Involvement of Interleukin-2 and Growth Factors in Chronic Kidney Allograft Rejection in Rats

P. Hamar, A. Szabó, V. Müller, and U. Heemann

The most important obstacle to organ transplantation today is the ill-defined process of chronic rejection. Chronically rejecting allografts are infiltrated with mononuclear cells, including T lymphocytes. As IL-2- and IL-2R-positive lymphocytes are constantly detected in chronically rejecting kidneys, they may determine the pace of chronic rejection. The development of interstitial fibrosis in chronic rejection has been attributed to local TGF-β and PDGF production. As it has been well described that cyclosporin A (CyA) induces TGF-β1 in vitro, we hypothesized that a suppression of the IL-2 pathway by this drug may be harmful over the long-term. To test this hypothesis, we studied how a continuous inhibition of the IL-2 pathway by either CyA or Tac interfered with the pace of chronic kidney allograft rejection in rats. We put a particular emphasis on their effects upon PDGF and TGF-β.

Materials and Methods

For renal transplantation naive male inbred Lewis (LEW) rats were used as donors and Fisher (F-344) rats as recipients. Total graft ischemia was less than 30 minutes. Rats received 20 mg/kg cephratixone daily during the first 10 postoperative days to prevent infectious complications and 1.5 mg/kg body weight CyA to prevent an initial episode of acute rejection. At day 10 the right native kidney was removed, and the animals were divided into three treatment groups (n = 15/group) receiving either 1.5 mg/kg bw CyA, 0.16 mg/kg bw Tac, or vehicle on a daily basis. Drug dosages were determined in a pilot study. In the present study the highest dosages without side effects were applied. Body weight was matched at the time of operation (260 ± 15 g). Two controls and one Tac-treated recipient were excluded from the analysis due to complications of grafting (hydronephrosis, stones). After 16 or 24 weeks, rats were anesthetized with diethylether, and intraaortic blood pressure was measured. Rats were bled thereafter, and the transplanted kidney was removed for PCR analysis and light microscopy. Urinary protein and creatinine clearance were determined with standard methods. Histologically, the number of glomeruli with sclerotic lesions was expressed as percentage of the total number of glomeruli counted. Additionally, CD5 + T lymphocytes and ED-1 + macrophages were counted. The mRNA expression for TGF-β1, PDGF-AB, and IL-2 was estimated by densitometric comparison with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) internal control following RT-PCR. Data are presented as mean ± standard deviation (SD). Differences between the three groups were assessed using Fisher’s least square distance test as appropriate.

Results

In all recipients, proteinuria progressed over time. A proteinuria of more than 30 mg/24 hours developed in controls by week 16, in CyA-treated recipients by week 20, and in Tac-treated ones by 24 weeks. The deterioration of kidney function further manifested in a decreased crea clearance and increased serum crea levels. Suppression of the IL-2 pathway, both by CyA and Tac, improved kidney function by week 24. Paralleling the decline of graft function, mean arterial blood pressure (MAP) increased from week 16 to week 24 in controls more rapidly than in treated rats. Morphological findings correlated with kidney function. In controls, more than 40% of glomeruli were sclerosed at week 16. Interstitium and vessels walls were infiltrated by large numbers of leukocytes, identified by immunohistology as ED1 + macrophages and CD5 + T cells. Inhibition of the IL-2 pathway reduced the extent of GS and cellular infiltration. At week 24, cellular infiltration had markedly decreased, giving way to extensive interstitial fibrosis, intimal proliferation of the arteries, and more extensive GS in controls. IL-2 treatment ameliorated these changes. In controls, IL-2 mRNA synthesis paralleled the number of infiltrating mononuclear cells. IL-2 mRNA synthesis was intense at week 16 and decreased by week 24. Not surprisingly, both CyA and Tac significantly inhibited IL-2 synthesis. TGF-β1 synthesis was highly up-regulated in controls at week 16. At week 24, when cellular infiltration had been replaced by interstitial fibrosis and GS, TGF-β1 mRNA was markedly reduced. Suppression of the IL-2 pathway reduced TGF-β1 synthesis at both time points. In contrast to TGF-β1, PDGF synthesis in controls did not decrease from week 16 to week 24. While considerably lower expressed in treated rats at week 16, the synthesis of PDGF increased in both treated groups thereafter (Table 1).

From the Department of Nephrology (P.H., A.S., V.M., U.H.), University Hospital, Essen, Germany, and Institute of Pathophysiology (P.H.), Faculty of General Medicine, Semmelweis University, Budapest, Hungary.

Address reprint requests to Dr med Peter Hamar, Institute of Pathophysiology, Faculty of General Medicine, Semmelweis University, Nagyvárad tér 4, Budapest 1089, Hungary.
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tion. In the present study, we tested whether the inhibition of the IL-2 pathway by both agents reduced the pace of deterioration. A few recent studies have suggested beneficial effects of a continuous treatment with CyA on the process of chronic rejection in experimental and clinical transplantation. However, none of these studies focuses on the role of the IL-2 pathway. Our data support the hypo-
thesis that chronic rejection, at least in part, has to do with permanent alloantigen recognition. On the other hand, the involvement of the IL-2 pathway may be interpreted as part of the ongoing unsppecific inflammatory reaction. Further studies are needed to elucidate this question. Tissue remodeling processes such as chronic rejection of renal allografts may be mediated by growth factors. TGF-β1 plays a pivotal role in fibrinogenesis and is an important mediator of fibrosis. TGF-β is produced by graft-invading macrophages and T lymphocytes. In our study an intense up-regulation of TGF-β mRNA was observed in controls. This up-regulation had a time course relationship with the intensity of cellular infiltration. Further, TGF-β synthesis was minimal in immunosuppressed animals, which could explain the reduction of GS and interstitial fibrosis observed. CyA might stimulate TGF-β1 expression in T cells in vitro. However, no in vivo data are available. Thus, the stimula-
tory effect of CyA upon local TGF-β synthesis may be minor compared to the inhibition of cellular infiltration, and thus the elimination of TGF-β-producing mononuclear cells. There is a growing body of evidence that besides TGF-β, PDGF synthesis is important in tissue remodeling.

Table 1. Functional Parameters, Immunohistology, and RT-PCR Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CyA</th>
<th>Tac</th>
<th>CSA vs Controls</th>
<th>Tac vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Glomerulosclerosis (%)</td>
<td>42 ± 1.5</td>
<td>4</td>
<td>30 ± 0.6</td>
<td>5</td>
<td>22 ± 1.6</td>
</tr>
<tr>
<td>Crea clearance (Ml/min)</td>
<td>1.8 ± 0.1</td>
<td>4</td>
<td>2 ± 0.1</td>
<td>5</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Serum crea (mg/DL)</td>
<td>1.5 ± 0.2</td>
<td>4</td>
<td>1.3 ± 0.1</td>
<td>5</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Systemic RR MAP (mm Hg)</td>
<td>2.1 ± 0.2</td>
<td>9</td>
<td>1.7 ± 0.2</td>
<td>10</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Macrophages (ED1) (cells/fv)</td>
<td>68 ± 4.1</td>
<td>9</td>
<td>47 ± 4.6</td>
<td>10</td>
<td>37 ± 2.3</td>
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<tr>
<td>Lymphocytes (CDS) (cells/fv)</td>
<td>65 ± 6.6</td>
<td>4</td>
<td>45 ± 4.9</td>
<td>5</td>
<td>40 ± 4.4</td>
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<tr>
<td>IL-2 mRNA (U)</td>
<td>49 ± 3.3</td>
<td>9</td>
<td>33 ± 4.2</td>
<td>10</td>
<td>27 ± 4.6</td>
</tr>
<tr>
<td>TGF-β1 mRNA (U)</td>
<td>1.6 ± 0.3</td>
<td>4</td>
<td>0.4 ± 0.2</td>
<td>5</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>PDGF mRNA (U)</td>
<td>0.2 ± 0.1</td>
<td>9</td>
<td>0.01 ± 0.01</td>
<td>10</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>4.5 ± 1.8</td>
<td>9</td>
<td>0.05 ± 0.01</td>
<td>10</td>
<td>0.05 ± 0.01</td>
<td>9</td>
</tr>
<tr>
<td>0.08 ± 0.04</td>
<td>4</td>
<td>0.02 ± 0.03</td>
<td>5</td>
<td>0.02 ± 0.02</td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>0.09 ± 0.04</td>
<td>9</td>
<td>0.07 ± 0.04</td>
<td>10</td>
<td>0.05 ± 0.04</td>
</tr>
</tbody>
</table>

Statistical significance within rows was analyzed with Fisher’s least square distance test. Densitometric comparison of IL-2 or GF bands with GAPDH bands. fv, field of view at 400× magnification; U, relative unit.

DISCUSSION
Presently, the ill-defined process of chronic rejection is the most important cause of allograft loss. Chronic rejection may be the result of a cell-mediated immune reaction based on T-cell recognition of the permanently present alloanti-
gen or alloantigen-independent events. Immunohistochem-
ical analysis of renal transplants undergoing chronic rejec-
tion revealed an infiltrate of both T cells and macrophages. Injury or local hemodynamic factors, such as hyperfiltra-
tion, may activate graft endothelial cells, resulting in an unspecified emigration of mononuclear cells into the al-
lograft. As lymphocytes are activated during extravasation, all theories suggest an involvement of the IL-2 pathway in chronic rejection. In the present rat model, the synthesis of IL-2 peaked in controls (simultaneously with mononuclear cellular infiltration) at week 16. Krams et al also demonstrated the presence of IL-2R mRNA in human nephrec-
tomy samples with pathologic evidence for chronic rejec-
tion. In the present study, we tested whether the inhibition of the IL-2 synthesis with either CyA or Tac ameliorates the process of chronic rejection in renal allografts. The inhibi-
tion of the IL-2 pathway by both agents reduced the pace of deterioration. A few recent studies have suggested beneficial effects of a continuous treatment with CyA on the process of chronic rejection in experimental and clinical transplantation. However, none of these studies focuses on the role of the IL-2 pathway. Our data support the hypo-
cial influence upon PDGF synthesis in our study, their influence vanished over time. Since PDGF is produced not only by platelets but by mesangial cells as well, it is suggested that glomerular cell proliferation may become self-perpetuating by an autocrine mechanism involving mesangial cell production of PDGF. Therefore, PDGF may be more important for the later progress of chronic rejection than TGF-β. Furthermore, while CyA and Tac are beneficial in the early phase of chronic rejection, once renal scarring has occurred, alloantigen-independent processes may become dominant in the self-perpetuating process, and therefore there may be no further use of IL-2 suppression. In summary, continuous suppression of the IL-2 pathway may reduce the pace of chronic rejection of rat kidney allografts.

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REFERENCES