The involvement of activated T cells and growth-factor production in the early and late phase of chronic kidney allograft nephropathy in rats

Abstract T cells are thought to play a regulatory role in chronic allograft nephropathy (CAN). Thus, we investigated whether lymphocyte inhibition influences CAN. Fisher rat (F-344) kidneys were transplanted orthotopically into Lewis rats. Animals received cyclosporin A (1.5 mg/kg per day, s.c.) for 10 days and were treated daily with either cyclosporin A (1.5 mg/kg), tacrolimus (0.16 mg/kg), or a vehicle thereafter (n = 15 per group). Kidneys were harvested at 16 or 24 weeks. Interleukin-2 (IL-2) and interleukin-2 receptor β (IL-2Rβ) mRNA synthesis were intense at 16 weeks and decreased thereafter. Unsurprisingly, both cyclosporin A and tacrolimus significantly inhibited IL-2 and IL-2Rβ at both time points. Proteinuria increased more rapidly in controls than in treated animals. Morphologically, over 40% of glomeruli were sclerosed by 16 weeks in controls, and ED-1+ macrophages and CD5+ T cells infiltrated the graft. IL-2 mRNA synthesis paralleled the number of infiltrating cells. Inhibition of T-cell proliferation significantly reduced glomerulosclerosis and leukocyte infiltration at both time points. Transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF) synthesis were highly upregulated in controls at 16 weeks, the time of peak infiltration. At 24 weeks, as cellular infiltration was replaced by scar formation, TGF-β1 mRNA returned to normal, while PDGF did not. Inhibition of T cells prevented the upregulation of TGF-β1 at both time points; however, PDGF was suppressed only at week 16. These results indicate a beneficial effect of continuous suppression of T cells in CAN. T cells are probably more important in the early, inflammatory phase.

Keywords Chronic allograft nephropathy · T cells · Interleukin-2 · Transforming growth factor-β · Platelet-derived growth factor

Abbreviations ED-1 monoclonal antibody against rat CD68 equivalent tissue-macrophage marker · ICAM-1 (CD54) intercellular adhesion molecule 1 · IL-2Rβ (CD122) 70 kDa beta protein (p70) of the interleukin-2 receptor · LFA-1 (CD11a) alpha chain of the leukocyte function associated antigen 1 · OX-19 monoclonal antibody directed to rat CD5+ equivalent pan-T-cell marker · TGF-β1 isof orm 1 of transforming growth factor beta · VCAM-1 (CD106) vascular cell adhesion molecule 1 · VLA-4 (CD49d) alpha integrin of very late activation protein 4
Introduction

Results of clinical transplantation have been markedly improved by the development of new immunosuppressive drugs. Despite these improvements, the rate of attrition over the long term remained constant [27, 45, 46]. The underlying cause is thought to be the ill-defined process of chronic allograft nephropathy (CAN). CAN has been hypothesized to be caused by cell-mediated permanently ongoing rejection of the present alloantigen [1, 43], recurrent intermittent acute rejection episodes [28, 36, 46], or alloantigen-independent processes that activate graft endothelial cells and induce an unspecific inflammatory response [21, 46]. The extent to which these mechanisms participate in the process of CAN is not yet understood.

Kidney grafts with CAN are infiltrated with mononuclear cells, including T cells. Interleukin-2 (IL-2), originally called T-cell growth factor [56], is responsible for the proliferation of T cells upon activation [1]. IL-2 and its receptor (IL-2R) are upregulated during the process of T-cell activation [41]. As IL-2- and IL-2R-positive lymphocytes have constantly been detected in chronically rejecting kidneys [18, 38], they may determine the pace of CAN [1, 10, 43], stressing the importance of alloantigen-dependent events. A number of recent studies deals with the role of T cells in CAN. Oligoclonal T cells [55] and T cells with antidonor specificity [25] were demonstrated in CAN of human heart allografts, indicating antigen-driven proliferation and clonal expansion of T cells. Furthermore, Kirk et al. have demonstrated that the number of lymphocytes infiltrating human kidney allografts and T-cell receptor turnover correlated with proteinuria, fibrosis, and late functional deterioration. It has also been described that CD8+ T cells induce apoptosis during chronic rejection [42, 52]. All these observations stress the importance of activated T cells in the process of CAN.

The development of interstitial fibrosis in CAN has been attributed to local transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF) production [46, 54]. In clinical biopsies of chronically rejecting kidneys, the TGF-β1 isoform predominated [53]. As it has been well described that both cyclosporin A and tacrolimus induce TGF-β1 in vitro and in vivo, it was hypothesized that continuous treatment with these drugs might be harmful over the long term. To test whether continuous immunosuppressive treatment is beneficial or harmful over the long term, we studied how inhibition of T cells by either cyclosporin A or tacrolimus interfered with the pace of kidney CAN in rats. We put a particular emphasis on their effects upon PDGF and TGF-β.

As re-transplantation experiments have shown earlier, CAN is reversible if re-transplantation is performed early after transplantation (within 8 weeks in the Fisher-to-Lewis rat kidney allograft model), but irreversible if performed later [51]. Thus, we hypothesized that at different times after transplantation, different mechanisms might operate during the process of CAN.

Materials and methods

Experimental animals

Naive male inbred Lewis (LEW) and Fisher (F-344) rats weighing 180–250 g were used throughout the experiment. All animals were obtained from Charles River, Munich, Germany, housed under standard conditions, and fed rat chow and water ad libitum. All animal experiments were carried out according to the principles of laboratory animal care [16], and the experimental protocol was reviewed and approved by a government animal care and research committee of Germany.

Renal transplantation

Fisher rats served as donors and Lewis rats as recipients. Transplantation was performed as previously described [21]. Briefly, the left donor kidney was perfused with Ringer’s lactate (4 °C), removed, and positioned orthotopically into the recipient, whose renal vessels had been isolated and clamped and from whom the native kidney had been removed. End-to-end anastomosis of renal artery, vein, and ureter was performed, using 10-0 Prolene sutures. Total graft ischemia was less than 30 min. To prevent infectious complications during the perioperative phase, we administered 20 mg/kg ceftriaxone (Rocephin) daily during the first 10 postoperative days, at which time the right native kidney was removed.

Experimental design

Rats received 1.5 mg/kg b.w. cyclosporin A (Calbiochem Biochemicals, Bad Soden, Germany) for the first 10 postoperative days to prevent an initial episode of acute rejection. After day 10, animals were divided into three treatment groups (n = 15 per group) and received either 1.5 mg/kg b.w. cyclosporin A, 0.16 mg/kg b.w. tacrolimus (Fujisawa, Japan) [28], or a vehicle, on a daily basis. Both immunosuppressants were suspended in Cremophor-ethanol, according to the manufacturer’s instructions and administered subcutaneously.

Drug dosages were determined in a pilot study. Transplanted rats chronically receiving 3.2 mg/kg/day cyclosporin A or 0.32 mg/kg/day tacrolimus for 2 months suffered from toxic side effects such as continuous loss of body weight and diarrhea. In the present study the highest dosages – without side effects – were applied. To exclude the influence of body weight on the extent of proteinuria, we matched body weight at the time of operation (260±15 g). Weight differences between the groups did not reach statistical significance throughout the experiment. Complications of grafting (hydronephrosis, stones) were observed in two controls and in one tacrolimus-treated recipient. Those animals were excluded from further analysis. After 16 or 24 weeks, rats were anesthetized with diethylether, and the intra-aortic blood pressure was measured with a DPT 3003-S/3 cc arterial transducer (Peter von Berg Medizintechnik, Germany). Rats were bled thereafter, and the intra-aortic blood pressure was measured with a DPT 3003-S/3 cc arterial transducer (Peter von Berg Medizintechnik, Germany). Rats were bled thereafter, and the transplanted kidney was removed. Samples were snap-frozen in liquid nitrogen for immunohistological staining and for polymerase chain reaction (PCR) analysis, or fixed in buffered formalin (4%) for light microscopy.