as the average of the 2 highest (not necessarily consecutive) measurements obtained at least 3 weeks apart, such measurements being made without the use of an inhaled bronchodilator preceding the study. The *baseline date* is defined as the date of the first measurement used to compute the baseline. The values used to compute the baselines for FEV_1 and for $FEF_{25–75}$ may be obtained on different days. Because spirometric values may increase with post-operative time, the baseline should be recalculated using the highest values achieved. The definition of baseline, and hence of BOS stages, is expected to be more accurate as more functional tests are performed.
4. Definition of confounding conditions: Patients are evaluated under this system only after evaluation of other conditions that may alter graft function and after treatment of these conditions if found. Interpretation of changes in lung function should take into account confounding conditions, which are discussed below.
5. Definition of variables: In the original staging system, a ≥20% decrease in FEV_1 from previous baseline was used to diagnosis BOS. Studies of intrasubject variability of spirometry in lung transplant recipients indicate that using a 10% to 15% decrease in FEV_1 may be more appropriate for early detection of BOS.[5–7] In addition, evidence suggests that $FEF_{25–75}$ deteriorates before FEV_1 in most bilateral and heart–lung transplant recipients with BOS.[4–6] Therefore, a *potential-BOS stage* (BOS 0-p), defined by a 10% to 19% decrease in FEV_1 and/or by a ≥25% decrease in $FEF_{25–75}$ from baseline is added to the original staging system. This potential-BOS stage alerts the physician to the need for close functional monitoring and in-depth assessment, which might include surrogate markers for BOS (see below).
6. Definition of BOS stages: For the purpose of staging, a significant decrease in FEV_1 or $FEF_{25–75}$ will be determined by the average of 2 measurements made at least 3 weeks apart, without patient use of an inhaled bronchodilator. Patients having a single measurement of decreased FEV_1 or $FEF_{25–75}$ are not evaluated until a second measurement is obtained at least 3 weeks after the initial data point. Because BOS is meant to represent a persistent alteration in lung function, additional values of FEV_1 or $FEF_{25–75}$, which may be obtained during this 3-week period,

TABLE I Original and proposed classifications of BOS

Original classification		Current proposition	
BOS 0	FEV_1 80% or more of baseline	BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25-75} > 75\%$ of baseline
		BOS 0-p	FEV_1 81% to 90% of baseline and/or $FEF_{25-75} \leq 75\%$ of baseline
BOS 1	FEV_1 66% to 80% of baseline	BOS 1	FEV_1 66% to 80% of baseline
BOS 2	FEV_1 51% to 65% of baseline	BOS 2	FEV_1 51% to 65% of baseline
BOS 3	FEV_1 50% or less of baseline	BOS 3	FEV_1 50% or less of baseline

BOS, bronchiolitis obliterans syndrome; FEF_{25-75}, mid-expiratory flow rate; FEV_1, forced expiratory volume in 1 second.

should also show a significant decrease from baseline value. The date at which a patient enters the new BOS stage is the date of the first of the 2 measurements used to confirm the stage. In case of a concomitant decrease in vital capacity (VC) and FEV_1, a restrictive ventilatory defect should be excluded before categorizing the patient in a new BOS stage (see confounding conditions discussed below).

7. Definition of functional decline: Because a universal table for converting the absolute value of FEV_1 and FEF_{25-75} to "percent predicted" does not exist, a fractional decrease in FEV_1 and FEF_{25-75} should be determined from absolute values. The fractional decrease in FEV_1 and FEF_{25-75} shall be expressed as the percent of decrease from the previously established baseline, i.e., the highest previous baseline value is used for all subsequent calculations.
8. Definition of staging system: A proposed staging system is outlined in Table I. Within each of the staging categories is an "a" and a "b" subcategory. These relate to histologic findings of biopsy specimens. This staging system is intended to describe the recipient's current status. Although BOS is considered irreversible, a minority of patients may show improvement in lung function over time. When a patient experiences such improvement in BOS stage, the worst stage that the patient has ever achieved may be noted in parentheses, if desired for study purposes. Therefore, BOS 1(2) will indicate a patient currently in BOS 1 who has been in BOS 2 at some point in the past.

BOS CONSIDERATIONS IN PEDIATRIC PATIENTS

Background

Approximately 2.5% of lung transplant candidates are ≤17 years of age. In terms of the number of transplants, number of patients on the waiting list, and number of active centers, pediatric lung transplantation lags behind adult lung transplantation and other pediatric solid-organ transplantation. Published reports indicate an incidence of BO similar to that of adults,[10–12] except in children <3 years old, in whom it may be lower.[10]

Airway inspection is particularly important in children to assess for stenosis and/or malacia at the anastomotic site. In general, the BOS criteria can be used in children who can perform pulmonary function tests reproducibly (usually at least 5 years of age). However, in defining functional decline, a decrease in percent predicted rather than a change in absolute value (see 7 above) should be used. The use of percent predicted values for FEV_1 and FEF_{25-75} should be a more accurate indicator in children because absolute values of lung function should increase with the child's growth. In older children who can perform reproducible respiratory maneuvers, the adult criteria with the use of predicted values should be easily applied. Because of the difficulty in performing pulmonary function studies in some pediatric patients, surrogate markers for BOS may assume more importance. Infants and young children require lung function testing by other techniques, most commonly through the rapid compression technique. The combined use of forced expiratory flow at functional residual capacity, normalized by the measured functional residual capacity, is a useful technique to separate anastomotic complications from peripheral airflow obstruction. Techniques for lung function testing in infants and young toddlers provide tools for performing serial lung function testing in lung transplant recipients of this age.[13,14] Experience with such techniques is limited to 1 pediatric lung transplant center,[15] and further clinical research with newer techniques is clearly indicated.

Recommendations

1. Pediatric patients suspected of having BO should undergo bronchoscopic examination of the airways and transbronchial biopsy when possible. On occasion in young patients or in those with obscuring clinical or large airway pathology, an open lung biopsy to assess for histopathology may facilitate early therapeutic intervention.
2. In general, the criteria for BOS can be applied in children who can complete pulmonary function tests satisfactorily. However, declines in function should be expressed in terms of percent predicted instead of absolute values because of lung and airway growth. Newer techniques facilitate measurements in infants and have been used to assess for BOS.

RISK FACTORS FOR BOS

Background

Many factors have been reported as risk factors for BOS. However, quality of data is often a problem because almost all existing information derives from retrospective studies with no control groups and reflects the experience of single centers. Numbers are small and often difficult to interpret. In some cases, risk factors seem to have been more important in the earlier years of lung transplantation, e.g., cytomegalovirus (CMV) infection. This may reflect a change in the risk environment because of the use of prophylactic antimicrobial regimens, changing immunosuppressive approaches, or the increasing experience of transplant management teams.

Alloimmunologic injury directed against endothelial and epithelial structures have been thought to mediate BOS, but non-alloimmunologic inflammatory conditions including viral infections or ischemic injury may also play a role. Risk factors reported in the literature will be designated as (1) probable risk factors, (2) potential risk factors in need of further analysis, and (3) hypothetic risk factors.

Probable Risk Factors

Acute rejection and lymphocytic bronchitis/bronchiolitis belong to this category. Six separate publications document the increased incidence of BOS in patients with acute rejection episodes, especially when multiple and/or long-lasting and/or high-grade episodes occur.[16–21] Two additional publications document the role of late acute rejection in the development of BOS.[22,23] Five publications report that lymphocytic bronchitis/bronchiolitis is a risk factor for BOS, when infection has been excluded as a cause of an inflammatory airway process.[18,20,24–26]

Medication non-compliance is a known risk factor for rejection and graft loss after kidney, heart, and liver transplantation.[27–30] Medication non-compliance also is perceived as a risk factor after lung transplantation, although results supporting this have not been published.

Cytomegalovirus is difficult to interpret as a risk factor for 2 main reasons: the pattern of CMV has changed with the widespread use of prophylactic strategies directed against the virus and with varying definitions of infection, disease, and pneumonitis among institutions. Eight reports consider CMV a risk factor for BOS,[16,19,22,25,31–34] whereas 4 other studies reported no impact of the virus.[18,20,21,35] Four other studies document a decreased risk of CMV in the development BOS—either decreased incidence or delay in onset—after the use of CMV prophylaxis.[17,36–38] However, data from the pre-prophylaxis era in which CMV pneumonitis was more prevalent strongly correlates pneumonitis as a BOS risk factor.

Potential Risk Factors

Potential risk factors are so designated because of conflicting data, suggestive but not definitive data, or differences in definitions of the specific risk factor between centers so that available data cannot be interpreted. These factors include (1) organizing pneumonia; (2) bacterial, fungal, and non-CMV viral infection; (3) older donor age; (4) longer graft ischemic time; and (5) donor antigen-specific reactivity.

Two centers report that organizing pneumonia is a risk factor for BOS. One of these centers reported that it was a univariate risk factor for BOS. The data are from small numbers and not complete enough to designate it a probable risk.[18,19]

A surprisingly small body of data has been published that report the impact of bacterial, fungal, and non-CMV viral infections. One center reported bacterial and P carinii pneumonia as risks during the period before broad-spectrum prophylaxis in lung transplantation.[17] In a more recent report, bacterial or fungal pneumonia was not associated as an univariate risk with an increased rate of BOS, but did increase the acute rejection score in a multivariate model.[18] A peak incidence of BOS onset in the respiratory virus season suggested to one set of authors that common respiratory viral infections may trigger the complication.[39] Treatment of respi-

ratory syncytial and parainfluenza viruses decreased the incidence of BOS in one center.[40]

Donor age did not correlate with BOS in a large population in the United Kingdom; however, the ISHLT 2000 Registry identified donor age as a risk factor.[19,41] The Registry identified graft ischemic time as a second donor risk factor, a finding also differing from the findings of the UK study.

Persistent donor antigen-specific reactivity has reportedly led to increased rates of BOS, and conversely, donor-specific hyporeactivity was reported as protective.[42,43] Preliminary experience from the Pittsburgh Transplant Group has shown that the infusion of donor bone marrow in combination with lung transplantation increases donor cell chimerism and donor antigen-specific hyporeactivity, and is associated with a lower incidence of BOS.[44]

Hypothetic Risk Factors

Hypothetic risk factors include factors supported by theoretical considerations but having scanty clinical evidence to date. These factors include (1) underlying disease, (2) genotype of the recipient for certain cytokine gene polymorphisms, (3) HLA-mismatching, and (4) gastroesophageal reflux with aspiration.

Two studies suggested that underlying diagnosis is a risk factor and that patients with pulmonary hypertension may be more at risk of BOS; in a third study, this was not the case.[17,25,33] The ISHLT 2000 Registry identifies emphysema patients as having the best survivals but does not identify freedom from BOS as the reason.[41]

Data are emerging on the potential role for genotypic susceptibility to development of BOS. Cytokine gene polymorphisms of tumor necrosis factor (TNF)–α, interferon γ, IL-10, IL-6, or TGF-β genes may play a role.[45] Available data are scant and conflicting.[46]

Data also conflict on HLA mismatching, with most series showing no association.[17,18,20] One institution has documented an increased risk of BOS with the development of anti-HLA Class I antibodies.[47] Confusion in this area arises in part from the small number of transplantations performed in individual centers and because no attempt at HLA matching is made. Therefore, it is uncommon for any center to have more than a few HLA-matched recipients. In the largest study yet reported that involves HLA matching, 3,549 lung transplantations were reviewed using the United Network for Organ Sharing (UNOS)/ISHLT Registry database. Only 164 patients had 2 or fewer mismatches. No significant association could be found between HLA mismatching and BOS development.[48]

TABLE II Risk factors for BOS

Probable risk factors:
Acute rejection
Lymphocytic bronchitis/bronchiolitis
CMV pneumonitis
Medication non-compliance
Potential risk factors:
CMV infection (without pneumonitis)
Organizing pneumonia
Bacterial/fungal/non-CMV viral infection
Older donor age
Longer graft ischemic time
Donor antigen-specific reactivity
Hypothetic risk factors
Underlying disease
HLA-mismatching
Genotype of recipient
Gastroesophageal reflux with aspiration

BOS, bronchiolitis obliterans syndrome; CMV, cytomegalovirus.

Case reports and small series have suggested an incremental risk from gastroesophageal reflux disease with aspiration and from impaired mucociliary clearance.[49–52]

Several additional factors, including history of smoking or asthma in the donor, head injury as cause of death, airway ischemia, and diffuse alveolar damage (reperfusion injury), have been proposed as risk factors for late organ dysfunction. However, convincing data to support the role of these factors are lacking.[20,53–56]

A differential in the prevalence of BOS among unilateral, bilateral, and heart–lung grafts has not been documented.

Recommendations

1. Many factors have been reported as potential risk factors for BOS, but proven causal relationships are difficult to establish.
2. Based on available information, Table II summarizes the probable, potential, and hypothetic risk factors.

PATHOLOGY OF BO

Background

Bronchiolitis obliterans is a cicatricial process that affects the small airways of the allograft lung. Conceptually, BO is thought to result from chronic lung rejection, although not exclusively. It progresses through a sequence of lymphohistiocytic-mediated

cytotoxicity directed at the respiratory epithelium. The initial process is a lymphocytic infiltrate of the sub-mucosa of the airways with migration of lymphocytes through the basement membrane into the epithelium.[57] At this site, epithelial cell necrosis occurs with denudation of mucosa. A secondary cascade of non-specific inflammatory mediators and cytokines attracts other cells, including neutrophils. The reaction stimulates migration of fibroblasts and myofibroblasts into the luminal exudate. Formation of an intraluminal fibromyxoid granulation tissue polyp results. In some instances, macrophage collagenases may dissolve the polyp. The diagnostic fibrous scarring can be eccentric with formation of a fibrous plaque in the wall of the airway; concentric with the interposition of a "donut" of collagen tissue; or the granulation tissue may completely obliterate the lumen of the airway, reducing the air passages to stenotic cords of scar tissue ("vanishing airways disease").[58] At the time of histologic diagnosis, the airway injury may be temporally heterogeneous with some airways showing only cellular infiltrates, some displaying active fibroplasia, and others demonstrating inactive fibrosis.

Bronchoscopy may exclude other causes of deteriorating lung function, but diagnosing BO with transbronchial biopsy specimens may be extremely difficult. It requires multiple, large fragments, and even then, diagnostic lesions may be missed. Trichrome and elastic tissue stains may assist in recognizing the damaged or obliterated airway. When the clinical diagnosis is unclear and transbronchial biopsy specimens have not offered an unequivocal answer, open lung biopsy may be necessary.

The initial document describing BOS used an "a" sub-category to designate no pathologic evidence of BO (or no pathologic material for evaluation) and a "b" sub-category to mean that pathologic evidence of BO was obtained. The usefulness of these designations has not yet been validated.

Recommendations

1. Histologic activity may not reflect the clinical activity monitored by pulmonary function tests.
2. The term *bronchiolitis obliterans* should be used only when histology demonstrates dense fibrous scar tissue affecting the small airways.
3. The presence of only lymphocytic sub-mucosal infiltrate or intraluminal granulation tissue is not sufficient for a diagnosis of BO.
4. If the obliterative lesion is associated with a mononuclear infiltrate, it is defined as active; fibrosis without inflammatory cells is defined as inactive.
5. An "a" sub-category designates no pathologic evidence of BO (or no pathologic material for evaluation). A "b" sub-category means that pathologic evidence of BO has been obtained.

SURROGATE MARKERS FOR BOS

Background

The diagnostic criteria for BOS are based on a decrease in lung function. Various indirect measures or analyses have been undertaken to identify alternative early markers of a decrease in graft performance. Perhaps these markers can provide a surrogate means of predicting disease or of monitoring disease activity, with the aim of enabling early therapy to block a relentless decrease in lung function.

Bronchoalveolar lavage (BAL) analysis

A number of cross-sectional studies[59–64] and 3 prospective studies[7,60,64] indicate an association between BOS and BAL neutrophilia, and they indicate that this alteration may actually precede the 20% decrease in FEV_1 required for the spirometric diagnosis of BOS.[7,60,64] In addition, a persistent increase in BAL neutrophilia is an independent predictor of mortality after lung transplantation.[65] Other preliminary studies implicate various BAL markers or mediators in the pathogenesis of BOS (e.g., IL-8, markers of oxidative stress, neutrophil elastase, TGF-β, platelet derived growth factor (PDGF), collagen I/III, insulinlike growth factor-1). Although these markers may provide useful concepts for exploring the mechanisms behind development of chronic allograft rejection, they are not yet sufficiently robust tests to contribute to the clinical diagnosis of BOS.

Exhaled nitric oxide

Exhaled nitric oxide (eNO) provides a potentially useful tool in diagnosing acute and chronic allograft rejection in lung transplant recipients. Several lung transplant centers have evaluated eNO and found it to be reproducible, repeatable, and reflective of NO levels in the lower airways.[66,67] The source of eNO in allograft pathology remains to be identified, but potential sources include epithelial cells and infiltrating leukocytes;[67–69] eNO has a close link with BAL neutrophilia.[67] A cross-sectional study of 104 lung transplant recipients noted elevated eNO in

lymphocytic bronchitis and BOS Stage 1 but not in BOS Stages 2 and 3.[70] Other studies have reported a variable association between increased eNO and BOS.[71,72]

Air trapping shown on expiratory computerized tomography scans

Imaging is a potentially simple and repeatable means of assessing BOS. High-resolution computerized tomography (CT) scanning is the most accurate imaging tool for diagnosing BOS. On inspiratory scans, several abnormalities have been associated with BOS, including bronchial dilatation, bronchial wall thickening, and mosaic perfusion pattern, although these findings lack sensitivity.[73–76] In contrast, the presence of air trapping on expiratory CT scans is an accurate indicator of the bronchiolar obliteration underlying BOS.[77–80] In patients with BOS, the pulmonary lobules that have normal airways increase in density during the expiratory phase, whereas areas with diseased airways cannot empty and remain radiolucent secondary to the obstructive bronchiolar inflammatory and fibrotic changes. In a recent prospective study that included 111 expiratory CT scans in 38 heart–lung transplant recipients, the presence of air trapping >32% had a 87.5% sensitivity and specificity for the diagnosis of BOS, and in some patients this preceded the spirometric criteria for BOS.[79] Conversely, having <32% of air trapping had a high negative predictive value until the fifth post-operative year. In another, smaller study, an air-trapping score provided a sensitivity of 74% and a specificity of 67% for histopathologically proven OB.[80]

Bronchial hyper-responsiveness.

Bronchial hyper-responsiveness has been reported in patients who have undergone lung transplantation, although some studies have been negative for this finding.[81–89] In a recent longitudinal study that included 111 patients undergoing bilateral lung transplantation, Stanbrook and Kesten[89] reported that 30% of patients had a positive methacholine challenge at 3 months after transplant and were significantly more likely to have BOS; the mean time to development of BOS was 16.9 months. A retrospective study of 94 lung transplant recipients showed that the presence of a bronchodilator response at low lung volume had a sensitivity of 51%, a specificity of 87%, and a positive predictive value of 81% for the diagnosis of BOS.[90] This study also noted that the bronchodilator response may precede BOS by months.

Distribution of ventilation.

Two recent prospective studies have shown that indices of ventilation distribution (e.g., the alveolar plateau slope obtained for nitrogen or helium during single-breath washout) may detect BOS earlier than do conventional pulmonary function tests.[6,7] Reynaud-Gaubert et al[6] considered a nitrogen slope >3% as abnormal, whereas Estenne et al[6] considered significant a 100% increase above baseline.

Problems with and quality of data.

In addition to the limitations that clinical trials in lung transplant recipients frequently encounter (small sample size, retrospective study, lack of adequate control group), 3 specific limitations should be mentioned in the context of the surrogate markers for BOS:

1. Many of the markers discussed above have been used and validated primarily in recipients of heart–lung and double-lung grafts, e.g., air trapping on expiratory CT and indices of ventilation distribution. No clear effect on eNO caused by the type of surgical procedure or the type of disease in the native lung has been demonstrated in transplant recipients who are stable or who have BOS. This point deserves further study.
2. Specificity of the markers discussed here for the diagnosis of BOS is low, e.g., BAL neutrophilia may be caused by infection, and eNO or indices of ventilation distribution may increase in acute rejection or infection.
3. Thresholds indicating a significant alteration from the stable state, particularly for BAL neutrophilia and eNO, have not been clearly established. These thresholds must be determined on the basis of standardized baseline values[91] using intrasubject coefficients of variation.

Recommendations

1. BAL neutrophilia and elevated cytokine levels, eNO, air trapping on expiratory CT scans, bronchial hyper-responsiveness, and measures of an altered distribution of ventilation have all been identified as early markers of BOS. However, none is specific or sensitive enough to be used reliably for diagnosing BOS.
2. The presence of an abnormal level of a surrogate marker should alert the clinician to the potential for BOS onset.

CONFOUNDING FACTORS IN DIAGNOSING BOS

Background

Lung function is exquisitely sensitive to complications that affect the allograft, such as rejection, infection, and anastomotic complications. These complications often produce some degree of airflow obstruction and may lead to a pattern of functional deterioration, which is qualitatively similar to that seen in BOS. In addition, several complications that affect the native lung and disease progression in the native lung may contribute to changing pulmonary function. This section addresses (1) confounding factors in the graft that apply to all types of transplants, (2) confounding factors that affect the native lung in single lung transplants, and (3) confounding factors that cause a restrictive ventilator defect.

Factors that affect the graft.

- Infection and rejection: Symptoms characteristic of infection frequently herald the onset of BOS, and a community-acquired respiratory bacterial or viral infection may be documented. Similarly, some patients with recurrent or refractory acute rejection (including acute cellular rejection and lymphocytic bronchitis/bronchiolitis) progress to BOS. Therefore, the presence of infection or acute rejection, which may produce airflow obstruction,[92] does not exclude the diagnosis of BOS and may confound its early diagnosis. If the lung function change persists after appropriate treatment, the diagnosis of BOS can be made.
- Anastomotic complications: Complications at the site of the tracheal or bronchial anastomosis (e.g., stenosis, dehiscence, and malacia) may alter forced expiratory flows and volumes. Because these complications occur early after surgery, they are generally recognized before the diagnosis of BOS is suspected. Yet interpretation of functional changes in the presence of anastomotic complications may be difficult because it is not always easy to determine whether stenosis/malacia or the development of BOS is responsible for a decrease in lung function. The final diagnosis is left to the discretion of the individual physician.
- Disease recurrence: Some primary diagnoses have recurred in the lung graft. These include sarcoidosis, lymphangioleiomyomatosis, Langerhans cell histiocytosis X, alveolar cell carcinoma, desquamative interstitial pneumonitis, panbronchiolitis, and giant cell interstitial pneumonitis.[93–99] Disease recurrence may cause graft dysfunction, may confuse the diagnosis of BOS, or may coexist with BOS. In other cases, e.g., sarcoid, recurrent disease may have little functional effect. In the context of recurrent disease, the diagnosis of BOS must be made with caution unless histologic confirmation is available.
- Aging: In long-term survivors, the physiologic aging process of the lung is expected to significantly decrease both FEV_1 and $FEF_{25–75}$. However, making firm recommendations as to how to account for this factor is not possible because the rate of functional decline with age in an otherwise normal graft remains unknown.

Factors affecting the native lung.

- Native lung hyperinflation: Acute native lung hyperinflation is a complication reported in patients with emphysema who receive single lung transplants.[100–104] If acute native lung hyperinflation occurs early after surgery, it does not interfere with the diagnosis of BOS. However, intermediate- and long-term, progressive hyperinflation of the emphysematous lung may be associated with graft dysfunction.[105] Studies in stable recipients of single lung transplants for emphysema have shown that the total lung capacity of the graft is decreased to 66% to 79% of the predicted normal values.[106,107] In a small sub-set of patients, hyperinflation of the native lung may worsen over time and lead to clinical and functional changes similar to those produced by BOS (e.g., dyspnea, worsening airways obstruction, hypoxemia, accentuated radiologic shift of the mediastinum toward the graft, and V/Q mismatch). In this context, lung volume reduction or lobectomy of the native lung may improve lung function in selected individuals.[108–112] The mechanisms underlying delayed native lung hyperinflation have not been precisely identified, and more importantly, no easy means exist to distinguish between this complication and BOS. Moy et al[113] suggested that measuring lung resistance during inspiration may be helpful in this context, but further studies must validate the use of this variable. From a practical standpoint, if a patient with emphysema who has undergone single lung transplantation has worsening airflow obstruction without another specific cause, the patient should be considered to have BOS.
- Disease progression in patients without emphysema: Disease progression in the native lung may contribute partially to a change in overall lung function. However, because the native lung usually makes only a minor contribution to maximal expiratory flows and volumes, disease progression

is not expected to be a frequent confounding factor for the diagnosis of BOS.
- Other complications: Several complications may occur in the native lung and affect approximately 25% to 40% of the recipients.[114–117] Infectious complications are more frequent, and recipients who have emphysema seem to be at increased risk. However, complications affecting the native lung are easy to identify and generally do not interfere with the diagnosis of BOS.

Factors causing a restrictive ventilatory defect

Several diseases may decrease static and dynamic lung volumes in recipients of lung transplants. These conditions include increased body mass index,[118] respiratory muscle weakness unrelated[119] or related to generalized neuromuscular disorders, pleural effusion, rib fractures, chronic post-operative pain, and pulmonary edema. The functional impact is expected to be a decrease in both VC and FEV_1. Therefore, in the presence of a decreased FEV_1, an unchanged FEV_1/VC ratio should alert the clinician to exclude the above-mentioned conditions before considering the diagnosis of BOS. In the presence of a concomitant decline in VC and FEV_1 with an unchanged FEV_1/VC ratio, the baseline for FEV_1 and for $FEF_{25–75}$ may be reset to a lower value.

Recommendations

1. Infection, acute rejection, disease recurrence, and anastomotic complications can confound the diagnosis of BOS. These diagnoses should be excluded or treated before assigning a designation of BOS.
2. Following single lung transplant for emphysema, native lung hyperinflation occasionally results in a functional and physiologic picture similar to BOS. In this setting, a precise diagnosis may be impossible and each case should be judged on its individual characteristics.
3. A number of conditions can occur that cause decreases in both the VC and the FEV_1 (e.g., an increase in body mass index, muscular weakness, pleural effusion, etc.) without a decrease in the FEV_1/VC ratio. Such comorbidities must be excluded before assigning a diagnosis of BOS.

ASSESSING BOS RESPONSE TO THERAPY

Background

Although the fibrous obliteration of the bronchioles seen in BO probably is irreversible, the histologic lesions are often heterogeneous, with some airways showing inflammatory infiltrates potentially amenable to treatment. This probably explains why some patients show functional stabilization or improvement with treatment. Assessing response to therapy is difficult in individual patients because of the high variability of the disease response of an individual to an intervention.[9,120–125] This document proposes methods of assessing populations and study purposes. Retrospective and non-randomized designs, small sample size, absence of a control group, and relatively short follow-up have weakened published studies of treatment for BOS. Given the variable natural course of BOS, an appropriate number of patients in randomized studies with both a treated and a control arm is mandatory, and the method used to assess the response to therapy must be standardized. Designing multicenter studies with a large number of patients may allow stratification according to several factors that may affect response to therapy, e.g., BOS stage, association with acute rejection or lymphocytic bronchiolitis, rate of functional decrease, association with infection, time from transplantation to development of BOS, etc.

Recommendations

1. Assessing response to therapy should be based on the diagnostic criteria for BOS, i.e., FEV_1. Absolute values of FEV_1 measured before and after the therapeutic intervention should be plotted over time, and the slopes should be obtained by linear regression analysis. At least 3 measurements with a negative slope, obtained over 1 to 3 months, should be used to compute the slope before treatment. This slope should be calculated using all the data points obtained in the 1 to 3 months before initiation of treatment; the first point used should be the first measurement below the BOS threshold. The slopes after treatment should include all data points obtained after initiation of treatment and for at least a period of 6 months (see Appendix). A decrease in the rate of functional decline after initiation of treatment may be coincidental (i.e., reflect the natural history of the disease) and may not reflect a therapeutic benefit. This underscores the difficulty in interpreting the response in individual patients and emphasizes the need for control groups in prospective studies.
2. Stability may occur spontaneously after onset of BOS. This results in a flat FEV_1 slope (instead of a negative slope), and assessment of therapeutic intervention is problematic. Because this course of the disease occurs relatively frequently, pro-

spective studies assessing intervention probably will require large numbers of patients and prolonged study periods.
3. Comparisons of frequency of occurrence and progression through BOS grades are appropriate end-points for assessing therapy. In individuals, improvement in BOS grade is not expected or consistent with the current understanding of this syndrome.

FUTURE STUDIES

The committee recognizes that although BOS is the most common complication leading to chronic graft dysfunction and death of lung transplant recipients, it remains poorly understood. However, the course of disease progression may be quite variable for individual patients, suggesting a heterogeneous pathogenesis. Although lung function may decrease rapidly, leading to respiratory failure and death in some patients, other patients may survive for years with either stable or slowly progressive loss of lung function. Therefore, we recommend use of this document to stimulate collection of data and to underlie prospective studies that will lead to better understanding of and eventually prevention of this devastating complication. We suggest the following research priorities.

Risk Factors

1. Collation of existing large data bases to better define risk factors
2. Collaborative prospective collection of data in a centralized database to subsequently correlate with development of BOS

Criteria for BOS

1. Prospective collaborative studies to validate the usefulness of the new BOS 0-p stage, in particular in recipients of single lung transplants.
2. Prospective collaborative studies to evaluate survival and quality of life after BOS onset at each stage.
3. Prospective collaborative studies to define different courses of disease progression, risk factors for disease progression, and time of onset.
4. Prospective collaborative studies to evaluate the relative impact on survival, quality of life, and exercise capacity in double vs single lung transplant recipients.

Surrogate Markers

1. Prospective collaborative studies comparing surrogate markers with lung function and ability to predict future decreases in lung function.
2. Prospective collaborative studies to establish normative data and thresholds for significant change in markers such as BAL neutrophilia and eNO; prospective collaborative studies correlating changes in different surrogate markers.

APPENDIX

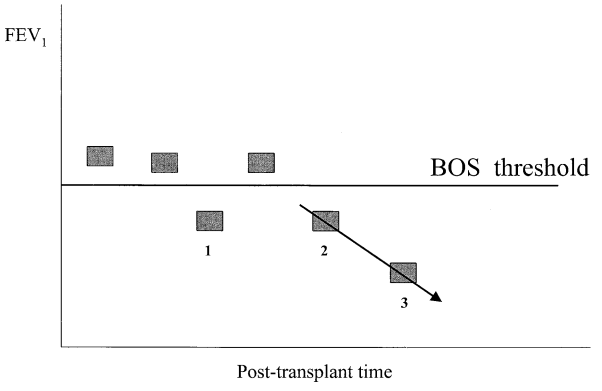

FIGURE 1 Event 1: drop below BOS threshold, not validated by second measurement. Event 2: first BOS measurement and time of onset of BOS defined by validating event #3. FEV_1 decline = slope of values 2 and 3 and any additional measurement over a 1–3 month period.

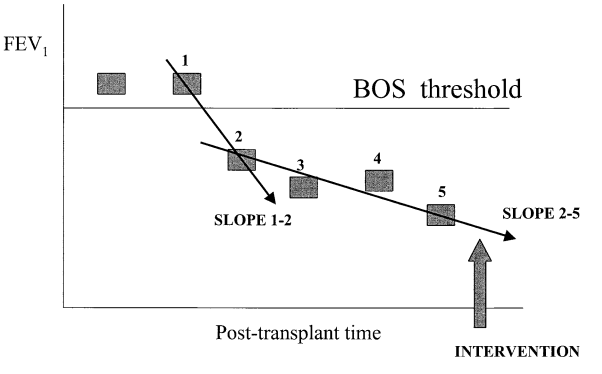

FIGURE 2 Though initial decline below BOS threshold shows a steep decline (slope 1–2), preintervention value 2 which defines BOS onset (and is validated by subsequent values) and subsequent values 3–5 define the slope prior to intervention. Benefit of therapeutic intervention will be defined by comparison with the slope 2–5.

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Pancreas

Grading of Acute Pancreas Allograft Rejection

Grade	Histopathology
Grade 0 (NORMAL)	Unremarkable pancreatic parenchyma without inflammatory infiltrates
Grade I (INFLAMMATION OF UNDETERMINED SIGNIFICANCE)	Sparse, purely septal mononuclear inflammatory infiltrates. No venous endotheliitis or acinar involvement identified
Grade II (MINIMAL)	Purely septal inflammation with venous endotheliitis (attachment of lymphocytes to the endothelium with associated endothelial damage and lifting of the endothelium from the basement membrane). In the absence of venous endotheliitis a constellation of at least 3 of the following 4 histologic features: a. Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes b. Eosinophils c. Acinar inflammation in rare (up to 2) foci d. Ductal inflammation (permeation of inflammatory cells through the ductal basement membrane)
Grade III (MILD)	Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes with associated acinar inflammation in multiple (3 or more) foci. Eosinophils, venous endotheliitis, ductal inflammation and evidence of acinar single cell injury may be seen depending on sampling. The latter is manifested as cellular drop-out (apoptosis-pyknotic cell death), or necrosis (oncotic cell death)
Grade IV (MODERATE)	Arterial endotheliitis and/or necrotizing arteritis (vasculitis). Features described in Grade III are usually present
Grade V (SEVERE)	Extensive acinar lymphoid or mixed inflammatory infiltrates with multicellular focal or confluent acinar cell necrosis. Depending on sampling vascular and ductal lesions may be demonstrated

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EVALUATION OF PANCREAS TRANSPLANT NEEDLE BIOPSY: Reproducibility and Revision of Histologic Grading System

[Clinical Transplantation]

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Outline

Graphics

Abstract

Background. **Tissue samples for the diagnosis of pancreatic allograft rejection are now obtained routinely through the application of the percutaneous needle biopsy technique. The availability of biopsy material (89% adequate for diagnosis in our setting) presents a challenge for pathologists who are asked to provide a fast and accurate diagnosis of rejection and its severity, while at the same time being able to differentiate rejection from other causes of graft dysfunction**.

Methods. **To differentiate rejection from other pathologic processes, 26 histologic features were assessed in 92 biopsies performed for confirmation of clinical diagnosis of rejection and the results were compared with 31 protocol biopsies, 12 allograft pancreatectomies with non-rejection pathology, and 30 native pancreas resections with various disease processes**.

Results. **Based on these comparisons, a constellation of findings relating to the vascular, septal, and acinar inflammation was identified for the diagnosis of rejection. Application of these features led us to revise our scheme for grading rejection (ranging from 0-normal to V-severe rejection) to include the categories of "inflammation of undetermined significance" and "minimal rejection." The scheme was used by five pathologist to grade 20 biopsies independently of any clinical data and the interobserver level of agreement was highly significant ([kappa]=0.83, *P*<0.0001). This grading scheme was applied blindly to all (183) biopsies from 77 patients with 6-52 months of follow-up. The correlation of the highest degree of rejection on each patient and ultimate graft loss (0% for grades 0-I, 11.5% for grade II, 17.3% for grade III, 37.5% for grade IV, and 100% for grade V) was highly statistically significant (*P*<0.002). The fraction of grafts lost due to pure immunologic causes increased proportionally to the grade of rejection (0, 50, 66, and 100% for grades II, III, IV, and V, respectively)**.

Conclusions. **This study provides strong support for the proposed pancreas rejection grading scheme and confirms its potential for practical use**.

The major cause of graft loss with pancreas transplants has been irreversible rejection (1). This finding is particularly true for pancreas after kidney (PAK *) and pancreas transplant alone (PTA) cases because the clinical diagnosis of rejection remains relatively nonspecific. When a simultaneous kidney transplant (SPK) is performed in a uremic diabetic, the cotransplanted kidney is thought to provide a reliable indicator for rejection through serial determinations of the recipient's serum creatinine. Isolated pancreas rejection in combined kidney pancreas transplantation is not unusual, however (2). Parameters used for the diagnosis of pancreas rejection include decrease in urinary insulin and C peptide (3, 4); increase in serum amylase, lipase, and anodal trypsinogen (5-7) and pancreas specific protein (8, 9); and cytologic evaluation of pancreatic juice (10, 11) and urine (12, 13). In bladder drained grafts, urinary amylase has been used as a measure of pancreas exocrine function (14-19). Other methods used for the diagnosis of pancreas rejection are ^{99m}Tc DTPA scintigraphy (20) and uptake of indiumlabeled platelets (21). None of these modalities is sufficiently specific to be used without the risk of occasional under- or overtreatment of rejection.

The 1-year pancreas graft survival for SPK transplants performed between 1992 and 1993 is

81% (22). In contrast, the results of pancreas transplants performed in patients who have had a prior successful kidney transplant (pancreas after kidney, PAK) or in patients who have never had uremia (PTA) lagged behind the results obtained in SPK cases, with a 1-year success rate of 61% for both PAK and PTA cases performed between 1992 and 1993 (22). The results of PAK and PTA collectively have continued to improve particularly since the general availability of the newer immunosuppressive agents, tacrolimus, and mycophenolate mofetil. The steady improvement in the success of PTA cases is in part due to the restriction of cases to superior HLA match between the donor and recipient. This strategy markedly decreases the possibility that recipients will experience a rejection episode; however, most patients will undergo a prolonged waiting period before this degree of match can be achieved, even with the existing pancreas sharing scheme that emphasizes HLA match (22). In most cases a compromise is necessary if the transplant is ever to be accomplished. Despite better immunosuppression and HLA matching some patients will experience allograft dysfunction that must be diagnosed.

The success of renal, hepatic, and cardiac transplantation, has been dependent on the ability to differentiate nonimmunologic causes for graft dysfunction from rejection by reliance on a confirmatory biopsy (23). Biopsy material from transplanted pancreas grafts was obtained in the past during laparotomy (23, 24) or through cystoscopically guided transduodenal pancreatic biopsy (25-27). Our center has applied a method for routine ultrasound guided percutaneous pancreatic biopsy under local anesthesia (28, 29). This approach has yielded tissue for histologic analysis in greater than 88% of attempts, with complications in fewer than 2% of cases (2, 30). We have previously proposed a system for grading pancreas allograft rejection that differs from other schemes (31, 32) in that it includes the diagnosis of milder forms of rejection in the absence of arterial vascular rejection. Our original report (32) was based on our experience with the application of the percutaneous needle biopsy technique on patients with graft dysfunction; subsequently protocol biopsies from patients with normal function were also available for evaluation, enabling us to test the specificity of the various morphological parameters. In this study we attempted to identify specific histologic features of milder as well as more severe forms of rejection and devised a detailed system for their practical application in the diagnostic process. The differential diagnosis of rejection is also discussed.

MATERIALS AND METHODS

Between July 2, 1992, and May 31, 1995, 129 bladder drained pancreatic transplants were performed at the University of Maryland Hospital. Of these 129 cases, 64 patients (44 SPK, 15 PAK, and 5 PTA) had 138 pancreatic biopsies, 123 (89%) of which were adequate for diagnosis (see criteria below). Biopsies were performed from 2 days to 48 months after transplantation (mean, 17.1 months) and originated from 43 males and 21 females. The ages ranged from 23 to 56 years (mean, 35 years). The number of biopsies per patient ranged from 1 to 7 (mean, 1.3). The biopsies were obtained using an 18-gauge automated biopsy needle with a 17-mm specimen notch.

The 123 biopsies were performed in two types of circumstances. Ninety-two biopsies were performed on 38 patients for confirmation of clinical diagnosis of rejection. This constitutes the main study group and was designated the Rejection biopsy (bx) group. The clinical indications prompting biopsy required fulfillment of one of the following criteria: (1) a twofold or greater increase in serum amylase (mean increase, 3.5-fold) or lipase (mean increase, 8.5-fold); (2) a sustained 40% or greater decrease in urinary amylase (mean, 45%); 3) loss of glycemic control. Baseline laboratory values were calculated as the means of all values obtained in the 4 weeks preceding the episode of rejection. Twenty-eight simultaneous renal and pancreatic transplant

biopsies were performed in combined kidney-pancreas allograft recipients. In 24 of these instances, concurrent acute increase in serum creatinine and abnormal pancreatic function were observed.

The first episode of minimal rejection (grade II) was treated with pulse steroids. Subsequent rejection episodes or mild, moderate, and severe rejection were treated with OKT3 i.p. The Protocol bx group consisted of 31 consecutive protocol pancreatic transplant biopsies. These biopsies were performed in the absence of any clinical sign of rejection as part of a randomized trial comparing tacrolimus and cyclosporine based immunosuppression in simultaneous pancreas-kidney transplants (33).

The Protocol bx group and two pancreatectomy control groups were used for comparison with the Rejection bx group. The Nonrejection tx pancreatectomy group consisted of 12 partial or complete transplant pancreatectomies for nonrejection related problems and included 2 cases of posttransplant lymphoproliferative disorders (PTLD), 4 cases of chronic pancreatitis (3 obstructive, 1 related to ethanol abuse), 2 of cytomegalovirus duodenopancreatitis, 2 cases of peripancreatic abscesses, and 2 cases of early graft thrombosis.

The Native pancreatectomy group consisted of 30 control native pancreases with various diseases. This group included tissue from partial pancreatectomies for recent traumatic injury from 5 young males, 17 partial pancreatectomies in cases of chronic pancreatitis (14 obstructive type, 3 calcifying type), 4 partial pancreatectomies for acute pancreatitis, 2 autopsy pancreases in cases of cystic fibrosis, and 2 partial pancreatectomies for acute infectious pancreatitis.

For the needle biopsies, three serial 4-µm-thick hematoxylin-and-eosin-stained sections from each case were examined. Biopsies with 2-3 mm^2 or more of pancreatic acinar parenchyma present/biopsy surface area were considered adequate. From the pancreatectomies 4-µm-thick hematoxylin-and-eosin-sections from two blocks per case were examined.

The presence of 26 histologic features was evaluated in the main study group and in the three control groups (Table 1). The results in the various groups were compared with those of the Rejection bx group, using the Fisher's exact test. The results were corrected for the absence of specific structures (ducts, vessels, islets, and nerves) in the needle biopsies.

	Rejection bx	Protocol bx	Native pancreas	Nonrejection Tx
Number of specimens	92	31	30	12
Septal inflammation[b]	85	7 (0.0001)	21 (0.006)	9 NS[c]
Acinar inflammation[b]	65	0 (0.0001)	3 (0.0001)	5 NS
"Activated lymphocytes"[a,b]	60	0 (0.0001)	3 (0.0001)	6 NS
Plasma cells	33	3 (0.005)	17 NS	6 NS
Eosinophils[b]	61	0 (0.0001)	6 (0.0001)	3 NS
Neutrophils	36	0 (0.0001)	6 NS	2 NS
Acinar single cell injury[b]	34	0 (0.0001)	0 (0.0001)	2 NS
Venous endotheliitis[b]	32	0 (0.0001)[d]	0 (0.0001)[d]	1 NS
Arterial endotheliitis[b]	11	0 NS[d]	0 NS[d]	0 NS
Arteritis[b]	8	0 NS[d]	0 NS[d]	0 NS
Confluent acinar necrosis[b]	7	0 NS	1 NS	2 NS
Ductal inflammation[b]	52	0 (0.0001)[d]	15 NS[d]	2 NS
Dilated ducts[e]	2	0 NS	18 (0.0001)	3 (0.006)
Angulated-compressed ducts[e]	0	0 NS	18 (0.0001)	2 (0.008)
Ductal cell necrosis	16	0 NS	4 NS	0 NS
Ductal epithelial prolif.[e]	0	0 NS	8 (0.0001)	1 NS
Ductal cell atypia	25	0 (0.0001)	3 NS	1 NS
Proliferation of small ducts[e]	0	0 NS	17 (0.0001)	1 NS
Ductal squamous metaplasia[e]	0	0 NS	6 (0.0001)	1 NS
Interstitial edema	27	5 NS	9 NS	3 NS
Nerve inflammation	10	0 NS[d]	13 NS[d]	0 NS
Lamellar fibrosis[e]	1	0 NS	23 (0.0001)	6 (0.0001)
Acinar atrophy[e]	1	0 NS	23 (0.0001)	4 (0.0002)
Acinar enzymatic necrosis[e]	0	0 NS	4 (0.004)	3 (0.001)
Calcification[e]	0	0 NS	3 (0.0002)	0 NS
Islet inflammation	10	0 NS	4 NS	0 NS

[a] Numbers in parentheses refer to the *P* value of comparison between the respective group with the Rejection bx group.
[b] Features used in diagnosis of rejection.
[c] NS, not significant.
[d] Corrected for the presence of the specific structure in the biopsies.
[e] Features used in diagnosis of pancreatitis.

Table 1. Histologic features[a]

For the evaluation of the histologic features, all cases were examined independently by two pathologists (C.B.D. and J.C.P.), who were blinded as to the clinical status of the patients. To test the reproducibility of the grading scheme, one hematoxylin and eosin section from 20 percutaneous pancreas biopsies were evaluated independently by five pathologists blinded to the clinical data. Three of them were transplant pathologists (L.C.R., C.B.D., and J.C.P.) whereas two had no previous experience with transplantation pathology (R.J.C. and O.B.I.). The results were evaluated statistically by the [kappa] analysis for the measurement of interrater agreement.

At the closing of this study (May 1996), the total number of patients with pancreas transplant biopsies for which at least 6 months of follow-up was available amounted to 77. The grading scheme was applied blindly to the 183 biopsies from these patients, and the highest grade of rejection on each patient was correlated with the fate of the pancreatic graft. The linear trend of decreasing graft survival was analyzed with the Pearson's chi square test.

RESULTS

Differentiation between rejection and other pathologic processes. As seen in Table 1, the occurrence of septal inflammation, acinar inflammation, activated lymphocytes, eosinophils, acinar single cell injury, and venous endotheliitis differs significantly between biopsies from patients with clinically suspected rejection (Rejection bx group), the Protocol bx group, and the Native pancreatectomies group. Arterial endotheliitis and arteritis are features that are associated almost exclusively with rejection, but occur far less frequently than the features above and for that reason they do not show statistical significance in the respective comparisons.

The presence of ductal inflammation is not significantly different between the cases with presumed rejection and the native pancreas diseases or nonrejection pancreatectomies. Significant differences are noted, however, between the Protocol bx group and the Rejection bx group.

The presence of plasma cells, neutrophils, ductal cell necrosis, ductal cell atypia, interstitial edema, and nerve and islet inflammation is not significantly different between biopsy samples from patients with clinical suspicion of rejection and samples from other pancreatic pathologic processes.

Spotty or diffuse coagulation necrosis of the acinar parenchyma with associated enzymatic fat necrosis was seen in the cases of early graft thrombosis; no significant inflammatory infiltrates were observed in these cases. In contrast, enzymatic necrosis associated with prominent predominantly neutrophilic infiltrates was seen in acute pancreatitis.

Dilated, angulated, and compressed ducts, ductal epithelial cell proliferation, small duct proliferation, ductal squamous metaplasia, lamellar interstitial fibrosis, acinar atrophy, acinar enzymatic necrosis, and calcification were notable for their rarity or virtual absence in the biopsies from the Rejection bx group. In contrast, these findings were commonly seen in the Native pancreatectomies group, yielding highly significant statistical differences. From the above statistical analysis, a constellation of histological features was identified, which was not seen in the diseases of the native pancreas or in the biopsies from patients with normal graft function (Table 1). The concurrent finding of a minimum of these features in biopsies from patients with clinically presumed rejection was considered as suggestive or diagnostic of rejection (see grading scheme, Table 2). Applying the same principle, a group of histological features remarkable for their absence in rejection but commonly seen in acute and chronic pancreatitis was identified and therefore considered useful for the differential diagnosis (Table 1).

Grade 0—Normal
Unremarkable pancreatic parenchyma without inflammatory infiltrates.

Grade I—Inflammation of Undetermined Significance
Sparse, purely septal mononuclear inflammatory infiltrates.
No venous endotheliitis or acinar involvement identified.

Grade II—Minimal Rejection
Purely septal inflammation with venous endotheliitis (attachment of lymphocytes to the endothelium with associated endothelial damage and lifting of the endothelium from the basement membrane).
In the absence of venous endotheliitis a constellation of at least three of the following four histologic features:
(a) Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes.
(b) Eosinophils.
(c) Acinar inflammation in rare (up to two) foci.*
(d) Ductal inflammation (permeation of inflammatory cells through the ductal basement membrane).

Grade III—Mild Rejection
Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes with associated acinar inflammation in multiple (3 or more) foci.*
Eosinophils, venous endotheliitis, ductal inflammation and evidence of acinar single cell injury may be seen depending on sampling. The latter is manifested as cellular drop-out (apoptosis-pyknotic cell death), or necrosis (oncotic cell death).

Grade IV—Moderate Rejection
Arterial endotheliitis and/or necrotizing arteritis (vasculitis). Features described in grade III are usually present.

Grade V—Severe Rejection
Extensive acinar lymphoid or mixed inflammatory infiltrates with multicellular focal or confluent acinar cell necrosis.
Depending on sampling vascular and ductal lesions may be demonstrated.

* Inflammatory focus is defined as a collection of at least 10 mononuclear cells.

Table 2. Grading scheme

Histologic features used for grading. In the Rejection bx group, septal inflammation (Fig. 1 and 2) was the most commonly observed finding (85/92, 92%) (Table 3).

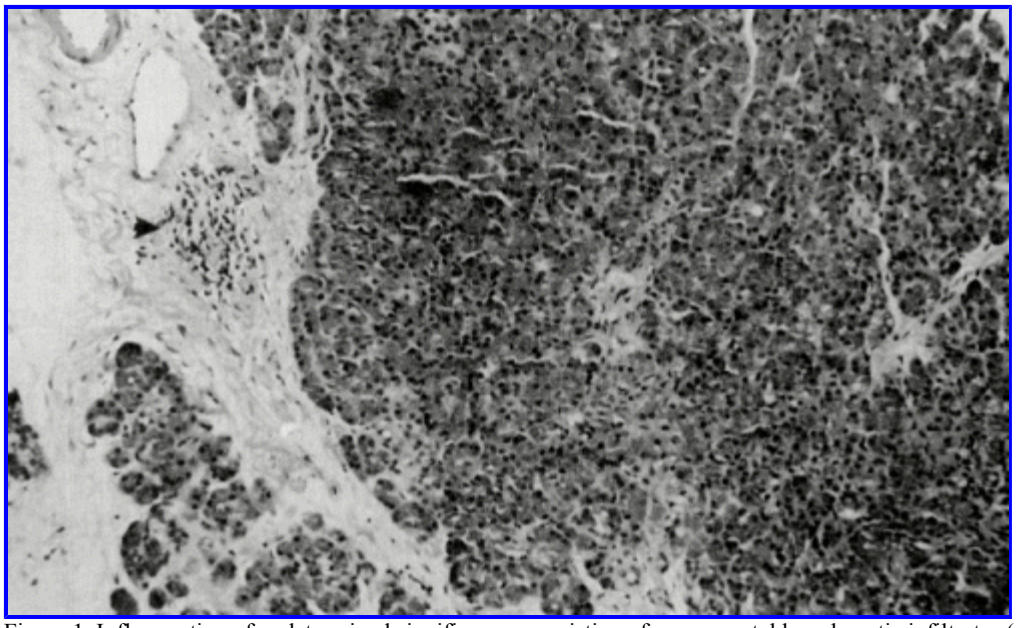

Figure 1. Inflammation of undetermined significance, consisting of sparse septal lymphocytic infiltrates (arrowhead). The acinar parenchyma and neighboring vessels are free of inflammation.

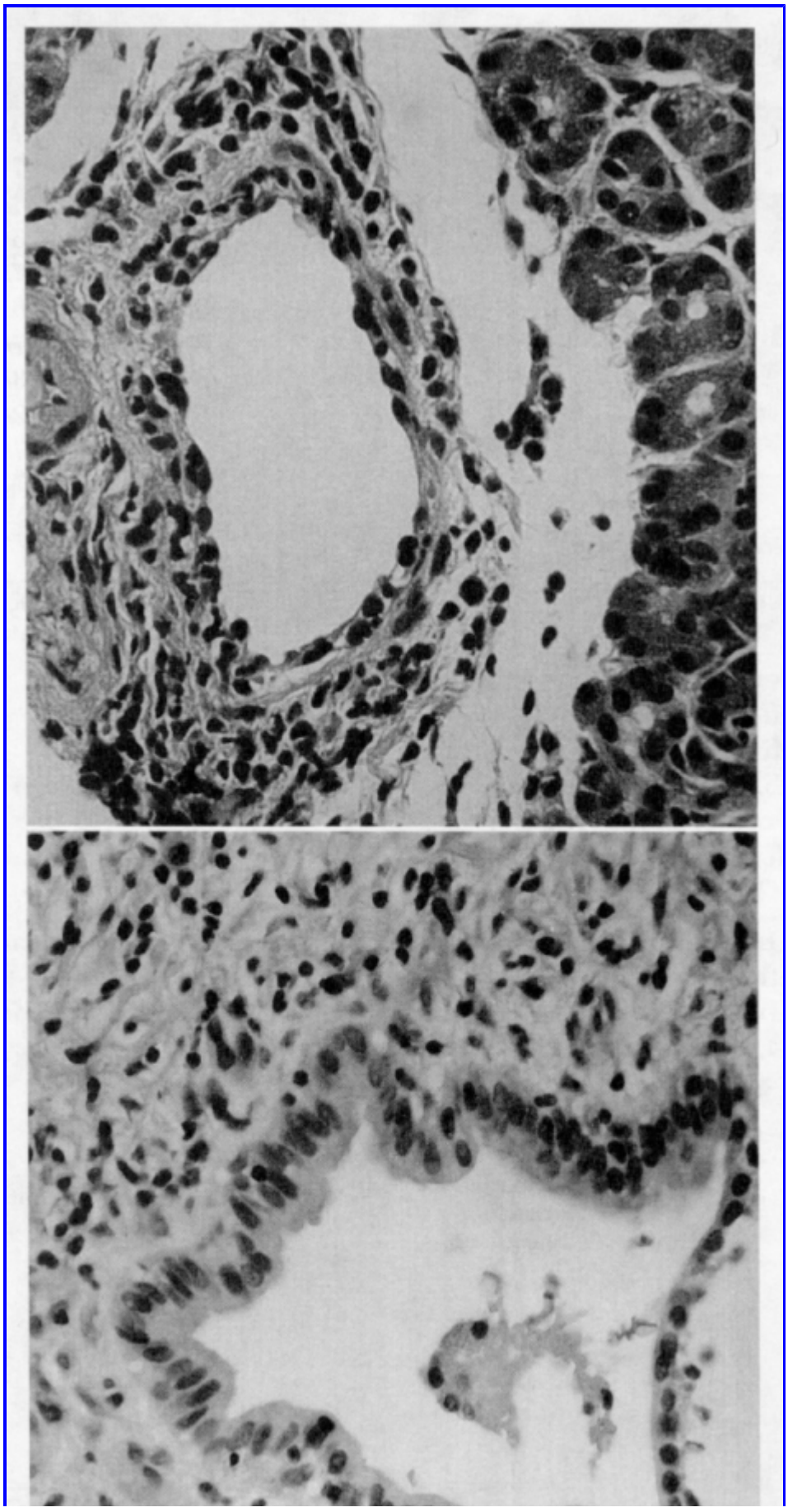

Figure 2. Septal infiltrates associated with venous endotheliitis (A) and ductal inflammation (B).

	Total	0	I	II	III	IV	V
Septal inflammation	85	—	14/14	29/31	27/27	8/8	7/7
Acinar inflammation	65	—	—	23/31	27/27	8/8	7/7
Ductal inflammation	53	—	—	19/31	23/27	7/8	4/7
Eosinophils (septal, acinar)	61	—	—	25/31	23/27	8/8	5/7
Activated lymphocytes	60	—	—	18/31	27/27	8/8	7/7
Venous endotheliitis	32	—	—	6/31	15/27	5/8	6/7
Acinar single cell injury	34	—	—	—	19/27	8/8	7/7
Arterial endotheliitis	11	—	—	—	—	8/8	3/7
Arteritis (vasculitis)	8	—	—	—	—	6/8	2/7
Confluent acinar necrosis	7	—	—	—	—	—	7/7

Table 3. Histologic features useful for grading of rejection (92 nonprotocol needle biopsies)

In 73 of 92 (79%) of the biopsy samples, the inflammatory infiltrates involved additional structures present in the tissue. Ductal inflammation and acinar inflammation were seen in 53 of 92 (64%) and 65 of 92 (71%) of the biopsy samples, respectively (Figs. 2B and 3). Concurrent ductal inflammation and acinar inflammation occurred in 49 of 92 (53%) biopsies. Acinar inflammation with unremarkable ducts was observed in 10 of 92 (11%) cases. Ductal inflammation in the context of septal inflammation and associated venous endotheliitis was seen in 5% of cases with no associated acinar involvement.

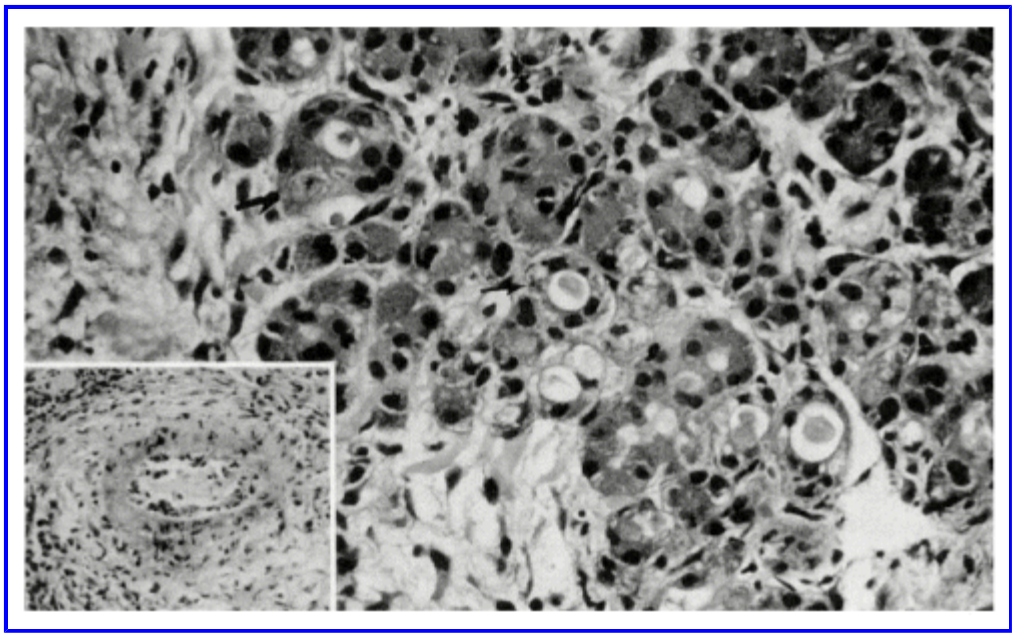

Figure 3. Acinar inflammation with associated acinar cell damage (arrows). Inset shows arteritis.

Eosinophils were identified in fibrous septa and acini in 82% of the biopsies with features of rejection. “Activated” appearing lymphocytes (showing enlarged and convoluted nuclei, increased amounts of cytoplasm) were observed in 60 of 92 cases (65%). Venous endotheliitis consisting of dense perivenular infiltrates with lifting and swelling of endothelial cells was seen in 32 of 92 (35%) biopsies (Fig. 2A). Acinar single cell injury in the form of necrosis (oncotic cell death) or drop-out (apoptosis) was observed in cases with significant inflammation (34/92, 37%, Fig. 3). Arterial endotheliitis, consisting of lifting-up and damage of endothelial cells by lymphocytic or mixed inflammatory infiltrates was seen in 11 of 92 (12%) biopsies. Permeation

of the arterial wall by inflammatory cells and associated fibrinoid necrosis (arteritis) was seen in 8 of 92 (9%) biopsies (Fig. 3, insert). Acinar multicellular necrosis and extensive (nonenzymatic) confluent necrosis were seen in severe rejection (7/92, 8% biopsies, Fig. 4). Necrosis in association with mixed inflammatory infiltrates was most typical of severe acute rejection.

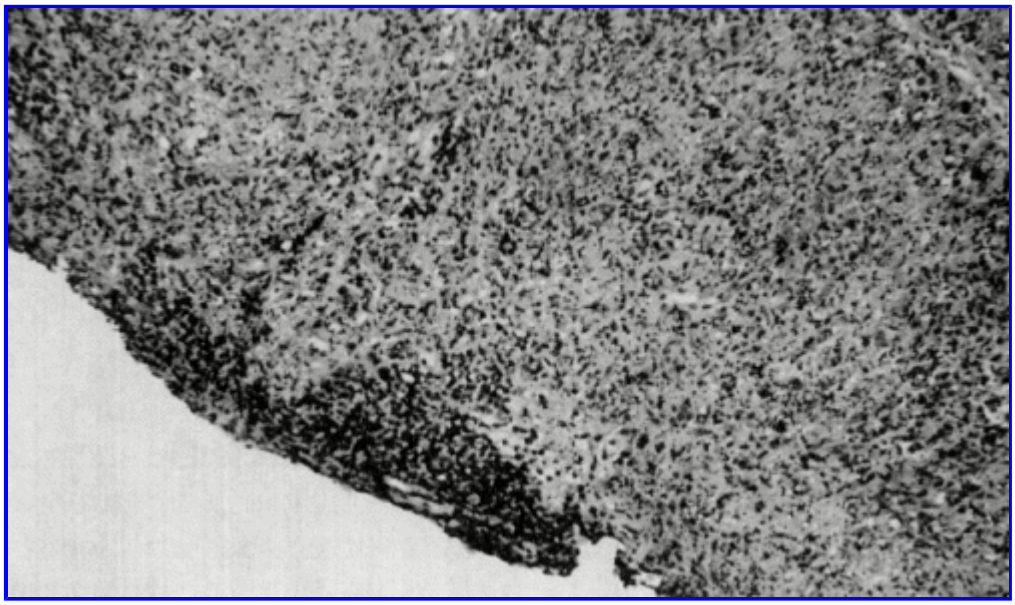

Figure 4. Extensive inflammatory infiltrates associated with confluent necrosis.

The rest of the histologic features evaluated were not useful in the diagnosis and grading of rejection. Interstitial edema, when present, was usually associated with acinar inflammation (22/27 cases). Lack of edema, however, was seen in cases with significant acinar inflammation.

Inflammation involving nerve branches was seen in 10 of 92 cases, and the degree of inflammation was proportional to the septal inflammation. In 10 biopsies, the acinar inflammation extended to involve the islets.

Additional features observed inconsistently in the biopsies from patients with clinical suspicion of rejection are listed in Table 1 and include presence of plasma cells, neutrophils, ductal epithelial cell necrosis, and atypia.

Correlation between grade of rejection and ultimate graft loss. After a mean follow-up (±SEM) of 19.3±1.95 months (range, 6-52 months) the percentage of ultimate graft loss in 77 patients was 0% for patients with biopsies of grades 0-I, 11.5% for grade II, 17.3% for grade III, 37.5% for grade IV, and 100% for grade V (Table 4). The linear trend for decreasing graft survival correlating with the histologic grade, was statistically significant ($P<0.002$) by the Pearson's chisquare method. No difference in graft loss was found between grade II cases with or without venous endotheliitis. The percentage of graft loss due to pure immunological causes increased proportionally to the histological grade (0, 50, 66, and 100% for grades II, III, IV, and V, respectively). Table 5 lists the correlation of immediate clinical outcome with corresponding biopsy grade.

Highest degree of rejection	Graft loss	Functioning	Total no. of patients
Severe (V)	7 (100%), 7[a] 0[b]	0 (0%)	7
Moderate (IV)	3 (37.5%), 2[a] 1[b]	5 (62.5%)	8
Mild (III)	4 (17.3%), 2[a] 2[b]	21 (82.7%)	23
Minimal (II)	3 (11.5%), 0[a] 3[b]	23 (88.5%)	26
Undetermined (I)	0 (0%)	3 (100%)	3
Normal (0)	0 (0%)	10 (100%)	10
Total			77

[a] Graft failure due to immunological causes (acute and/or chronic rejection).
[b] Graft failure due to nonimmunological causes (cardiac death, n = 4, drug overdose, n = 1, late graft thrombosis, n = 1).

Table 4. Ultimate graft loss/highest degree of rejection

Grade, no. of cases	No. of patients treated for rejection	Clinical response[a]	Persistent transplant dysfunction[b]
0, 7	0	—	2
I, 12	3	2	1
II, 31	31	22	9
III, 27	27	20	7
IV, 8	8	4	4
V, 7	7	0	7

[a] Graft function returned to baseline after antirejection treatment.
[b] Persistent graft dysfunction despite antirejection treatment.

Table 5. Correlation of immediate outcome with grade

Correlation of findings in simultaneous pancreatic and renal biopsies. The findings in 22 of the 28 simultaneously biopsied pancreas and kidney transplants correlated with each other (both organs showed minimal rejection in 18 cases and mild rejection in 4 cases). The noncorrelative biopsies showed isolated pancreas rejection in four cases and isolated renal rejection in two cases.

Correlation of number of rejection episodes with severity of rejection and ultimate graft survival. Patients with biopsies showing grades 0-III as highest degree had an average of 1.2 episodes of graft dysfunction (range, 1-2). Patients with one or more grade IV biopsies had an average of 2.1 rejection episodes (range, 1-7). Patients with one or more grade V biopsies had 3.4 rejection episodes (range, 3-5). Patients with failed grafts had 2.2 rejection episodes (range, 1-6), whereas patients with functioning grafts had 1.9 rejection episodes (range, 1-5).

Degree of interobserver agreement between pathologists. Overall high level of agreement was obtained by all five pathologists ([kappa]=0.83, $P<0.0001$). Similar [kappa] values were obtained

for grades I ([kappa]=0.85, $P<0.0001$) and IV ([kappa]=0.79, $P<0.0001$). The reproducibility of grades 0 and V was close to perfect ([kappa]=0.90, $P<0.0001$, and [kappa]=0.94, $P<0.0001$, respectively), whereas substantial levels of agreement were obtained for grades II and III ([kappa]=0.66, $P<0.0001$, and [kappa]=0.72, $P<0.0001$). Excellent discrimination between the diagnosis of rejection versus nonrejection was achieved ([kappa]=0.96, $P<0.0001$), with only one discrepancy by one pathologist who diagnosed one case as grade II (minimal rejection) and the other four pathologists diagnosed it as grade I (inflammation of undetermined significance).

DISCUSSION

By retrospectively examining failed and functioning pancreatic grafts, Nakhleh and Sutherland (30) identified histologic features associated with higher probability of graft failure and proposed a classification scheme for the grading of rejection. In this significant study, the authors indicated that to diagnose rejection vascular changes must be seen. In their classification the latter changes were indicated as arterial endotheliitis in mild rejection and vasculitis in severe rejection.

From our experience with pancreas transplant needle biopsies, however, we developed the concept that a significant number of milder forms of rejection will be underdiagnosed if the presence of arterial changes is a condition for the diagnosis of rejection. In our study we attempted to characterize the features of both mild as well as advanced degrees of pancreas rejection by analyzing needle biopsies from patients with and without clinical evidence of rejection. We compared these features with the morphologic features seen in nontransplant related pancreas diseases and prospectively evaluated their relevance in the graft outcome.

In designing this scheme for histological grading of rejection, we took into account a sequence of events that has been described in unmodified transplant rejection in canine and rodent animal experimental models. The first significant changes in this context occur on days 2 to 3 and consist of septal inflammation and perivascular infiltrates around veins and capillaries (34-36). Capillary-venous endotheliitis also starts to appear at that stage, although it can be somewhat more delayed in the presence of immunosuppression (35). Progressive involvement of the acinar parenchyma and arterial endotheliitis ensue in the following days (34-37). Our findings on needle biopsies support the concept that the most subtle evidence of rejection in human pancreas transplants consists of perivenular infiltrates (often associated with venous endotheliitis) and associated septal inflammatory infiltrates. The small vascular structures (venules) are the point of entry of lymphocytes into the affected tissues (38) and their endothelial cells can be the target of immune attack. Subsequently, the infiltrates involve other interlobular structures (ducts, nerves), as well as the acinar parenchyma and arterial branches, as is shown in the animal models (34, 36). In accordance with this concept, we based the grading of allograft rejection on the progressive involvement of septal, acinar, and arterial vascular structures. This grading concept does not, however, imply a necessary or exclusive pathophysiologic sequence in individual patients.

Our study shows that acinar inflammation is a very sensitive feature of rejection. Thus, it occurs far more often in the context of suspected rejection to a highly significant degree ($P<0.0001$). In particular, the identification of acinar single cell injury secondary to immune attack is a specific feature of rejection ($P<0.0001$), that can be compared with venous endotheliitis, arterial endotheliitis, and vasculitis. In this study, the diagnostic value of eosinophils was again confirmed, as we had previously shown for kidney allograft rejection (39).

Although ductal inflammation shows a significant clinicopathological correlation and appears to be an integral component of pancreas rejection (35, 40) (as is the case with hepatic ducts and

renal tubules in liver and kidney allograft rejection, respectively), it is also often seen in chronic pancreatitis. Ductal inflammation by itself is therefore considered nonspecific if it is not associated with acinar inflammation, venous endotheliitis, eosinophils, or "activated" appearing lymphocytes.

Taking into account the sampling variations and limitations inherent to small needle biopsies, as pancreatic biopsies typically are, we propose a grading scheme that uses a combination of the various useful histological parameters, to maximize the sensitivity and specificity of the diagnosis of minimal rejection (grade II, Table 2). Previous studies have emphasized the specificity of endotheliitis (30, 31)-in particular arterial endotheliitis-for the diagnosis of rejection. Endotheliitis, however, was shown to be related to the degree of inflammation present (30) and therefore would be seen in general in more advanced stages of rejection. The absolute requirement for its identification for the diagnosis of rejection would lead to histological diagnosis of mostly the higher grades whereas the lower grades would be probably treated empirically according to the clinical findings.

From our results we conclude that biopsies with grades II and III identify populations of patients with milder forms of cellular rejection that in the appropriate clinical context should be treated. All 31 biopsies diagnosed as minimal evidence of rejection (grade II) corresponded to patients with acute allograft dysfunction and clinical suspicion for acute rejection (18 cases from this group had concurrent renal biopsies showing mild rejection by the Banff criteria (41). The laboratory abnormalities reversed to baseline after antirejection treatment in 71% of the cases diagnosed as minimal rejection (Table 5). In 19% of the cases there was persistent graft dysfunction, and despite antirejection treatment a higher degree of rejection was seen in a subsequent biopsy. All of our protocol biopsies from patients with normal graft function failed to show features of rejection, being either totally normal (grade 0) or showing inflammation of undetermined significance (grade I).

In the lower grades (0-II), due to the focality of the early rejection process, sampling may cause potential diagnostic problems. Two patients with normal biopsies (grade 0) had persistent transplant dysfunction and showed rejection in subsequent biopsies.

In our initial grading scheme (32), we considered the presence of any degree of interstitial infiltrates as borderline changes (in an analogous manner to the borderline category in the Banff scheme for grading of kidney rejection) (41). With the possibility of evaluating protocol biopsies it became evident that this finding is nonspecific. In rare cases, however, sparse septal infiltrates preceded the development of rejection or occasionally were noted in resolving treated rejection. Thus, we renamed the grade I as inflammation of undetermined significance. Recent papers have addressed the potentially ambiguous meaning of inflammation in grafts, particularly in patients without clinical evidence of organ dysfunction (42).

The evaluation of multiple controls and the statistical analysis described above led us to divide the grades in a slightly different fashion from the original classification in search of increased specificity and sensitivity in the diagnosis of rejection. We introduced the "minimal" category, which is diagnosed by the observation of well defined venous endotheliitis in the context of purely septal infiltrates. To increase the sensitivity of the system we also diagnose minimal rejection in the absence of venous endotheliitis but with the concurrent presence of the constellation of other features described in Table 2. Biopsies that fall short of fulfilling these criteria are classified as inflammation of undetermined significance.

The higher grades (III-V) in this scheme correspond to unequivocal allograft rejection and

therefore pose no problem from a differential diagnostic point of view. Less pronounced acinar or septal inflammation not associated with definite vascular changes should, however, be differentiated from other conditions affecting the graft. The statistical analysis indicates that it should be possible in most cases to differentiate mild rejection from drainage related problems associated with chronic pancreatitis. The latter cases display fibrosis, acinar atrophy and ductal dilation and proliferation, as is the case with chronic pancreatitis in general. On the other hand, it may be impossible to differentiate only on a morphological basis cellular rejection from other conditions acutely affecting the graft. A high degree of caution should therefore be applied to all biopsy samples to rule out the viral cytopathic changes typical of cytomegalovirus pancreatitis, the presence of the atypical infiltrates (particularly atypical immunoblasts) that may suggest PTLD or any other feature indicative of a disease process different from a pure rejection reaction (43). The presence of peripancreatic abscesses or other similar conditions that affect the surgical bed may cause significant changes in the superficial pancreatic tissue, which is the portion usually sampled with the percutaneous biopsy technique. These changes consist of significant mixed septal inflammatory infiltrates associated with early septal fibrosis and occasional acinar involvement. In a different clinical setting many of these features would be indicative of cellular rejection (43). The algorithm proposed here (Fig. 5) emphasizes the importance of first correctly diagnosing rejection before assigning the appropriate grade. Strict adherence to the proposed guidelines leads to highly reproducible results that have clinical significance.

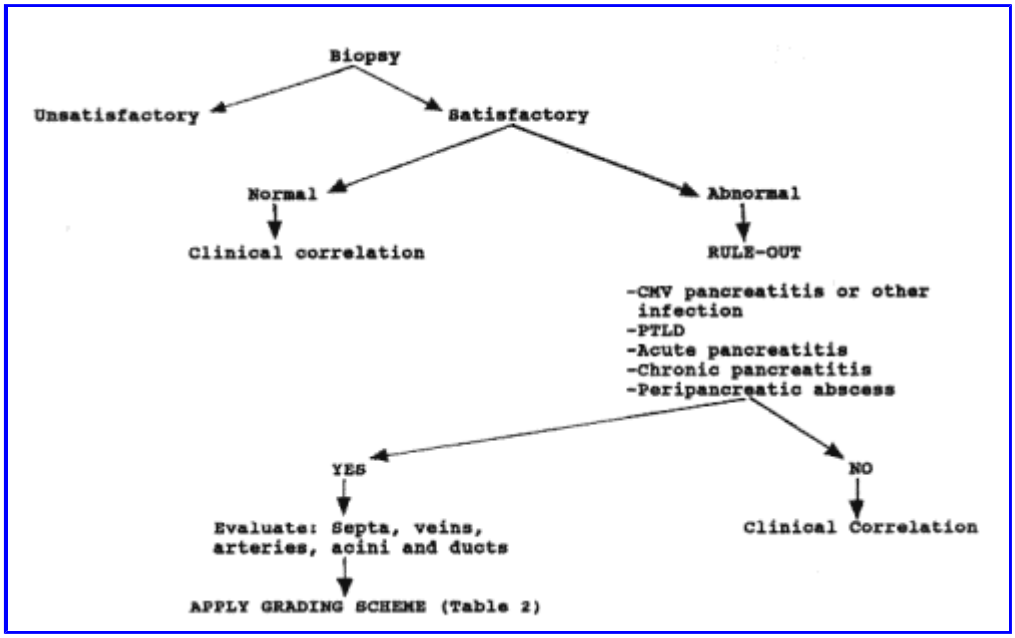

Figure 5. Algorithm for the evaluation of pancreas allograft biopsies.

Although our study deals mainly with diagnosis of acute rejection, it should be stressed that findings indicating chronic rejection (increased septal fibrosis, acinar parenchymal loss, and chronic transplant vasculopathy) have additional prognostic implications as previously described (44), and should be stated in the pathology report. We were not able to demonstrate evidence of primary isletitis or of recurrent diabetes mellitus (45).

In summary, in addition to the well established concept that acute arterial vascular changes are diagnostic of advanced pancreas allograft rejection, we propose that early rejection starts in the connective tissue septa often with associated venular inflammation. With the subsequent

infiltration by a mixed population of inflammatory cells, ducts and acinar parenchyma are affected. These early changes should be recognized and treated as clinically indicated. The progressive morphologic findings leading to our grading system are supported conceptually by experimental studies in animals and further confirmed by the clinical outcome in this group of patients.

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Footnotes [Context Link]

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PANCREAS TRANSPLANTATION: THE HISTOLOGIC MORPHOLOGY OF GRAFT LOSS AND CLINICAL CORRELATIONS

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Background. **Graft losses due to leaks, bleeding, thrombosis, infections, and early pancreatitis are grouped together under the category of technical failure. Among these complications, massive vascular thrombosis continues to be the most important cause of early graft loss due to technical failure. Pathological evaluation of most allografts lost early in the posttransplantation period shows vascular thrombosis with associated proportional parenchymal necrosis. The morphological findings in allografts that are considered to be lost due to technical failure has not been systematically addressed. In particular, the role of acute rejection in early graft loss has not been well studied.**

Methods. **Seventy-four consecutive pancreas graft pancreatectomies were studied histologically to evaluate for thrombosis (recent versus organized), type of vessel involved by thrombosis (arteries, veins, or both), acute rejection grade, chronic rejection grade, endotheliitis, transplant arteritis, coagulation necrosis, acute pancreatitis, presence of infectious organisms, transplant (obliterative) arteriopathy, neoplasia, relative proportions of alpha and beta islet cells, and immunoglobulin and complement deposition. The histological findings were correlated with donor and recipient data as well as clinical presentation.**

Results. **In 23 out of 39 grafts lost in the first 4 weeks posttransplantation, the only pathological changes found were vascular thrombosis and bland ischemic parenchymal necrosis. In these cases, no underlying vascular pathology or any other specific histological change was identified. Most of these grafts (78%) were lost in less than 48 hr and all in the first 2 weeks posttransplantation. Massive vascular thrombosis occurring in an otherwise histologically normal pancreas was the most common cause of graft loss in the first 4 weeks posttransplantation (59%). In most of the remaining cases (33%), although the clinical presentation suggested technical failure, there was clear histological evidence that the massive thrombosis resulted from vascular injury due to immune damage (acute and hyperacute rejection). Increased incidence of early graft thrombosis was seen in grafts from older donors and longer cold ischemia times. After the first month posttransplantation, graft pancreatectomies revealed a wider variety of pathological processes that included severe acute rejection, combined acute and chronic rejection, chronic rejection, and infections. Acute and chronic vascular thrombosis in large and small vessels was commonly seen at all times posttransplantation; chronic, organized thrombosis was strongly associated with chronic rejection.**

Conclusions. **(a) Early acute thrombosis occurring in a histologically normal pancreas defines a true technical failure. This study showed that acute rejection leading to massive thrombosis, which clinically simulates technical failure, results in a significant proportion of early graft losses. (b) Systematic histological evaluation of failed grafts is absolutely necessary for the accurate classification of the cause of graft loss. (c) There is morphological evidence that chronically ongoing thrombosis is an important, common, contributing factor for late graft loss.**

INTRODUCTION

Refinement of surgical techniques, potent immunosuppressive drugs, accurate diagnosis of rejection, better treatment of infections, and careful selection of donors and recipients have all resulted in the widespread use of pancreas transplantation with improving long-term results (*1–24*).

Whereas excellent rates of 1-year graft survival have been achieved in recent years for all types of technically successful pancreas transplants (simultaneous pancreas kidney (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK)) (*1*, *21*), a significant obstacle in pancreas transplantation continues to be the high incidence of graft loss in the early postimplantation period, due to a variety of surgical complications (*13*, *15*, *25–36*). As reported by the Pancreas Transplant Registry/UNOS, the marked decrease in the rejection rate has caused the relative risk of graft loss to be higher for technical failures than for rejection (*5*). Among the peritransplantation complications, thrombosis continues to be the leading cause of nonimmunological graft loss (5.8–16.4%) with higher rates seen in PAK and PTA cases with enteric drainage (*5*).

The pancreas has intrinsically a low blood flow compared with other solid organs. Perioperative inflammation and edema (*29*), as well as microvascular and endothelial damage relating to donor factors and organ preservation, all contribute to further compromise blood flow in the early posttransplant period (*34*), which leads to thrombosis. Correspond-

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ingly longer cold ischemia times have been associated with increased incidence of graft thrombosis (*30*, *36*).

Abnormalities in coagulation factors have been found in association with increased risk of thrombosis (*37*, *38*), and anticoagulation has been proposed to prevent clotting (*29*, *39*). This treatment, however, seems to be less important than better surgical technique and decreased pretransplantation graft damage in diminishing the risk of graft thrombosis (*13*, *15*).

Acute rejection is suspected to play a role in some patients with early graft thrombosis (*5*, *32*). HLA mismatches seem not to impact on the incidence of graft loss due to technical failures, however, HLA mismatch does have an overall negative impact on graft survival (*6*, *40*, *41*).

In this study, we performed a detailed, histological evaluation of all the pancreatectomies performed during the first 8 years of the pancreas transplant program at the University of Maryland. The objective of this study was to correlate the morphological findings with the clinical course. Specifically, we attempted to (a) determine the morphological changes associated with early graft thrombosis (up to 4 weeks); (b) evaluate the spectrum of morphological changes in pancreatectomies performed at later times, with emphasis on the relationship between graft loss and acute rejection, chronic rejection, and acute and chronic thrombosis; and (c) determine the overall pattern of graft loss in relationship to posttransplant time and specific pathological processes (e.g., rejection, infection, PTLD).

MATERIALS AND METHODS

Between April 1, 1991 and April 1, 1999, 301 pancreas transplants were performed at the University of Maryland Hospital (154 SPK, 114 PAK, 32 PTA). During the same time, 74 pancreas graft pancreatectomies were performed in 69 patients (5 retransplants). In all but two patients, the transplants were performed for insulin-dependent diabetes mellitus type I. Two patients had diabetes secondary to chronic pancreatitis. Five to fourteen routinely prepared hematoxylin and eosin stained sections from each case were reviewed by two pathologists, who were blinded to any clinical data. Available frozen tissue from 10 organs that failed within 7 days posttransplantation were evaluated by immunofluorescence studies for deposition of immunoglobulins and complement (IgG, IgM, IgA, C3). Immunoperoxidase stains for insulin and glucagon (Dako, Carpinteria, CA) were performed on 1 paraffin section from each of the 74 cases to determine the proportion and distribution of alpha and beta cells.

The histological parameters that were evaluated are thrombosis (recent and organized), type of vessel involved by thrombosis (arteries, veins, or both), acute rejection grade, chronic rejection grade, endotheliitis, transplant arteritis, coagulation necrosis, acute pancreatitis, presence of infectious organisms, transplant (obliterative) arteriopathy, neoplasia, and proportion of alpha and beta islet cells on peroxidase stains.

Data made available after the histological evaluation included pertinent clinical history, recipients' age and gender, dates of transplant and pancreatectomy, first serum amylase, and peak lipase in the first 24 hr posttransplantation. Donors' age, gender, weight, serum amylase, serum lipase, cause of death, and HLA mismatch were also recorded.

The diagnosis of infectious pancreatitis was based on the morphological pattern and the microbiology culture results. The diagnosis of acute rejection, chronic rejection, and posttransplant lymphoproliferative disorder was based on the previously described criteria (*42–44*).

The immunosuppression schemes were as follows: all patients received 10–14 days of induction therapy with either ATGAM (Upjohn, Peapack, NJ) or OKT3 (Orthoclone, OrthoBiotech, Raritan, NJ). Maintenance therapy was initiated when the nasogastric tube was discontinued (PAK, PTA) or when a dropping serum creatinine clearly indicated renal transplant function (SPK). Triple maintenance therapy consisted of either cyclosporine (Sandimune or Neoral) or tacrolimus (Prograf), prednisone and azathioprine (Immuran), or mycophenolate mofetil (Cellsept). Target blood concentration of cyclosporine or tacrolimus in the immediate postoperative period were 300–400 ng/ml and 12–20 ng/ml, respectively; by 1 year, the target levels were tapered to 200 ng/ml and 8–10 ng/ml, respectively. Rejection episodes were treated with 500 mg of intravenous methylprednisolone followed by a taper over 2 weeks. ATGAM (Upjohn) or OKT3 (Orthoclone, OrthoBiotech) were administered at standard doses for 10–14 days according to clinical parameters. The first episode of minimal (grade II) or mild (grade III) rejection was treated with corticosteroids. Recurrent episodes of rejection and moderate or severe rejection (grades IV and V) were treated with a combination of corticosteroids and ATGAM or OKT3.

Starting in 1998, all pancreas transplant patients received low doses of heparin for 5 days posttransplantation and then were placed on either ASA indefinitely (SPK) or Coumadin (PAK, PTA) for 3 months.

The morphological parameters were correlated with the patient and donor data and the time at graft loss and analyzed with the 2-tailed Pearson's correlation test and *t* test. Relative risks were calculated with the Cox regression analysis.

RESULTS

Demographic Data

The 74 graft pancreatectomies (including 5 retransplants) were performed on 69 patients; 29 females and 40 males; 7 African-Americans and 62 Caucasians; ages 26–59 with a mean of 28.5 years (SD 12.2 years). Time of graft pancreatectomy ranged from 1 hour posttransplantation to 81 months (mean of 6.4 months, SD 13.8 months). The pancreatectomies corresponded to 39 SPK, 21 PAK, and 14 PTA; 34 grafts were bladder drained and 40 were enteric drained. The donors had a mean age of 27.9 (range 10–54, SD 1.49); these were 14 African-Americans and 55 Caucasians; 25 females and 44 males. The donors' weights ranged from 110 to 229 lb (mean 149, SD 3.4); they had a mean serum amylase and mean serum lipase of 146.6 U/L and 164.4 U/L at the time of procurement (range 15–754, SD 164.4 and range 9–910, SD 72.04, respectively). The mean cold ischemia time was 1137 min (range 540–1950, SD 372 min). The degree of mismatch ranged from 0–6 (mean 3.38, SD 1.74). The cause of death was traumatic/accidental in 67 cases and cerebrovascular in 7 cases.

Histological Findings

Based on the histological findings the cases were classified in the following groups.

Pure vascular thrombosis in an otherwise normal pancreas. In 23 grafts, the only pathological changes found were vascular thrombosis and bland ischemic parenchymal necrosis. No underlying vascular pathology or any other specific histological change was identified in these cases (Fig. 1). The majority of these grafts (78%) were lost in less than 48 hr after transplantation (n=18) and all 23 were lost in the first 2 weeks posttransplantation.

Three of the 5 patients that required retransplantation lost both first and second grafts to this type of thrombosis. The other patients lost their first grafts to infection and

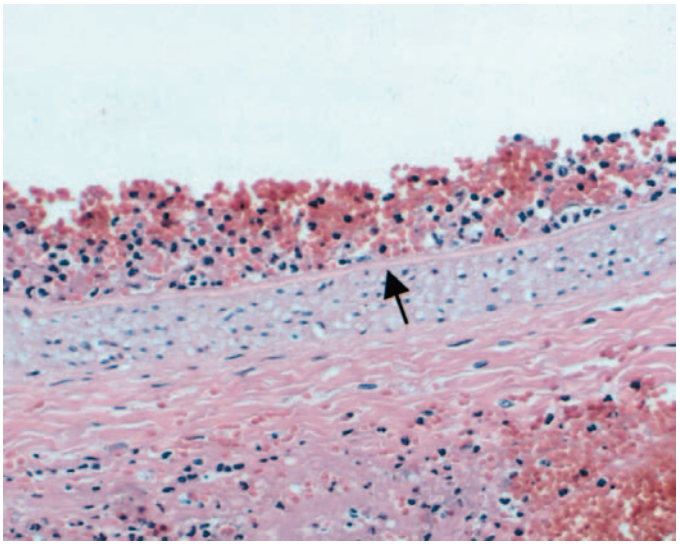


FIGURE 1. Acute thrombosis occurring in otherwise normal pancreas (early thrombosis). The vascular wall is intact. Arrow marks the internal elastic lamina, overlying the normal muscular layer.

rejection, respectively, and their second grafts to pure vascular thrombosis. The time intervals between the first and second grafts were 11, 12, 24, 8, and 7 months, respectively.

Hyperacute allograft rejection. Two pancreatectomy specimens, resected at 1 and 12 hr posttransplantation, respectively, showed fibrinoid necrosis of arteries and veins, indicating hyperacute allograft rejection (Fig. 2A); there was associated massive vascular thrombosis and parenchymal necrosis. Immunohistochemical studies indicated deposition of IgG and C3 in the wall of blood vessels (Fig. 2B).

The first patient with hyperacute rejection was a 50-year-old woman that underwent a PAK transplant. Within minutes of anastomosis, the graft became cyanotic, hemorrhagic, and had no blood flow. Repeat crossmatch and panel reactive antibodies were negative. Additional workup demonstrated (in the recipient's serum) antikeratinocyte antibodies reacting against 20% of a panel of samples.

The second patient with hyperacute rejection was a 40-year-old woman with history of high panel-reactive antibodies; she underwent plasmapheresis before a PAK. Despite an initially negative pretransplant crossmatch, within hours after transplantation the graft was tender, swollen, hemorrhagic, and lacked blood flow. The posttransplantation serum was positive for anti-HLA antibodies.

The cold ischemia time in these 2 patients was 1150 and 1740 min, with a mismatch 5 and 3 antigens, respectively. The donors' amylase and lipase were within normal limits.

Acute allograft rejection. The histological changes in 15 pancreatectomies resected between 1 week to 4 months posttransplantation (mean 5.1 weeks) consisted of endotheliitis and various degrees of necrotizing arteritis (acute rejection grade IV and V). Immunohistochemical studies in eight of these cases failed to show any significant immunoglobulin or complement deposition in the grafts.

Seven patients in this group (46.6%) had a posttransplantation course complicated by systemic and/or peripancreatic infections that required reduction of immunosuppression.

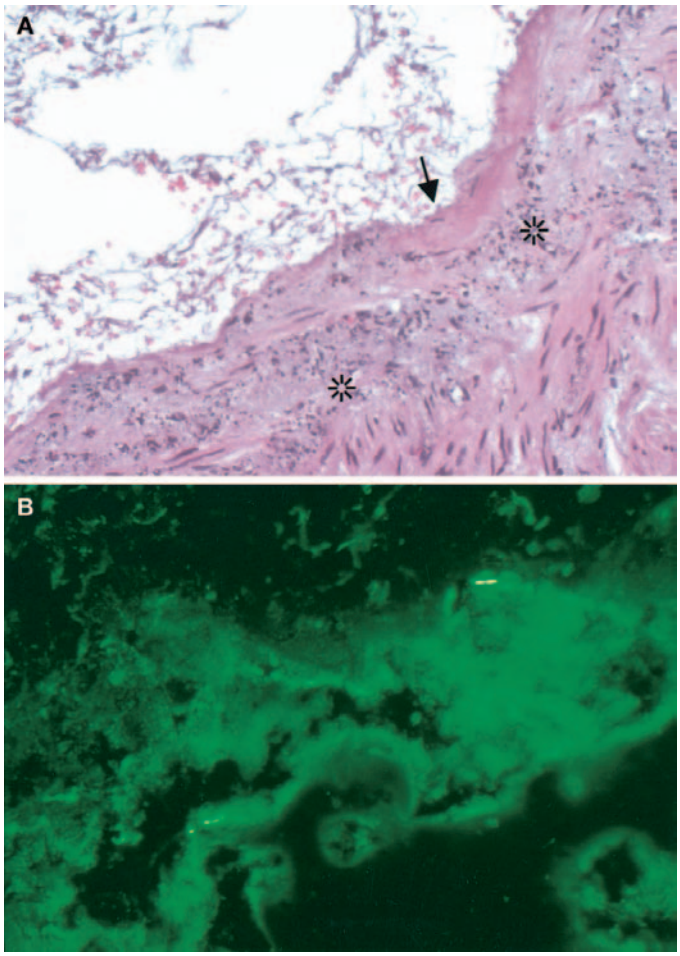


FIGURE 2. (A) Hyperacute allograft rejection with transmural necrosis of the arterial wall. Asterisks mark neutrophilic infiltrates. Early thrombus formation and fibrin strands are attached to the wall (arrow). (B) Hyperacute allograft rejection. Deposition of IgG is in the vascular walls.

Duodenal leaks were seen in five of these patients. Graft losses due to a combination of infection and rejection occurred between 3–6 weeks posttransplantation. In addition to the features of rejection, for patients with peripancreatic infection, the grafts showed increased fibrosis.

Acute and chronic rejection. Cases with persistent (biopsy proven) acute allograft rejection showed early interstitial fibrosis and acinar loss consistent with chronic rejection (*44*), starting in the second month posttransplantation. Six grafts showing these combined features of acute and chronic rejection were lost at times ranging from 6 weeks to 20 months (mean 6.6 months). Three patients that lost their grafts within 4 months posttransplantation due to acute rejection superimposed on accelerated chronic rejection received less than optimal immunosuppression due to persistent infectious complications.

Pancreatitis and peripancreatitis. Five grafts had necrotizing, infectious duodeno-pancreatitis and were resected at 1, 1, 2, 3, and 11 months, respectively (mean 3.6 months); these corresponded to 3 bacterial (*Enterobacter cloacae*, *Proteus mirabilis*, Methicillin resistant *Staphylococcus aureus* (MRSA)), 1 fungal (Candida glabrata), and 1 mixed infection

(Candida albicans/MRSA). Figure 3 demonstrates the wall of a pancreatic abscess.

Chronic rejection. Fifteen graft pancreatectomies showed extensive interstitial fibrosis and acinar atrophy in a pattern consistent with chronic rejection (Fig. 4) and were lost at a mean time of 28.6 months (range 4–81 months). These grafts did not show any significant concurrent acute rejection.

Posttransplant lymphoproliferative disorder. Epstein-Barr related posttransplant lymphoproliferative disorder (PTLD) resulted in five graft losses. Allograft pancreatectomies for PTLD were performed in four patients in the second month posttransplantation and in one patient in month 12 (mean 5 months).

Incidence of Vascular Thrombosis and Relationship with Rejection

Sixty-four grafts, out of the total of 74 (86.4%), displayed some degree of recent thrombosis; in 39 of these grafts, the recent thrombosis was extensive and was associated with focal or diffuse ischemic (coagulative) or hemorrhagic necrosis. Arteries and veins were affected in 44 grafts with recent thrombosis; venous thrombosis only was seen in 4 cases and arterial thrombosis only in 14 cases. There was no correlation between the time of graft loss and the type of vessel affected by the thrombosis.

Recent thrombosis was seen to some degree in all cases of early graft loss due to acute allograft rejection with vascular involvement (endotheliitis or arteritis) (Fig. 5A).

Eight functioning pancreas allografts had to be resected between 10 and 36 months posttransplantation due to acute (recent) thrombosis occurring in larger arteries. In these cases, the thrombosis always occurred in abnormal blood vessels, either showing transplant arteriopathy or lesions consistent with healing vasculitis/endotheliitis (Fig. 5B).

All cases with chronic rejection showed scattered vessels with thrombosis (acute and chronic in 13 cases and only chronic (old, organized) in 2 cases); this typically involved medium size to small arteries and veins (Fig. 5C).

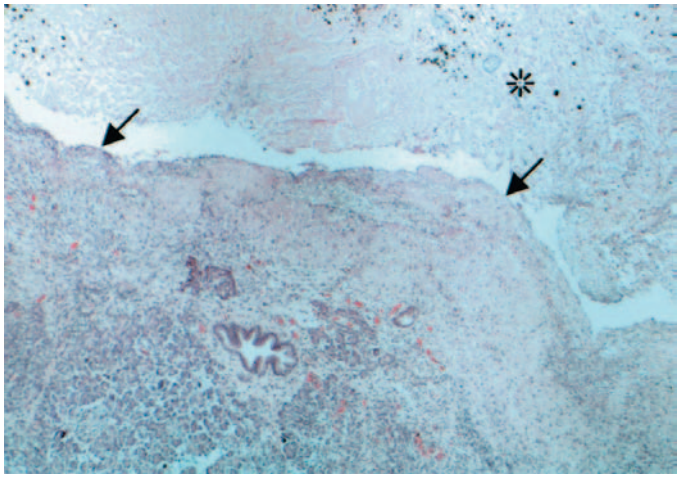


FIGURE 3. Necrotizing bacterial pancreatitis with abscess formation. The cavity of the abscess (asterisk) is lined by a wall composed of granulation tissue and acute and chronic inflammatory exudates (arrows).

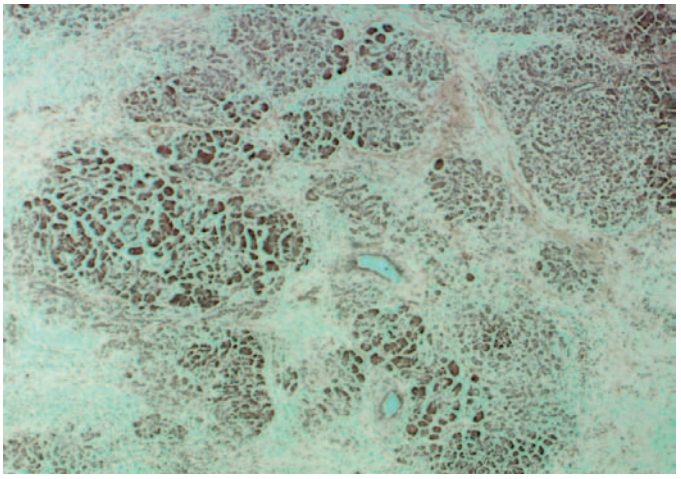


FIGURE 4. Chronic rejection is characterized by an increase in parenchymal fibrosis and concurrent atrophy of the acinar component.

Thrombosis was insignificant in the pancreatectomies performed for infectious processes.

Immunohistochemical Stains for Insulin and Glucagon

The viable components of pancreatectomies that showed no fibrosis or atrophy showed similar strength of staining and pattern of distribution for insulin and glucagon to the controls. Pancreatectomies with chronic rejection displayed fragmented or hyperplastic islets; alpha and beta cells were present in all cases as previously described (*45*). We did not find selective loss of beta cells or evidence of insulitis in any of the pancreatectomies.

Statistical Correlations Between Histological Findings and Other Data

Older donor age and longer cold ischemia time were associated with increased occurrence of early graft thrombosis ($r=0.240$, $p=0.01$, and $r=0.275$, $p=0.02$, respectively). Thrombosis overall occurred with increased frequency in grafts from older donors ($r=0.253$, $p=0.03$).

Higher donor amylase levels were associated with an increased overall incidence of acute rejection ($r=0.323$, $P=0.009$); high donor amylase levels were also associated with higher histological grades of acute rejection (grades IV and V, $r=0.260$, $P=0.03$). The mean donor amylase corresponding to grafts that did not show acute rejection was 98 U/L (SE 17.4), whereas the mean donor amylase corresponding to grafts that showed acute rejection was 216 U/L (SE 38.3), $P=0.003$.

Higher peak lipase values in the first day posttransplantation were associated with increased incidence of acute allograft rejection occurring within the first month posttransplantation ($r=0.377$, $P=0.006$).

The presence of transplant arteriopathy (one of the histological features of chronic rejection) was strongly associated with recent and organized thrombosis ($r=0.278$, $P=0.01$, and $r=0.469$, $P<0.000$, respectively). Correspondingly old (organized) thrombosis was seen almost invariably in pancreatectomies with chronic rejection ($r=0.378$, $P=0.001$). As expected, progressive graft fibrosis (correlating with increasing

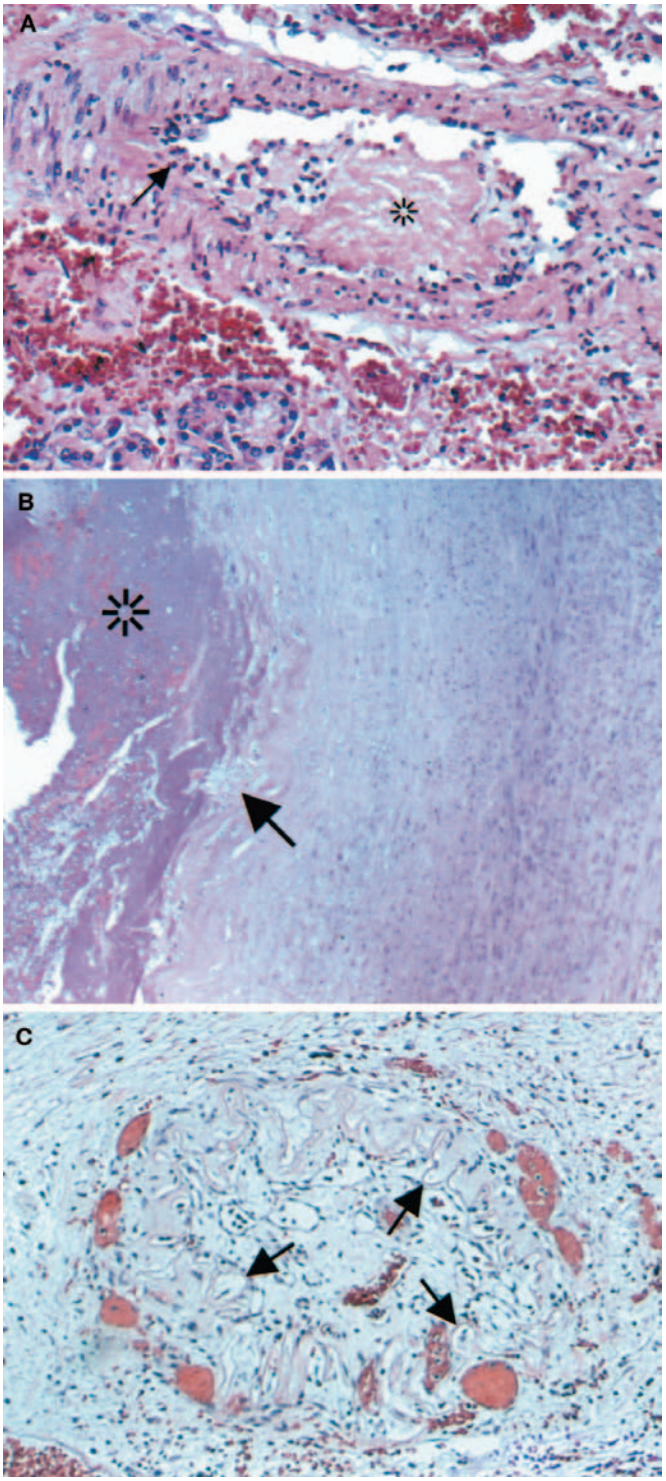


FIGURE 5. (A) Early endotheliitis (arrows) associated with recent fibrin and platelet thrombi (asterisk). (B) Recent (non-organized), massive thrombosis (asterisk) occurring 28 months posttransplantation. The thrombosis occurred in association with abnormal endothelium overlying transplant arteriopathy. The arterial wall is thickened, fibrotic, and there are clusters of subendothelial foam cells (arrow). (C) Organized thrombosis with recanalization in medium-size artery. Arrows mark the internal elastic lamina. Note multiple vascular lumina embedded in fibrous tissue occupying what used to be the original lumen. These changes are commonly seen in pancreatectomies with chronic rejection.

grades of chronic rejection) and the presence of transplant arteriopathy were directly related to the time elapsed after transplantation ($r=0.518$, $P=0.000$ and $r=0.699$, $P<0.0001$, respectively).

There were no significant differences in the donor and recipient data or type of transplant between the group of patients with simple (idiopathic) graft thrombosis occurring early posttransplantation, the group of patients with acute allograft rejection that lost their grafts within 6 months posttransplantation, and the group of patients with successful grafts at 12 months posttransplantation. With respect to drainage type, there was a trend suggesting an association between enteric drainage and idiopathic, early thrombosis, but this did not reach statistical significance ($P=0.057$). No correlation could be found between the type of death in the donor (traumatic versus cerebrovascular).

The type of graft loss in relationship to time of pancreatectomy is summarized in Table 1. Early thrombosis occurring in histologically normal organs and thrombosis due to acute/hyperacute rejection caused 92% of graft losses in the first month posttransplantation. Chronic rejection was the most important cause of graft loss after the first 6 months posttransplantation. Table 2 summarizes donor and recipient data for each histological group.

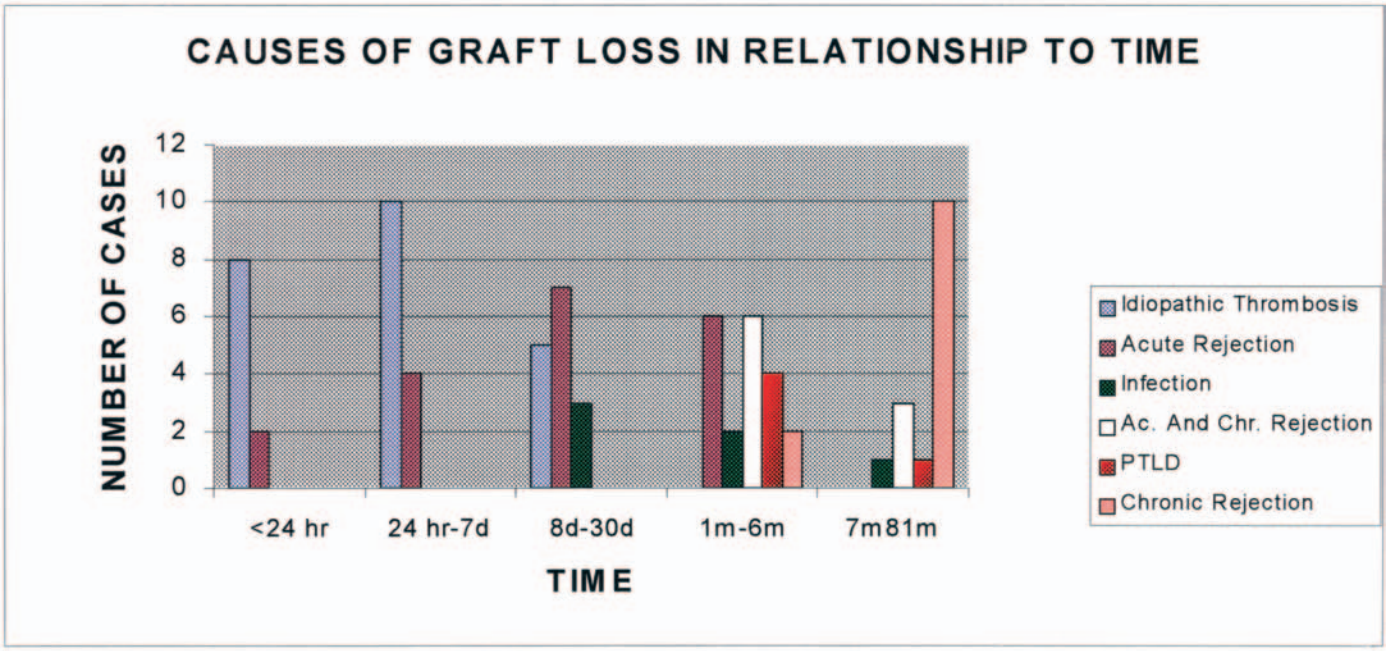
DISCUSSION

Graft losses due to leaks, bleeding, thrombosis, infections, and pancreatitis are grouped together under the category of technical failure. Among these complications, massive vascular thrombosis continues to be the most important cause of early graft loss due to technical failure (*5*). Many factors have been implicated in the occurrence of early graft thrombosis, including old donor age, long cold ischemia time, and poor surgical technique (*15*, *23*, *29*, *33*, *34*, *36*, *45*). The possibility of immunological factors involved in technical failures was raised in the 1994–1998 summary report from the International Pancreas Transplant Registry (*5*). The possibility of thrombosis occurring relatively late due to relatively early rejection arose from the fact that for PAK the risk for technical failure was "inexplicably high as the number of HLA mismatches increased," whereas apparent protection against technical failure was seen with the use of tacrolimus and mycophenolate versus other less potent immunosuppressants (*5*).

The morphological findings in allografts considered to be lost due to technical failure had not been systematically addressed. On the other hand, pathological evaluation of most allograft pancreatectomies performed in the first weeks posttransplantation period do show a common feature, which is the presence of some degree of vascular thrombosis with associated proportional parenchymal necrosis. In this study, we sought to define additional histological features that could help to better understand the mechanisms of graft failure requiring pancreatectomy.

The main histological feature present in the majority of the grafts lost in the first month posttransplantation was extensive vascular thrombosis. In 59% of these grafts, massive thrombosis occurred in otherwise structurally normal pancreas. Thrombosed pancreas with no underlying histopathological abnormalities represent the group of true technical failures.

TABLE 1



In contrast to the group showing early thrombosis in otherwise normal pancreas, in 33% of cases lost within 1 month posttransplantation, extensive vascular thrombosis occurred superimposed on immunological endothelial damage (acute rejection) in the form of endotheliitis or arteritis. Although the need for pancreatectomy in these cases was determined by the occurrence of massive vascular thrombosis, due to the presence of definite acute allograft rejection, these cases cannot be considered idiopathic in nature or true technical failures. The exact determination of the cause of graft loss is further complicated by the fact that in more than half of these patients, the immunological graft loss resulted not from rejection refractory to treatment but from lowered antirejection prophylaxis because of serious infections affecting these patients.

With the exception of rare cases of hyperacute allograft rejection, immune-type losses occurred more often after the second week posttransplantation. This contrasted with the timing of graft pancreatectomy in idiopathic graft thrombosis (true technical failure) that occurred very early in the posttransplantation period, usually within 48 hr and always within the first 2 weeks.

Hyperacute allograft rejection in pancreas transplantation is considered a rare occurrence (*46*). The morphological features in the two cases in this series correspond to the findings described in experimental hyperacute allograft rejection in the pancreas. Hyperacute rejection in the pancreas is indistinguishable from hyperacute rejection affecting other organs (*47*), and it is characterized by necrosis of arteries and veins with secondary massive and immediate thrombosis and parenchymal necrosis.

Thrombosis in pancreas allografts complicates not only the early posttransplantation period but may also occur at later times (*33*). In this study, we found a clear relationship between thrombosis and the presence of acute or chronic damage to vascular walls at all posttransplantation times; the vascular damage occurs in the form of endotheliitis/arteritis in acute rejection and as transplant (obliterative) arteriopathy in chronic rejection. Other more subtle forms of endothelial damage, seen with high levels of cyclosporine, have been implicated in the formation of thrombi in pancreas allografts in one study (*33*).

In the case of early thrombosis, the lack of obvious histological changes associated with the thrombosis does not rule out ultrastructural or subtle functional damage in these organs, because older donor age and longer cold ischemia times were associated with increased risk for early thrombosis. Our findings confirm other studies that showed an association between increasing donor age with long cold ischemia time and technical failures (*33*, *36*, *48–50*). Increased risk for graft thrombosis with older donor age is probably related to preexisting vasculopathy (i.e., atherosclerotic disease). This idea is supported by the fact that donors' cardiovascular disease is associated with worst graft outcome (*33*).

Previous studies have shown that increase in donor amylase levels have no significance for graft survival or immediate function (*47*, *51*, *52*). In this study, we found that there was a statistical association between high donor amylase levels and acute rejection. Although at this time specific data are lacking, it may be speculated that increased exposure of cellular antigens secondary to cellular damage during procurement and preservation could increase the risk of acute rejection.

In this study, there was a strong statistical association between organized (old) thrombosis and the presence of interstitial fibrosis and acinar loss (histological parameters that define chronic rejection). We believe that minor but repetitive episodes of vascular thrombosis contribute to graft sclerosis due to chronic ischemia. Judging by the histological appearance of these vessels, the process seems to be cyclic with initial thrombosis occurring in blood vessels damaged by endotheliitis or transplant arteriopathy followed by organization/recanalization and further formation of clots in the now markedly narrowed blood vessels. This process, which is inherently chronic, tends to

TABLE 2. Donor and recipient data for each histological group

	All cases	Early th[a]	AR early[b]	AR late	AR+CR[c]	PTLD[d]	Infection	CR
Cases	74	23	13	6	9	5	6	12
Gender	29F[e]	9F	7F	3F	2F	2F	2F	5F
Mean age	28.5	39.5	42.1	40.3	39.4	35.4	39.7	39.7
Race	7A,[f] 62C[g]	2A, 21C	1A, 6C	6C	1A, 8C	2A, 4C	1A, 5C	1A, 11C
Mean time of loss	6.4m	3.3d	2.8w	3.8m	6.6m	5m	4.2m	28m
Tx type	21pak,[h] 14pa,[i] 39spk[j]	7pak, 9pa, 7spk	1pak, 7pa, 4spk	1pak, 5spk	1pa, 8spk	1pak, 1pa, 3spk	1pa, 5spk	4pak, 2pa, 6spk
Drainage	34B,[k] 40E[l]	3B, 10E 3B, 10E	3B, 3E	7B, 2E	4B, 1E	3B, 3E	8B, 4E	
Mean 1st amylase	292.2	270.4	426.2	434	237.7	156.2	203.2	237
Mean 1st lipase	1918	2009.4	1920.2	4638	1742.5	843	1793.4	1068.9
Mean Donor age	27.9	32	25	28	29	22	25	26
Mean Donor amylase	146.6	84	100	283	183	313	140	142
Mean Donor lipase	61	59	56	56	99	101	57	40
Mean CIT[m]	1137	1204	970	884	1039	766	1083	1064
Mean MM[n]	3.3	3.8	3	4	4	3.8	3.3	3.5

[a] Early th, thrombosis occurring in normal pancreas.
[b] AR, acute rejection.
[c] CR, chronic rejection.
[d] PTLD, posttransplant lymphoproliferative disorder.
Early, <4 weeks.
Late, >4 weeks.
[e] F, female.
[f] A, African American.
[g] C, Caucasian.
[h] pak, pancreas after kidney.
[i] pa, pancreas alone.
[j] spk, simultaneous pancreas kidney.
[k] B, bladder drained.
[l] E, enteric drained.
[m] CIT, cold ischemia time.
[n] MM, HLA mismatch.

disproportionately affect medium and small sized arteries and veins. In contrast, abrupt graft dysfunction and thrombosis requiring pancreatectomy that occurs in grafts with vessels damaged by transplant arteriopathy or ongoing endotheliitis affects the main arteries. Thus, thrombosis is a common histological finding associated with most forms of graft injury during the whole posttransplantation period.

After the first month posttransplantation, graft pancreatectomies were performed as the result of a wider variety of pathological processes that included severe acute rejection, combined acute and chronic rejection, chronic rejection, and infections; the latter included bacterial, fungal, and EBV-related lymphoproliferative disorders. From the clinical perspective, a similar pattern of graft loss has been reported previously by Stratta (*17*, *18*).

Summary

(a) Massive vascular thrombosis is the most common cause of pancreas allograft loss. It can be present in completely normal pancreas or can result from immunological damage to blood vessels. In the early posttransplantation period, both of these processes can clinically present as technical failures. Acute early thrombosis occurring in normal pancreas represents the morphological definition of a true technical failure; this type of idiopathic thrombosis was never seen after 2 weeks posttransplantation. (b) Systematic histological evaluation of failed grafts is necessary for accurate classification of the cause of graft loss. Minimum histological sampling should include cross-sections of all large vessels and several sections from the parenchyma to include an adequate number of medium size and small vessels. (c) The consistent presence of recent and organized thrombosis in pancreas allografts with chronic rejection underscores the importance of acute and chronic thrombosis as a contributing factor for late graft loss. Further studies are necessary to establish the practical significance of these findings and to determine if some form of long-term anticoagulation therapy can be potentially useful to prolong pancreas allograft survival.

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A SCHEMA FOR HISTOLOGIC GRADING OF SMALL INTESTINE ALLOGRAFT ACUTE REJECTION

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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

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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.

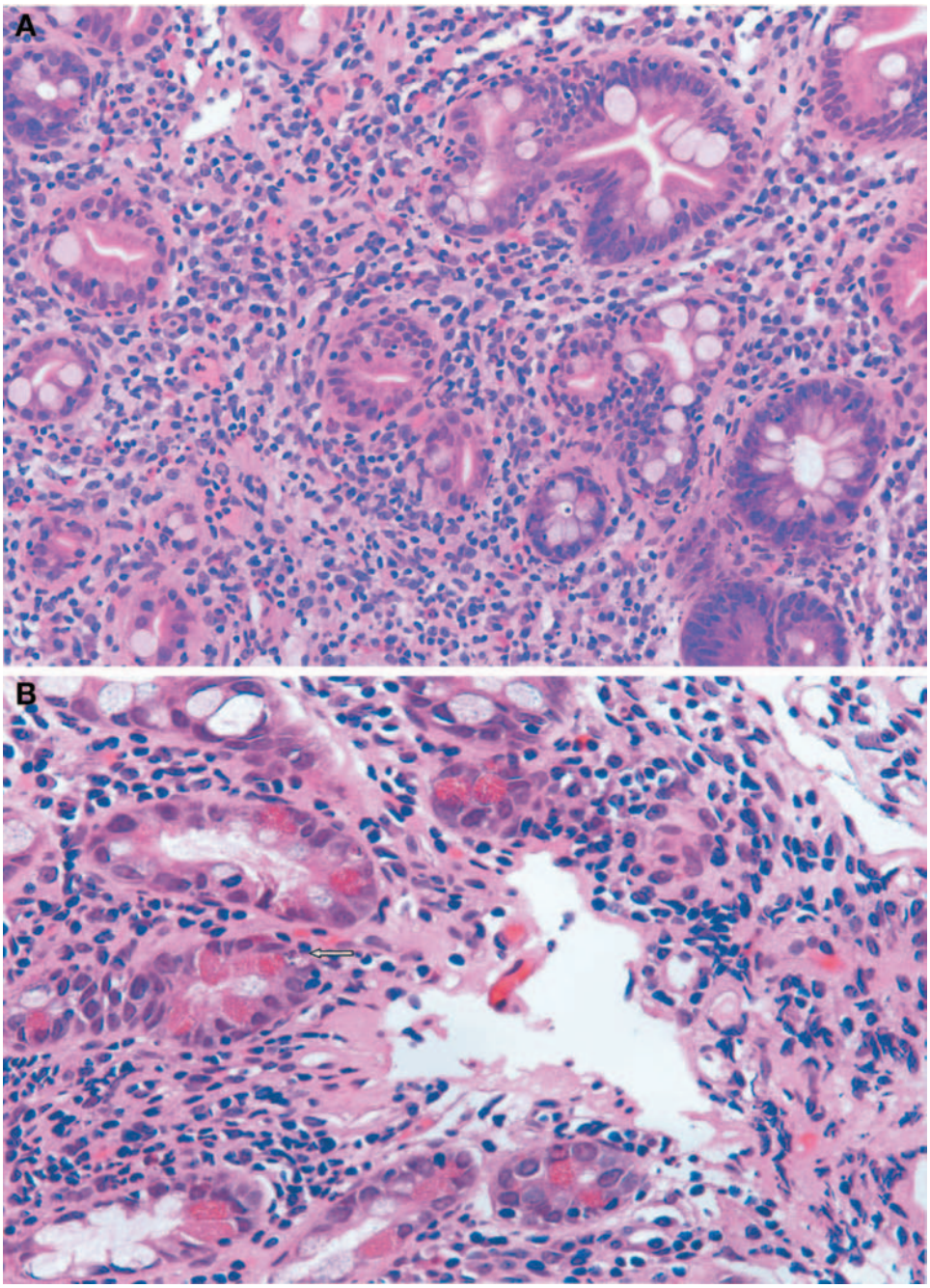

FIGURE 1. Indeterminate for acute rejection. The lamina propria is infiltrated by a heterogeneous population of mononuclear cells composed of blastic and small lymphocytes, plasma cells, and plasmacytoid lymphocytes. There is focal minimal crypt damage and apoptotic bodies (*arrow*) (hematoxylin-eosin; 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

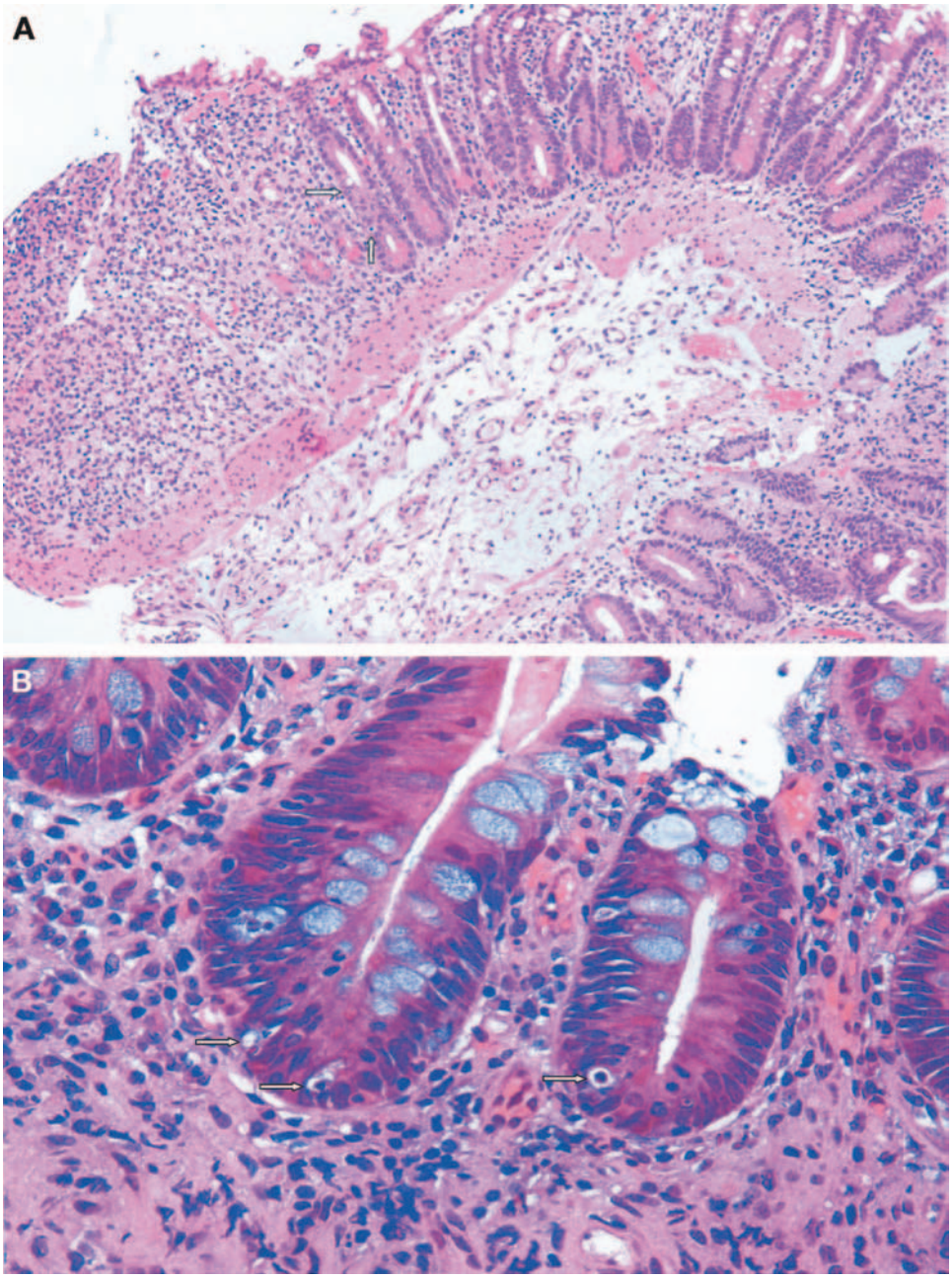

FIGURE 2. Mild acute rejection. (A) The villi are shortened and the architectural features are distorted because of expansion of the lamina propria by the heterogeneous mononuclear cell infiltrate (*left*). The crypts exhibit features of epithelial injury and scattered apoptotic bodies (*arrows*) (hematoxylin-eosin; 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-

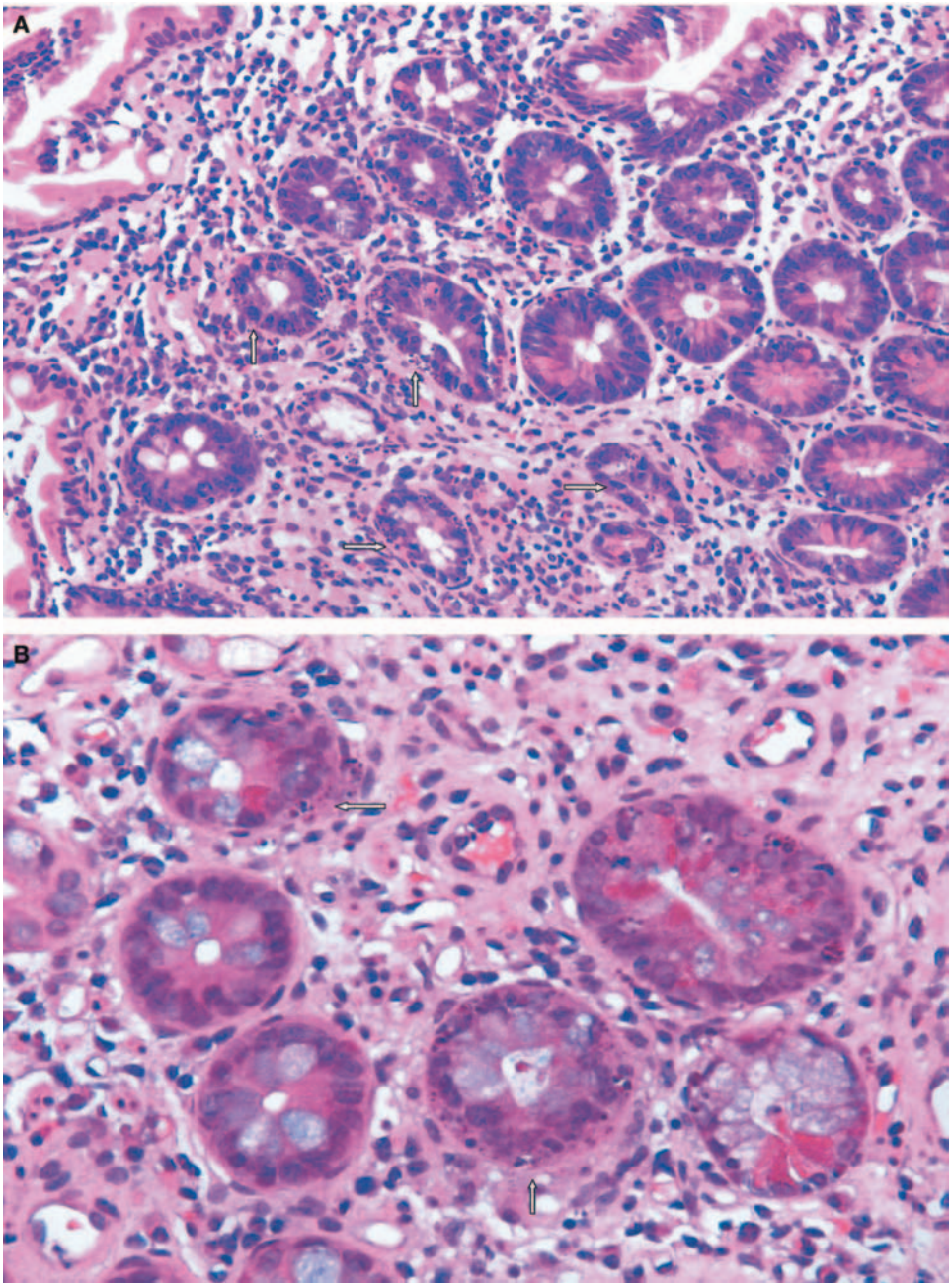

FIGURE 3. Moderate acute rejection. Crypt damage and apoptosis are distributed more diffusely than in mild acute rejection. The number of apoptotic bodies is greater than in mild acute rejection, with focal confluent apoptosis (*arrows*). The mucosa is usually intact, without ulceration (hematoxylin-eosin; 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

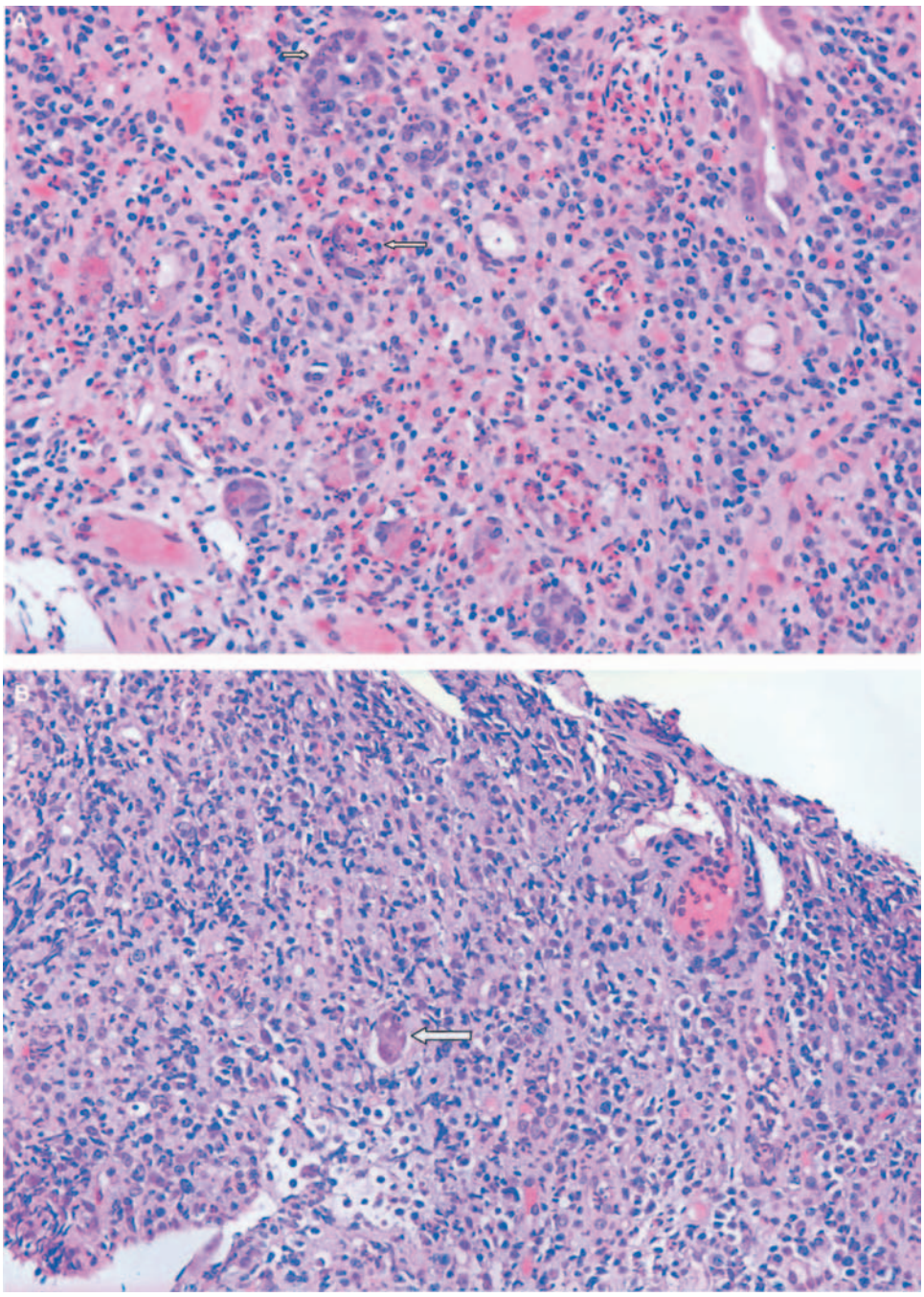

FIGURE 4. Severe acute rejection. There is extensive mucosal destruction, with loss of crypts, mucosal ulceration, and mixed lymphoplasmacytic, eosinophilic, and neutrophilic infiltration. The residual crypts, if present, often exhibit marked epithelial injury and apoptosis (*arrows*) (hematoxylin-eosin; 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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(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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PTLD Classification of 1997 (Harris et al.)

Category	Examples	Histopathology	Clonal Status	Oncogene, Tumor Suppressor Gene Changes	Comments
"Early" lesions	Reactive plasmacytic hyperplasia, (atypical lymphoid hyperplasia)*, infectious mononucleosis-like	Some architectural preservation; numerous plasma cells and lymphocytes; many immunoblasts may be present; atypia slight	Polyclonal; Minor clones may be present	(None)	Often regress with reduced immunosuppression, severe cases may be fatal
PTLD-Polymorphic	(Polymorphic B cell hyperplasia)*, (Polymorphic B cell lymphoma)*, (Polymorphic PTLD)*	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	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
PTLD-Monomorphic	**B-cell lymphomas:** Diffuse large B-cell lymphoma (e.g., immunoblastic, centroblastic, anaplastic subtypes), Burkitt's or Burkitt-like lymphoma **T-cell lymphomas:** Peripheral T-cell	Majority of cells are transformed with cytologic atypia, prominent nucleoli, basophilic cytoplasm, underlying architectural	Monoclonal	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; T cell antigens CD43 and CD45RO may be

	lymphoma, unspecificed type (usu. large cell), Anaplastic large cell lymphoma (T or null cell), Other types (eg, T-NK)	effacement/destruction			present on B cells in these cases; Some B cell tumors may regress with reduced immunosuppression; T cell PTLD had longer time to onset and did not regress in this sample.
PTLD-Other	a) T-cell rich/Hodgkin's disease-like B-PTLD, b) Plasmacytoma-like PTLD	a) Diffuse background of small lymphocytes with scattered Reed-Sternberg-like cells; b) diffuse infiltrate of mature plasma cells	Monoclonal	None described	T cell-rich/HD-like B-PTLD may regress with reduced immunosuppression
*Cases included in classification but examples not presented for review at Society for Hematopathology Workshop					

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Neoplasia

PT-LPD Classification of 1995 (Knowles et al.)			
Category	**Histopathology**	**Clonal Status**	**Oncogene or Tumor Suppressor Gene Changes**
1	Plasma cell hyperplasia	Polyclonal, occasional faint clone	No
2	Polymorphic B cell hyperplasia or Polymorphic B cell lymphoma	Monoclonal	No
3	Pleomorphic immunoblastic lymphoma, plasmacytoid immunoblastoid lymphoma, or multiple myeloma	Monoclonal	Yes
Note: Observed molecular alterations in Category 3 included rearrangement of c-myc, mutation of N-ras and mutation of p53 in individual cases.			

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Neoplasia

PTLD Classification of 1988 (Nalesnik et al.)						
Category	**Lymphocyte Heterogeneity (Polymorphism)**	**Plasmacytic Cells**	**Necrosis**	**Underlying Architecture**	**Clonality#**	**Comments**
Reactive Diffuse Plasma Cell Hyperplasia	Minimal	Marked	No	Intact	Not determined	Described, but relationship to PTLD undefined
Polymorphic PTLD	Prominent	Variable	Variable may be prominent	Disrupted*	17/31 (55%) with Monoclonal component	Includes Polymorphic Hyperplasia and Lymphoma of Frizzera et al.
Minimally Polymorphic PTLD	Minimal	Prominent	Variable	Disrupted	18/22 (82%) with Monoclonal component	Term not widely applied; synonymous with plasmacytoma
Monomorphic PTLD	None	Rare to None	Variable	Disrupted	5/5 (100%) Monoclonal	Synonymous with non-Hodgkin's lymphomas
Notes: *Early infiltrative lesions may not have recognizable architectural destruction; #Clonal composition was based on molecular or immunoperoxidase studies in individual cases.						

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Neoplasia

PTLD Classification of 1981 (Frizzera et al.)						
Category	**Follicular Center Cells**	**Plasmacytic Cells**	**Large Lymphoid Cells**	**Atypical Immunoblasts**	**Invasion**	**Necrosis**
Nonspecific Reactive hyperplasia	++ (Germinal Centers)	++	+/++	No	No	No
Polymorphic Diffuse B Cell Hyperplasia	++ (Diffuse)	++	++/+++	No	+	No
Polymorphic Diffuse B Cell Lymphoma	++ (Diffuse)	+	++/+++	+/+++	+	+++
Immunoblastic Sarcoma of B Cells	No	+	+++	+/+++	+	+

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Review article

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The diverse pathology of post-transplant lymphoproliferative disorders: the importance of a standardized approach

Key words:
organ transplantation; lymphoma; lymphoproliferation; post-transplant lymphoproliferative disorders; Epstein–Barr virus

Abstract: Post-transplant lymphoproliferative disorders (PTLD) are a diverse group of abnormal lymphoid growths that include both hyperplasias and neoplasias. They have been divided into several general pathologic categories that have prognostic significance. These include early or hyperplastic PTLD, polymorphic PTLD, and lymphomatous or monomorphic PTLD. The majority of PTLDs are of B-cell origin and contain Epstein–Barr virus (EBV). However, PTLDs of T- or NK-cell origin have been described, and late-arising EBV-negative lymphoid tumors are becoming more frequently reported in this population. Other lymphoid neoplasms, such as those arising from mucosal-associated lymphoid tissue (MALTomas), have recently been recognized in transplant patients, and their relationship to PTLD is uncertain. Multicentric PTLD may represent either advanced-stage disease or multiple independent primary tumors. Likewise, recurrent PTLD may represent true recurrence or the emergence of a second primary tumor. Transplant patients are also at risk for other opportunistic neoplasms, including EBV-associated leiomyosarcomas that may be seen alone or in conjunction with PTLD. This underscores the necessity for pathologic diagnosis of mass lesions in this patient population. The pathologist should strive to categorize the form of post-transplant lymphoproliferation in accordance with currently accepted criteria. The diagnosis should incorporate the histopathologic appearance, cell phenotype, clonal status, and EB viral status. The pathologist may play a special role in guiding therapy by ascertaining the presence of such markers as CD20 on tumor cells. Specialized techniques, such as molecular analysis of oncogenes/tumor suppressor genes and evaluation of host:donor status of PTLD, may play important roles in diagnostic evaluation in the future.

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The proper management of post-transplant lymphoproliferative disorder (PTLD) is aided greatly by precise pathologic diagnosis. Transplant patients, like other patients, may develop non-neoplastic mass lesions, or they may develop tumors other than PTLD (1). Episodes of suspected allograft rejection may in reality represent allograft-restricted PTLD, or may be due to concurrent acute rejection and PTLD (2–5). Clinical recurrence of PTLD may reflect re-emergence of the same tumor, development of a separate tumor

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clone, or the appearance of a completely unrelated neoplasm (6, 7).

The importance of pathologic diagnosis to management of PTLD is further emphasized by the variety of lymphoid tumors that comprise this syndrome (8). Some of these tumors are prone to regression and some are not, some contain the Epstein–Barr virus (EBV) and some do not, some are monoclonal and some are not, some are of B-cell origin whereas others may be of T-cell or NK-cell origin.

The classical role of the pathologist is to first describe disease, then to categorize variants of the disease and finally to explain the events underlying disease emergence, progression and resolution. Progress in these areas does not proceed uniformly, and advancement in one area often impacts or redefines the others. It is helpful to keep these principles in mind as we summarize the various developments that have contributed to our current understanding of PTLD, and as we relate these lessons to the practical questions that surround the pathologic diagnosis of these disorders.

Development of PTLD classification systems

Pathologic interpretation of PTLDs was predated by the clinical observation that transplant patients appeared prone to develop lymphomatous growths (9). The seminal studies defining the concept that a range of lymphoproliferations could occur in the post-transplant setting were performed by Frizzera et al. (10) on lesions that arose in kidney transplant patients. In 1981, there were no monoclonal antibodies to type lymphocytes, and neither routine flow cytometry nor molecular clonal analysis with which to analyze tissues. Using histologic analysis alone, this group was able to define two conditions that arose as abnormal growth of B lymphocytes. These lesions were named polymorphic diffuse B-cell hyperplasia and polymorphic diffuse B-cell lymphoma. These proliferations had in common a diffuse, invasive growth pattern and a variety of cell sizes, representing a wide range of lymphoid maturation, on microscopic examination. The two conditions were distinguishable from each other on the basis of large bizarre cells and necrosis that were present in cases of polymorphic lymphoma, but not in polymorphic hyperplasia. Based on immunoglobulin staining results in paraffin sections, it was determined that the polymorphic hyperplasias were polyclonal and therefore reactive, and that the polymorphic lymphomas contained an emerging monoclonal population of cells. The authors presumed that the large bizarre cells in such cases represented this emerging monoclonal malignancy. Since concomitant studies from this group also identified the presence of the Epstein–Barr virus (EBV) in all of these lesions, it was concluded that the polymorphic B-cell hyperplasias most likely represented virus-driven growths and that this process could ultimately give rise to malignant neoplasms. On this basis it was proposed that antiviral therapy might prove useful in cases of polymorphic diffuse B-cell hyperplasia, whereas antineoplastic therapy would be more appropriate for the polymorphic lymphomas. Importantly, the authors also observed that the behavior of a given tumor could not always be predicted from the histopathology alone.

Clonal analysis of lymphoid growths became clinically available shortly thereafter. The Stanford group applied this approach to lymphoproliferations that arose in their transplant patients and concluded that all of the lesions were monoclonal, regardless of histologic appearance (11). Study of additional cases from this series, particularly cases of multiple concurrent tumors, revealed that separate monoclonal tumors could arise from separate clones, representing multiple independent primary tumors. The philosophy of reduced immunosuppression guided therapy of PTLD in the Pittsburgh transplant series (12). This approach had the side effect of allowing follow-up study of the behavior of PTLDs of different histopathologic appearances in the setting of partially restored host immune competence (13). Both polymorphic diffuse B-cell hyperplasias and polymorphic B-cell lymphomas were identified, and both were observed to regress in a number of cases following reduction or discontinuance of immunosuppression. We therefore elected to group these conditions together under the heading of "polymorphic PTLD" (14). This had an added practical benefit in that it simplified analysis of small biopsies in which the histologic differentiation between polymorphic hyperplasia and polymorphic lymphoma was problematic. Some lesions in this series continued to progress despite reduction of immunosuppression. A number of these had in common a more uniform appearance of lymphoid cells. Similar tumors had been recognized by the Minnesota group as immunoblastic sarcomas and had been separated from the polymorphic lesions in their series (10). However, we also observed tumors that did not fit the criteria of immunoblastic sarcomas (using the then-current terminology) but were also of uniform cell type and progressed despite therapy. As we were able to detect the EBV in these tumors, the relationship of such tumors to standard non-Hodgkin's lymphomas was unknown. We therefore elected to descriptively group these lesions together as "monomorphic PTLD" (14).

As more PTLDs arose, limitations of the descriptive pathologic classification systems began to appear. Lesions with features intermediate between polymorphic B-cell hyperplasia and polymorphic B-cell lymphoma were described (15). The ability to detect EBV

within routine pathologic samples improved (16) and gave rise to nosological questions. Was the presence of EBV necessary for the diagnosis of PTLD? What was to be done with lesions that contained EBV but fell short of the histologic criteria for inclusion into one of the categories defined for PTLD?

The answer to the first question had been given from a practical standpoint all along. Although EBV was recognized as an important cofactor of PTLD, none of the classification systems had required the presence of the virus for a diagnosis of PTLD. The answer to the second question will be considered under the heading of PTLD and EBV, below.

In the Pittsburgh series it was recognized that a small percentage of PTLDs contained rearrangements of the c-myc proto-oncogene, and that such tumors had a worse outcome than the overall PTLD population (17). However, it remained for Knowles et al. (18) to apply molecular genetic techniques to the study of PTLD in a uniform fashion, and to integrate it into their classification system.

Their findings, based on their experience with PTLD in heart transplant patients, supported and extended earlier studies. Using molecular clonal analysis they found that all lesions that appeared histologically as polymorphic B-cell hyperplasias were actually monoclonal proliferations. Thus the term polymorphic hyperplasia is somewhat of a misnomer, as hyperplasias do not typically contain monoclonal components. They confirmed the observation that polymorphic hyperplasias and polymorphic lymphomas often, but not always, regress with conservative therapy, and they also noted that histologic forms intermediate between these two categories existed. Nevertheless, they retained these two terms under the general category of polymorphic PTLD (or PT-LPD as an alternative acronym). Their molecular analysis of PTLD showed that anomalies described in standard lymphomas, such as p53 or N-ras mutations or c-myc rearrangements, occurred only in the category of PTLD that histologically resembled lymphomas, i.e., the monomorphic PTLDs. They recommended that the term monomorphic be dropped and replaced by standard lymphoma nomenclature for these lesions. They also recognized a benign-appearing diffuse growth of mature plasma cells, so-called plasma cell hyperplasia, as representative of an early form of PTLD. Such lesions were invariably polyclonal or contained at most a minor clonal component. These workers also suggested that such early cases, which commonly appeared in the head and neck region, represented most or all of the cases in the Pittsburgh series that underwent regression. (In passing, we note that regression in our series was not limited to such cases). Thus, by 1995, the general categories of PTLD had been distilled into hyperplasias, polymorphic PTLDs, and lymphomatous (monomorphic) PTLD.

Society of Hematopathology classification system of PTLD

Neither the classification system of Knowles et al. (18) nor any of the earlier systems (10–14) incorporated entities such as T-cell lymphomas or Hodgkin's disease, which were also being described in transplant patients (19, 20). In 1996 the Society of Hematopathology (SH) convened a meeting to review a collected series of PTLD cases and to attempt to incorporate the entities into a common classification system (8). Their system, although more inclusive than previous systems, likewise recognized three major categories in this family of disorders: a) lymphoid hyperplasias, or "early" lesions, b) polymorphic PTLDs, and c) lymphomatous or monomorphic PTLDs. They also included an "Other" category to descriptively include some of the more recently described variants of lymphoid neoplams observed in transplant patients. The classification is important because it represents a consensus among hematopathologists as to how to approach such lesions. Despite the fact that only one of the principals of the three prior classification systems participated in the discussions, the outcome was remarkably similar to prior attempts to classify these disorders. This classification works from the strengths of a greater prior experience with the range of PTLDs at this point in time, and from the expertise of the panel in standard lymphoma classification. A modified version of this classification is given here (Table).

Early lesions in this classification system include plasma cell hyperplasia, lesions resembling infectious mononucleosis, and other forms of atypical lymphoid hyperplasias characterized by preservation of the underlying architecture. Such growths are usually polyclonal but may contain one or several minor clonal subpopulations as well. These lesions often regress following reduction of immunosuppression, but, like infectious mononucleosis itself, may on occasion act in an aggressive fashion and lead to the death of the patient. This underscores an important point. Many of these lesions are rapidly growing proliferations that require some form of intervention for control. The fact that a particular PTLD may prove to be "benign" in a pathological sense does not imply that it will not progress if untreated.

Polymorphic PTLDs represent destructive lesions that infiltrate and destroy underlying tissue. The emphasis in the SH classification was placed on recognition of a wide range of B-cell maturation in these cases, as opposed to the uniform appearance characteristic of lymphomas. The features originally used to differentiate polymorphic B-cell hyperplasia from polymorphic lymphoma, i.e., necrosis and bizarre cells, were recognized, but subdivision into polymorphic hyperplasia or lymphoma was not considered essen-

tial, as it was recognized that these lesions tend to behave similarly. Whereas immunocytochemical staining may give variable results in terms of clonality, molecular studies show that virtually all of these tumors are monoclonal. The response to therapy is variable in polymorphic PTLD. Some tumors regress following reduction of immunosuppression and some do not. At the time that the SH classification was proposed there was no way to predict the behavior of an individual polymorphic tumor. Similar to the finding of Knowles (18), this panel of investigators concluded that abnormalities of oncogenes or tumor suppressor genes were not a feature of polymorphic PTLD.

The term monomorphic was retained and used for PTLDs that morphologically resembled non-Hodgkin's lymphomas. The SH group recommended using standard lymphoma nomenclature (i.e., the Revised European American Lymphoma system [21]) to categorize such lesions, while including the term "PTLD" in the diagnosis. Most such tumors were of the diffuse large B-cell lymphoma subtype, although other entities such as Burkitt lymphoma were also seen. In some cases, molecular studies could show abnormalities of the ras or p53 genes as noted above. In this collected series, reduction of immunosuppression was reported in six patients. Two underwent sustained remission, two went into remission but developed tumor relapse, and two did not respond to this therapy. Thus it appeared that at least some examples of lymphomatous forms of PTLD could respond to reestablishment of host immunologic surveillance, although clear-cut responsiveness was seen in only a minority of these advanced lesions.

In addition to B-cell lymphomas, the existence of post-transplant T-cell lymphomas was also recognized and was placed within the category of monomorphic PTLD. In contrast, lesions that resembled Hodgkin's disease or B-cell lymphomas with a large number of T cells were placed in the "Other" category, as were post-transplant plasma cell neoplasms such as plasmacytoma or multiple myeloma.

Categories of post-transplant lymphoproliferative disorders[1,2]

Hyperplastic PTLD ("early lesions")
Reactive plasmacytic hyperplasia
Infectious mononucleosis
Atypical lymphoid hyperplasia with architectural retention
Polymorphic PTLD
Lymphomatous PTLD ("monomorphic PTLD")
B-cell lymphoma
Diffuse large B-cell lymphoma (immunoblastic, centroblastic, anaplastic)
Burkitt/Burkitt-like lymphoma
MALToma
T-cell lymphoma
Peripheral T-cell lymphoma, unspecified type (usually large cell)
Anaplastic large cell lymphoma (T or null cell)
Hepatosplenic gamma-delta T-cell lymphoma
Other (e.g. T-NK)
Other
Plasmacytoma
Myeloma
T-cell rich/Hodgkin's disease-like large B-cell lymphoma

[1] Adapted from refs. 8, 10, 14, 18, 27, and 40.
[2] Diagnostic line should incorporate histologic appearance, cell phenotype, clonal and EB viral status.

Table

Specific topics in PTLD pathology

EBV and PTLD

None of the classification systems described above has required documentation of intratumoral EBV for a tissue diagnosis of PTLD. The SH classification acknowledges that a proportion of PTLDs, approximately 10%, fail to show evidence of the virus using presently available methods of detection. Nevertheless, that group felt that it was appropriate to include all such EBV-negative cases under the umbrella term of PTLD. Thus, at present, any lymphoma that occurs in the post-transplant patient population is by definition considered to be a variant of PTLD. This highlights a current limitation of our knowledge. Clearly some spontaneous lymphomas will arise in this population and be diagnosed as lymphomatous/monomorphic PTLDs. Most likely (although not necessarily) these tumors will not contain the EBV. However, we are not currently able to distinguish such tumors from those whose emergence is linked in some way to the immunosuppressed post-transplant state. Indeed, it may be that even spontaneously arising lymphomas become clinically evident at an earlier stage in their evolution within this patient population due to a decrease in host immune surveillance.

Leblond et al. (22) reported a series of 11 transplant patients who developed EBV-negative PTLD. Tumors arose at a median time of 60 months, with the earliest tumor arising at 6 months post-transplant. In contrast, EBV-positive tumors arose at a median of 6 months, with the earliest arising 1 month post-transplant. Median survival in the virus-negative group was 1 month, compared to 37 months in the EBV-positive patient subset. Dotti et al. (23) also noted that a high proportion of late-arising PTLDs in their series appeared to be EBV-negative. They reported that 56% of PTLDs arising 22 months or later post-transplant failed to show evidence

of intratumoral EBV. Such tumors were associated with a median survival time of 7 months.

A pattern of late-onset EBV-negative PTLD was also observed in our own series, in which virus-negative cases occurred at a median time of 50 months post-transplant as compared to 10 months for EBV-positive PTLD (24). Interestingly, we observed that EBV-negative tumors accounted for only 2% of PTLD prior to 1991, but for 23% of PTLD after that time in our series. In contrast to other reports, we did find that occasional cases responded to reduction of immunosuppression despite the absence of EBV in the tumors. The mechanism by which such putative regression may occur is obscure. However, it should be stressed that the majority of these tumors are reportedly recalcitrant to therapy and are associated with a worse prognosis than EBV-positive tumors as a group (22, 23).

T-cell, NK-cell PTLD, Hodgkin's-like PTLD, plasma cell dyscrasias, and MALT lymphomas in transplant patients

Both T-cell and NK-cell PTLD are included under the monomorphic category in the SH classification system (8). Post-transplant T-cell lymphomas were reported at least as early as 1987 and the absence of EBV in index cases indicated that not all post-transplant tumors could be shown to be related to this virus (25). Dockrell et al. (20) estimated that only 38% of T-cell PTLDs contained the EBV. Prognosis has generally been considered poor. However, Kim et al. (26) reported a T-cell PTLD that arose approximately 2 months post-transplant and underwent regression following a reduction of immunosuppression. In this case the tumor was negative for both EBV and HTLV-1, and clonal rearrangements of the gamma chain of the T-cell receptor were demonstrated. Gamma/delta T-cell lymphomas tend to have a hepatosplenic distribution and have also been described in transplant patients (27). Other examples of post-transplant T-cell lymphoma demonstrate clonal rearrangement of the T-cell receptor beta gene (28).

Hsi et al. (29) recently reported a case of NK-cell PTLD in a renal transplant patient. This tumor had a monomorphic appearance, was EBV-negative and was unresponsive to therapy. Kwong et al. (30) reported a similar dismal outcome in a renal transplant patient who developed a disseminated EBV-positive NK-derived PTLD. In contrast, Mukai et al. (31) used combination chemotherapy to successfully manage a nasal NK cell lymphoma that arose in a renal transplant patient.

Post-transplant Hodgkin's disease has been uncommonly reported in transplant recipients. Rowlings et al. (32) noted an increased frequency of this tumor following allogeneic bone-marrow transplantation. Five of six such cases contained EBV and arose more than 2.5 years post-transplant. We observed a case of late-arising Hodgkin's-like lymphoma in a liver transplant patient (33). In this case chemotherapy was required and the patient has enjoyed long-term remission. Smets et al. (34) also noted that Hodgkin's-like forms of PTLD appeared to be unresponsive to immunomodulation and required antineoplastic chemotherapy for control.

A possibly related T-cell-rich B-cell PTLD was reported by Grosso et al. (35). The precise relationship of such B-cell tumors to spontaneous Hodgkin's disease is unknown at present.

In contrast to reactive post-transplant plasmacytic hyperplasias, malignant post-transplant plasma cell dyscrasias are recognized as virulent forms of PTLD. Knowles et al. (18) incorporated multiple myeloma into their most advanced category of disease and the SH classification includes both multiple myeloma and plasmacytoma-like PTLD within the "Other" category. These tumors may be EBV positive or negative (36). In some cases the PTLD may present as an extramedullary plasmacytoma and later manifest as multiple myeloma (37). In other cases it may present as an ascites-type tumor (38). One post-transplant extramedullary plasmacytoma in our series presented as an aural polyp. This was successfully excised but was followed several months later by a monomorphic large B-cell PTLD of the lower extremity (39). This latter tumor responded to autologous lymphokine-actived killer (LAK) therapy.

Low-grade gastric B-cell lymphomas compatible with lymphomas that arise in mucosa-associated lymphoid tissues (MALT lymphomas) were first observed by Wotherspoon et al. (40), who noted that such lesions were associated with *Helicobacter* but not with EBV. Hsi et al. (41) recently described several post-transplant MALT lymphomas in their series. These late-appearing B-cell tumors arose on the average 7 years after transplantation. The histologic appearance was that of low-grade marginal zone lymphoma. No evidence of EBV was seen but *Helicobacter* was observed in gastric cases. All patients were alive at last follow-up and the authors stress that proper recognition of these lymphomas would help to avoid unnecessarily aggressive therapy.

Host versus donor origin PTLD

At least 90% of PTLD that occur in solid organ transplant patients arise from recipient cells (42), and the opposite applies in the case of bone marrow transplantation. Donor-derived PTLD in organ transplant patients may have a predilection for the allograft (43). Some authors have suggested that they may have a worse (2), and some a better (44), prognosis than recipient origin PTLD. Further studies are needed in this area.

BCL-6 mutations and PTLD

It has been notoriously difficult to predict the behavior of polymorphic PTLDs. Penn (45) estimated that approximately 31% of PTLD overall resolve under reduction or withdrawal of immunosuppression alone, whereas others have placed this figure nearer 50% (46). In our experience, the majority of PTLDs responsive to this form of therapy include the early and polymorphic categories (47). However, the behavior of individual polymorphic tumors is usually determined by clinical trial, and the distinction between histologic subtypes within this group is not helpful in predicting behavior.

In their approach to this problem, Cesarman et al. (48) studied bcl-6 mutations in PTLDs. The bcl-6 gene encodes a transcriptional repressor (49) that is rearranged in approximately 35–40% of diffuse large B-cell lymphomas (50, 51). Cesarman et al. (48) found mutations of this gene in 40% of polymorphic PTLD and concluded that the presence of this mutation predicted refractoriness to the effect of reduced immunosuppressoion as well as shorter survival. Further studies are needed to support this important contention. Since the bcl-6 gene is also mutated in 30% of normal germinal center B cells (52), the interpretation of this finding is unclear. Assuming that this observation is upheld, it is not clear if the gene mutation itself contributes to tumor behavior, or if bcl-6 mutation acts as a surrogate marker for a particular stage of B cell development that is less responsive to host immune control mechanisms. Our own studies (Nalesnik et al., manuscript in preparation) suggest that EBV-negative tumors express bcl-6 protein but not CD138, consistent with a germinal center stage of development, and that EBV-positive tumors are usually bcl-6 negative, CD138 positive, suggestive of a postgerminal center phenotype. We have not been able to correlate bcl-6 protein expression itself with tumor behavior.

EBV-positive leiomyosarcomas in immunodeficiency states

In 1995 Lee et al. (53) reported the existence of EBV-positive sarcomas in several pediatric transplant patients. These tumors, which have been called "post-transplant spindle cell tumors," have been shown to be leiomyosarcomas by immunohistochemical analysis. Similar tumors have been described in AIDS patients (54). Cells explanted from one of these tumors showed low levels of the EBV receptor CD21 and expressed lytic EB viral proteins (55), confirming the fact that active intracellular EBV infection was present.

In at least some cases the tumors contained clonal EBV (53). In several cases these EBV-associated leiomyosarcomas have arisen in the setting of prior PTLD. In one case it was shown that the same clone of EBV was present in both the PTLD and the post-transplant leiomyosarcoma (56). Such examples confirm the necessity of pathologic diagnosis of mass lesions in transplant patients.

Recurrent PTLD

Clinical recurrence of PTLD has been estimated to occur in approximately 5% of cases (6). Wu et al. (6) examined a series of 11 such patients and found that the recurrent tumors comprised a heterogeneous assortment. In some cases the recurrence was morphologically and clonally identical to the original tumor. In several cases PTLD recurred in a more aggressive form. For example, patients with mononucleosis-like PTLD could present later with polymorphic PTLD, and patients whose original disease was polymorphic PTLD might later develop one of the lymphomatous forms of PTLD. In one case, as noted above, a recurrent "PTLD" was found on biopsy to actually be post-transplant leiomyosarcoma (56). For this reason biopsy of such recurrences is encouraged.

Suggested pathologic evaluation of PTLD

General comments to the pathologist

The diagnosis of PTLD is best made on tissue biopsy. Cytological preparations are useful, particularly in the analysis of effusions (57), and can provide adequate diagnostic material particularly if ancillary studies such as phenotypic, clonal and viral analysis are also performed.

Since PTLDs may contain large areas of necrosis, excision of involved lymph node or tumor is preferred over a needle biopsy; however, in many cases a needle biopsy may be the only source of tissue available. If such specimens are compromised by extensive necrosis it may still be possible to read through the necrotic areas and identify the cells as mononuclear in origin. Usually recuts will show at least a few spared tumor cells that resemble the cell ghosts present in areas of coagulative necrosis. In these cases we find that immunostain for EBV latent membrane protein may still provide useful information. *In situ* hybridization for Epstein–Barr early RNA (EBER), which is usually more sensitive than EBV immunostain in paraffin sections, is essentially worthless in necrotic areas in our experience.

Although it would be ideal to sample each tumor in cases of multicenter PTLD, this is seldom possible and the surgeon should be encouraged to sample the largest or most clinically ominous lesion. Although each tumor may represent a separate clone, most lesions in multicentric PTLD have a similar appearance. The histologic grade of tumor may be underestimated in multicentric cases if a marginally enlarged lymph node is sampled on the rationale that it is the easiest site to biopsy. In this case the surgeon runs the

risk of having sampled a reactive node that may contain evidence of EBV infection, while the primary lymphomatous PTLD lies elsewhere. As the response to therapy is followed, it is also useful to consider biopsy of any lesion that responds in an atypical fashion, particularly if regression is documented in other concurrent lesions.

Histologic evaluation should first ascertain whether the lesion fits into any of the accepted categories of PTLD. In some cases it may happen that EBV is demonstrated within what otherwise appears to be an innocuous inflammation. The approach of Finn et al. (5) provides a model that we find useful in handling such cases. Those investigators examined the frequency of EBER-positive cells in intestinal allograft biopsies in a pediatric population. A continuum of alterations was observed, beginning with low-grade EBER-positive inflammation and extending through EBER-positive monomorphic PTLD. They found that those patients with low-grade EBER-positive inflammation were at risk for the development of PTLD, but reserved the term PTLD for those cases in which frank histologic evidence of polymorphic or monomorphic PTLD was present.

Diagnostic reporting of PTLD

The nomenclature proposed by the Society of Hematopathology provides the most comprehensive and uniform basis for diagnosis and we recommend its use for routine reporting. A revised version of this system, incorporating recently described variants, is given (Table). It may be difficult to apply this categorization to small specimens or those with extensive necrosis. At the least, an effort should be made to differentiate those lesions with retention of underlying architecture from those with invasive growth patterns. The former most likely represent a form of "early" or hyperplastic PTLD, and it is important that the responses of such lesions be separated from those of polymorphic or lymphomatous PTLDs when the results of different therapeutic interventions are compared.

Phenotypic evaluation should be performed as part of the workup of PTLD. The form that this takes is dependent upon local resources. At a minimum we recommend that phenotypic identification of B- or T-cell tumor origin be undertaken. Therapeutic monoclonal antibodies directed against the B cell antigen CD20 appeared to produce some examples of impressive remissions in early studies (58). Fortuitously, the immunostain for CD20 can be performed on routinely processed paraffin embedded tissues and should be part of the workup of all PTLDs.

Clonal analysis of immunoglobulin genes may be used to support the diagnosis of PTLD. In some cases it provides the information required to differentiate hyperplastic (early) PTLD from more advanced forms of this disorder. However, we stress that both polymorphic and monomorphic (lymphomatous) PTLD are almost always monoclonal, and the mere demonstration of monoclonality does not itself predict tumor behavior in response to modulation of immunosuppression or other forms of therapy. In the case of multicentric or recurrent tumors, clonal analysis may be helpful in staging the disease since histologically identical tumors may actually represent separate independent primary tumors (7).

Clonal analysis may also be required to establish a diagnosis of lymphomatous PTLD of T-cell origin. The beta chain of the T-cell receptor appears to be most commonly rearranged; however, evaluation of the gamma chain may also be required, and it is noted that gamma-delta T-cell lymphomas usually have a hepatosplenic distribution. We recommend that a comment regarding tumor clonality be appended to the diagnostic line of PTLD. If no clonal studies were performed, this should also be stated.

We believe that it is important to make an effort to identify the EBV within PTLDs, and that this should be part of routine pathologic evaluation. As more EBV-negative PTLDs are uncovered it becomes important to document their pathologic features and response to therapy. In some cases cytotoxic T-cell therapy may be considered, and since this treatment is actually directed against the virus-infected tumor cells, the presence of viral targets is necessary for this form of therapy to be effective.

Molecular analysis of oncogenes and tumor suppressor genes will undoubtedly play an increasingly important role in predicting behavior. At present, these techniques are not widely available and only a few genes have been analyzed. As high throughput microarray analysis becomes a reality we can anticipate that the characterization of PTLD will become much more precise and treatment regimens will be designed for more uniformly defined subpopulations of this disease. At present we consider this to be an optional technique for routine clinical practice. Likewise, we do not consider evaluation of host versus donor origin of PTLD to be necessary from a clinical standpoint except in those cases in which cell therapy is to be employed, since HLA matching of effector and target tumor cell is necessary.

In summary, we stress that it is important for the pathologist to be aware that the diagnosis of post-transplant lymphoproliferations covers a large but knowable number of conditions that can be placed into several general categories. Pathologic diagnosis should incorporate the histologic form of disease in as precise a manner as possible, phenotype of involved cell, and a determination of clonal and EB viral status of the lesion. Clinicopathologic correlation should seek to stage the disease using standard systems such as the Ann Arbor Staging Classification with Cotswald Modifications (59). Only in this way will we get a clear picture of the range of abnormal lymphoid growths in immunosuppressed transplant patients and be in a position to design rational strategies for these diverse conditions that exist under the generic heading of PTLD.

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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

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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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