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Uwe W. Heemann
Stefan G. Tullius
Haruhito Azuma
Nicholas L. Tilney

The relationship between reduced functioning kidney mass and chronic rejection in rats

U. W. Heemann · S. G. Tullius · H. Azuma
Surgical Research Laboratory,
Harvard Medical School,
260 Longwood Ave.,
Boston, MA 02115, USA

N. L. Tilney
Department of Surgery,
Brigham and Women's Hospital,
75 Francis St., Boston, MA 02115, USA

Abstract Interest has recently increased in the role of alloantigen-independent factors in chronic rejection. In this context, we examined the long-term effects of reduced functioning kidney mass in a F344 → LEW allograft (A) model. Animals were divided into three groups depending upon the amount of retained kidney. Renal arterial branches in the hilus were ligated so that one-third or two-thirds of the graft remained viable (1/3 and 2/3 groups, respectively); organs were left intact in the third (3/3) group. Urine protein concentrations were determined 4, 6, 8 and 10 weeks after engraftment and organs (five/group/time) were harvested and examined morphologically and immunohistologically. Proteinuria increased progressively in all 1/3, 2/3 and 3/3A animals, but faster in those with reduced kidney mass. This functional decline correlated well with increasing numbers of macrophages followed by interstitial fibrosis and glomerular sclerosis, which had become prominent by week 6 in group 1/3A and by 8 weeks in groups 2/3A and 1/3I (I, isografted), with animals beginning to die. IL-1, IL-6 and TNF production correlated well with the location and number of macrophages in all groups. These results suggest that kidney mass exerts a significant alloantigen-independent influence on chronic rejection. Allogenicity of the graft accelerates and amplifies the process.

Key words Chronic rejection
Kidney allograft · Rats
Kidney mass

Introduction

Chronic rejection has long been thought to be a unique entity with features dependent upon allogenic differences similar to those important for more acute forms of rejection. However, alloantigen-independent factors have been implicated increasingly in late graft failure. These include injuries of warm and cold ischaemia, surgical trauma, reperfusion and kidney size [2, 3]. The goal of the present study was to examine the importance of functioning kidney mass as a risk factor for later graft deterioration.

Materials and methods

Animals

Naive male 200–250 g inbred rats (Harlan Sprague-Dawley, Indianapolis, Ind.) were used throughout the experiments. Lewis rats (LEW, $RT1^{l}$) acted as graft recipients, and Fisher (F344, $RT1^{lv1}$)

animals as donors. F344 → F344 served as isograft and naive controls.

Experimental groups

Bilaterally nephrectomized recipients bearing orthotopically placed allografts (A) and isografts (I) were studied in parallel and separated into three groups depending upon the amount of functioning renal tissue ($n = 5$/group per time point). Kidneys with one-third and two-thirds functioning mass (1/3 and 2/3 groups, respectively) were prepared by selectively ligating appropriate arterial branches; the kidney was left intact in the third (3/3) group. All recipients were sustained by their transplanted organ; those dying immediately following removal of the contralateral kidney at 10 days were not included in the series. All animals were treated with low-dose cyclosporine (1.5 mg/kg per day) for the first 10 days after engraftment to suppress an initial acute rejection episode occurring in the allografted recipients.

Tissue preparation

Reduced-mass kidneys (1/3 and 2/3) from all groups were harvested at 4, 6, 8 and 10 weeks after transplantation, and intact organs (3/3) at 2, 4, 8, 12, 16 and 24 weeks. All tissues were snap-frozen in liquid nitrogen and stored at −70 °C, or fixed in 10% formalin for haematoxylin/eosin and periodic acid-Schiff (PAS) staining.

Functional measurements

Every 2 weeks 24-h urine samples were collected. Protein excretion was determined by measuring precipitation following interaction with 3% sulphosalicylic acid.

Antibodies

Monoclonal antibodies (mAbs) against ICAM-1 (1A29) were provided by Prof. M. Miyasaka (Tokyo, Japan) [7]; those against T cells (CD5-OX-19) and monocytes/macrophages (ED-1) were obtained from BPS, Indianapolis, Ind., and those against TNFα, PDGF and IL-6R from Genzyme, Boston, Mass.

Immunohistology

Cryostat sections of frozen tissue were stained individually with mAbs from the above panel using alkaline phosphatase–anti-alkaline phosphatase (APAAP) or immune peroxidase (PAP). Stained cells were then counted using an ocular grid and expressed as cells per field of view (c/FV) ($\times 600$, > 30 fields counted per specimen, 2 or 3 specimens per kidney). The intensity of tissue staining was evaluated on a sclae of 1–4.

Statistics

Results were evaluated using Student's t-test.

Results

Function

Deteriorating renal function, as assessed by proteinuria, became manifest in all animals with a 1/3 kidney within 4–6 weeks (about 40 mg/24 h vs < 5 mg in 3/3I, $P < 0.001$), deteriorating progressively before death. Proteinuria also developed in all animals sustained by a 2/3 kidney, albeit later (8–10 weeks), and developed by 16–20 weeks in allografted recipients with intact (3/3) grafts, increasing thereafter, but did not develop in 3/3 isografted during the period of observation.

Morphological changes

Morphological events associated with chronic rejection occurred in predictable sequence in the 3/3A model evolving eventually into sclerosis. Large numbers of cells infiltrated the kidneys transiently during a reversible episode of actue rejection at 1–2 weeks. By 16 weeks, sclerosis of the interstitium, vessels and $> 25\%$ of the glomeruli was becoming intense in most animals, commensurate with the onset of proteinuria and the death of a few recipients. Similar patterns developed earlier in mass-reduced kidneys (4–6 weeks in 1/3A and 1/3I, 6–8 weeks in 2/3A). Negligible changes occurred in 2/3I and 3/3I kidneys during the observation period.

Immunology

Macrophage infiltration peaked in similar fashion in 1/3A and 1/3I animals during weeks 4–6 (88/FV) decreasing thereafter (40/FV at week 10). In contrast, numbers of lymphocytes remained relatively stable, higher in allografted than in isografted animals (A 88/FV vs I 33/FV, $P < 0.01$). Both macrophages and lymphocytes peaked in group 2/3A by 6–8 weeks (120/FV and 113/FV respectively). Macrophages peaked in group 3/3A at 16 weeks (120/FV); there was no increase in groups 2/3I and 3/3I.

The presence of IL-6R, TNFα and PDGF correlated strongly with the onset and location of macrophage infiltration in the individual groups, and was particularly striking in perivascular areas and in glomeruli. PDGF and TNFα were also strongly associated with vascular endothelium and related to the degree of glomerulosclerosis, invariably being found in those kidneys with $> 30\%$ and not in those with $< 20\%$ sclerosis. IL-6R was

present in areas around or in glomeruli, always correlating with the presence of macrophages, while MHC class II and ICAM-1 expression corresponded to the number of cells infiltrating the graft.

Discussion

Late failure of transplanted organs has been attributed to alloantigen-mediated activities, although alloantigen-independent factors have also been implicated [5, 6]. The progressive functional and morphological deterioration that occurs in chronically rejecting renal grafts mimics those changes developing in kidneys failing progressively from chronic glomerulonephritis, hypertensive nephropathy, and other conditions [1, 6].

These findings have been unified into a theory focusing on the relative lack of functioning kidney mass [4], which suggests that compensatory hyperfunction of the remaining nephrons leads to sclerosis, hyalinosis, and eventual dropout. These progressive events reinforce the stimulus of hyperfiltration, thereby establishing a self-sustaining vicious cycle. A single transplanted kidney with twice the workload of the two kidneys in a normal patient increases its glomerular filtration rate (GFR) and develops structural hypertrophy [3]. In addition, further nephron loss may potentially occur through the injurious effects of warm and cold ischaemia, reperfusion, episodes of acute rejection and cytomegalovirus infection [2].

In the present transplant model, the influence of functioning mass on the process of functional and morphological deterioration was striking. In those uninephrectomized hosts in which the kidney mass was reduced to one-third, non-alloantigen-dependent events resulted in changes that made the differentiation between allografts and isografts difficult. Conversely, with a kidney mass of two-thirds and with intact kidneys in uninephrectomized rats, alloantigen-dependent factors gained importance, with more striking manifestations developing in allografts.

In conclusion, a reduction in functioning kidney mass regardless of allogenicity correlates with the onset of changes characteristic of chronic rejection in kidney allografts. These results suggest that kidney mass exerts a significant alloantigen-independent influence which may contribute to ultimate dysfunction and sclerosis. Allogenicity of the graft accelerates and amplifies the process.

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References

1. Andres GA, Accini L, HSU KG, Penn I, Porter KA, Rendall JM, Seegal BC, Starzl TE (1970) Human renal transplants III. Immunopathological studies. Lab Invest 22:588–604
2. Basadonna GP, Matas AJ, Gillingham KJ, Payne WD, Dunn DL, Sutherland DER, Gores PF, Grüssner RWG, Najarian JS (1993) Early versus late acute renal allograft rejection: impact on chronic rejection. Transplantation 55:993–995
3. Brazy PC, Pirsch JD, Belzer FO (1992) Factors affecting renal allograft function in long-term recipients. Am J Kidney Dis 19:558–566
4. Brenner BM (1983) Hemodynamically mediated glomerular injury and the progressive nature of glomerular disease. Kidney Int 23:647–655
5. Brenner BM, Cohen RA, Milford EL (1992) In renal transplantation one size may not fit all. J Am Soc Nephrol 3:162
6. Feehally J, Harris KPG, Bennet SE, Walls J (1986) Is chronic rejection a non-immunological phenomenon. Lancet 2:486–488
7. Tamatani T, Kotani M, Miyasaka M (1991) Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. Eur J Immunol 21:627–633