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Host leukocytes and their products in chronic kidney allograft rejection in rats

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Abstract Bilaterally nephrectomized Lewis recipients of Fisher 344 (F344) kidney allografts, treated with CyA (1.5 mg/kg/day × 10), develop progressive changes of chronic rejection. Treated F344-to-F344 acted as isograft controls. Proteinuria was determined sequentially. Grafts were harvested 8, 12 and 16 weeks after transplantation ($n = 9$/group/time period). Infiltrating host cells and their products were assessed in chronically rejecting grafts by histology and immunostaining using mAbs for monocyte/macrophages, T-cells, ICAM-1, LFA-1 and cytokines. For in vitro binding studies, snap-frozen sections of transplanted kidneys were incubated with monocytes/macrophages and lymphocytes isolated from peripheral blood (PBL) of naive animals. For in vivo migration studies, naive cell populations were labeled with Bis-Benzamide and transferred i.v. to grafted animals at weeks 8, 12 and 16 ($n = 3$/group); grafts were harvested 24 h later and cell localization assessed under immunofluorescence. Increasing numbers of ED1 + monocytes/macrophages in allografted kidneys peaked at 16 weeks, localizing preferentially in glomeruli, where IL-1, IL-6 and TNF-α expression had also become intense and correlated with progressive glomerulosclerosis. Binding studies corroborated these results. In vitro, a few monocytes/macrophages bound to glomeruli and vessels at 8 weeks; by 12 weeks, binding to glomeruli was high (72% of cells). In vivo, large numbers of transferred labelled monocytes/macrophages were found in kidney allografts at 12 weeks (23%, isografts; < 7%, $P < 0.01$). In contrast T cells (primarily CD4+) were a consistent feature in allografts elevated as compared to isografts and correlating with in vitro and in vivo binding patterns; associated cytokines included IL-2, IFN- and TNF-α. Functional data followed these results: urine protein excretion by allograft recipients increased from baseline at 8 weeks (12 mg/day) to > 50 mg/day at 16 weeks at which point animals were beginning to die of renal failure; proteinuria in isografted rats did not increase during this time period. These results suggest that monocyte/macrophage and CD4+ T cells and their products are important in chronic kidney allograft rejection, contributing to the progressive sclerosis and fibrosis.

Key words Chronic rejection
Macrophages · Cytokines

Introduction

Although the mechanisms of chronic allograft rejection are unknown, host leukocyte populations and various cytokines and adhesion molecules have been thought to be critical in the evolution of the process [1, 2]. We used an established rat model of chronic rejection of kidney allografts, and compared morphological and immunohistological findings with in vitro and in vivo patterns of binding to define the presence of these cells and factors. Macrophages and their products may contribute especially to the characteristic changes of chronic rejection.

Methods

Lewis recipients of Fisher 344 (F344) kidney allografts were treated briefly with low dose cyclosporin A (1.5 mg/kg per day × 10 days) to reverse an initial acute rejection episode, then followed-up for 24 weeks. F344-to-F344 acted as isograft controls. Function was assessed by sequential measurement of urine protein excretion. Grafts were harvested 8, 12, 16, and 24 weeks after transplantation ($n = 9$/group per time). Morphology was assessed serially using hematoxylin and eosin (H&E) and PAS staining. The infiltrating populations and their products were identified by immunohistology. Snap-frozen sections of grafts were stained individually with mAbs using the APAAP or immunofluoresence methods. Cells were counted on an ocular grid and expressed as cells per field of view (c/FV) or cells per glomerulus; the intensity of tissue staining was graded 1–4+. MAbs against macrophages (ED-1), PMN (FITC-PMN), T lymphocytes (CD5–OX19), IL-1, IL-6, TNF-α, and ICAM-1 were used.

For in vitro binding studies, snap-frozen sections of transplanted kidneys were incubated with monocyte/macrophages (Mϕ) from the peripheral blood and lymphocytes from lymph nodes of naive animals (LNL), isolated using Percoll gradients. For in vivo migration studies, naive peripheral blood Mϕ, PMN, and lymphocytes (PBL) were labeled with bis-benzamide and transferred IV to grafted animals 8, 12, and 16 weeks after transplantation ($n = 3$/group per day). Grafts were harvested 24 h later and cell localization assessed under immunofluorescence.

Results

Allograft recipients developed significant proteinuria by week 16 (> 30 mg/24 h vs. c. 5 mg/24 h excreted by isografted recipients, $P < 0.01$), excreting progressively greater amounts thereafter (> 50 mg/day at 24 weeks). After this period, animals in the former group began to die of renal failure; the isograft recipients continued to survive normally.

Histologically, by 8 weeks after engraftment, chronically rejecting allografts had developed extensive cellular infiltrates associated with early interstitial damage, fibrosis, and arteriolar inflammation. Between 12 and 16 weeks, partial collapse and sclerosis of glomeruli (20–30%), marked interstitial fibrosis with tubular atrophy, and varying degrees of intimal proliferation and luminal obliteration of vessels were seen. By 24 weeks, generalized fibrosis and sclerosis of glomeruli became increasingly intense. No changes occurred in isografts during this period.

Immunohistologically, small numbers of ED1+ macrophages were present in allografted kidneys by 8 weeks, but increased dramatically between weeks 12 and 16 (> 60 c/FV at 12 weeks vs. < 5 c/FV in isografts kidneys at any time point, $P < 0.001$) and diminished thereafter. By 16 weeks, this population localized preferentially in glomeruli (72% of cells or 7.6 cells/glomerulus vs. 0.5 cells/glomerulus in isografts). Intense IL-1, IL-6, and TNF-α expression was associated with the presence of glomerular macrophages; both glomeruli and infiltrating cells strongly expressed ICAM-1 at this stage. By 24 weeks, the macrophage population had infiltrated more generally throughout the grafts, particularly in areas of interstitial fibrosis. The infiltrating patterns of PMN was similar to that of macrophages, although their numbers were lower. At 12 and 16 weeks they localized specifically (70% vs. < 45%) and in higher numbers (c. 4 vs. < 0.5 cells/glomerulus) in glomeruli of allografted kidneys as compared to isografts. Numbers of T lymphocytes remained relatively stable although elevated as compared to isografts (c. 20 c/FV vs. c. 10 c/FV). In contrast to macrophages and PMN, lymphocytes were almost never encountered in glomeruli and were distributed throughout the interstitium.

Binding studies corroborated these results. In vitro, a few Mϕ bound to allograft glomeruli and vessels at 8 weeks. Binding peaked by 12 weeks (17c/FV vs. < 6c/FV in isografted kidneys at all time points; $P < 0.001$), decreasing thereafter (at 16 weeks, 10 c/FV). These cells bound particularly to glomeruli (> 60% of cells bound vs. < 5% in isografts at 12 weeks). By 24 weeks, Mϕ distribution changed, with more frequent binding to areas of interstitial fibrosis. LNL binding to allografts was consistently elevated as compared to isografts (> 100 vs. < 26 c/FV). They homed specifically to tubules at week 8, increased slightly and bound preferentially to vessels by week 12, then declined after 16 weeks, localizing to both vessels and atrophic tubules but never to glomeruli.

In vivo, large numbers of transferred Mϕ migrated into allografts and peaked at 12 weeks (23% of recovered cells vs. < 7% in isografts; $P < 0.01$). Migration patterns of PMN showed parallel trends peaking at 12 weeks (> 30% vs. < 5% in isografts). The pattern of in vivo lymphocyte binding was relatively stable over the period

observed, although the number of cells migrating into allografts was consistently elevated as compared to isografts (c. 8% vs. < 4% at 12 weeks).

Discussion

Characteristic morphological changes of chronic rejection in kidney allografts include progressive arterio- and glomerulosclerosis, tubular atrophy, and interstitial fibrosis. Although the pathophysiological mechanisms producing the vascular lesions have been thought to result from repetitive endothelial injury, with intimal proliferation and gradual luminal obliteration, the etiologies of the other manifestations of the process are unknown [2].

We showed that the immunohistological features of infiltrating cells in our chronic rejection model correlated closely with findings from in vitro and in vivo binding experiments. Graft infiltration by macrophages and neutrophils peaked at 12 weeks and localized specifically in glomeruli and perivascular areas; cell presence was associated with intense expression of IL-1, IL-6, TNF-α, and with coincident proteinuria and increasing glomerulosclerosis [3]. ICAM-1 staining was associated with cell-binding patterns. Lymphocyte infiltration remained stable. In vitro and in vivo binding studies supported these findings. TNF-α, among several other functions, may be important to promote proliferation of intimal and smooth muscle cells leading to vascular obliteration and glomerulosclerosis [4]. IL-1 may act similarly, while IL-6, a potent mitogen for mesangial cells, may be critical in development of glomerulosclerosis. In conclusion, the dramatic increase in macrophages and their products in allografted kidneys at 12–16 weeks in this model seem important in the process of chronic rejection. Although the role of lymphocytes and PMN are less clear, they also may contribute.

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