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Long-Term Kidney Isografts Develop Functional and Morphologic Changes That Mimic Those of Chronic Allograft Rejection

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Objective
This study examined antigen-independent factors in the pathogenesis of chronic rejection of organ transplants.

Summary Background Data
In addition to alloantigen-dependent events, antigen-independent factors can influence chronic rejection of organ allografts. Initial injury, including early ischemia and acute rejection, may contribute.

Methods
Kidney isografts were transplanted orthotopically into bilaterally nephrectomized rat recipients and studied functionally, morphologically and immunohistologically, at serial intervals up to 72 weeks after transplantation. Controls included chronically rejecting kidney allografts using a well-established model, non-nephrectomized and uninephrectomized animals with a native kidney that had undergone initial ischemia and uninephrectomized rats whose remaining kidney had been manipulated operatively.

Results
Allograft recipients developed progressive proteinuria after 12 weeks, with gradual renal failure ultimately leading to death. At the same time. morphologic changes, including progressive arteriosclerosis and glomerulosclerosis, tubular atrophy, and interstitial fibrosis, developed. Immunohistologically, macrophages infiltrated glomeruli during this period and cytokines became upregulated. Comparable changes occurred in isografts, but later, beginning after week 24 and progressing thereafter. The single ischemic kidney in uninephrectomized controls also developed the same lesions; no comparable changes were noted in other control kidneys.

Conclusions
Antigen-independent functional and morphologic changes occur in long-term kidney isografts that resemble those appearing considerably earlier in allografts that reject chronically. Initial injury and extent of functioning renal mass may be important factors for such late changes.

Despite progressive improvements in early success of clinical organ transplantation, the rate of attrition over the long term has remained constant.[1,2] Increasingly detailed knowledge of the immunobiology of transplant rejection or more effective immunosuppression have not prolonged the half-life of organ allografts; only half of cadaver kidneys, for instance, continue to function 6 years after transplantation.

Chronic rejection of kidney transplants has been defined as progressive functional deterioration, occurring months or years after grafting and associated with morphologic changes that include vascular obliteration, glomerular sclerosis, tubular atrophy, and interstitial fibrosis. The actual mechanisms leading to this enigmatic phenomenon are unknown.[3-5] Two working hypotheses have evolved to explain its etiology. First, the chronic process primarily is an antigen-dependent phenomenon influenced by early immunologic injury and continuing host alloresponsiveness.[6-10] Second, alloantigen-independent factors contribute to the progressive changes. For instance, functional and morphologic changes that resemble those of chronic rejection have been observed in response to reduced renal mass in experimental models;[11-13] clinically, a low ratio of functioning renal parenchyma to body weight may explain the influence of donor age, gender, and race on decreased long-term survival.[14] Duration of ischemic time, surgical manipulation, and perfusion and reperfusion injury may be additional early influences on long-term outcome.[15-17]

This study was designed to assess the impact of nonimmunologic factors on chronic deterioration of long-functioning kidney isografts by evaluating and comparing functional, morphologic, and immunohistologic alterations to those occurring in allografts and in naive and surgically manipulated controls. The progressive changes characteristic of kidney allografts that reject chronically also develop in long-term isografts and native uninephrectomized, age-matched animals subjected to an initial ischemic injury, albeit considerably later in time.

MATERIALS AND METHODS

Animals

Inbred male rats (Harlan Sprague—Dawley, Indianapolis, IN) were used throughout the experiments. The rats weighed 200 to 250 g and were 2 to 3 months of age at the beginning of the experiment.

Operative Techniques

Renal grafts in all groups were transplanted orthotopically to the left recipient renal vessels and ureter by end-to-end anastomoses, using 10-0 Prolene, after the left kidneys had been mobilized and removed. Right nephrectomies were performed 10 days later.

Experimental Groups

Lewis (Lew, $RT1^{l}$) recipients of orthotopic Lew kidney isografts either were untreated or received initial low doses of cyclosporin A ([CyA], 5 mg/kg/d × 10 days: n = 24/group). Treated Lew recipients of Fisher 344 (F-344, $RT1^{lvl}$) kidney allografts (n = 50) were used as control subjects, using an established model of chronic rejection in which the transient immunosuppression was necessary to reverse an acute rejection episode within the first 10 days.[18,19]

The effects of ischemia, operative manipulation, and renal mass on later kidney function and structure also were ascertained in age-matched control Lew animals (n = 12/group). These included unilaterally nephrectomized rats whose remaining kidneys were subjected to initial ischemic injuries by temporarily clamping the renal vessels for 30 minutes of cold and 30 minutes of warm ischemic time, simulating the situation that occurs during actual kidney transplantation. To produce cold ischemia, the isolated and clamped kidney was packed in ice. One of the kidneys of a comparable series of nonnephrectomized rats was treated in similar fashion; the other kidney remained untouched. Other control groups included naive unilaterally nephrectomized and non-nephrectomized animals whose kidneys had not been subjected to ischemia. The possible impact of the operative procedure on later changes was examined in non-nephrectomized naive animals in which the renal artery, vein, and ureter of the experimental kidney were individually clamped, dissected, divided, and consecutively reanastomsed, or autografts were placed to study the effect of denervation on any long-term changes (n = 12/group). Functional, morphologic, and immunohistologic evaluations of kidneys were performed 4, 8, 12, 16, 32, 40, 52, and 72 weeks after engraftment; the manipulated kidney was compared to its normal counterpart.

Functional Studies

Urinary protein excretion was determined by measuring precipitation after interaction with 3% sulfosalicylic acid. Turbidity was assessed by absorbance at a wave-

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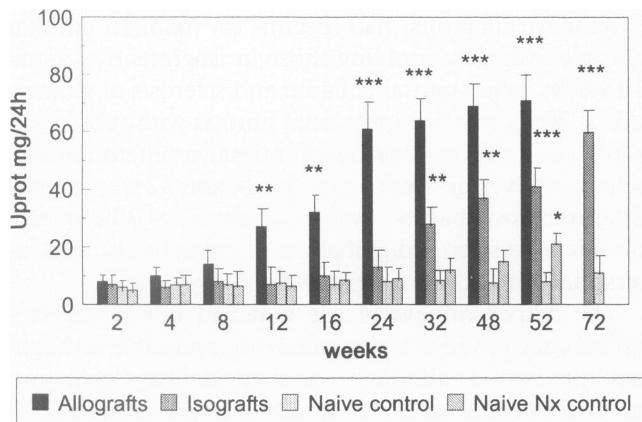

Figure 1. Serial urinary protein excretion is shown in isografts, allografts, and controls. Protein excretion in isografts remained at baseline levels until week 32, but increased progressively thereafter; proteinuria developed in allograft recipients earlier after week 12 increasing to >60 mg/24 hr by 24 weeks. Protein excretion increased modestly in uninephrectomized naive rats after one year; *p < 0.05, **p < 0.01, ***p < 0.001 *vs.* naive controls.

length of 595 nm using a Coleman Junior II spectrophotometer.[20]

Histology

Kidneys were removed at serial intervals and fixed in 10% buffered formalin. Paraffin sections were stained with hematoxylin and eosin, periodic-acid-schiff, Trichrome and Silver-Masson, and assessed by light microscopy.

Immunohistology

Representative pieces of allografts, isografts, and control kidneys were snap frozen in liquid nitrogen, cut (4 μm), fixed in acetone for 10 minutes, air-dried, and stained with mouse monoclonal antibodies. After staining, the sections were interacted with rabbit anti-mouse immunoglobulin G by the alkaline phosphatase anti-alkaline phosphatase (APAAP) or peroxidase-anti-peroxidase (PAP) methods;[21,22] sections were counterstained with hematoxylin. Cell and cell surface labelling was assessed using monoclonal antibodies to CD-5+ T cells (OX-19), CD4+ T helper cells (OX-4), CD8+ T cytotoxic/suppressor cells (OX-8), macrophages (ED-1), major histocompatability complex (MHC) class II (OX3) (BPS, Indianapolis, IN), and intracellular adhesion molecule (ICAM)-1 (IA29) (courtesy of Professor M. Miyasaka, Tokyo, Japan).[23] Cytokine labeling was performed using monoclonal antibodies to TNF-α (J2D10, courtesy of Dr. I. Mackenzie, Melbourne, Australia), Interferon-Γ (DB-10, courtesy of Dr. P. van der Meide, Rijwijk, Holland), transforming growth factor (TGF)-β and epidermal growth factor ([EGF], British Biotech., London, United Kingdom), Interleukin (IL)-1β (Olympus, Lake Success, NY), IL-2 (1D10),[17] IL-4, IL-6, IL-7, IL-8, platelet-derived growth factor (PDGF)-AA/PDGF-BB (Genzyme, Boston, MA), and IL-2R (CD25, ART-18, courtesy of Dr. T. Diamantstein, Berlin, Germany). Deposition of immunoglobulin M, immunoglobulin G, C3, and fibrin was ascertained using specific polyclonal monoclonal antibodies (Cappel, West Chester, PA). Major histocompatability complex class II and ICAM-1 expression was quantified on a 0-to-4+ scale (4+ =dense); positive cell counts were expressed as mean $\pm$ SD of cells/field of view (c/fv); > 20 fv/section/specimen were evaluated at 600 $\times$. Cytokine and adhesion molecule expression was quantified by their cellular or endothelial labeling.[22,23]

RESULTS

Functional Studies

Isograft recipients developed significant proteinuria by 32 weeks after engraftment, which worsened progressively thereafter compared with naive nephrectomized and non-nephrectomized control subjects; by 72 weeks, protein excretion had increased to > 60 mg/24 hr *versus* baseline of approximately 10 mg/24 hr in naive non-nephrectomized animals (Fig. 1). cyclosporin A treatment did not influence protein loss. In contrast, rats sustained by a kidney allograft developed obvious proteinuria by week 12, excreting progressively greater amounts of protein thereafter. After week 24, animals in this group began to die of renal failure, although a few survived as long as 1 year. Interestingly, protein excretion also increased modestly in uninephrectomized control rats after 1 year (approximately 20 mg/24 hr, $p < 0.05$) when compared to naive non-nephrectomized controls. Ischemia of the single native kidney in uninephrectomized hosts also produced strikingly late functional deterioration, with such animals excreting approximately 50 mg/24 hr of protein (Fig. 2) by 52 weeks, significantly ($p < 0.001$) more than baseline values of non-nephrectomized rats with single ischemic kidney (<11 mg/24 hr). Animals receiving an autograft or those undergoing other operative manipulations never showed significantly increased protein excretion (Fig. 3).

Morphology

For the first 24 weeks after engraftment, the morphology of kidney isografts remained relatively unremarkable except for a persistent minor perivascular and periglomerular mononuclear cell infiltrate. However, by 32 weeks, tubular atrophy had become increasingly evi-

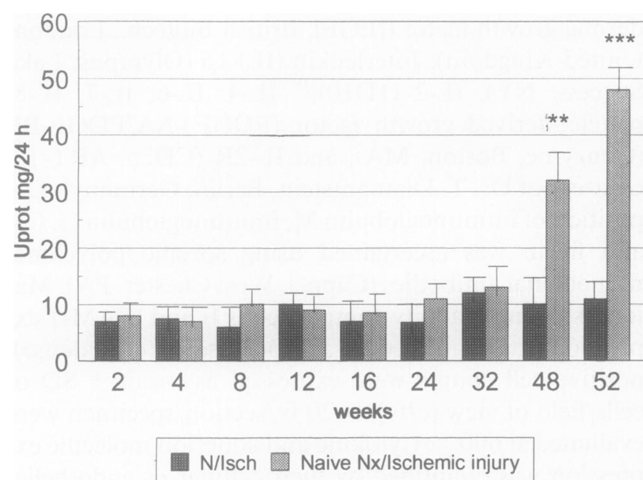

Figure 2. Late functional deterioration occurred in naive uninephrectomized animals subjected to an initial ischemic injury (naive Nx/Ischemic injury); by 52 weeks, such animals excreted >50 mg/24 hr of protein, significantly ($p < 0.001$) more than non-nephrectomized rats with single ischemic kidney (N/Isch)

dent. Focal areas of intimal hypertrophy in interlobular and cortical arteries were associated with smooth muscle cell proliferation in the arterial media. By 52 weeks, almost all vessels showed marked to severe luminal narrowing. Glomerular alterations varied, with some glomeruli exhibiting focal and segmental collapse and others becoming enlarged and hypercellular. These lesions progressed further by week 72, with > 30% of glomeruli developing segmental and global sclerosis and almost all arteries showing severe luminal narrowing; tubular atrophy and interstitial fibrosis had become obvious. Cyclosporin A treatment did not effect these changes.

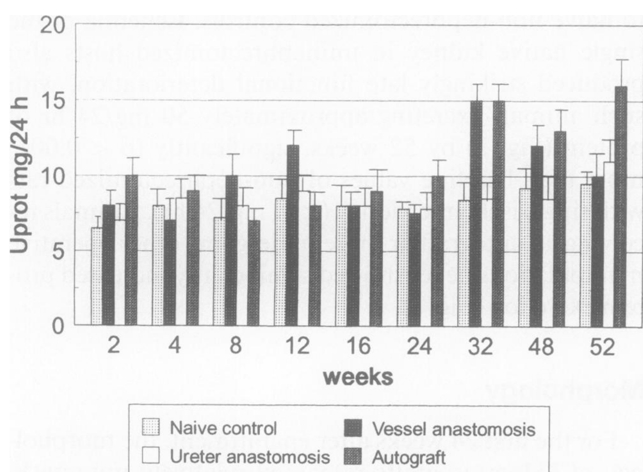

Figure 3. No significant increase or differences in protein excretion occurred in animals receiving an autograft or those undergoing operative manipulations.

Kidney allografts had begun to manifest similar chronic lesions resembling those in isografts by 12 and 16 weeks, when partial collapse and sclerosis of glomeruli (> 20%), marked interstitial fibrosis with tubular atrophy, and varying degrees of intimal proliferation and sclerosis of vessels were noted; by 24 and 32 weeks, graft arteriosclerosis had become generalized, > 30% of glomeruli were sclerosed globally and segmentally, and interstitial fibrosis was widespread.

The native kidneys of age matched non-nephrectomized control subjects remained unremarkable throughout this period, although the single kidney from uninephrectomized hosts eventually (at approximately 72 weeks) demonstrated minor smooth muscle proliferation in the arterial media and a few segmentally sclerosed glomeruli distributed in a patchy fashion. In contrast, kidneys in uninephrectomized hosts which had undergone initial ischemia developed marked periglomerular and perivascular mononuclear cell infiltration by 24 weeks, with 5% of glomeruli showing globular and segmental sclerosis; by 52 weeks, 25% to 30% of glomeruli had become sclerosed segmentally and globally, tubular atrophy and interstitial fibrosis had become widespread, and some vessels showed luminal obliteration with intimal and medial thickening; these alterations correlated with worsening proteinuria exhibited by animals in this group after 48 weeks (Fig. 2). In contrast, such changes were virtually absent in the ischemic or operatively manipulated kidneys in non-nephrectomized animals.

Immunohistology

Minor perivascular and periglomerular mononuclear cell infiltrates developed in isografts within 1 week after transplantation, consisting primarily of macrophages (> 80%) and small numbers of CD4+ T cells; neither cytokine nor IL-2R expression was detected (Table 1). Little immunoglobulin M, immunoglobulin G, or C3 was seen at this or any subsequent time period. The cell infiltrate persisted during the first 4 weeks, associated with focal interstitial labeling for EGF, TGF-β, PDGF, and small amounts of TNF-α. No further immunohistologic changes were noted in isografts until week 32, at which time ED-1+ macrophages had entered vessel walls and adjacent perivascular areas; TNF-α, PDGF, and TGF-β were expressed in the same areas (Fig. 4). By week 52, considerable numbers of intraglomerular macrophages were noted associated with intense TNF-α staining. Mononuclear cells (monocytes/macrophages and CD8+ and CD4+ T lymphocytes) infiltrated periglomerular and perivascular areas; IL-1β, IFN-Γ, IL-4, IL-6, and IL-8 were expressed on mononuclear leukocytes and endothelial cells (Table 1). The numbers of cells infiltrating the isografts were significantly higher (ED-1+ macro-

Table 1. MORPHOLOGIC CHANGES, CELLULAR INFILTRATES, AND CYTOKINE EXPRESSION IN ISOGRAFTED AND ALLOGRAFTED RAT KIDNEYS

	Isografts				Allograft
Marker	Week 1	Week 4	Weeks 8–24	Weeks 32–52	Week 12–16
Histology	Normal morphology	Normal morphology	Normal morphology	Increasing arteriosclerosis (initimal and SM proliferation), tubular atrophy, focal and segmental collapse of glomeruli	20–30% of glomeruli sclerosed, tubular trophy, interstitial fibrosis, vascular luminal obliteration
Leucocytes	Trace MNC	Trace MNC	Small MNC foci	Dense MNC aggregates	Intense MNC infiltrate
IL-2R+ cells	—	—	—	<5% MNC	>10% MNC
IL-2, IL-7	—	—	—	—	<5% MNC
IL-1β, IFN-γ	—	—	—	<5% MNC and EC	<5% MNC and EC
IL-4	—	—	—	<5% MNC and EC	10–20% MNC
IL-6, IL-8	—	—	—	<5% MNC and EC	<5% MNC and EC
TNF-α	—	<5% MNC	<5% MNC, focal EC	>50% MNC and EC	20% MNC, EC, glom
PDGF	—	<5% MNC	<5% MNC, focal EC	>50% MNC and EC	50% MNC, focal EC
TGF-β, EGF	—	<5% MNC	<5% MNC,	10–20% MNC and EC focal EC	50% MNC, focal EC

EC = endothelial cells; MNC = mononuclear cells.

Kidney morphology of naive non-nephrectomized controls remained unremarkable with minimal MNC infiltrates and no cytokine expression; naive kidneys from uninephrectomized hosts eventually demonstrated minor arterial narrowing with smooth muscle proliferation and a few segmentally sclerosed glomeruli (c. 72 wks).

Cytokine labelling was judged semiquantitively <5%, >10%, 10–20%, ~20%, ~50% of indicated cells.

phages/monocytes, $p < 0.001$; CD-5 T cells, $p < 0.01$; CD-4+ Th-cells and CD-8+ Tc/s cells, $p < 0.05$) than in naive non-nephrectomized controls that showed minimal cell infiltration and no cytokine expression throughout the period of observation. Interestingly, ICAM-1 and MHC II expression in both isografts and uninephrectomized controls was elevated significantly ($p < 0.01$) at this period when compared to naive non-nephrectomized rats (Table 2). Cyclosporin A treatment did not alter the immunohistologic findings.

Allografted kidneys that rejected chronically showed similar changes, albeit more intensely and considerably earlier than those noted in the isografts. Within 4 weeks of transplantation, after the initial reversible acute rejection episode at approximately 1 week, a dense generalized infiltrate of T cells and macrophages became evident; 10% to 15% of these were IL-2R+. Massive amounts of IFN-Γ, IL-2, IL-4, IL-6, IL-7, IL-8, TNF-α, TGF-β, EGF, and PDGF were present within the interstitium. Arterial smooth muscle cells stained strongly positive for most cytokines, especially IL-7 and TGF-β; By 8 weeks, the extent of infiltration had diminished, with cells localizing primarily to perivascular and periglomerular areas. The relatively few interstitial mononuclear cells and intertubular capillaries labeled for IL-4, PDGF, EGF, and TGF-β, cytokines that seemed to be associated with increasing interstitial fibrosis and inflammation. By 12 to 16 weeks, IL-2R and IL-4 expression had increased in mononuclear cells infiltrating the allograft interstitium; those entering glomeruli, predominantly macrophages, were associated with labelling for TNF-α, EGF, TGF-β, IL-6, and IL-7; > 50% of glomeruli showed mesangial proliferation (Table 1). Numbers of infiltrating cells, particularly ED1+ monocyte/macrophages, were higher in allografts at week 16 than in isografts at week 52 ($p < 0.001$); however, monocyte/macrophage infiltration in isografts was significantly ($p < 0.001$) higher than in naive controls (Table 2). Similarly, infiltration of CD-5+ T cells was higher in allografts (not significant) as compared to isografts, although significantly higher numbers ($p < 0.01$) were found in isografts compared with age-matched naive controls. Numbers of infiltrating CD4+ and CD8+ T cells and ICAM-1 and MHC class II expression were relatively similar in allografts and isografts, although they were elevated significantly when compared with naive non-nephrectomized controls (Table 2).

After week 24, macrophages (17 ± 5 c/fv) and T cells (21 ± 6 c/fv) also were found to infiltrate the initially ischemic kidney in uninephrectomized hosts. By 52 weeks, ED1+ macrophages had increased slightly (21 ± 6 c/fv) and CD-5+ T cells (26 ± 8 c/fv) had infiltrated peritubular and perivascular areas. Major histocompatibility complex class II (3+) and ICAM-1 (2+) were expressed on endothelial cells of vessels, glomeruli, and tubules; PDGF labelling was noted on >50% of mononuclear cells and endothelial cells. In contrast, only a few infiltrating macrophages and T cells were scattered through the kidneys of non-nephrectomized groups subjected to either mechanical or ischemic damage, whereas

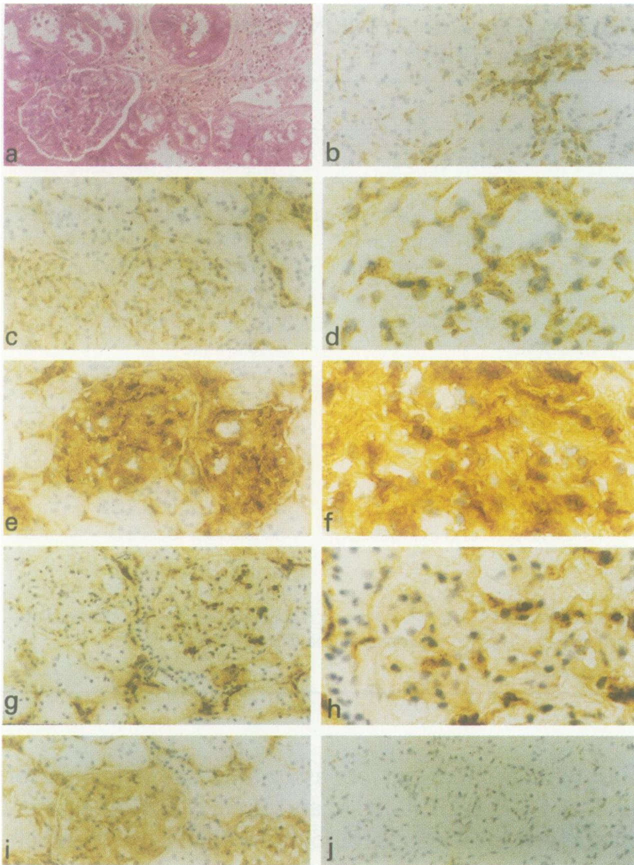

Figure 4. Immunopathology of isografts harvested at 32 weeks after transplant. (a) H&E showing extensive tubular injury, glomerular sclerosis, perivascular mononuclear leukocytes infiltrate and focal intimal and diffuse medial thickening of vessels. (b) ED-1+ macrophages were distributed diffusely within interstitial areas and increased within glomeruli. (c) IL-6 labelling was detected in interstitial areas and glomeruli. (d) IL-6 (high power of glomerulus) staining of cells within the mesangial and capillary walls was observed. (e) Platelet-derived growth factor-$\beta\beta$ labelling was present within interstitial areas and glomeruli. (f) Platelet-derived growth factor-$\beta\beta$ (high power of glomerulus) dense and diffuse staining of glomerular cells and mesangial matrix was noted. (g) TNF-α was seen as discrete labelling of cells within glomeruli and as more diffuse labelling of cells within intertubular areas. (h) TNF-α (high power of glomerulus) labelling of mononuclear leukocytes within mesangial areas and weaker staining of glomerular capillary walls was dense. (i) Transforming growth factor-β was observed within interstitial and glomerular areas. (j) Transforming growth factor-β absorption control: reabsorption of antibody with recombinant protein (Genzyme, #1838-01) at 100 ng/nL abolished all labelling. (H&E of paraffin section, (×250); panels b, c, e, g, h (×250); high magnification of glomeruli in panels d, f, h, and i (×630).

the other naive controls demonstrated no immunohistologic changes during the period studied.

DISCUSSION

The inexorable progression of chronic rejection of organ allografts is well recognized clinically and in experimental systems. For instance, in long-standing kidney allografts experiencing the condition, gradual functional decline is associated with vascular obliteration, glomerular sclerosis, tubular atrophy and fibrosis.[3,4] Macrophages seem particularly important because they infiltrate glomeruli and blood vessels in large numbers and are associated with the expression of various cytokines, which are thought to contribute to the progressive morphologic changes.[18] The etiology of the phenomenon is not known, but it seems to be multifaceted,[3–5] with both alloantigen-dependent and independent factors implicated.[12–18,24] We have attempted to evaluate the importance of these latter events by examining kidney isografts for prolonged periods, noting progressive functional (proteinuria) and morphologic changes similar to but occurring considerably later than the characteristic lesions of chronic rejection in long-surviving allografts.[18,19]

The persistent nephrotoxic effects of CyA may effect late fibrotic changes in long-term kidney allografts and influence the features of chronic rejection.[25,26] Although multiple reports have documented the nephrotoxicity of CyA, its impact on hypertension, low-density lipoprotein levels, intimal thickening of vascular endothelium, and the development of graft arteriosclerosis, others have not found evidence of chronic CyA nephrotoxicity when the agent is used at a low dose or in combination with other drugs.[27–31] Initial host treatment with CyA in low doses in these experiments did not influence late changes in kidney isografts because no differences were noted between grafts in recipients who underwent treatment and those who did not.

The initial investigators believed that the most common cause of late failure of human kidney transplants between identical twins was secondary to recurrent nephritis, most likely a manifestation of the underlying disease that led to transplantation in the first place.[32,33] This process developed in the isografted kidneys between 2 months and 16 years after engraftment and was characterized by proteinuria and progressive renal deterioration. Hypercellularity and immunoglobulin deposition in the glomeruli were prominent relatively early, whereas obliterative arterial changes and glomerulosclerosis became obvious later. However, it also was suggested that such changes observed in human isografts may have been a consequence of transplantation injury per se.[34] The progressive morphologic alterations noted in the experimental isografts in this study show similarities to those described clinically in the identical twin grafts emphasize the effects of alloantigen-independent influences on this long-term process rather (or in addition to) than recurrence of the original disease.

Factors independent of the host immune responses directed against alloantigen have not been well defined but may include early injury secondary to prolonged isch-

Table 2. CELLULAR INFILTRATES, MHC II AND ICAM-1 EXPRESSION IN RENAL ALLOGRAFTS, ISOGRAFTS, AND NAIVE CONTROLS

Marker/Cells	Week 16 Allograft	Week 52 Isograft	Week 52 Naive Control UniNx	Week 52 Naive Control
ED-1+ monocytes/macrophages	95 ± 12	36 ± 7	12 ± 3	8 ± 2
CD 5+ T lymphocytes	38 ± 8	26 ± 5†	8 ± 3	5 ± 3
CD 4+ T helper cells	24 ± 4†	19 ± 4‡	11 ± 4	8 ± 3
CD 8+ T c/s cells	17 ± 4†	14 ± 3‡	8 ± 2	5 ± 2
MHC class II	2.5†	2.0†	2.0†	1.0
ICAM-1	3.5±†	3.3†	3.0†	1.5

Cells counts are expressed as mean ± cells/field of view; >20 fields/section were counted at 600×.

MHC II and ICAM-1 expression were quantified on a 0–4 scale (4 = dense); $p < 0.001$, † $p < 0.01$, and ‡ $p < 0.05$ vs. naive controls.

Tc/s = T cytotoxic/suppressor cells; ICAM-1 = intercellular adhesion molecule-1.

emic time and reperfusion,[15] the long-term effects of a diminished ratio of functioning kidney mass to body weight,[14] damage through hyperfiltration,[11,35] or the effects of surgery per se.[16] Prolonged ischemia has been implicated as a clinical risk factor for chronic rejection[30,36] and correlates with initial nonfunction.[37] In one series, the 1-year survival of grafts with warm ischemic times >50 minutes was 40%, whereas that of grafts with a warm ischemic time <50 minutes decreased by 1% with every 1-minute increase of warm ischemia; the ischemic period also correlated with the number of reversible rejection episodes in grafts functioning longer than 1 year, suggesting a lasting effect of the initial injury.[38] In experimental models, reduction in ischemic time significantly ameliorated later vascular and glomerular changes; conversely, creatinine levels increased steadily over time in rat kidney allografts subjected to prolonged ischemia.[15] Recovery from ischemia upregulated MHC expression, particularly class II, possibly rendering postischemic allografted tissue more immunogenic.[39] Early ischemia of the single kidney in naive unilaterally nephrectomized rats showed worsening proteinuria and morphologic alterations comparable to those observed in isografts after 1 year. Because surgical manipulation, renal denervation, and the presence of two kidneys (one ischemic) did not produce such changes, early ischemia of a single kidney may explain many of the later changes noted in the isografted organs, as well as those developing more rapidly in "chronically rejecting" allografts.

The slightly elevated protein excretion levels with significant higher ICAM-1 and MHC II expression noted in unilaterally nephrectomized naive control animals emphasizes findings in models of reduced renal mass. Hyperfiltration and progressive fibrosis occurring in those models may be influential on the long-term outcome of kidney transplants and may possibly explain the influence of donor age, race, and gender.[11-14,35,40]

Thus, initial damage, primarily from ischemia and amplified by diminished renal mass, may lead to chronic changes in a response-to-injury mode;[41] these may induce upregulation of adhesion molecules, cytokine expression and cellular infiltrates. Such stimuli may combine with or amplify continuing antigen-directed activity to produce chronic rejection of allografts. Thus, pathophysiologic mechanisms of the complex phenomenon of this process may include both alloantigen-dependent and alloantigen-independent factors.

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Discussion

DR. ALI NAJI (Philadelphia, Pennsylvania): Dr. Tilney has provided us with the results of a series of experimental work critical to the future evolution and progress of clinical transplantation. The message from this body of experimental work is important and is in accord with the mission of our Association set forth by President Schwartz in his "Reflections."

The field of solid organ transplantation has attained remarkable maturity, and as Dr. Tilney presented, the early graft and patient survival of recipients of hepatic, renal and pancreatic transplants has steadily improved. However, further reflection has brought about the realization that the long-term survival and salvage of solid organ allografts remain disappointingly poor. The primary reason for long-term loss of solid organ transplants has been attributed to chronic rejection. Speculation on the basis of this chronic destructive process has focused on the inadequacy of current immunosuppressive agents to halt the immune attack and immunogenetic disparities of donor and recipient for histocompatibility antigens. However, as presented by Dr. Tilney, the long-term failure of the historic cases of identical twin renal isografts at Brigham has challenged the view that genetic disparity or inadequate immunosuppression may have contributed to the long-term failure of renal isografts. The first task to study this complex biologic phenomenon in an experimental setting required recapitulation of the process in an animal model. It appears that Dr. Tilney has done this remarkably well and the renal grafts in the selected rat