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DIALYSIS - TRANSPLANTATION

Contribution of early acute rejection episodes to chronic rejection in a rat kidney retransplantation model

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Contribution of early rejection episodes to chronic rejection in a kidney retransplantation model. Chronic graft rejection represents the single most important risk factor for unsatisfactory long-term results after organ transplantation. In addition to various alloantigen dependent and independent factors, acute rejection episodes have been cited as a major immunological risk factor. However, the effects of acute rejection episodes on long-term graft outcome remains unknown. To examine the influence of a single early rejection event on ultimate graft outcome, acutely rejecting rat kidney grafts were retransplanted sequentially into syngeneic rats and their functional and structural behavior assessed over time. LEWxBNF$_1$ kidney allografts and LEW isografts were removed from their LEW recipients after three, four, five and seven days (N = 12/group/time period) and retransplanted into donor strain hosts. The grafts were followed functionally and harvested four, eight, and 32 weeks later. Urinary protein excretion was measured weekly. Kidneys were examined morphologically and immunohistologically using monoclonal antibodies (mAbs) against macrophages (ED-1), T cells and their subsets (CD5, CD4, CD8), MHC class II expression (OX3) and adhesion molecules (ICAM-1 and LFA-1α). The mean standard time ± SD of non-retransplanted allografts was 14.5 ± two days; isografts functioned indefinitely. At five and seven days, acutely rejecting allografts showed massive cellular infiltrates associated with extensive necrosis. These changes could not be reversed by retransplantation and the syngeneic recipients later died of renal failure. In contrast, most allografts retransplanted earlier in the process recovered completely when retransplanted after three (12 of 12 allografts) and four (7 of 12 allografts) days. During the subsequent follow-up period, urinary protein excretion was comparable in retransplanted allografts and isografts. The increased mononuclear cell infiltration in non-retransplanted allografts seen at three and four days was only occasionally observed during the follow-up period after retransplantation. Only a few sclerosed glomeruli (~15%), mild arterial changes and minimal cellular infiltrates were observed by 32 weeks, which were similar to that seen in retransplanted isografts. A single acute rejection episode was completely reversible and did not progress to chronic rejection if retransplanted into syngeneic donors when the inflammatory changes are still early. Those results demonstrate the critical effect of alloantigen-dependent events on chronic graft deterioration, and indicate that prompt and aggressive treatment of initial acute rejection episodes are beneficial to protect against late deleterious changes in the graft.

Key words: acute rejection episodes, retransplantation, chronic graft dysfunction.

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Although early results of clinical transplantation have improved progressively, the ultimate goal of organ transplantation, which is to provide long-term treatment for irreversible organ failure, has not yet been achieved. Chronic rejection has been cited as the most important cause of late graft loss [1–4]. Although the functional and morphological findings are well characterized, the pathophysiological mechanisms leading to graft deterioration secondary to the evolution of this process are poorly understood. Potential risk factors include alloantigen-dependent factors such as early acute rejection [5–9] and HLA-matching [10], and alloantigen-independent components including initial ischemia and reperfusion injury, inadequate functional nephron mass, viral infections and drug nephrotoxicity [2, 11–14]. These events may affect the process either alone or in concert.

The influence of early acute rejection on long-term graft function has been shown in several clinical series [5–8]. However, the characteristics (morphological changes, time of occurrence and success of treatment) of acute rejection episodes, including mechanisms resulting in long-term graft dysfunction, remain unknown. Two explanations have been offered for this relationship: first, alloreactivity may persist despite putatively satisfactory maintenance immunosuppression. As a consequence, indirect presentation of donor MHC peptides may become increasingly important over time [15]. Secondly, predominantly alloantigen-independent events may gain influence over the long term. The ongoing immunological injury caused by repeated subclinical acute rejection episodes may additionally lead to progressive reduction of functioning organ mass, causing hyperfunction of the remaining nephron units which themselves gradually fibrose over time. The influence of reduced organ mass on long-term function has been demonstrated in several experimental and clinical settings [3, 13, 16].

Previous experimental studies demonstrated that early acute rejection episodes are reversible by retransplantation back into a donor strain animal. However, those experiments did not follow retransplanted grafts long-term [17–19]. Thus, it remains unknown if the severity of acute rejection and the subsequent damage to the graft may contribute to chronic graft failure even in the absence of an ongoing immunological stimulus. Complete reversal of acute rejection by syngeneic retransplantation in the absence of the development of chronic graft deterioration may

support the critical role of alloantigen-dependent effects during the process and the concept to treat acute rejection episodes early and aggressively.

We attempted to examine the contribution of a single acute rejection episode on the development of long-term graft changes by sequential retransplantation of acutely rejected rat renal allografts into donor strain recipients, and studied their changes in a long-term follow-up.

METHODS

The model

Male inbred 200 to 250 g rats (Charles River, Sulzbach, Germany), were used throughout the experiments. Lewis Brown Norway F_1 (LEWxBNF$_1$,RT$^{1/n}$) kidney allografts were grafted into Lewis (LEW, RT1^1) recipients using standard microsurgical techniques; LEW kidneys placed in LEW rats served as isograft controls. Grafts were transplanted orthotopically to recipient renal vessels and ureter by end-to-end anastomosis using 10-0 prolene. Both native kidneys were removed during transplantation in all experimental groups. Graft tissues were harvested after three, four, five, and seven days ($N = 5$ allografts, $N = 3$ isografts/time interval). Survival times of unmodified allograft recipients was tested in a separate group ($N = 6$).

Allografted and isografted kidneys from a parallel series of recipients were retransplanted into bilaterally nephrectomized donor strain rats (LEWxBNF$_1$ or LEW, respectively) three, four, five and seven days after their initial engraftment ($N = 12$ allografts, $N = 3$ isografts/time interval). Operative technique and duration of ischemic time (~30 min) were similar to the first transplants. Retransplanted grafts were harvested after four, eight, and 32 weeks. Native and uninephrectomized LEW and LEWxBNF$_1$ controls were examined in parallel throughout the observation period. All kidneys were assessed serially for function, morphology and immunohistology.

Functional studies

Urine samples (24 hr) were collected serially at two week intervals. Protein excretion was measured by precipitation with 20% CCl_3COOH. Turbidity was assessed at a wavelength of 415 nm using a Hitachi 911 analyzer.

Histology

Kidneys were removed at serial intervals in experimental and control groups and fixed in 5% buffered formalin. Paraffin sections were stained with hematoxylin and eosin, periodic acid-Schiff, Trichrome and Silver-Mason, and assessed by light microscopy. To determine the extent of glomerulosclerosis, > 100 glomeruli/kidney were counted and the ratio of sclerosed glomeruli/total glomeruli expressed as a percentage.

Immunohistology

Portions of representative kidneys in experimental and control grafts were snap frozen in liquid nitrogen, cut (4 μm), fixed in acetone for 10 minutes, air dried and stained with mouse monoclonal antibodies (mAbs) to CD5+ T-cells (OX-19), CD4+ T helper cells (W3/25), CD8+ T cytotoxic/suppressor cells (OX-8), macrophages (ED-1), MHC class II (OX3; Serotec, Wiesbaden, Germany), intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 1α (LFA-1α; both Genzyme, Wiesbaden, Germany). After specific mAb staining, the sections were then interacted with rabbit anti-mouse IgG following a mouse anti-alkaline phosphatase complex (APAAP), and the sections were then counterstained with hematoxylin. MHC class II and ICAM-1 expression was quantified on a 0 to 4+ scale (4+ = dense); positive cell counts were expressed as mean ± SD of cells/field of view (c/FV); > 20 FV/section were evaluated at ×400.

Statistics

Statistical significance was ascertained using ANOVA and the Student's *t*-test.

RESULTS

Survival data

Allograft recipients ($N = 6$) died of renal failure 14.5 ± two days (mean ± SD) after transplantation while isografted hosts survived indefinitely.

The majority of allograft recipients (23 of 24 animals) that were retransplanted five and seven days after the original kidney engraftment, a period when graft destruction was far advanced, died 3 ± 1 days later; only one animal that had been retransplanted after five days survived for 28 days. However, if retransplantation was performed earlier in the course of the process, most allograft recipients recovered completely (12 of 12 and 7 of 12 retransplanted after 3 and 4 days, respectively). Several (5 of 12) kidney allografts retransplanted four days after their initial engraftment failed after two to three weeks, having sustained their recipients during that period before deteriorating completely, which suggests that these animals sustained progressive ongoing and irreversible injury (Fig. 1). All recipients of retransplanted isografts ($N = 12$) survived indefinitely.

Function

Urinary protein excretion in non-retransplanted control allografted hosts peaked by 11 days (86 ± 10 mg/24 hr), diminishing rapidly thereafter as the grafts failed and animals began to die of renal failure a few days later.

Allografts retransplanted after seven days never regained function and remained anuric. Those retransplanted at five days also never recovered, with the exception of the single animal who died with progressive proteinuria (162 mg/24 hr) at 28 days.

Allograft function returned to stable values after retransplantation at three and four days, which was similar to retransplanted isograft recipients or uninephrectomized native controls. However, proteinuria increased slightly in retransplanted allografts (23 ± 5 mg/24 hr and 28 ± 7 mg/24 hr by days 3 and 4), isografts (42 ± 11 mg/24 hr) and native uninephrectomized controls (26 ± 6 mg/24 hr; P = NS) by 32 weeks, and was increased as compared to native non-nephrectomized controls (12 ± 3 mg/24 hr, $P < 0.05$) presumably as a consequence of ischemic injury and reduction of functioning kidney mass. The five allograft recipients eventually dying after retransplantation four days after the initial engraftment demonstrated progressive proteinuria at 18 ± 3 days (146 ± 12 mg/24 hr; Fig. 2).

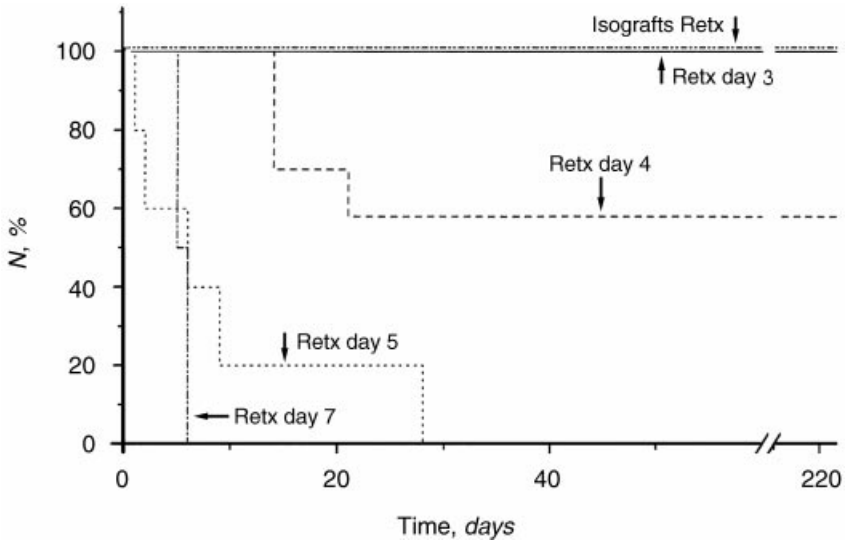

Fig. 1. Survival data of allografts retransplanted after 3, 4, 5, and 7 days are shown. While all animals retransplanted after three days survived indefinitely, most grafts (7 of 12) retransplanted after four days survived the observation period. Animals never recovered when retransplantation was performed after 5 and 7 days.

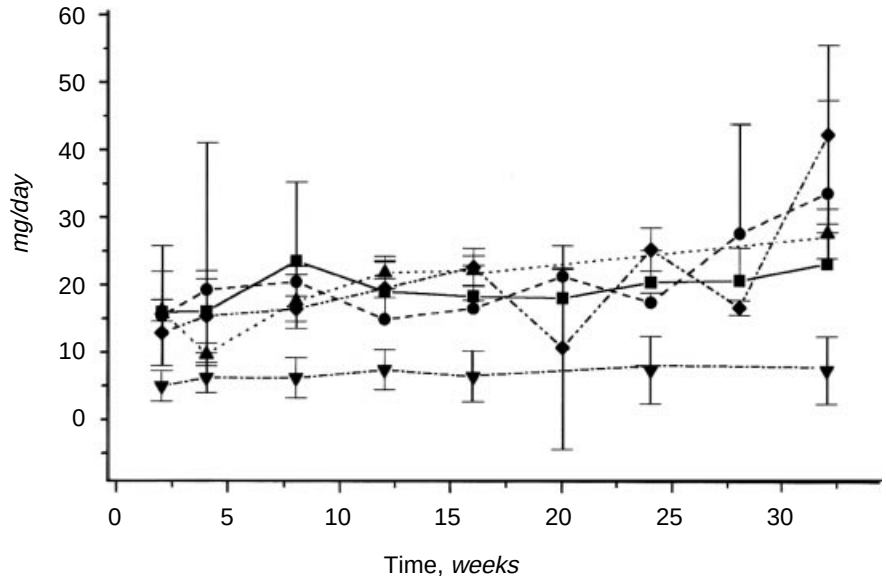

Fig. 2. Urinary protein excretion in allografts retransplanted three (■) and four (●) days after initial engraftment, retransplanted isografts (♦), uninephrectomized (▲), and untreated native controls (▼) are shown. Proteinuria did not increase significantly in experimental groups as compared to naive uninephrectomized controls. However, a minor increase was observed compared to non-nephrectomized native controls by 32 weeks ($P < 0.05$).

Morphology

Progressive alterations were observed in the non-retransplanted allografted kidneys. By three days mononuclear cells were scattered around vessels and tubules, and by four days the numbers of infiltrating cells had increased. Cellular infiltration peaked by five days, which declined slightly by seven days. Associated structural changes developing during the later stages of acute rejection included interstitial inflammation, loss of tubules, endothelialitis, and finally, as the animals exhibited renal failure, increasing areas of edema and necrosis.

The events of acute rejection occurring by five and seven days could not be reversed by retransplantation. Most of the recipients (23 of 24) died shortly thereafter with extensive and continuing graft destruction. In contrast, in those allografts that recovered completely after retransplantation at three and four days, only a few mononuclear cells were observed around tubules and vessels at 32 weeks, glomerulosclerosis remained minor (15%), and arteries showed smooth muscle cell proliferation only occasionally. Morphological changes characteristic of chronic rejection were not observed. The allografts of the five animals that did not recover completely after retransplantation at four days demonstrated major cellular infiltrates, and loss of tubules with extensive areas of necrosis when examined after two to three weeks.

Regrafted isograft controls showed normal morphology with few mononuclear cells in the interstitium and around tubules. However, by 32 weeks few cellular infiltrates with minor glomerulosclerotic and arteriosclerotic changes were observed, similar to that in allografts recovering after retransplantation by three and four days. Native kidneys in uninephrectomized animals remained normal throughout the observation period (Fig. 3).

Immunohistology

Monocyte/macrophages and T-lymphocytes infiltrated allografts increasingly within three and four days after transplantation

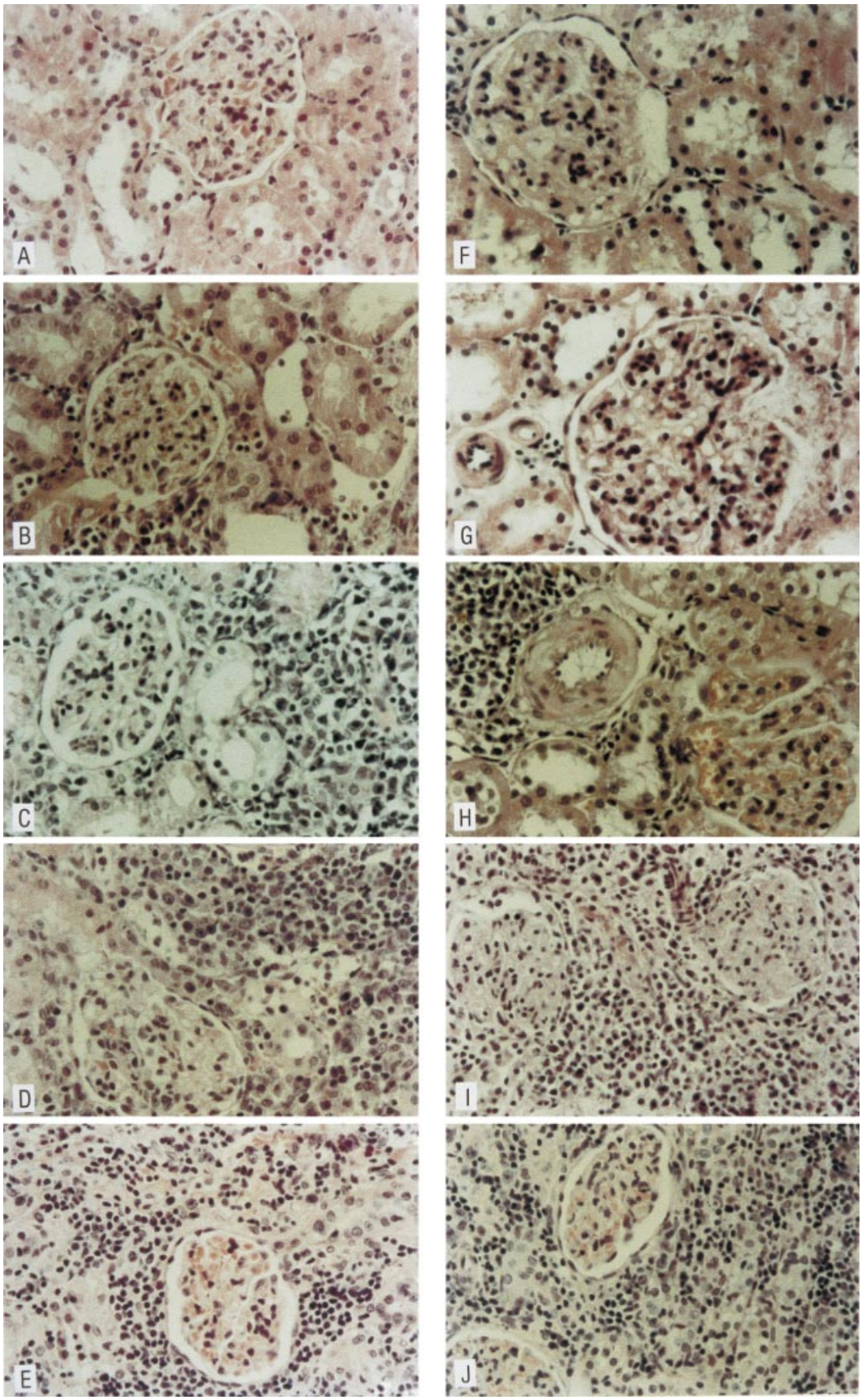
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Fig. 3. Photomicrographs (magnification ×600) of hematoxylin and eosin stained renal allografts and isografts are shown. While the left column represents isografts (*A*; LEW→LEW by three days) and acutely rejected renal allografts of the LEWxBNF1→LEW model at progressive stages of the rejection process (*B*, day 3; *C*, day 4; *D*, day 5; *E*, day 7), the right column represents retranplanted iso- and allografts, respectively (*F*, isograft retansplanted by three days and evaluated after 32 weeks; *G and H*, allografts retransplanted after 3 and 4 days, respectively, and harvested 32 weeks later). (*I*) A retransplanted allograft by day 5, evaluated when the animal died of renal failure after 28 days. (*J*) Allograft retransplanted by seven days and dying two days later. Isografts demonstrated a normal morphology during the observation period with rare cellular infiltrates. Retransplanted isografts (F) showed minor glomerulosclerotic and arteriosclerotic changes with few infiltrating MNC 32 weeks after retransplantation. Mononuclear cell infiltrates increased progressively in allografts from days 3 to 4 (B, C), while allografts retransplanted after those time intervals and evaluated 32 weeks later (G and H) demonstrated only minor changes similar to retransplanted isografts (F). Cellular infiltrates were stronger in allografts retransplanted by day 4, and the minor structural changes were comparable to those in retransplanted isografts and allografts retransplanted by day 3. On the contrary, animals retransplanted 5 and 7 days after the original transplantation never recovered completely and died of renal failure shortly thereafter. Major cellular infiltrates with extensive areas of necrosis present prior to retransplantation by 5 and 7 days (D, E) never recovered, as the animals died of renal failure after syngeneic retransplantation (I and J).

(ED-1, 12 ± 6 and 18 ± 5 c/FV; CD-4, 10 ± 5 and 18 ± 7 c/FV; CD-8, 15 ± 4 and 19 ± 8 c/FV, respectively; P = NS; LFA-1α, 16 ± 4 and 18 ± 6 c/FV, respectively). MHC class II expression (3+) was found on infiltrating and endothelial cells, and ICAM-1 was strongly expressed on vascular cells and in the interstitium (3+, days 3 and 4). Few mononuclear cell infiltrated isografts were harvested after three and four days (ED-1, 4 ± 2 and 3 ± 1 c/FV; CD-4, 5 ± 2 and 6 ± 4 c/FV; CD-8, 3 ± 2 and 2 ± 2 c/FV, respectively; $P < 0.05$), while few cells in the interstitium and around vessels stained for LFA-1α (4 ± 2 and 3 ± 1 c/FV, respectively, $P < 0.05$). Vascular endothelium and interstitial cells stained for MHC II and ICAM-1 (2+ and 3+, respectively, P = NS).

By five days, allografts demonstrated peak cellular infiltrates with slightly declining numbers by seven days (ED-1, 54 ± 7 and 42 ± 6 c/FV; CD-4, 28 ± 4 and 20 ± 6 c/FV; CD-8, 42 ± 8 and 36 ± 5 c/FV, respectively). LFA-1α (13 ± 4 and 5 ± 2 c/FV, respectively; $P < 0.01$ vs. allografts by 3 and 4 days) and MHC II expression (3+) were found on infiltrating cells, in the interstitium, on glomeruli and around vessels. ICAM-1 was expressed on tubules, vascular endothelial cells and additionally on glomeruli, (3 to 4+ and 3+, days 5 and 7, respectively). These changes, associated with the major structural changes, could not be reversed by retransplantation. The immunohistological changes observed in the retransplanted allografts were comparable despite absence of the continuing host immunological challenge.

When allografts were retransplanted after 3 and 4 days and harvested 4, 8 and 32 weeks later, the widespread cellular infiltrates decreased in number, as grafts examined at 32 weeks showed (ED-1, 6 ± 3 and 8 ± 2 c/FV; CD-4, 8 ± 3 and 11 ± 4 c/FV; CD-8, 6 ± 3 and 5 ± 2 c/FV, respectively; $P < 0.01$ vs. non-retransplanted allografts at 3 and 4 days). Few interstitial cells and glomeruli stained for ICAM-1 (2+). MHC II expression (2+) and few LFA+ cells (4 ± 2 and 6 ± 3 c/FV, $P < 0.01$) were found on cells in the interstitium and glomeruli. Of the five animals retransplanted on day 4 and dying of renal failure two to three weeks later, the allografts continued to demonstrate major cellular infiltration (ED-1, 55 ± 8 c/FV; CD-4, 36 ± 9 c/FV; CD-8, 42 ± 5 c/FV; $P < 0.01$ vs. non-retransplanted allografts on day 4; $P < 0.01$ vs. allografts retransplanted by day 4 evaluated 32 weeks later) with strong expression of ICAM-1 and MHC II (3 to 4+ and 3+, respectively) on infiltrating cells, around tubules and in glomeruli.

Few cells (ED-1, 3 ± 1 c/FV; CD-4, 4 ± 2 c/FV; CD-8, 1 ± 1 c/FV) were observed in isograft controls by 32 weeks, while only occasional glomeruli and interstitial cells stained for ICAM-1 and MHC class II [2+]. Retransplantated isografts also demonstrated minor cellular infiltrates 32 weeks after retransplantation (ED-1, 7 ± 2 c/FV; CD-4, 8 ± 4 c/FV; CD-8, 2 ± 1 c/FV; P = NS vs. retransplanted allografts retransplanted by days 3 and 4) regardless of the time of the second engraftment. Similarly, low grade expression of ICAM-1 (1 to 2+) and OX-3 (2+) was found around tubules and in glomeruli (Fig. 4).

DISCUSSION

Chronic graft rejection represents a major cause of attrition of organ transplants over time. Although the pathophysiology of this process is only incompletely understood, putative alloantigen-dependent and -independent risk factors include acute and subacute rejection episodes [5–7], initial ischemia and reperfusion injury [11–14], organs from marginal donors, and reduced functioning renal mass [4, 16], viral infections [20, 21] and drug toxicity [22, 23]. Acute rejection episodes seem to be critical. In one clinical report, for instance, cadaver kidney recipients never experiencing acute rejection demonstrated a five-year survival rate of 92% compared to 45% in recipients who had undergone at least one rejection episode. Other studies have been confirmatory [5–7].

To further study the contribution of acute rejection episodes on chronic graft rejection, we retransplanted acutely rejecting rat renal allografts sequentially into donor strain animals to remove the ongoing host immunological drive. When retransplantation was performed at early stages of the rejection process (days 3 and 4) most grafts recovered completely and did not progress to chronic graft failure. Progressive deterioration in some animals by four days was irreversible after retransplantation. Both the concept of an already advanced acute rejection process and the synergistic action of alloantigen dependent and independent events (damage by ischemia and reperfusion injury) seem to be plausible explanations. Further studies into those events are currently being performed. The low grade cellular infiltration and the development of minor signs of glomerulosclerosis observed both in retransplanted allografts and isografts (3 and 4 days) over the long-term probably were an expression of ischemic damage and reperfusion injury during engraftment [13]. When the injury was advanced (days 5 and 7) it could not be reversed by retransplantation, and the recipients began to die shortly thereafter of kidney failure from progressive destruction.

Although differing from the clinical situation, no immunosuppressive treatment was used in our experimental acute rejection model, as we attempted to emphasize the reversal of acute rejection episodes and long-term outcome in the absence of a host

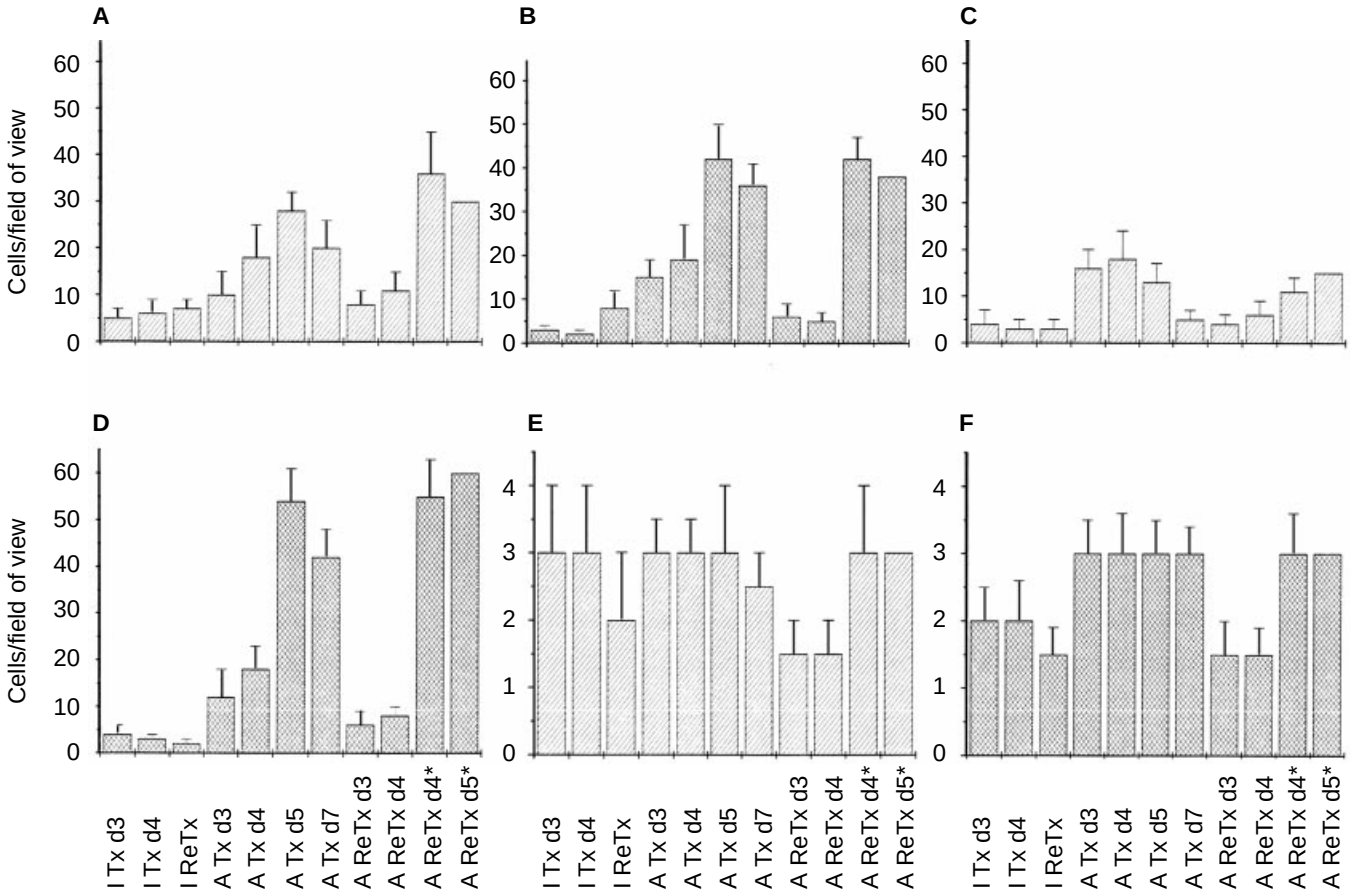

Fig. 4. Quantitated immunohistologial findings for isografts harvested after 3 and 4 days (ITxd3, ITxd4), retransplanted isografts (IReTx, retransplanted by day 3 and evaluated 32 weeks later), non-retransplanted allografts by 3, 4, 5 and 7 days (ATx d3 - d7) and retransplanted allografts (AReTx d3 - d5, *retransplanted allografts by days 4 and 5 dying 2 to 4 weeks after retransplantation) are shown. Cell counts are quantitated as cells/field of view and expressed as mean ± SD; MHC and ICAM-1 are quantitated on a 0 to 4+ scale. Cellular infiltrates were significantly different between non-retransplanted allografts and isografts by 3 and 4 days ($P < 0.05$) and between allografts by 4 and 5 days ($P < 0.01$); no significant differences were found between allografts retransplanted by 3 and 4 days and retransplanted isografts evaluated 32 weeks later. In addition, no significant differences were observed for the expression of MHC II and ICAM-1 in any of the experimental groups. However, during the early phase of the acute rejection process episode (days 3 and 4) MHC II and ICAM-1 were found predominantly on endothelial cells and in the interstitium, while an additional glomerular expression has been observed during later stages (day 5) and in retransplanted allografts and isografts. (*A*) CD3+ T cells; (*B*) CD8+ T cells; (*C*) LFA-α; (*D*) ED-1 + monocytes/macrophages; (*E*) ICAM-1; (*F*) MHC class II.

immunological drive. In addition, our model did not directly correlate to clinical situations, as retransplantation removes the ongoing allogeneic drive completely. The potential contribution of a graft-versus-host reaction was not studied in our model. However, similar changes in retransplanted allografts and isografts seem to exclude 'passenger' LEW cells as influential after retransplantation.

Analyzing such factors as the relationship between the time of the occurrence of acute and chronic rejection, morphological characteristics, severity and success of treatment seems to be important [7–9]. It has been noted that early vascular rejection resulted in a significantly decreased one- and five-year graft survival rate, while patients experiencing an interstitial process or no rejection showed comparable short- and long-term graft function [7]. It seems particularly interesting to find in our experimental studies that effectively treated early acute rejection episodes (< 3 months) did not affect the rate of development of chronic graft failure, while late acute episodes were highly influential on long-term graft outcome. Those latter observations may also reflect a delayed diagnosis or more advanced stages of renal failure secondary to inadequate follow-up [10, 11]. In addition, as characteristics of cellular infiltrates varied during early and late acute rejection episodes different mechanisms might apply [21]. Several reports associated frequent and early acute rejection episodes with a poor long-term outcome [5–8, 24–26]. When HLA-identical renal grafts were analyzed over the long-term, chronic rejection rarely was noted, despite a high incidence of early acute rejection episodes successfully reversed by pulse steroid treatment [27]. Evaluating the association between acute and chronic rejection, we suggest that the impairment seen after the initial treatment was adversely related to long-term function, while acute rejection episodes that were completely reversed had no impact on the development of chronic graft dysfunction [28].

Although retransplantation experiments have been performed before in transplantation settings, the impact of initial acute rejection events has not been studied over the long-term [17–19, 29, 30]. Early stages of acute rejection [17–19] could be obviated by replacement of the graft into donor strain animals; however, no

long-term follow-up has been reported after successful reversal of acute rejection, with the exception of one early report that described thickening of arterial walls in a dog kidney graft one month after retransplantation [19]. Retransplantation experiments in chronic rejection models have been studied before by us and others [29, 30]. Graft coronary arteriosclerosis did not develop when rat hearts were retransplanted back into a donor strain animal within three days. However, when the same procedure was performed later (> 3 days), graft vasculopathy eventually developed [30]. When heart allografts were retransplanted into an animal of donor origin at early (14 days) and late stages (50 days), mononuclear infiltration but not myointimal proliferation were reversed [31]. Retransplantation experiments in a chronic kidney graft model demonstrated complete reversal of functional and morphological changes if sclerosis had not become too advanced [29]. Rat strain and treatment differences including duration of ischemia and preservation techniques in the systems used may explain these discrepancies. Although progressive mononuclear cell infiltrates were present in the early stages of the present acute model, retransplantation could remove the inflammatory process and the graft did not progress to chronic changes. On the other hand, the already advanced acute condition remained irreversible after retransplantation.

Minor cellular infiltrates were noted in both retransplanted allografts and isografts, while minor glomerulosclerosis occurred over time. The development of characteristic findings of chronic graft dysfunction similar to those occurring in allografts at earlier stages has been shown in clinical and experimental studies in isografts, although generally at a period later than 32 weeks [32, 13]. Alloantigen-independent factors such as ischemia and reperfusion injury and their interaction with alloantigen-dependent factors may be of particular importance in this context and need further study. These factors may explain the sustained, slightly elevated levels of proteinuria noted in these experiments.

In summary, we found that early acute rejection episodes were completely reversible after syngeneic retransplantation and did not lead to chronic progression of renal disease. Retransplantation at more advanced stages of acute rejection were irreversible. Those observations demonstrate the critical contribution of alloresponsiveness on the development of chronic graft deterioration, and indicate that prompt and sufficient treatment of acute rejection episodes may improve long-term graft outcome and ameliorate synergistic events of alloantigen dependent and independent events.

While several clinical studies have associated delayed graft function with increased acute rejection episodes [31, 33], it has to be determinated whether an additional ischemic at more advanced stages of the rejection process may support the concept of a synergistic action of alloantigen dependent and independent events.

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APPENDIX

LEW, Lewis rat strain; LEWxBNF$_1$,RT$^{l/n}$, Lewis Brown Norway F$_1$ rat strain; mAb, monoclonal antibody; OX-19, CD5+ T-cells; W3/25, CD4+ T helper cells; OX-8, CD8+ T cytotoxic/suppressor cells; ED-1, macrophages; OX-3, MHC class II; ICAM-1, intercellular adhesion molecule 1; LFA-1α, lymphocyte function-associated antigen 1α; APAAP, anti-alkaline phosphatase complex; c/FV, cells per field of view.

REFERENCES

1. Tilney NL, Whitley WD, Diamond JR, Kupiec-Weglinski JW, Adams DH: Chronic rejection–An undefined conundrum. *Transplantation* 52:389–398, 1991
2. Paul LC, Benediktsson H: Chronic transplant rejection: Magnitude of the problem and pathogenic mechanisms. *Transplant Rev* 134:5–19, 1993
3. Tullius SG, Tilney NL: Both alloantigen-dependent and -independent factors influence chronic allograft rejection. *Transplantation* 59:313–318, 1995
4. Lemström K, Koskinen P, Häyry P: Molecular mechanisms of chronic allograft rejection. *Kidney Int* 48(Suppl 52):S2–S11, 1995
5. Matas AJ, Gillingham KJ, Payne WD, Najarian JS: The impact of an acute rejection episode on long term renal allograft survival (t1/2). *Transplantation* 57:857–859, 1994
6. Tesi RJ, Matas A, Gillingham K, Dunn DL, Payne WD, Gores P, Gruessner R, Najarian JS: Risk factors for chronic renal allograft recipients. *Transplantation* 55:752–757, 1993
7. van Saase JLCM, van der Woude FJ, Thorogood J, Hollander AA, van Es LA, Weeningg JS, Van Bockel JH, Bruijn JA: The relation between acute vascular and interstitial renal allograft rejection and subsequent chronic rejection. *Transplantation* 59:1260–1285, 1995
8. Massy ZA, Guijarro C, Kasiske B: Clinical predictors of chronic renal allograft rejection. *Kidney Int* 48(Suppl 52):S85–S89, 1995
9. Braun WE, Popwniak KL, Nakamoto S, Gifford RW, Stratton RA: The fate of renal allografts functioning for 20 years (level 5 A)–Indefinite success or beginning of the end. *Transplantation* 60: 784–790, 1995
10. Opelz G, Schwarz V, Engelmann A, Back D, Wilk M, Keppel E: Long-term impact of HLA matching on kidney graft survival in cyclosporine treated recipients. *Transplant Proc* 23:373–375, 1991
11. van Es A, Hermans J, Van Bockel JH, Persjin GG, Van Hoof JP, De Graeff J: Effect of warm ischemic time and HLA (A and B) matching on renal cadaveric graft survival and rejection episodes. *Transplantation* 36:255–217, 1983
12. Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G, Artors KE, Messmer K: The benifical effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation* 57:211–217, 1994
13. Tullius SG, Heemann U, Hancock WW, Azuma H, Tilney NL: Long-term kidney isografts develop functional and morphological changes which mimic those of chronic allograft rejection. *Ann Surg* 220:425–435, 1994
14. Yilmaz S, Paavonen T, Häyry P: Chronic rejection of rat kidney allografts. II. The impact of prolonged ischemia on transplant histology. *Transplantation* 53:823–827, 1992
15. Shoskes DA, Wood KJ: Indirect presentation of MHC antigens in transplantation. *Immunol Today* 15:32–38, 1994
16. Brenner BM, Cohen RA, Milford EL: In renal transplantation, one size may not fit all. *J Am Soc Nephrol* 3:162–169, 1992
17. Murray JE, Balankura O, Greenberg JB: Reversibility of the kidney homograft reaction by retransplantation and drug therapy. *Ann NY Acad Sci* 99:768–782, 1962
18. Kupiec-Weglinski JW, Araujo JL, Towpik E, Tilney NL: Host-graft relationship: The systemic nature of allograft rejection. *Surgery* 98:259–266, 1985
19. Osteen RT, Klebanoff G: The vascular determinants of reversible rejection. *J Surg Res* 10:245–251, 1970
20. Hoskinen PK, Krogerus LA, Nieminen MS, Mattila SP, Häyry

PJ, LAUTENSCHLÄGER IT: Cytomegalovirus infection-associated generalized immune activation in heart allograft recipients: A study of cellular events in peripheral blood and endomyocardial biopsy specimens. *Transplant Int* 7:163–171, 1994
21. REINKE P, FIETZE E, ODE-HAKIM S, PROSCH S, LIPPERT J, EWERT R, VOLK HD: Late acute renal allograft rejection and symptomless cytomegaloovirus infection. *Lancet* 344:1737–1738, 1994
22. ROSS J, NEWTON L: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 311:699–705, 1984
23. GARCIA DL, RENNKE HG, BRENNER BM: Corticosteroid therapy amplifies glomerular injury in rats with renal obliteration. *J Clin Invest* 80:867–874, 1987
24. PIRSCH JD, PLOEG RJ, GANGE S, D'ALESSANDRO AM, KNECHTLE SJ, SOLLINGER HW, KALAYOGLU M, BELZER FO: Determinants of graft survival after renal transplantation. *Transplantation* 11:1581–1586, 1996
25. FOSTER MC, ROWE PA, DENNIS MJ, MORGAN AG, BURDEN RP, BLAMEY RW: Characteristics of cadaver renal allograft recipients developing chronic rejection. *Ann R Coll Surg Engl* 72:23–26, 1990
26. MATAS A: Chronic rejection in renal transplant recipients–Risk factors and correlates. *Clin Transplant* 8:332–335, 1994
27. BALTZAN MA, SHOKER AS, BALTZAN RB, GEORGE D: HLA-identity—Long-term renal graft survival, acute vascular, chronic vascular, and acute interstitial rejection. *Transplantation* 61:881–885, 1996;
28. OPELZ G: Critical evaluation of the association of acute with chronic graft rejection in kidney and heart transplant recipients. *Transplant Proc* 29:73–76, 1997
29. TULLIUS SG, HANCOCK WW, HEEMANN U, AZUMA H, TILNEY NL: Reversibility of chronic renal allograft rejection. *Transplantation* 58:93–99, 1994
30. IZUTANI H, MIYAGAWA S, SHIRAKURA R, MATSUMIYA G, SEIZAH N, SHIMAZAKI Y, MUTSADA H: Evidence that graft coronary arteriosclerosis begins in the early phase after transplantation and progresses without chronic immunoreaction. *Transplantation* 60:1073–1079, 1995
31. NICHOLSON ML, WHEATLEY TJ, HORSBURGH T, EDWARDS ML, VEITCH PS, BELL PRF: The relative influence of delayed graft function and acute rejection on renal survival. *Transplant Int* 9:415–422, 1996
32. GLASSOCK RJ, FELDMAN D, REYNOLDS EG: Human renal isografts: A clinical and pathological analysis. *Medicine* 47:411–454, 1968
33. TROPPMANN C, GILLINGHAM KJ, BENDETTI E, ALMOND S, GRUESSNER RWG, NAJARIAN JS, MATAS AJ: Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. *Transplantation* 59:962–968, 1995