Immunologic Enhancement of Rat Renal Allografts

III. Immunopathologic Lesions and Rejection in Long-Surviving Passively Enhanced Grafts

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Immunologic enhancement of renal allografts from (Lewis × Brown Norway) F_1 to Lewis rats was achieved by administering a single dose of antidonor serum at the time of transplantation. A series of grafts functioning for 1 to 4 months after transplantation were examined by light and immunofluorescence microscopy to evaluate the long-term protective effects of the enhancing serum and to determine if previously unobserved lesions appeared in long survivors. Despite the absence of detectable circulating cytotoxic alloantibody, long-term allografts showed necrotizing glomerular and arterial lesions which resembled those seen in acutely rejecting grafts and were compatible with humoral rejection. Thus, in this model, there is a late decline in the ability of passive enhancement to inhibit humoral rejection. Long-term grafts also developed tubular lesions with deposition of immunoglobulin and complement on the tubular basement membranes (TBM). Anti-TBM antibodies were demonstrated in recipients' sera and found to be organ specific but not major histocompatibility antigen or species specific. This tubular lesion is therefore a unique form of allograft injury in which the immune response is directed against tissue antigen(s) which are distinct from the major histocompatibility antigens that induce rejection. (Am J Pathol 79:255–270, 1975)

IMMUNOLOGIC ENHANCEMENT is the prolonged survival of foreign tissue or tumor grafts mediated by antibodies directed against their histocompatibility antigens. Renal allograft survival in inbred rats has been prolonged greatly by preimmunizing recipients with donor histocompatibility-antigen-specific cells or subcellular fractions (active enhancement) [1,2] and/or treating recipients with hyperimmune antidonor serum (passive enhancement).[3] We have shown earlier that passive enhancement of (Lewis × Brown Norway) F_1 hybrid (LBN F_1) to Lewis kidney grafts represents a significant abrogation of the humoral antibody-mediated rejection response and that this abrogation is crucial to graft survival.[4,5] Although survival is prolonged, renal

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function is not entirely normal, and moderate functional impairment has been reported in long-surviving recipients of both passively and actively enhanced renal allografts.[6,7] It is not known whether these functional abnormalities are due to the late development of the lesions of acute rejection which were suppressed in the early life history of these transplants or to the development of other lesions not present in short-term grafts. Studies have been carried out in several laboratories to define the immunologic status of rats bearing long-surviving enhanced kidney grafts, but different findings have been reported in different strains combinations. Thus, Biesecker *et al*[6] have observed that long-surviving enhanced Lewis recipients of LBN F_1 grafts have levels of donor-specific lymphocyte cytotoxicity (a reflection of cellular immunity) and cytotoxic and hemagglutinating antibodies (humoral immunity) that are comparable to those observed in unmodified grafts. In contrast, Mullen *et al*[8] have described diminished cellular immunity (as measured by microcytotoxicity and skin graft rejection) associated with enhancement of (Lewis × Buffalo) F_1 to Lewis grafts. However, no attempts have been made to relate these *in vitro* tests for immunologic response to changes occurring in the grafted kidneys. Moreover, no descriptions are available of the morphologic changes in functioning long-term enhanced renal allografts performed across a major histocompatibility barrier.

The present study was designed to examine a series of enhanced LBN F_1 to Lewis grafts in the period of 1 through 4 months. Enhancement was achieved by treating recipients with hyperimmune anti-Brown Norway (BN) serum at the time of transplantation. The time of 1 month was defined as representing prolonged survival. We have described and quantified the anatomic lesions, compared them with those seen in acute unmodified rejection and in short-term enhanced grafts,[4,5] and have evaluated the role of antibodies in the evolution of the lesions by the use of immunofluorescent technics. Moreover, we have related the lesions in the long-term grafts to humoral and cellular immunity as defined by *in vitro* technics. The latter studies will be reported in detail elsewhere.[9]

Materials and Methods

Renal transplants were performed from LBN F_1 rats of major histocompatibility type Ag-B1, 3 to bilaterally nephrectomized Lewis rats of Ag-B1 type. Both strains were obtained from Microbiological Associates, Walkersville, Md. The technics of transplantation, and the preparation and administration of enhancing antiserum have been described previously.[4] A single pool of serum was used, and all recipients received 0.5 ml of hyperimmune anti-BN serum intravenously immediately after

transplantation. Three functioning grafts each were examined at 1 month (30 to 35 days), 2 months (59 to 63 days), 3 months (87 to 93 days) and 4 months (119 to 120 days) after grafting. The kidneys were hemisected, and half of each was fixed in 10% formalin and embedded in paraffin. Sections of each kidney were stained with hematoxylin and eosin, the periodic-acid Schiff technic, Mallory's trichrome, Verhoeff–van Gieson elastic tissue stain, and the Frazer Lendrum stain for fibrin. The remaining half of each kidney (except one at 3 months) was processed for immunofluorescence studies as described previously.[5] For immunofluorescence, 4- to 6-μ cryostat sections were cut and stained with fluorescein-isothiocyanate-conjugated (FITC-conjugated) rabbit antisera to rat immunoglobulin G (IgG), C3 (β1C-globulin, the third component of complement), fibrinogen and albumin (Cappel Labs, Downington, Pa). Fluorescence microscopy and photography have been described earlier.[5]

Quantitation of Extent of Involvement

Histologic sections were examined by light microscopy, and the extent of involvement by the following lesions was graded independently by two observers according to criteria described below. The nature of the alterations are described in detail under "Results."

Glomerular Lesions

Involvement by acute necrotizing lesions was graded under four categories: 0, no involvement; 1+, glomeruli with 1 to 25% of the tuft involved; 2+, 26 to 75% of the tuft involved; 3+, 76 to 100% of the tuft involved. One hundred consecutive glomeruli were counted in a section of each graft, and the percentage of each category was tabulated.

Arterial Lesions

All the arteries visible in one or more sections of the hemisected kidney were counted, and the percentage involved by acute and chronic lesions was recorded separately.

Tubular Atrophy

The percentage of the area of the cortex in which tubules were atrophic was estimated by examination of one or more hemisections of each graft.

Mononuclear Cell Infiltration of the Interstitium

The percentage of the cortex involved by cellular infiltrates was determined in a manner similar to tubular atrophy.

The series of readings recorded independently for each morphologic category by the two observers were compared by correlation coefficients and found to correlate significantly ($P < 0.01$ or 0.05 in all cases).

Indirect Immunofluorescence Studies on Recipients' Sera

The following sera were available for the studies: two each from recipients that were 1, 3 and 4 months and one from 2 months after grafting (total of 7). The technic of indirect immunofluorescence has been described earlier.[5] Briefly, each serum was layered on a cryostat section of normal kidney for 45 minutes. The section was then washed, and stained with FITC-conjugated anti-rat IgG. Normal

kidneys were obtained from the following sources, with the major histocompatibility or AgB types of the inbred rat strains being listed according to the nomenclature of Palm and Black.[10]

LBN F_1 (AgB1,3), Lewis (AgB1), F344 (AgB1) and Buffalo (AgB6) (Microbiological Associates), Lewis (AgB1) and Sprague-Dawley (AgB6) (Charles River Labs, Wilmington, Mass) rats were used. Guinea pigs were of the Camm Hartley strain (Charles River Labs). Human tissues were obtained from a patient who underwent nephrectomy for renal cell carcinoma.

Functional and In Vitro Immunologic Studies

Weekly blood urea nitrogen (BUN) measurements were performed on all graft recipients. *In vitro* studies of immunologic function, namely assays of lymphocyte-mediated cytotoxicity and lymphocytotoxic antibodies are reported in detail elsewhere.[9]

Results

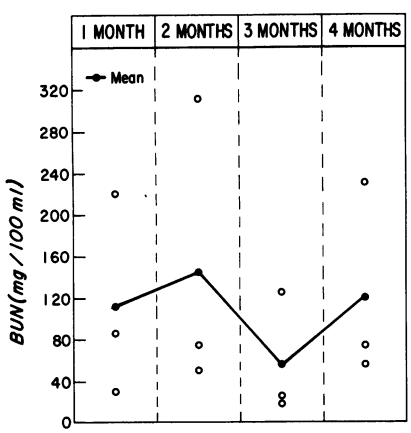
Functional Studies

Mild to moderate elevations and a wide variation in BUN values were characteristic of the long-term recipients. This was reflected in weekly measurements of BUN and in the terminal values obtained at the time of sacrifice (Text-figure 1). Although short-term enhanced recipients also showed some variation in BUN levels,[4] the heterogeneity was more prominent in the long-term group.

Morphology of the Long-Term Enhanced Allograft

Glomerular Lesions

At each of the four time periods of examination, a mean of 80 to 90% of the glomeruli showed acute lytic necrosis of endothelial and other cells associated with amorphous eosinophilic PAS-positive deposits which occluded capillary lumens (Figure 1). These deposits often



TEXT-FIG 1—Terminal blood urea nitrogen (BUN) values of the long-surviving allograft recipients. (BUN of individuals at the time of sacrifice, *open circles*; mean BUN, *solid circles*)

contained fibrin, which was also present occasionally in Bowman's space. Some glomeruli were enlarged and their tufts necrotic. These acute lesions varied in extent of involvement with severe (3+) lesions affecting a maximum of only 23% of the glomeruli, and they showed no consistent sequential progression from 1 through 4 months (Text-Figure 2).

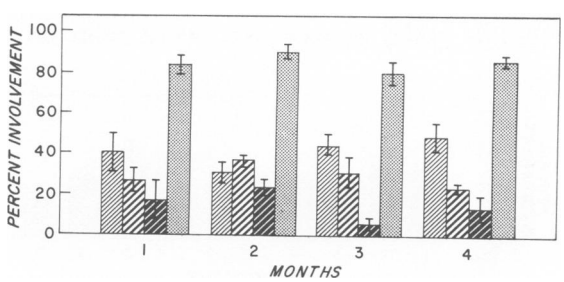
A mean of 10 to 20% of the glomeruli at each time interval appeared anatomically normal. Proliferation of glomerular cells was present at 1 month, and focal capillary wall thickening was observed in most grafts, but both these alterations involved relatively few glomeruli. Contracted hyalinized glomeruli indicative of healed injury were present in only three of the grafts and were few in number.

Arterial Lesions

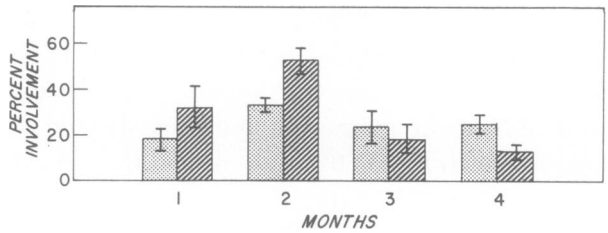
At each time interval, a mean of 18 to 33% of the arteries, arcuate or smaller in size, showed acute changes (Text-figure 3), manifested as "dropout" or loss of smooth muscle nuclei in the media, occasionally with fibrin deposition (Figure 2). Rarely, a more classic picture of acute necrotizing arteritis was present, with pyknosis and fragmentation of muscle nuclei, fibrin deposition and mild neutrophilic infiltration and fragmentation. This latter form of arteritis was at no stage severe or widespread enough to cause cortical necrosis. A mean of 13 to 52% of the arteries at each time interval showed evidence of chronic or healed injury (Text-figure 3) in the form of intimal and medial fibrosis, thinning of the media, proliferation of smooth muscle cells and disruption of elastic laminae (Figure 3). The extent of arterial involvement by either acute or chronic lesions did not progress in any consistent manner from 1 through 4 months (Text-figure 3).

Tubular Lesions

There was patchy tubular atrophy involving an average of 18 to 29% of the cortex at the intervals studied (Text-figure 4). Atrophy was



TEXT-FIG 2—Extent of involvement of glomeruli by acute necrotizing lesions from 1 through 4 months. (*Vertical lines* represent mean ± SE; *hatched columns* represent, from left to right, Grade 1+, 2+ and 3+ lesions and total lesions)



TEXT-FIG 3—Extent of involvement of arteries by acute (*dotted columns*) and chronic (*hatched columns*) lesions from 1 through 4 months. (*Vertical lines* indicate mean ± SE)

manifested by reduction in tubular size and thinning of the epithelium (Figure 4), and the basement membranes were thick, convoluted and occasionally partly split or disrupted. Moreover, epithelial cells showed degenerative changes, but no coagulative necrosis was present. The extent of tubular atrophy showed no sequential progression through 4 months (Text-figure 4).

Mononuclear Cell Infiltration

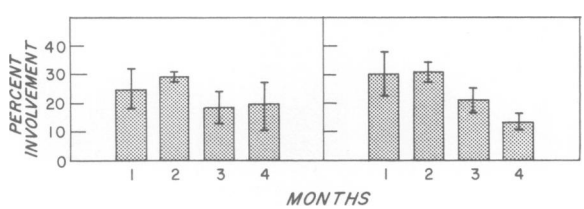
At 1 and 2 months, grafts showed extensive cellular infiltration (Figure 4) involving an average of 25 to 30%, of the cortex (Text-figure 4). At 3 and 4 months, the mean involvement was 21 and 13%, respectively (Text-figure 4). The cells consisted of an admixture of small lymphocytes, large lymphoid cells, plasma cells and macrophages. Invasion of tubular epithelium by presumably cytotoxic lymphoid cells was infrequently seen. Variable degrees of perivascular edema and interstitial fibrosis were also present.

Immunofluorescence Findings

Direct Immunofluorescence

In all 11 grafts examined (Table 1), most or all glomeruli contained deposits of IgG, usually in a granular and focal linear pattern along the capillary walls (Figure 5). There was never a diffuse linear deposition of IgG along the glomerular basement membrane, and no C3 was detected. Focal fibrinogen deposits were seen in necrotic glomeruli.

Nine of the 11 grafts showed deposits of IgG along the basement membranes of cortical tubules (Figure 6). The deposits were diffuse in all the grafts except one, were seen on the basement membrane of



TEXT-FIG 4—Extent of cortical tubular atrophy (*left*) and interstitial mononuclear cell infiltration (*right*) from 1 through 4 months. (*Vertical lines* indicate SE)

Table 1—Summary of Direct Immunofluorescence Findings

Deposits in renal allografts	IgG	C3
Glomeruli	11/11 (Focal granular and interrupted linear)	0/11
Tubular basement membrane	9/11 (8 diffuse, 1 focal)	9/11 (focal)

shrunken and dilated as well as normal-sized tubules, and appeared to involve both proximal and distal convoluted tubules. Deposits of C3 were relatively sparse and focal but were present in all 9 grafts. No fibrinogen or albumin was detected. The two kidneys that were negative for tubular basement membrane (TBM) deposits were seen at 1 and 2 months postgrafting.

In two kidneys (at 2 and 4 months), rare small arteries and arterioles contained medial and transmural deposits of IgG and fibrinogen but no C3 or albumin. In four grafts (three at 1 month and one at 2 months), there were scattered interstitial mononuclear cells with positive cytoplasmic staining for IgG.

Indirect Immunofluorescence

The sera of 7 recipients were examined by indirect immunofluorescence for the presence of IgG binding to normal kidneys (Table 2). Five contained IgG which was bound in a linear pattern to the TBM of normal LBN F_1 (donor strain), Lewis (Charles River), F344, Copenhagen, August, Buffalo and Sprague-Dawley strain rats but not to the kidneys of the Lewis strain (Microbiological Associates, Inc) used as graft recipients. This anti-TBM IgG was also bound to the TBM of normal guinea pig and human kidney. It did not bind to the basement membranes of LBN F_1 liver, lung or spleen. Of the two sera that were negative (1 and 3 months), one recipient's renal allograft (1 month) contained no IgG deposits on the TBM and the other's (3 months) had

Table 2—Summary of Indirect Immunofluorescence Findings

Binding of IgG in recipients' sera to:	Donor LBN strain	Recipient Lewis strain	Other rat strains	Guinea pig	Human
Glomeruli of normal kidneys	2/7	2/7	0/7	0/7	0/7
TBM of normal kidneys	5/7	0/7	5/7	5/7	4/6
BM of lung, liver, spleen	0/7	—	—	—	—

BM = basement membrane, TBM = tubular basement membrane.

only focal deposits. In addition, two sera contained IgG which was bound in a focal linear pattern to the glomerular capillaries of LBN F_1 (donor strain) and Lewis (recipient strain) rat kidneys; both these sera were also positive for the anti-TBM IgG.

Comparison With Lesions in Acutely Rejecting and Short-Term Enhanced Grafts

In the 1 to 9 days after grafting, the acutely rejecting graft showed lymphocytic infiltration, widespread glomerular necrosis and progressive necrotizing arteritis with deposition of IgG and C3 leading to cortical necrosis. Short-term enhanced grafts (in the 1- to 21-day period) showed comparable cellular infiltration but markedly reduced glomerular and arterial lesions and no cortical necrosis.[4,5] A comparison of the changes observed in these two groups with those in the long-term enhanced grafts is summarized in Table 3. Although the predominant glomerular lesions in the long-term grafts were essentially similar to those in acutely rejecting unmodified allografts, they were more variable and less extensive. Thus, by Day 9 nearly 75% of the glomeruli in unmodified grafts [4] had undergone extensive necrosis, while among the long-term grafts, severe (3+) acute injury involved a maximum of 23% of the glomeruli (Text-figure 2). The severe acute arteritis which characterized acute rejection and affected up to 25% of the arteries [4] was infrequent in enhanced grafts. In contrast, the main vascular change in the long-term grafts was nuclear loss in the media without IgG deposits and this was seen only transiently in unmodified allografts, prior to the development of arterial necrosis. Chronic arterial lesions were infrequent in the short-term grafts but were present in up to 52% of the arteries in the long-surviving transplants (Text-figure 4). Extensive tubular atrophy with deposition of anti-TBM antibody was present only in grafts surviving 1 month or more. The interstitial lymphocytic infiltrate showed not only diminution after 2 months (Text-figure 4) but also maturation of cells and less cytotoxic activity, as evidenced by invasion of the tubules by lymphoid cells.

In Vitro Immunologic Studies

These studies are described in detail elsewhere [9] and are only summarized here. Lymphocytotoxic alloantibody was assayed by the ability of recipients' sera to induce release of ^{51}Cr from labeled donor (BN) lymphocytes in the presence of rabbit complement. Anti-BN alloantibody was present in the sera of both acutely rejecting and short-term enhanced graft recipients but was not detected in the sera of enhanced animals beyond 9 days.

Table 3—Summary of Morphologic Lesions in Unmodified Allografts and Short-Term and Long-Term Enhanced Allografts

Lesions	Unmodified grafts (1–9 days)	Short-term enhanced grafts (1–21 days)	Long-term enhanced grafts (1–4 mons)
Necrotizing glomerular lesions	Severe, extensive, progressive after Day 5; IgG positive	Mild, involving few glomeruli after Day 5; IgG negative	Severe but less than in unmodified grafts, not progressive, IgG positive
Predominant arterial lesion	Acute necrotizing arteritis, IgG and C3 positive; associated with cortical tubular necrosis after Day 6	Minimal changes (focal acute arteritis), IgG negative, no cortical necrosis	Medial nuclear loss, chronic arterial lesions; generally IgG negative; no cortical necrosis
Tubular atrophy	Absent	Focal and mild after Day 14; IgG negative	Extensive (with some variability); IgG and C3 positive
Interstitial lymphocytic infiltrate	Intense, peak at Day 5 abates	Intense, peak at Day 7, persistent	More variable, abates after 2 months

Lymphocyte-mediated cytotoxicity, the assay of cellular immunity, was measured by the ability of graft recipient (Lewis) spleen lymphocytes to effect release of ^{51}Cr from labeled target lymphocytes of the histoincompatible donor (BN) strain. This activity peaked at about 40% specific release at 5 days in the unmodified and at 7 days in the enhanced animals, then diminished to about 10% and persisted through the fourth month in the enhanced group. A factor(s) which blocked specific cell-mediated cytotoxicity was detected in the sera of enhanced animals surviving beyond 9 days, and was present in all of the long-term recipients.

Discussion

The extended survival of (Lewis × Brown Norway) F_1 to Lewis renal allografts induced by antidonor antibody is accompanied by the development of some allograft lesions which appeared unique to the long-term graft and others which resemble the lesions seen in unmodified acutely rejecting grafts. In spite of the use of genetically inbred animals and a uniform method of inducing immunologic enhancement, the lesions in most of the allografts showed considerable variation and no sequential progression. This is in contrast with the uniform and sequential lesions observed in unmodified graft recipients [4]; it suggests that other as yet undefined factors are involved in determining the long-term response of the enhanced host to a kidney transplant.

Advanced tubular atrophy with deposition of IgG and complement on the TBM is a lesion encountered only in the long-term grafts. Similar tubular lesions have been produced in rats, guinea pigs and rabbits by immunization with kidney homogenates and have been attributed to anti-TBM autoantibodies.[11,14] An organ-specific anti-TBM antibody was detected in the sera of long-surviving recipients and found to be broadly reactive against TBMs of different rat strains as well as other species (Table 2), thus proving to be without major histocompatibility or species specificity. Failure of the antibody to bind to liver, lung and spleen indicates that it has organ specificity, but anti-TBM antibodies produced by immunization with renal tissue show limited cross-reactivity with other organs, namely choroid plexus and intestine.[11] This antibody probably results from an initial tubular injury due to cellular rejection in the early course of the graft [4] causing TBM antigens to be released into the circulation, to which the Lewis host responds by producing an anti-TBM antibody. Anti-TBM antibodies have been reported in human renal allografts [15,16] and in rejecting grafts in the same rat combination we have used, *ie*, LBN F_1 to Lewis.[17] It has been shown

that the development of these antibodies reflects the absence of the relevant TBM antigen in the graft recipient,[16,17] and our observations with regards to specificity further indicate that such antigenic differences between donor and recipient are not related to the major histocompatibility system.[18] Moreover, the appearance of tissue-specific antibodies in functioning long-surviving enhanced allografts demonstrates that although the acute rejection response (presumably directed against major histocompatibility antigens) is abrogated following enhancing serum treatment, an immune response to other graft antigens can still be manifested.

The necrotizing vascular lesions that are the hallmark of humoral antibody-mediated acute rejection[19] were rare in the long-term enhanced grafts. However, the medial nuclear loss and chronic obliterative arterial changes that were present in these allografts may be mild forms or late stages of humoral rejection, respectively. The presence of IgG deposits in the arteries of two long-term grafts (but not in any enhanced grafts before 21 days[5]) also suggest that humoral antibody-mediated vascular injury does occur in long survivors, albeit at a frequency and intensity far less than what is seen in unmodified allografts. Definitive evidence that the vascular lesions represent humoral rejection would require isolation and precise identification of the deposited immunoglobulins, and this is not feasible with present technics.

Glomeruli displaying immunoglobulin deposits and lytic necrosis of endothelial cells were present in the long-term grafts, and these lesions were also qualitatively similar to those observed in unmodified acutely rejecting grafts[4,5] (Table 3). Such a recapitulation of the changes typical of acute rejection in the same experimental model suggests that the acute glomerular injury, like the vasculitis, is due to rejection episodes. We are investigating this problem further by attempting to elute antibodies from the grafts and reproduce the lesions by passive transfer. Two recipients' sera contained an antiglomerular antibody which reacted against kidneys of both donor and recipient strains (Table 2), and is therefore not histocompatibility specific. Similar antibodies were detected in the sera of unmodified graft recipients,[5] but it is unlikely that they play a major role in the long-term grafts for the following reasons: a) the majority of the glomerular IgG deposits were of the granular type, which suggests localization of immune complexes and is against antiglomerular antibody deposition,[20] and b) organ-specific antiglomerular antibodies were present in only two of seven long-surviving recipients' sera even though IgG-positive glomerular lesions were present in all the grafts examined.

The mononuclear cell infiltration probably represents continuing cell-mediated rejection, which peaked at 5 days in the unmodified and 7 days in the enhanced animals.[4,9] The peak infiltration corresponded with maximum titers of spleen lymphocyte cytotoxicity as measured in the *in vitro* assay.[4] The abatement of cellular infiltration after 2 months may be due to a) a reduced total population of donor-specific cytotoxic lymphocytes and/or b) the presence of circulating factor(s) which block cellular responses against donor antigens. Such blocking factors have been identified by *in vitro* technics in the sera of long-surviving rat [6] as well as human [21,22] recipients of renal allografts, but their precise nature remains unknown. Although blocking factors may be involved in abrogating cellular rejection *in vivo,* their role is unclear, especially since we have observed in the LBN F_1 to Lewis model that they appear in the serum at 9 days after grafting, which is well before the abatement of cellular rejection is evident in the grafted kidney.

The appearance of glomerular and arterial lesions (which are probably manifestations of humoral rejection) in the long-surviving enhanced grafts indicates that the protective effect of the single dose of enhancing serum is incomplete. In these grafts, humoral rejection appears to occur in the absence of detectable lymphocytotoxic alloantibodies, suggesting that an *in vitro* assay for such antibodies is not an accurate index of rejection, at least in immunologically enhanced recipients. We have previously reported the converse dissociation between antibody titers and survival in short-term enhanced grafts—that is, abrogation of humoral rejection in the presence of circulating lymphcytotoxic antibody.[4] Other investigators have reported a similar lack of correlation between early graft function and antidonor antibody responses in immunologically enhanced recipients.[6,23,24] Such a dissociation suggests that the abrogation of humoral rejection in the early stages may not be due to a central effect on the immune system, which would be reflected in a reduction of circulating antibody titers. It is more consistent with a peripheral action of enhancing antibodies—*ie,* the critical early abrogation of humoral rejection may be due to "coating" of antigenic sites in the grafted kidney by enhancing antibodies.[25] Catabolism and loss of the antibody with regeneration of new antigens might allow sites to be reexposed, leading to binding of cytotoxic antibodies and the occurrence of rejection in the long-term grafts. It is also possible that the lesions of humoral rejection in the long-term grafts, especially the glomerular lesions, are due to the deposition of immune complexes of transplantation antigens and antibodies.[25,26] The presence of circulating complexes would account for the failure to detect free

cytotoxic alloantibody in the serum. Whether the abatement of cellular rejection seen after 2 months is due to peripheral mechanisms or whether a central immunosuppressive component plays a significant role are questions that remain to be answered.

In summary: Our observations lead to two main conclusions which are important in assessing the ultimate fate of renal allografts in immunologically enhanced recipients—first, that graft injury may result from relatively late immune responses to tissue antigens that are organ specific but major histocompatibility antigen unrelated like the TBM antigen, and second, that lesions characteristic of humoral rejection may occur at a late stage and show no correlation with titers of serum cytotoxic antibody.

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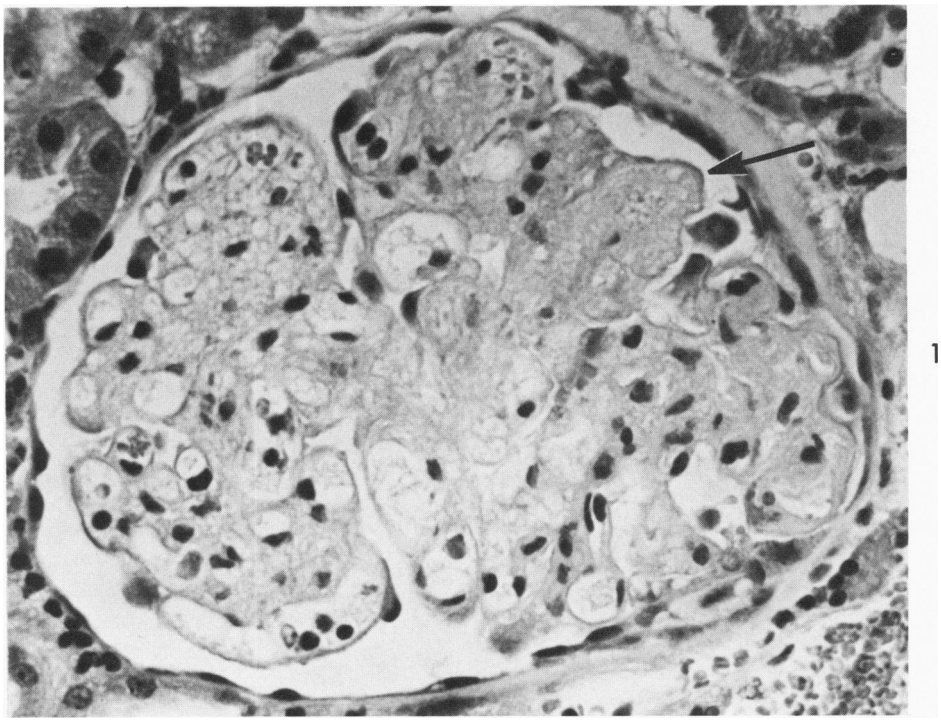

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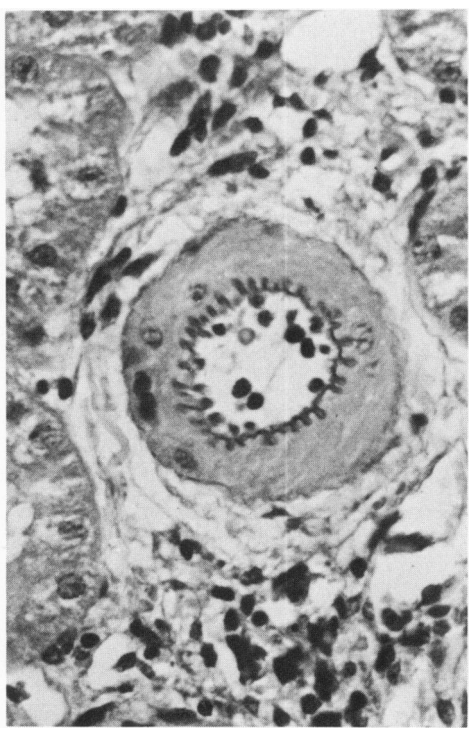

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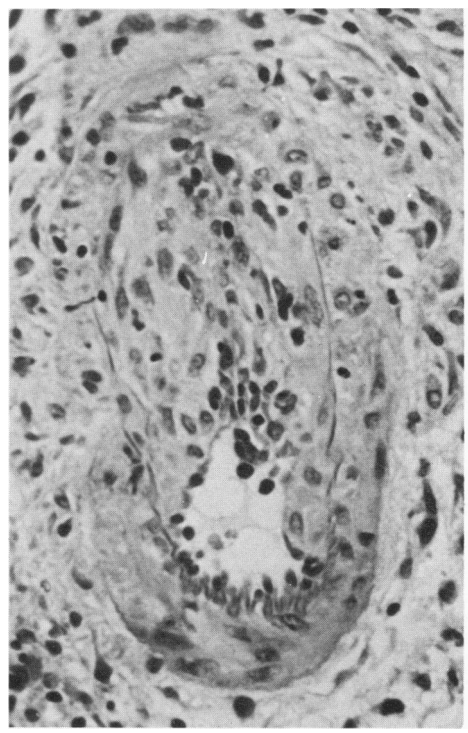
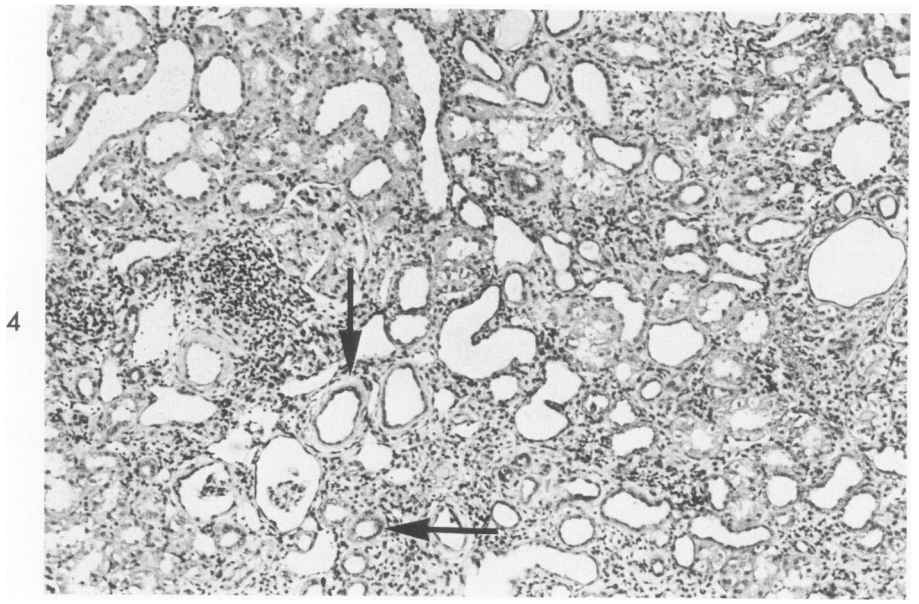
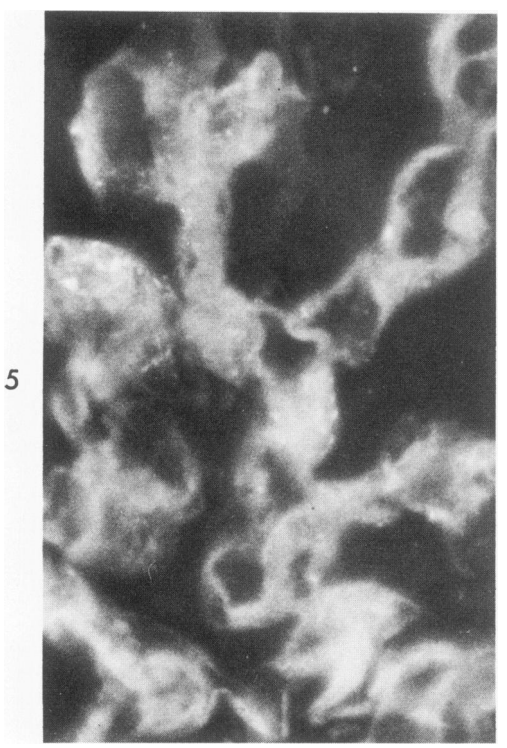


3

Fig 1—Glomerulus in a 4-month allograft, with extensive necrosis of endothelial cells and amorphous material occluding capillary lumens *(arrow)* (H&E, × 200). **Fig 2**—Interlobular artery in a 3-month allograft, showing loss of nuclei from large areas of the media (H&E, × 200). **Fig 3**—Arcuate artery in a 1-month allograft, with cellular infiltration, intimal and medial fibrosis and disruption of internal elastic lamina (H&E, × 200).





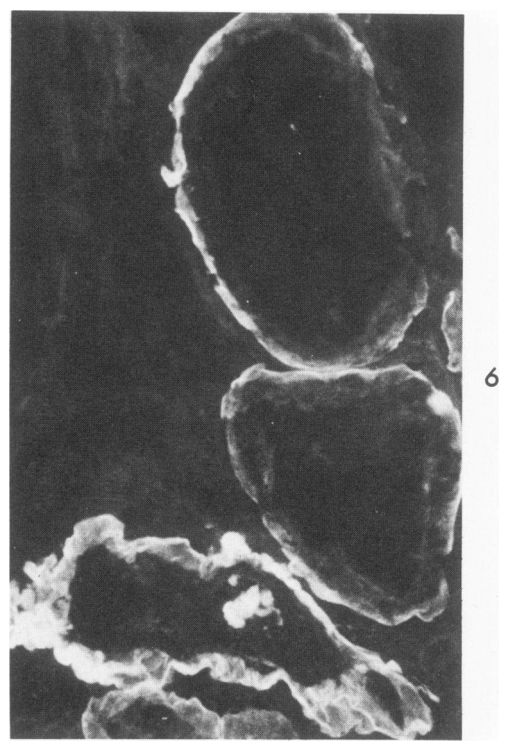


Fig 4—Tubular atrophy *(arrows)* and interstitial mononuclear cell infiltration in the cortex of a 2-month allograft (H&E × 32). **Fig 5**—Segment of glomerular tuft in a 2-month allograft, showing immunofluorescent deposits of IgG along the capillary walls in a granular and focal linear pattern (× 540). **Fig 6**—Linear immunofluorescent deposits of IgG on the tubular basement membranes of a 2-month allograft (× 250).