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ORIGINAL ARTICLE

Chronic allograft nephropathy in athymic nude rats after adoptive transfer of primed T lymphocytes

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Summary

The impact of presensitized T lymphocytes on the development of chronic allograft nephropathy (CAN) was investigated in nude athymic LEW.RNU recipients of F344 renal allografts. The recipients ($n = 8$) were reconstituted with 5×10^7 T lymphocytes primed against donor skin grafts to induce graft rejection. LEW.RNU ($n = 8$) and euthymic LEW recipients ($n = 6$) which underwent no further intervention after transplantation served as control groups. Adoptive transfer of primed T cells induced CAN in LEW.RNU rats. Their kidney function decreased progressively. After 90 days a moderate glomerulopathy, tubular atrophy and interstitial fibrosis were observed, vascular changes were only mild or absent. Cellular infiltrates were predominated by CD4+ T cells and ED1+ macrophages. Deposition of tenascin and laminin was enhanced. Grafts of euthymic recipients displayed only mild signs of CAN according to the Banff criteria. These data implicate an important role for the cellular immune response in the development of CAN.

Introduction

Chronic allograft nephropathy (CAN) is the major obstacle to the long-term survival of renal allografts. It has been reviewed extensively [1–3] and is described as a progressive decline in renal graft function because of pathological tissue remodeling typically associated with hypertension and proteinuria. Histological findings comprise glomerulopathy, tubular atrophy, interstitial fibrosis and arterial fibrous intimal thickening [4–7].

Both alloantigen dependent and independent factors are thought to contribute to this process [8], but the mechanisms are not yet fully understood. Although the various immunosuppressive agents that are available today have different targets and effects and thereby are efficient in reducing the number of acute rejections they seem to be unable to prevent this chronic progredient loss of graft function. So far the therapy for CAN remains insufficient and only consists in diminishing the risk factors. Finally returning to dialysis and waiting for re-transplantation cannot be avoided.

Alloantigen dependent mechanisms such as HLA-mismatching, acute rejection episodes and alloantigen independent mechanisms, namely ischemic/reperfusion injury, donor age, dyslipidemia of the recipient, hypertension, diabetes mellitus and cytomegalovirus infections are mostly intertwined in patients and it is difficult to separate the relative impact of immunologic or nonimmunologic factors on the development and progression of CAN.

In order to focus on the influence of presensitized T cells on the development of CAN we have established a new experimental model in congenic nude athymic Lewis rats (LEW.RNU) rats. Athymic nude rats lack a normal thymus and functionally mature T cells [9] and therefore accept major histocompatibility complex (MHC) mismatched organ transplants for several months. With increasing age

they develop lymphocytes expressing CD3 and T cell receptor, however those cells are mostly CD4- and CD8- and do not show alloreactivity *in vivo* [10]. It has been previously demonstrated that a reconstitution of T cells obtained from euthymic rats of the same genetic background leads to acute rejection in nude rat recipients of fully mismatched or of MHC class I-disparate solid organ allografts [11,12]. In this process CD4+ T cells seem to play a crucial role.

The aim of our study was to investigate the effect of reconstitution of primed T lymphocytes on the development of CAN in LEW.RNU recipients of MCH Ib and non-MHC disparate renal grafts from F344 donors. The extent of functional and morphological changes was compared with euthymic LEW recipients of F344 grafts.

Animals, material and methods

Animals

Male congenitally athymic Lewis rats (LEW.RNU, *RT1.C*^{l}) and euthymic Lewis rats (LEW. *RT.C*^{l}) acted as recipients for Fisher (F344, *RT.C*^{lv1}) kidneys. To analyze donor specific antibodies, peripheral blood lymphocytes (PBL) of additional rat strains were used (kindly provided by K. Wonigeit, Hannover, Germany): LEW.1LV3 (*RT1.C*^{lv3}, *RT6*^{a}) and LEW.6B (*RT.C*^{l}, *RT6*^{b}). Both rat strains are bred on a LEW background and share all other characteristics with the kidney recipient. *RT.C*^{lv3} is cross reacting with *RT.C*^{lv1} in the F344 rat.

All animals were bred and maintained at the Zentralinstitut für Versuchstierzucht, Hannover, Germany. They were housed under standard conditions and fed water and rat chow *ad libitum*. All animal experiments were carried out according to the principles of laboratory animal care (NIH publication No. 85-23, revised 1996) as well as the German Law on the Protection of Animals.

Kidney transplantation and experimental groups

F344 kidneys were transplanted to LEW.RNU and LEW recipients. The left donor kidney together with a patch of the aorta, the renal vein and the ureter was removed after perfusion with heparinized Ringer's lactate, cooled in iced isotonic saline and positioned heterotopically in the host. The recipient's aorta and vena cava were separated between the origin of the renal vessels and the aortic bifurcation, followed by anastomosis of the donor aortic patch and abdominal aorta and donor renal vein and inferior vena cava respectively. Donor and recipient ureters were attached end-to-end without stenting. Simultaneously the left native kidney was excised whereas the right kidney was not removed until day 6 after transplantation.

All nude rat recipients received a prophylactic antibiotic treatment with enrofloxacine (Baytril®, Bayer, Leverkusen, Germany) for a period of 10 days postoperatively. One group ($n = 8$) underwent no further intervention after transplantation and will be referred to as LEW.RNU control group. The LEW.RNU T cell group ($n = 8$) received an intraperitoneal injection of 5×10^7 purified T lymphocytes (presensitized and isolated as described below) 7 days after transplantation and a subsequent low dose (1.5 mg/kg/day) cyclosporine (Sandimmun®, Novartis, Basel, Switzerland) immunosuppression for 10 days to prevent early acute rejection. Euthymic LEW recipients of F344 renal allografts (LEW control group, $n = 6$) also were given 1.5 mg/kg cyclosporine (Sandimmun®) for 10 days post-transplantation.

Ninety days after transplantation, or earlier if renal function deteriorated severely, the animals were sacrificed and grafts were harvested for further morphological and immunochemical analysis.

Preparation and adoptive transfer of T lymphocytes

Lymph node cells (LNC) were prepared from pooled cervical, axillary and mesenteric lymph nodes obtained from euthymic LEW (*RT1.C*^{l}) rats that had been immunized by the application of two full skin allografts from F344 (*RT1.C*^{lv1}) rats 3 weeks apart. After skin graft rejection the lymph nodes were excised and dissociated in medium 199 (AppiChem, Darmstadt, Germany). To enrich T cells the cell suspension was passed over nylon wool columns according to the method of Julius *et al.* [13]. Cell recovery and cell viability counts were performed using trypan blue staining. The purity of LNC was checked by FACS analysis and was consistently >95% T cells (70% CD4+ T Cells, 25% CD8+ T cells) and contaminated with <2% B cells. A 5×10^7 of the obtained T lymphocytes were diluted in medium 199 and administered as ascites, by intraperitoneal injection.

Renal function

Serum creatinine was determined colorimetrically every other week with a Beckman Creatinine Analyzer (Beckmann Instruments, Inc., Galway, Ireland). Every 4 weeks the rats were placed into metabolic cages for determination of 24 h urine albumin excretion. Urine albumin concentrations were quantified by an enzyme-linked immunosorbent assay specific for rat urinary albumin (Nephrat II, Exocell, Inc., Philadelphia, PA, USA).

Antibodies

The following antibodies, (Serotec, Bicester, UK) and maintained in our laboratory, except where noted, were used in this work: G 4.18 (anti-CD3; BD, Becton

Dickson, San Jose, CA, USA), R73 (anti-rat TCR αβ constant determinant) a kind gift of T. Hünig, (Institut für Virologie und Immunbiologie, Universität Würzburg, Germany) [14], W3/25 (anti-CD4) [15], MRC OX8 (anti-CD8α) [15], 3.4.1. (CD8β), 3.2.3 (NK cells) [16], MRC OX12 (anti-rat κ chain) [17], Ki-B1R (rat pan B cell marker) (Dianova, Hamburg, Germany), ED1 (rat tissue macrophages, monocytes and dendritic cells) [18], polyclonal rabbit anti-rat fibronectin (Calbiochem, Bad Soden, Germany). An anti-rat tenascin rabbit antiserum was a kind gift of R. Chiquet-Ehrismann, (Friedrich Miescher Institut, Basel, Switzerland). D. Schuppan (Innere Medizin, Universität Erlangen-Nürnberg, Germany) kindly provided an anti-rat laminin rabbit antiserum (E 1b).

Flow cytometry analysis

Cell counts of lymphocyte subpopulations were assessed every other week. Peripheral blood lymphocytes (PBL) were obtained by treatment of ethylenediaminetetraacetic acid (EDTA) blood samples with commercially available erythrocyte lysis buffer (Ortho Diagnostics, Neckargemünd, Germany). Samples of 0.5×10^6 to 1.0×10^6 cells were washed with staining buffer [phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA)/0.1% NaN_3] and incubated with 50 µl primary mAb for 30 min at 4 °C. The cells were washed twice again and stained with 50 µl of the FITC-/PE-/SAPE conjugated goat-anti-mouse secondary antibody (Dianova). For two color immunofluorescence cells were incubated with mAb OX8, W3/25 or R73 followed by incubation with the FITC-conjugated goat-anti-mouse secondary antibody. A blocking step with normal mouse serum was ensued by incubation with biotinylated G4.18. Its binding was finally revealed with a PE-streptavidin conjugate (BD). Fluorescence analysis was performed on a Becton Dickinson FACScan and analyzed using a Consort 30 computer program. The Student's *t*-test was used to determine statistical significance in changes of cell counts.

Measurement of alloantibodies

Recipient sera of two representative animals of the T cell group and four animals of the LEW.RNU control group were tested for donor specific antibodies by flow cytometry. Cell suspensions of PBL from LEW (negative control), F344 (donor), LEW.6B (donor RT6) and LEW.1LV3 (donor MHC I) rats were each incubated with 100 µl animal serum. Serial samples were used including a pretransplant serum as an additional negative control. After washing a DTAF-conjugated goat-anti-rat IgG (H + L) secondary antibody (Dianova) was applied and fluorescence analysis was carried out.

Immunohistochemistry

Renal tissue was snap frozen in liquid nitrogen, cut into 5 µm thin sections, air-dried overnight and fixed in acetone for 10 min immediately before use. Single and double staining techniques were performed as described previously [19]. All steps were performed at room temperature. The sections were blocked using 10% heat-inactivated normal rat serum for 20 min. Subsequently the primary antibody was applied for 60 min in appropriate dilution. For negative control the primary antibody was replaced by PBS or an irrelevant antibody. After washing the sections were incubated for 60 min with the secondary antibody (Dianova): peroxidase-coupled goat anti-mouse immunoglobulins or alkaline phosphatase (AP) coupled goat anti-rabbit immunoglobulins respectively (diluted 1:50 in PBS containing 5% normal rat serum). The peroxidase enzyme label was visualized with 3-amino-9-ethyl-carbazole (AEC) in acetate buffer (pH 5.2) containing hydrogen peroxide, the alkaline phosphatase label was detected with naphtol AS-MX-phosphate and fast red dye (Sigma-Aldrich, Munich, Germany) in the presence of levamisole (Sigma-Aldrich). The sections were counterstained with Mayer's hemalaune and mounted in glycerol-gelatin.

Semiquantitative evaluation

According to a commonly used semiquantitative evaluation method [20], immunoreactivity was assessed by a scale rated from negative (−) to strongly positive (+++) by two independent investigators, corresponding to the intensity of staining or relative to the number of positive stained cells.

Histopathology

For morphological studies light microscopy was performed. Kidney grafts were fixed in buffered formalin. Paraffin-sections following staining with hematoxylin-eosin, periodic acid-Schiff and Masson's trichrome were evaluated according to the criteria of the Banff 97 classification. Additionally tissue from the renal cortex of all specimens was fixed in glutaraldehyde and further analyzed by electron microscopy following standard protocols.

Results

Median survival time (MST), kidney function and course of T lymphocyte populations in peripheral blood of graft recipients

Congenitally athymic LEW.RNU (*RT1.C*^{l}) recipients of allogeneic F344 (*RT1.C*^{lv1}) kidneys ($n = 8$) were unable

to reject their grafts and achieved prolonged survival with a MST >90 days. The graft function was normal (Fig. 1a and c). Flow cytometry analysis of lymphocyte populations in peripheral blood revealed no significant changes in T cell counts over the whole course of the experiment (Fig. 2a and b).

The LEW.RNU rats injected 5×10^7 highly purified presensitized T lymphocytes showed a deteriorating kidney function with increased serum creatinine and urine albumin levels (Fig. 1b and d). Five animals suffered from renal failure and were sacrificed before day 90 post-transplantation, thereby the group had a lower MST of 60 days. After T lymphocyte reconstitution the number of CD3+ T cells went up significantly from counts of 310 ± 212 to 1645 ± 399 cells/μl after 4 weeks ($P = 0.016$) and remained on that higher level throughout the experiment (1586 ± 243 cells/μl after 12 weeks). Especially the CD4+ subpopulation showed a remarkable and significant ($P < 0.001$) increase from 152 ± 118 to 1387 ± 350 cells/μl after 4 weeks (Fig. 2a), whereas the CD8+ subpopulation remained on low levels and did not show a significant difference from the number of CD8+ T lymphocytes of the control group (Fig. 2b). NK cells are known to be over represented in athymic rats. However, NK cell counts did not differ significantly between the LEW.RNU control group and the LEW.RNU group after adoptive T cell transfer (640 ± 82 vs. 1207 ± 527 cells/μl 4 weeks, and 817 ± 162 vs. 1339 ± 543 cells/μl 10 weeks after transplantation).

Euthymic LEW rat ($RT1.C^l$) recipients ($n = 6$) achieved a MST >90 days and displayed a stable kidney function during the investigation period. Lymphocyte cell counts in peripheral blood varied only mildly throughout the experiment. The number of CD3+ T cells was 4621 ± 523/μl before and 4209 ± 480/μl 12 weeks after transplantation. CD4+ T lymphocytes remained equally stable with 3190 ± 457 cells/μl before and 3228 ± 528 cells/μl 12 weeks after transplantation. Only the CD8+ T lymphocyte population decreased somewhat from 1608 ± 241 to 1230 ± 196 cell/μl 12 weeks after transplantation. The B cell population was similar in all three groups and did not differ significantly throughout the experiment.

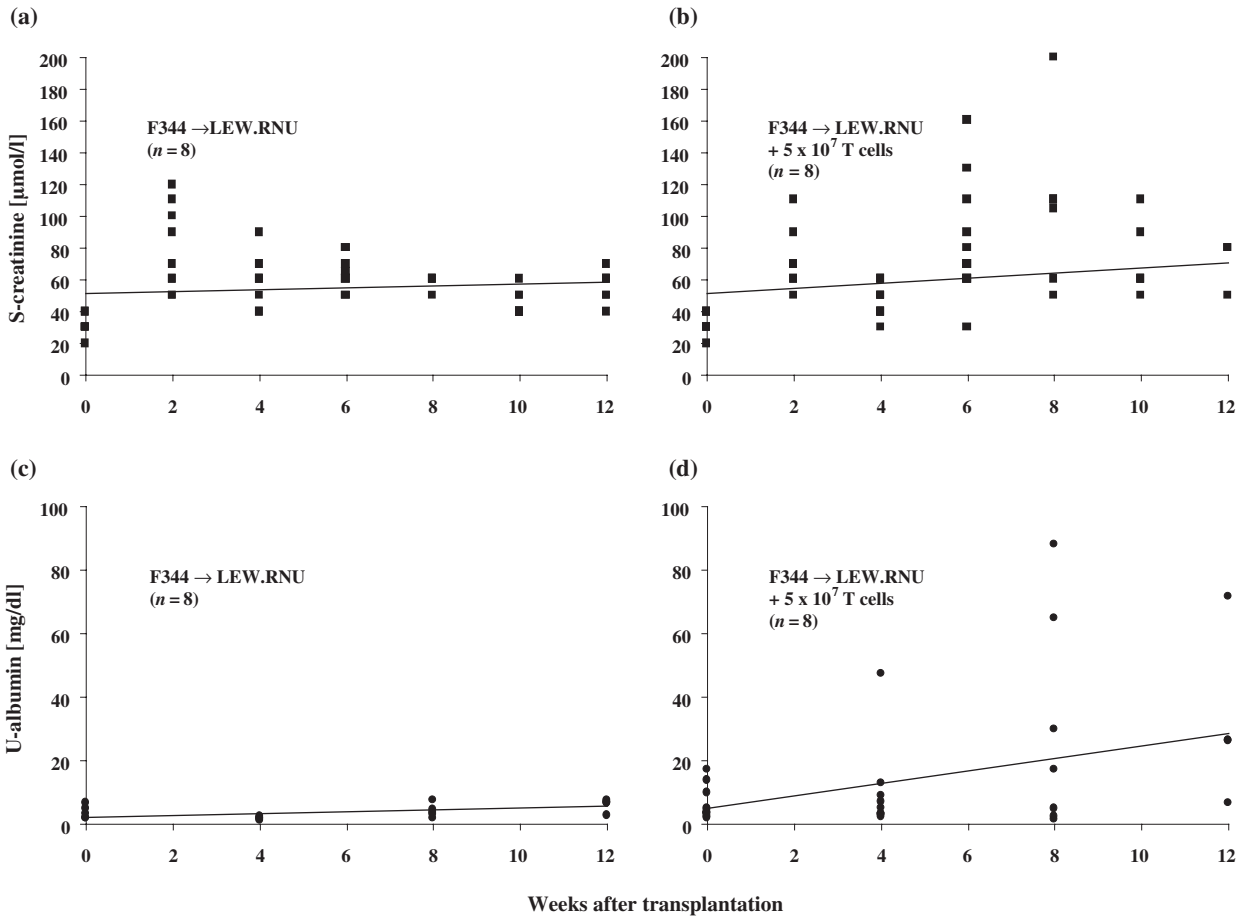

Figure 1 Serum creatinine (a, b) and urine albumin (c, d) after renal transplantation.

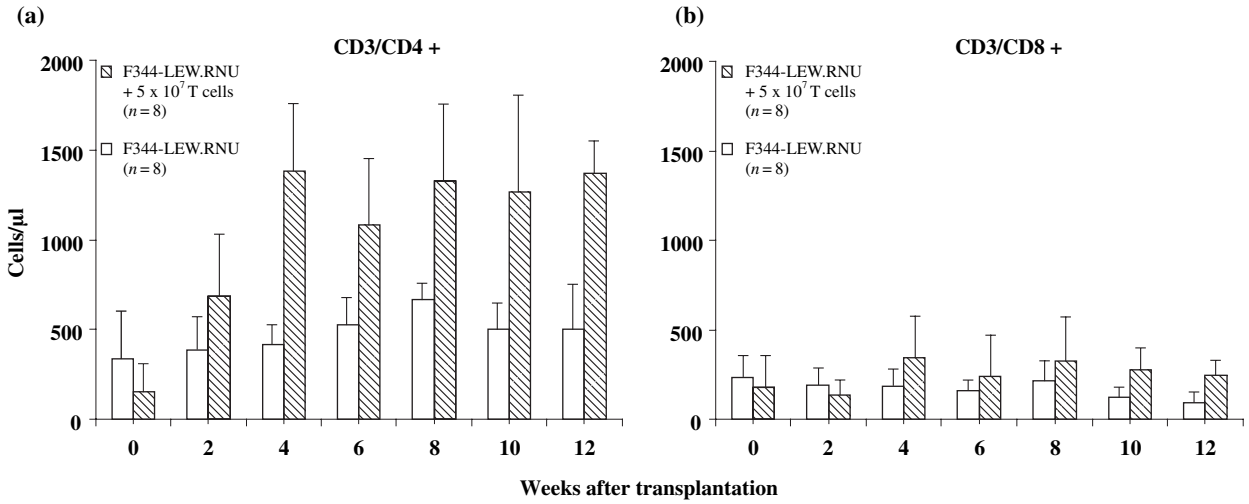

Figure 2 T lymphocyte subpopulation counts in peripheral blood of LEW.RNU renal transplant recipients. (a) A significant increase of CD4+ T lymphocytes can be noted after 4 weeks ($P < 0.001$) in T cell reconstituted recipients, whereas the CD8 population is only mildly elevated (b).

Antibody formation

Pooled lymph node cells of particular rat strains were used as antigen carriers to differentiate between donor specific antibodies in general, anti MHC class I and anti RT6 alloantibodies. The two animals investigated after adoptive T cell transfer developed a donor specific reactivity against F344 and LEW.6B cells, in one recipient antibodies against the donor MHC were detected (Fig. 3). Reactivity against F344 and LEW.6B cells was also found

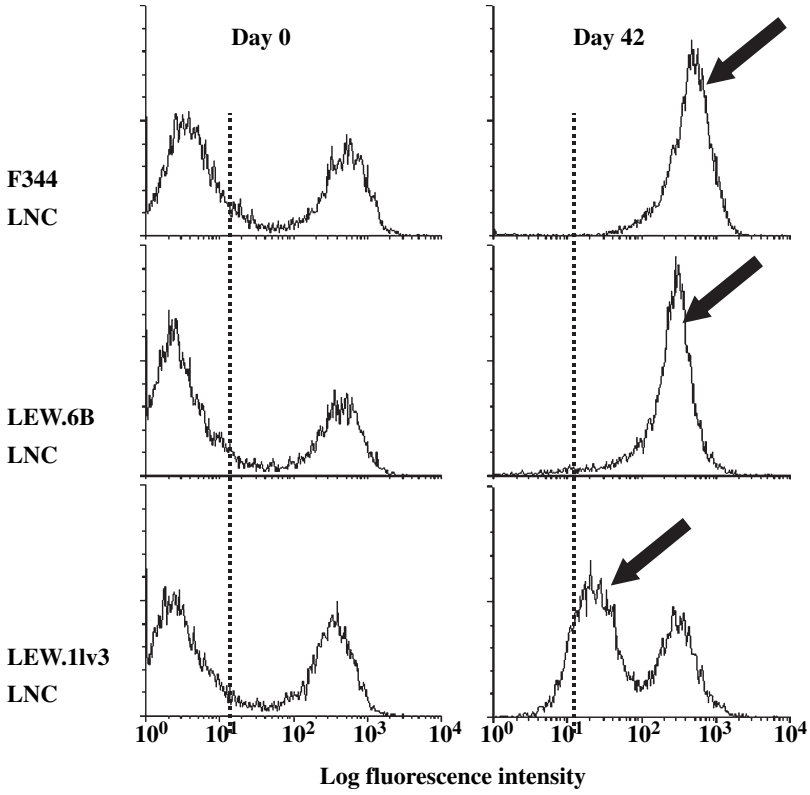

Figure 3 Detection of anti MHC class I and anti RT6 alloantibodies (arrows) in a LEW.RNU renal allograft recipient after adoptive T cell transfer. Antibody presenting cells (e.g. B cells) were excluded from analysis.

in four animals of the LEW.RNU control group, but no anti MHC antibodies.

Histopathological observations in nonrejecting and rejecting renal allografts

All kidneys were investigated by light and electron microscopy and evaluated according to the criteria of the Banff working classification of kidney transplant pathology [2,3]. F344 kidney grafts of the LEW.RNU control group displayed a normal morphology with no signs of acute or chronic rejection (Table 1).

The LEW.RNU T cell group showed a moderate CAN and in most cases inflammatory mononuclear cell infiltrates (Table 1). Predominant histological findings were interstitial fibrosis with tubular atrophy as shown in Fig. 4c, as well as glomerulopathy with mesangial matrix increase, segmental duplication of basement membrane and glomerulosclerosis (Fig. 5c). Electron microscopy revealed thickening of the basement membrane with extension of the subendothelial space and segmental duplication of the basement membrane. Endothelial cells are focally swollen with loss of fenestration and a segmental effacement of the podocyte foot processes can be appreciated (Fig. 5d). Vascular changes were present in some animals, but completely absent in four animals suggesting that in this model of chronic rejection the interstitial and glomerular damage dominates. Ninety days after transplantation F344 kidney grafts from euthymic LEW recipients displayed a mild interstitial fibrosis (Table 1).

Immunohistochemical characterization of infiltrating cell populations and extracellular matrix proteins

Table 2 summarizes the median expression patterns of cells and connective tissue components in F344 renal allografts of LEW.RNU and LEW recipients. In kidney grafts of the LEW.RNU control group only solitary T lymphocytes and macrophages and even less B lymphocytes and NK cells were detected. Immunohistochemical differentiation of the mononuclear cell infiltrates in grafts of the LEW.RNU T cell group showed a very strong accumulation of ED1+ macrophages and T lymphocytes particularly in the tubulointerstitium and in perivascular areas (Fig. 4a and b). Differentiation of CD4+ and CD8+ T cells by double immunolabeling revealed a ratio of 5:1, resembling the CD4/CD8 ratio of peripheral blood T lymphocytes (data not shown). Distribution of B lymphocytes was mildly increased within the glomeruli and moderately enhanced in the tubulointerstitium. NK cell infiltration was mildly increased.

In euthymic LEW recipients tubulointerstitial cellular infiltrates were increased, particular T cells and ED1+ macrophages, but not as strongly as in T cell reconstituted nude rats. The extracellular matrix components tenascin, fibronectin, laminin and collagen IV were all detectable in the tubulointerstitium of nonrejected kidney allografts of LEW.RNU and LEW recipients forming a thin continuous layer along tubular walls. Staining intensity differed, with fibronectin and collagen IV showing the strongest and laminin and tenascin a somewhat lesser

Table 1. Histopathological characteristics of the allografts according to the Banff criteria.

	Acute rejection	cg	ci	ct	cv	Chronic allograft nephropathy
F344 - LEW.RNU						
LEW.RNU ($n = 4$)	No rejection	0	0	0	0	No CAN
F344 - LEW.RNU + 5×10^7 presensitized T lymphocytes ($n = 8$)						
LEW.RNU 1	Borderline	1	2	2	1	II b
LEW.RNU 2	II b	2	0	1	3	II b
LEW.RNU 3	II b	1	1	0	2	II b
LEW.RNU 4	Borderline	2	2	2	0	II b
LEW.RNU 5	II a	1	1	1	3	I b
LEW.RNU 6	No rejection	0	2	2	0	II a
LEW.RNU 7	II a	1	2	2	0	II b
LEW.RNU 8	II a	1	1	1	0	I b
F344 - LEW ($n = 6$)						
LEW 1	No rejection	0	0	0	0	No CAN
LEW 2	No rejection	0	1	0	0	No CAN
LEW 3	No rejection	0	1	0	0	No CAN
LEW 4	No rejection	0	1	0	0	No CAN
LEW 5	No rejection	0	0	0	0	No CAN
LEW 6	No rejection	0	0	0	0	No CAN

F344, Fischer rats (*RT.C*^{lv1}); LEW, Lewis rats (*RT.C*^{l}); LEW.RNU, nude athymic Lewis rats (*RT.C*^{l}).
0, 1, 2, 3 = no, mild, moderate, severe. cg, chronic transplant glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy and loss; cv, fibrous intimal thickening.

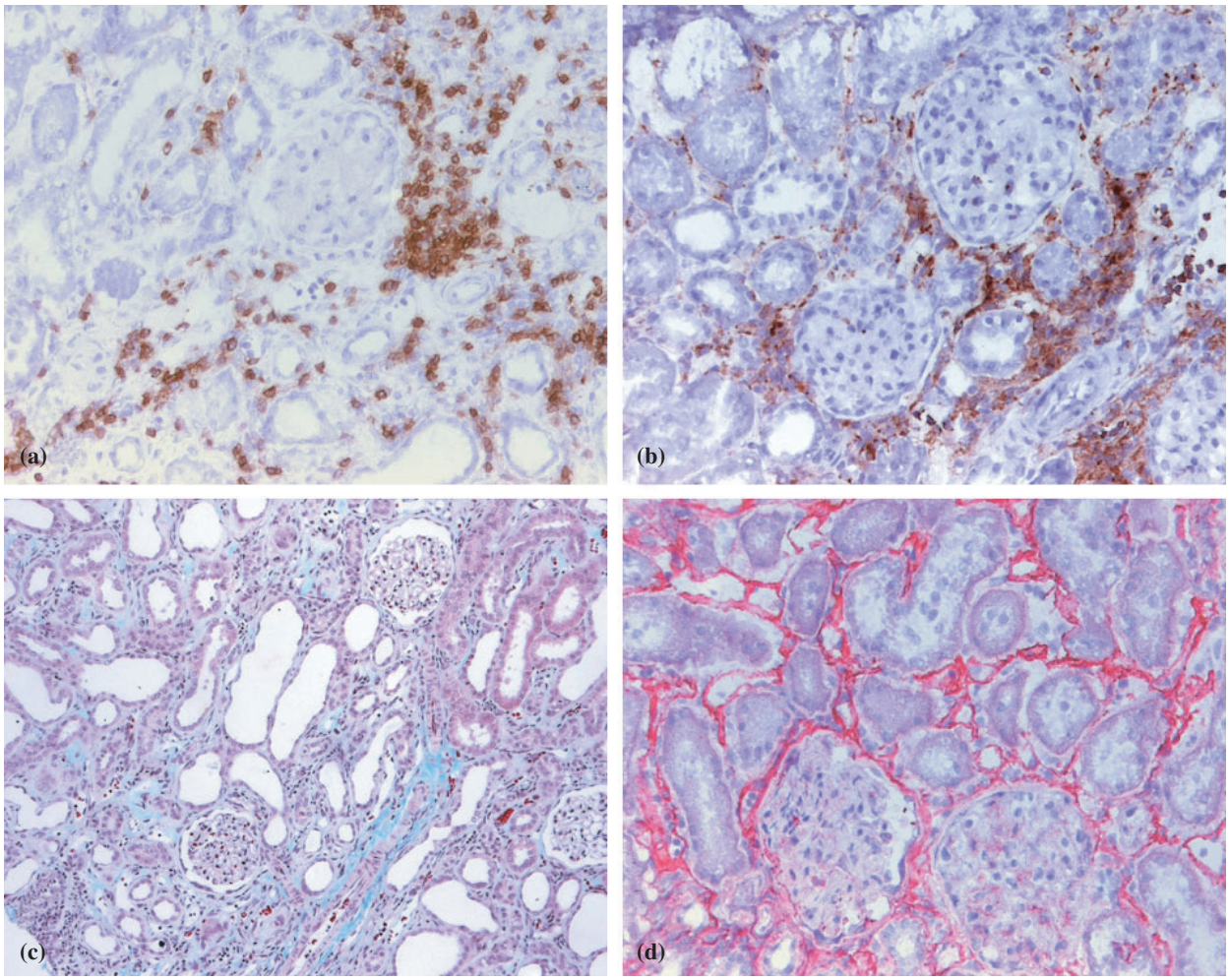

Figure 4 Single immunostaining for (a) αβ T cell receptor (original magnification ×200) and (b) ED1 macrophages (original magnification ×200) in grafts of T cell reconstituted LEW.RNU rats. (c) Light micrograph (Masson's trichrome, original magnification ×100): tubular atrophy and loss with enhanced accretion of connective tissue in the tubulointerstitium of LEW.RNU recipients after adoptive T cell transfer. (d) Single immunostaining for ECM component tenascin (original magnification ×200): enhanced tubulointerstitial and mesangial deposition of tenascin in kidneys with chronic rejection.

grade of immunoreactivity. Laminin and collagen IV were also detectable in the glomerular mesangial matrix. In kidneys with CAN of T cell reconstituted LEW.RNU rats the deposition of laminin, tenascin (Fig. 4d) and collagen IV was clearly enhanced within the widened intertubular space indicating interstitial fibrosis. Immunoreactivity for fibronectin however was not markedly stronger than in nonrejected grafts.

Discussion

Chronic allograft nephropathy is the major limiting factor for the long-term survival of renal transplants. The incidence of late graft failure in transplanted kidneys is about 5% each year post-transplantation. Interestingly, the effect of HLA matching on kidney graft survival remains essentially unchanged after 30 years even with remarkable improvements in immunosuppression [21]. Mild CAN is already to be observed months after transplantation and progresses thereafter. Predictors of the grade of CAN 1–12 months after transplantation are acute tubular necrosis on the one hand and previous episodes of clinical and also subclinical acute rejection on the other hand [22]. Patients presensitized by previous transplantations, pregnancies or blood transfusions have a higher risk to loose their grafts [23]. The development and progression of CAN clearly is a multifactorial process, but we can demonstrate here that immunological factors play a pivotal role.

The F344 to LEW kidney transplant model has been used by many investigators to study chronic rejection.

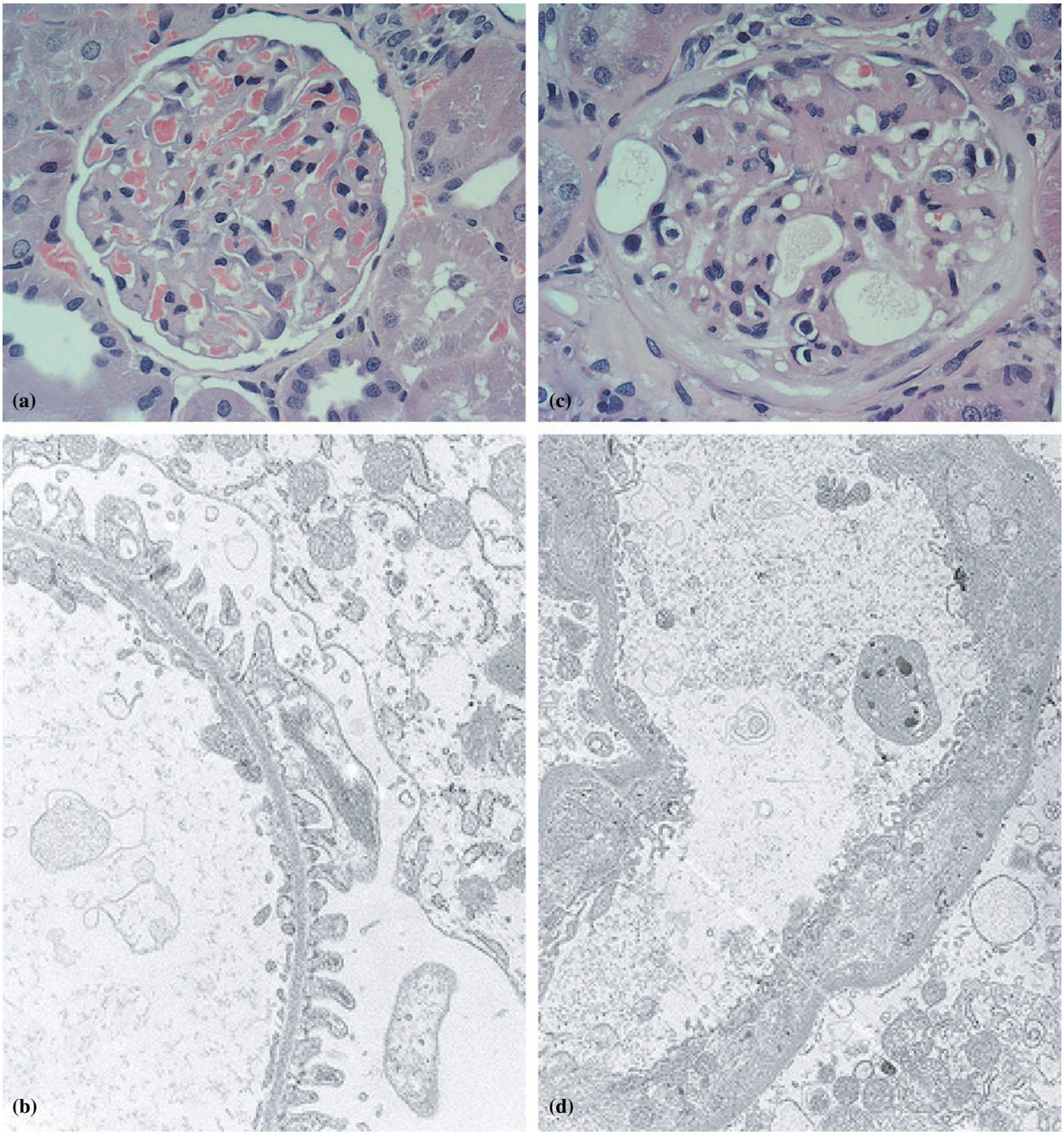

Figure 5 (a) Light micrograph (HE, original magnification ×400) and (b) electron micrograph (original magnification ×1200) with a normal glomerular structure in F344 grafts of LEW.RNU control animals. Characteristic appearance of transplant glomerulopathy on day 90 after transplantation in T cell reconstituted LEW.RNU recipients. (c) Light micrograph showing increased capillary wall thickness and enhanced mesangial matrix interposition (HE, original magnification ×400). (d) Electron micrograph displaying subendothelial accumulation of electron lucent flocculent material and reduplication of the basement membrane as well as podocyte loss (original magnification ×1200).

Both strains are phenotypically identical for the major MHC loci RT1-A and RT1-B but differ at the RT1-C locus and multiple minor loci. In the present study a new model of CAN was established in athymic LEW. RNU ($RT.C^{l}$) recipients of F344 ($RT.C^{lv1}$) renal allografts by adoptive transfer of 5×10^{7} primed T lymphocytes obtained from euthymic LEW ($RT.C^{l}$) rats.

In T cell reconstituted LEW.RNU allograft recipients the MST was reduced because of renal failure. The grafts displayed morphological changes typical of CAN and also

Table 2. Median expression patterns of cellular components and extracellular matrix proteins in F344 kidney allografts.

	Glomeruli			Tubulointerstitium		
	LEW.RNU ($n = 8$)	LEW.RNU + T cells ($n = 8$)	LEW ($n = 6$)	LEW.RNU ($n = 8$)	LEW.RNU + T cells ($n = 8$)	LEW ($n = 6$)
Cellular components						
αβ T cell receptor	–	–	–	(+)	++	+
CD4	–	(+)	–	(+)	+++	+
CD8	–	–	–	(+)	+	(+)
B cells	–	(+)	–	–	+	(+)
NK cells	–	(+)	–	–	(+)	–
ED1+ macrophages	(+)	(+)	(+)	(+)	+++	+
Extracellular matrix proteins						
Tenascin	–	–	–	(+)	++	+
Fibronectin	(+)	(+)	(+)	+	+	+
Laminin	(+)	+	(+)	(+)	++	(+)
Collagen IV	+	+	+	+	++	+

LEW.RNU, nude athymic Lewis rat recipients (*RT.C*^{l}); LEW.RNU + T cells, nude athymic Lewis rat recipients (*RT.C*^{l}) after adoptive T cell transfer; LEW, euthymic Lewis rat recipients (*RT.C*^{l}); Staining intensities: –/(+)/+/++/+++.

some histological signs of acute rejection, and these findings occurred earlier and were stronger than in euthymic LEW recipients. Euthymic LEW rats which had received cyclosporin A (CsA) in a low dose for 10 days displayed only mild interstitial inflammation and fibrosis 90 days after transplantation with no impairment of kidney function. Joosten *et al.* [24] observed similar findings in LEW recipients of F344 renal allografts and a 4 week low dose CsA immunosuppression, LEW recipients without immunosuppression however display characteristic lesions of CAN as well as functional alterations 100 days post-transplantation.

F344 grafts of LEW.RNU recipients after adoptive T cell transfer displayed characteristic morphological features consistent with CAN in human allografts according to the Banff criteria. Particularly glomerulopathy and interstitial fibrosis with tubular atrophy predominated. Vascular changes often emphasized as the hallmark of chronic rejection however were not present in four animals indicating that in kidney grafts glomeruli and tubuli are the structures most vulnerable to immunological injury and that interstitial fibrosis in renal allografts can be caused by isolated chronic parenchymal cellular rejection and not as a consequence of reduced blood flow and ischemia because of chronic vascular rejection as it is the case in liver and cardiac allografts [25].

All LEW.RNU rats investigated developed donor specific antibodies. We observed an anti RT6 antibody formation not only in post-transplant sera of T cell reconstituted animals but also in athymic controls, which was presumably caused by donor derived passenger lymphocytes. The RT6 antigen, a member of the mono-ADP-ribosyltransferase/NAD glycohydrolase family, is only expressed on mature T lymphocytes, a subset of NK cells and may also exist in a soluble form [26]. This antibody formation is possible without efficient T helper cells, but does not affect kidney allograft survival as the RT6 gene product is not expressed in kidneys.

A specific anti donor MHC antibody response was observed in one T cell reconstituted recipient. Unfortunately, MHC class I antibody formation cannot be correlated with glomerulopathy or vasculopathy because of limited numbers in this study. But as published recently [27] both MCH and glomerular basement membrane antibodies are supposed to play a role in the development and progression of CAN.

Immunohistochemical studies revealed enhanced deposition of extracellular matrix proteins such as tenascin, collagen IV, fibronectin and laminin particularly in the tubulointerstitium of F344 grafts with CAN. Chiefly tenascin expression was strongly enhanced. Laminin and fibronectin showed a similar staining intensity that was only mildly increased. These molecules are involved in tissue remodeling and are found in a variety of conditions such as acute and chronic rejection, lupus or IgA nephritis and diabetic nephropathy. Tenascin is a large oligomeric glykoprotein and plays an important role in nephrogenesis. In normal adult kidneys tenascin expression is limited to the medullary interstitium. However it reappears in all kinds of pathological conditions of the kidney and may as well provide immunomodulatory effects at the level of cell adhesion on several immune cell types [28] and is therefore suspected to be an important participant in inflammatory and fibrogenic processes. It has been previously reported that kidneys with either acute or chronic rejection diffusely express tenascin in areas of either interstitial edema, mature interstitial fibrosis or organized interstitial fibrosis [29]. We found that

laminin expression was also slightly increased within the glomeruli of F344 kidneys with CAN. This is consistent with findings of studies in human chronically rejected renal allografts which revealed a significantly increased intraglomerular amount of laminin and a slight but not significant enhancement of fibronectin [30].

Strong cellular infiltrates, particularly CD4+ T cells and ED1+ macrophages were observed in rejecting kidneys. There is evidence that T lymphocytes, especially those activated by the indirect pathway of alloantigen recognition play a crucial role in the development of CAN [31,32]. In experimental models progression of CAN can be delayed in rat kidney allografts by continuous suppression of T cell activation and proliferation [33] or even interrupted by co-stimulation blockade [34]. Calcineurin inhibitors such as tacrolimus or cyclosporine A are not sufficient to prevent the progression of CAN and unfortunately have fibrogenic effects themselves. Possibly antiproliferative drugs, such as mycophenolate mofetil or the TOR inhibitors sirolimus and everolimus might be able to stop or even reverse the ongoing effects of activated T cells in this model. We hypothesize that in particular activated CD4+ T lymphocytes induced and promoted tissue injury by attracting and stimulating macrophages, B cells and NK cells which finally resulted in irreversible structural alterations and tissue remodeling. Whether indirect or direct antigen presentation play the most important role in this model remains to be elucidated by further experiments. This model of chronic rejection gives proof of the pivotal role presensitized T cells play in initiating and promoting CAN. It allows interesting investigations of immunological pathomechanisms in chronic rejection and also offers a variety of possibilities to test different regimen of immunosuppression as well as new immunosuppressive agents.

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