Overview of the Oral Presentations at the International Symposium "Chronic Rejection in Experimental and Clinical Transplantation: New Strategies in Research and Therapy"

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Introduction

Organ transplantation has been lifesaving for patients with end stage heart, liver, and lung disease and has ameliorated substantially the quality of life for patients with end stage renal disease. However, chronic rejection of allografts, leading to graft loss, still remains a major problem. The ultimate goal for patients with end stage renal, liver, heart, or lung disease is to achieve a state of tolerance to their 1st transplant—without the need of lifelong immunosuppression. In this overview, we summarize novel and fascinating studies, unraveling donor-associated and alloantigen-dependent and -independent risk factors, donor-associated factors, and new therapeutic approaches to chronic rejection from the oral presentations at the International Symposium "Chronic Rejection in Experimental and Clinical Transplantation: New Strategies in Research and Therapy" held in Würzburg, Germany, in 2001.

New Animal Model for Chronic Rejection

Chronic rejection is a major cause of long-term graft loss after liver transplantation; therefore, an animal model to study chronic liver allograft rejection is of great value. A new model for chronic liver allograft rejection was developed in the rat strain combination Dark Agouti (donor) → Lewis (recipient), with arterialized orthotopic liver transplantation, and different dosages of tacrolimus from day 0 to 13 days posttransplant. Two forms of chronic rejection were identified: a more common ductoproliferative form with proliferation and periductal fibrosis of bile ducts and a less common ductopenic form analogous to the human situation. Both forms evolve from persistent cellular "acute rejection" and can be prevented by immunosuppression.

Donor-Associated Risk Factors in Chronic Rejection

The observation that kidneys from living-related donors perform in a consistently superior manner compared with cadaver donor transplants has persisted over time. In this respect, brain death has been shown to clearly have an adverse effect on kidney transplantation in humans and in different rat models. New, and of particular interest for the use in cadaver donor grafts of potentially diminished quality, is recombinant soluble P-selectin glycoprotein ligand (rPSGL-Ig). In a brain death rat kidney allograft model, rPSGL-Ig inhibited donor brain death-associated early inflammatory processes. RT-PCR analysis showed higher transcription of ICAM-1, IL-1β, MCP-1, TNF-α, TNF-β, IFN-γ, IL-2, Il-3, Il-4, Il-5, Il-6, and TGF-β in untreated brain-dead controls before transplantation, whereas rats treated with rPLGL-Ig were virtually normal. More important, as a consequence, chronic allograft dysfunction of these grafts was prevented.

This finding was confirmed in another study, where non-heart-beating rat donors treated with rPSGL-Ig were found to have significantly lower serum creatinine and less inflammatory cell infiltrates in their kidney transplant, compared with controls without treatment.

In a study on the impact of donor hypertension on chronic cardiac allograft rejection in rats, mononuclear cell infiltrates were found only in recipients of cardiac transplants whose donor had renal artery hypertension but not in recipients without donor hypertension. The mononuclear cell infiltrates later developed into widespread fibrosis. Furthermore, expression of IL-2R and cytokines (TGF-β and PDGF) was significantly greater in recipients of a donor heart with hypertension than without hypertension. This implies that hearts from donors with hypertension appear to induce alloresponsiveness in the recipient. Consequently, chronic rejection develops more rapidly in hearts from hypertensive donors. Likewise, donor hypertension intensifies chronic changes associated with allogeneic kidney transplantation through increased immunogenicity of the graft. RT-PCR revealed significant up-regulation of proinflammatory mediators before transplantation, which increased progressively after transplantation in kidney allografts of hypertensive donors as compared with normotensive donors. These results suggest the need for aggressive immunosuppressive treatment for allograft recipients of hypertensive donors.

Alloantigen-Independent Risk Factors in Chronic Rejection

Ischemia/reperfusion injury after renal transplantation leading to acute renal failure continues to be an important clinical problem and is influenced, among other factors, by gender and sex steroids (or hormones). After infliction of the same ischemic injury to male and female rats, 8% of males as compared with 75% of females survived more than 7 days. The expression of prepro-endothelin gene in the kidneys was increased 5 min after reperfusion and was significantly higher 2 h after ischemia in males but not in females. The postischemic recovery of renal blood flow was delayed owing to a dramatic increase in renal vascular resistance in male versus female rats. Ovariectomy had no impact on the course of renal failure in females. However, pretreatment of male rats with endothelin receptor A antagonist provided indistinguishable survival rates between male and female rats after warm renal ischemia. Therefore, androgens, rather than estrogens, are responsible for the different response of kidneys in male and female rats. The effect of androgens upon ischemic kidney damage seems to be mediated by endothelin-mediated vascular changes. This was confirmed in a different study where male recipients of orthotopic transplanted kidneys were either treated with the antiandrogen flutamide or with vehicle. Antiandrogen treatment reduced glomerulosclerosis as well as tubulointerstitial fibrosis and transplant vasculopathy. Furthermore, diminished macrophage infiltration and reduced mRNA expression of TGF-β, PDGF-A and -B chain were found in these allografts. Therefore, testosterone receptor blockade seems to ameliorate chronic allograft nephropathy in rats and may be a future therapeutic option in humans.

The current view on transplant vasculopathy is that donor-derived medial vascular smooth muscle cells of affected arteries migrate and proliferate into the subendothelial space resulting in luminal narrowing. Following this concept, the vascular smooth muscle cells are of donor origin. To study the origin, donor versus recipient, of endothelium and neointimal vascular smooth muscle cells, the cells are analyzed in 2 experimental transplant models. Immunohistochemical analysis showed that donor (female) endothelium, as well as smooth muscle cells, are completely replaced by host (male)-derived endothelial and smooth muscle cells in aortic allografts in a rat model. However, cardiac allograft endothelium cells present in arteriosclerotic lesions are still of donor origin whereas smooth muscle cells are of recipient origin. Like in aortic allografts, neointimal vascular smooth muscle cells are of recipient origin. The clinical implications of these findings are yet to be determined.

Vascular changes in chronic transplant rejection show many histological parallels to atherosclerotic lesions. Lipoprotein A is a well-known independent risk factor for atherosclerosis, but its role in chronic rejection has not been investigated. Lipoprotein A is an LDL-like particle with an additional apoprotein, apoprotein A. Apoprotein A occurs in multiple, genetically determined isoforms of different molecular weights (400-800 kD). In a retrospective

study, apoprotein A isoforms were measured in frozen sera of 327 renal transplant patients with more than 2-year graft survival. Regardless of the number of HLA mismatches, gender, or immunosuppressive treatment, young (< 35 years old) transplant patients with low molecular weight isoforms had a significantly shorter, 9.9 years (95% CI, 8.5-11.5), long-term graft survival when compared with patients with high molecular weight apoprotein A isoforms, 13.2 years (95% CI, 21.1-14.4). Although it is not certain if this finding is a primary cause or the consequence of chronic transplant rejection, it may lead to new therapeutic possibilities.

Alloantigen-Dependent Risk Factors in Chronic Rejection

Long-term allograft survival is dependent on numerous alloantigen-dependent and -independent factors. To study the relative importance of some of these factors, rat recipients of a renal allograft received either an additional secondary specific T-cell activating event (donor-specific skin graft) or additional ischemia/reperfusion injury. Skin grafting after renal transplantation resulted in significantly decreased survival rates compared with rats without secondary manipulation. Numbers of ED-1+Mϕ, $CD4^+$, and $CD8^+$ T-cells increased significantly following skin grafts, while remaining unchanged after secondary ischemia/reperfusion injury. These results demonstrate that secondary specific T-cell activating events (skin grafting) accelerate chronic graft rejection and that the consequences of secondary ischemia/reperfusion injury are less detrimental as a secondary event compared with the initial ischemia/reperfusion injury in the context of allotransplantation.

T-cells can recognize intact allo-MHC (major histocompatibility complex) molecules on the surface of donor cells via the direct pathway of allorecognition, or as processed allopeptides on the surface of self-antigen presenting cells via the indirect pathway of allorecognition. There is evidence that indirect allorecognition plays an important role in chronic allograft rejection. To study the influence of priming T-cells in vivo via the indirect pathway in experimental small bowel and kidney transplantation, rats were immunized with class II MHC allopeptide or a nonimmunogenic control peptide before transplantation. Animals immunized with the allopeptide rejected their grafts at an accelerated rate in 4 days, whereas nonimmunized animals rejected their grafts acutely after 8 days. Furthermore, animals immunized with allopeptide and treated with cyclosporin rejected their small bowel graft after 34 days, whereas animals immunized with the control peptide and treated with cyclosporin rejected their graft after 44 days. On the other hand, animals without immunization but with cyclosporin survived for more than 250 days. Lymphocytes from spleen and lymph nodes from rejecting animals demonstrated specific reactivity to the allopeptide. Cytokine expression in acutely and accelerated rejected allografts, determined by RNase protection assay, showed up-regulation of IL-2, IFN-γ, TNF-α/β, -4, -5, and -6. In contrast, allografts from tolerant animals expressed only TNF-β. These data demonstrate that T-cells primed in vivo via the indirect pathway of allorecognition can mediate a specific immunoresponse.

Infiltration and activation of macrophages are associated with the development of chronic renal allograft rejection. Macrophages can be activated in a classical pathway, which is characterized by tissue destruction and TNF-α expression, and an alternative pathway, which is associated with fibrogenesis and expression of alternative macrophage activation-induced CC-chemokine 1 (AMAC-1). To elucidate which type of macrophage is involved after kidney transplantation in a rat model, histological analysis, as well as RT-PCR assays for TNF-α and AMAC-1 mRNA, was performed. Infiltrated macrophages in this rat model were classically activated early posttransplantation; however, they take the alternative activation pathway at later stages posttransplantation. This finding suggests that alternative activation of macrophages may be associated with chronic allograft rejection.

New Therapeutic Approaches in Chronic Rejection

Cardiac allograft arterial disease, limiting long-term survival of heart transplant recipients, is characterized by intimal thickening composed of proliferative smooth muscle cells. Proliferating-cell nuclear antigen (PCNA) and cycline-dependent kinase 2 kinase (cdk2) play a pivotal role in the cell cycle regulatory genes involved in smooth muscle cell proliferation. In a murine cardiac allograft study, a single intraluminal administration of antisense oligonu-

cleotide against cell-cycle regulatory genes prevented graft neointimal formation, without systemic effects. This approach may become clinically feasible for attenuation of cardiac allograft arteriosclerosis.

Adenovirus-mediated transfer of immunoregulatory molecules in the graft prior to transplantation may be a novel tool to prevent chronic allograft rejection. To study this hypothesis, renal allografts transduced ex vivo with the immunoregulatory genes for IL-10, TNFRp55-Ig, and IL-12p40 were transplanted in a rat model. Creatinine clearance after 12 weeks was significantly higher in the treated group, and histology after 24 weeks revealed markedly less glomerulosclerosis and fibrosis in the treated group as compared with control groups. Therefore, it was concluded that adenovirus-mediated gene transfer of immunoregulatory molecules could partially inhibit chronic allograft nephropathy in rats. Additional studies are needed to further improve prevention of chronic allograft rejection by gene therapy.

Heparin-binding epidermal growth factor (HB-EGF), a newly identified member of the epidermal growth factor family, is a mitogen and chemoattractant for smooth muscle cells and plays an important role in the progression of coronary arteriosclerosis. Because of the common features between arteriosclerosis and chronic allograft vasculopathy, the expression of HB-EGF and HB-EGR-receptor in chronically rejecting heart and intestinal transplants in rats was studied. HB-EGF and HB-EGF-receptor were strongly expressed during chronic allograft vasculopathy but not in control syngeneic heart transplants. HB-EGF was also seen in mesenteric arteries in a chronically rejecting intestinal transplantation model. Abrogation of chronic allograft vasculopathy by cyclosporin and FTY720 was accompanied by a dramatic reduction of HB-EGF expression after heart transplantation.

T-cell immunosuppressive therapies have not had a major impact on chronic rejection, so non-T-cell mechanisms might be involved. Locally synthesized complement C3 was studied in a mouse renal transplantation model to investigate its role in chronic allograft nephropathy. For this purpose, C3 knockout kidneys or wild-type kidneys were transplanted in mice differing only in a single MHC class II. To prolong survival, all mice were pretreated with depleting anti-mouse CD4 and anti-CD8. The mice with the wild-type kidneys all developed severe chronic allograft nephropathy with widespread tubular atrophy and arteriosclerosis, whereas the recipients of C3-/- kidneys had significantly less tubular atrophy and arteriosclerosis. Interestingly, immunohistology revealed dense Ig deposits in glomeruli of the C3-/- kidneys. This may indicate that clearance of therapeutic antibody or immune complexes was impaired in the absence of local C3 synthesis. These data suggest that locally produced C3 may be a causative factor in chronic allograft nephropathy not previously identified and could therefore be a possible target for therapy or prevention of chronic rejection. The accumulation of administered antibody in glomeruli of a C3-/- transplanted kidney may have led to an underestimation of the protective effect of absent local C3 synthesis.

Another interesting area of research is to try to reduce immunosuppressive therapy by simultaneous transplantation of a liver with a small bowel allograft. In a fully MHC-incompatible rat strain combination, a heterotopic or orthtopic small bowel transplantation was combined with liver transplantation, with or without immunosuppressive therapy. Acute rejection could not be completely eliminated by immunosuppression or immunomodulation by a liver allograft. Although small bowel allografts were placed at 2 different anatomic positions, only severity of the parenchymal cell damage after heterotopic small bowel transplantation predicted later development of chronic rejection. As compared with small bowel transplantation alone, a simultaneous liver allograft can help to reduce the immunosuppressive load but does not eliminate transplant vasculopathy and thus chronic rejection in general.

Conclusion

Exciting new studies further elucidating different risk factors responsible for the induction of chronic rejection, and therapeutic approaches aimed to circumvent this process of chronic allograft rejection, were presented at the International Symposium "Chronic Rejection in Experimental and Clinical Transplantation: New Strategies in Research and Therapy." You will find more detailed reports in this issue.