The pathobiology of chronic allograft nephropathy: Immune-mediated damage and accelerated aging

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The pathobiology of chronic allograft nephropathy: Immune-mediated damage and accelerated aging. Chronic allograft nephropathy includes chronic calcineurin nephrotoxicity, recurrent and de novo glomerulonephritis and a group of disorders with graft dysfunction of unknown etiology designated chronic rejection. Review of risk factors of the latter category show that the chronic rejection lesions emerge in organs that have undergone injury. Despite the relevance of nonalloantigen-dependent progression factors in the tissue injury, alloantigen-dependent factors predominate in the pathogenesis. Lately, B cell responses have received increasing interest in transplant rejection and include responses against both major histocompatibility complex (MHC) and tissue-specific antigens, mainly on the endothelium and in the glomeruli. These humoral responses are thought to be involved in the development of vascular and glomerular lesions. Furthermore, at the tissue level, markers of senescence are found in the tubular epithelium contributing to the lesions of tubular atrophy and interstitial fibrosis.

Despite improvements in outcome in the early post transplant period, improving the long-term survival of kidney transplants remains a challenge as up to 50% of grafts are lost from chronic allograft nephropathy. The time to failure of a graft is determined by the initial function achieved, the number and severity of insults, and a number of tissue characteristics. The insults a graft usually encounters include ischemia/reperfusion injury, rejection episodes, hypertension, smoking, drug nephrotoxicity, and hyperlipidemia [1, 2]. The tissue susceptibility to injury and the ability to repair damage are important tissue characteristics.

Chronic allograft nephropathy is a descriptive term for histologic lesions in a renal allograft and includes atherosclerosis, glomerulosclerosis, interstitial fibrosis, and tubular atrophy [1]. Clinically, chronic allograft nephropathy can lead to chronic transplant dysfunction, characterized by a decline in function and the development of proteinuria [3]. The lesions of chronic allograft nephropathy can originate from various causes and the differential diagnosis includes chiefly recurrent and de novo glomerulonephritis, chronic calcineurin inhibitor nephrotoxicity, and a group of lesions of unknown origin called chronic rejection [3]. The diagnosis of chronic rejection is verified by histology: the presence of glomerular and/or vascular lesions against a background of interstitial fibrosis and tubular atrophy. Before establishing the diagnosis other known causes have to be ruled out. Immunofluorescence and electron microscopy are useful tools in addition to histology to establish the diagnosis.

Multivariate analysis to identify risk factors for chronic rejection revealed an important role for acute rejection episodes after the first 3 months. Peak panel reactive antibodies (PRA), PRA at the time of implantation, and donor age have also been identified as important risk factors [3–5]. Major histocompatibility complex (MHC) class I histoincompatibility is associated with the occurrence of late acute rejection episodes. Indirect presentation, which is the likely antigen presentation route in these conditions, can result in activation of CD4+ Th2 T cells, leading to B-cell activation and production of alloantibodies [6].

Previous experiments using severe combined immunodeficiency (SCID) (lack B- and T-cell responses) or immunoglobulin heavy chain (IGH) knockout (lack functional B cells) mice as recipients, have shown that chronic rejection was only induced upon transfer of alloantibodies [7, 8] reactive with donor MHC antigens or with non-MHC antigens.

Antibodies reactive with MHC antigens might be most harmful to the endothelium. The endothelium is the first site of contact between antibodies and the foreign MHC antigens. Transplant vasculopathy might be the result of anti-MHC antibodies binding in the large vessels leading to damage.

Antibodies reactive with non-MHC antigens may result in damage to different cell types in the graft, including endothelial cells and organ-specific cells. In
addition, antibodies can be reactive with the renal basement membranes.

**HUMORAL IMMUNE RESPONSE IN CHRONIC REJECTION**

In the Fisher (F344) to Lewis (LEW) rat model of chronic rejection we identified an antibody response reactive with antigens in the glomerular basement membrane (GBM) [9]. Antibodies were only detected posttreatment in LEW recipients of chronically rejecting F344 grafts but not in the long-term surviving, chronic rejection-free F344 recipients of LEW grafts or in syngeneic transplantations. The antigens were identified using proteomics techniques, and included the basement membrane heparan sulfate proteoglycan perlecan and the α1 chain of collagen type VI in association with the α5 chain of collagen type IV [9].

In patients, transplant glomerulopathy, characterized by duplications of the GBM, is found in about 2% of kidney transplant recipients and in about 15% of patients with chronic rejection [10] [Sijpkins et al, in press]. Risk factor analysis revealed pretransplant sensitization and late acute rejection episodes, which are immunologic risk factors. Using a polyvalent antiserum raised against C4d peptides, C4d deposits in the glomeruli were demonstrated, indicating that humoral responses might be involved [Sijpkins et al, in press]. Recently, we found antibodies reactive with GBM isolates in patients with transplant glomerulopathy, supporting a role for humoral immunity in transplant glomerulopathy [Joosten et al, unpublished data].

In addition, responses against human leukocyte antigen (HLA) and non-HLA antigens on endothelial cells can be involved in the vascular lesions. Renal transplant recipients with anti-HLA antibodies were five to six times more likely to develop chronic rejection compared to patients without anti-HLA antibodies, patients who have antibodies against both class I and II HLA antigens pretreatment have the worst prognosis [11, 12].

**OLD DONOR AGE**

An important factor in the decline of function after acute rejection is the quality to start with, determined by donor age and previous damage to the kidney [4]. Kidneys from older donors result in more acute rejection episodes, mainly due to an increase in the number of interstitial rejection episodes. Furthermore, if an acute rejection episode occurs in an older donor kidney, it results more often in graft loss compared to acute rejection episodes in young kidneys [13]. The lesions observed in aging kidneys are very similar to the lesions observed in chronic allograft nephropathy. Therefore, the “aging hypothesis of chronic allograft nephropathy” was proposed [14, 15], stating that chronic allograft nephropathy might be the result of accelerated aging of the graft.

In normal somatic cell replication, cells lose a small part of their telomeres with each division, at least in vitro; the cells ultimately achieve the Hayflick and Moorhead limit [16]. The Hayflick and Moorhead limit is the point at which the cells do not divide anymore, and at which the cells can enter the replicative senescence state. Telomeres protect the ends of chromosomes from degradation and the telomeric repeats (TTAGGG) are extremely sensitive to oxidative damage [17]. Alterations in telomeric structures can activate several cellular pathways leading to an arrest of the cell cycle. Replicative senescence is an arrest of the cell cycle in the G1 phase, in which the cells maintain their normal metabolic functions but become desensitized to various external stimuli and thereby contribute to persistent inflammation and fibrosis [18]. Ischemia and reperfusion during transplantation result in the production of free oxygen radicals that can alter the telomeric structure leading to induction of senescence.

Recently, we demonstrated telomere shortening and the induction of replicative senescence in the F344 to LEW rat model of chronic rejection [19]. Ischemia and reperfusion of the kidney already induced telomere shortening, subsequently the cell cycle regulator p21 was transiently induced and the cell cycle regulator p16 accumulated in the tubular epithelium. All these features were found in both the rats that did develop chronic rejection (F344 to LEW) as well as in the rats that did not develop chronic rejection (LEW to F344), and appeared to be the effect of the transplantation procedure rather than a result of chronic rejection. Senescence-associated β-galactosidase (β-Gal) is a marker of senescent cells, which increases due to the accumulation of lysosomes, that keep proliferating even if the cell is arrested [20]. Senescence-associated β-Gal staining [21] was only found in tubular epithelial cells in the F344 to LEW combination, but not in the LEW to F344 transplants and therefore paralleled with the lesions of chronic rejection. Thus, telomere shortening was not sufficient to induce senescence. In addition, in kidneys of rats with increasing age, no telomere shortening was observed. However, senescence-associated β-Gal staining was present in kidneys obtained from rats of 24 months of age [22]. Together these studies revealed that shortening of telomeres is not sufficient and also not required for the induction of replicative senescence. Furthermore, the senescence-associated β-Gal staining as found in aging rat kidneys (9 to 24 months of age) can also be found in kidneys with chronic rejection (maximum 6 to 7 months of age) but at a much younger age, supporting the accelerated ageing hypothesis of chronic allograft nephropathy. The tubular epithelial cells are most sensitive for the induction of senescence, contributing to the development of tubular atrophy and interstitial
fibrosis as background lesions observed in chronic allograft nephropathy and aging kidneys.

CONCLUSION

The pathogenesis of chronic allograft nephropathy is multifactorial and comprehends progression factors that are alloantigen-independent and alloantigen-dependent factors that are crucial for the development of chronic allograft nephropathy. Figure 1 summarizes the most important factors involved in the development of chronic allograft nephropathy, ultimately resulting in the lesions described. Transplant vasculopathy is characterized by intimal proliferation and can ultimately result in luminal occlusion, antibodies reactive with endothelial cell antigens and HLA antigens are thought to be involved. Transplant glomerulopathy is characterized by duplications of the glomerular basement membrane, and according to our hypothesis antibodies reactive with GBM antigens are involved in the development of these lesions. Both glomerulopathy and vasculopathy are frequently found in a background of interstitial fibrosis and tubular atrophy. These background lesions are less well understood. Most of the progression factors, including donor age, seem responsible for the development of these lesions. Replicative senescence might be a mechanism by which tubular atrophy develops. Risk factor analysis for chronic allograft nephropathy in the absence of glomerular or vascular lesion revealed young recipient age, sensitization, and late acute rejection episodes, suggesting the alloantigen-dependent responses also play a role in this entity.

Although many factors have been associated with chronic allograft nephropathy/chronic rejection, their relative contribution remains to be elucidated. Future research on the role of humoral responses might open new possibilities for treatment or prevention of chronic transplant failure. In addition, more insights into accelerated ageing of renal allografts might result in novel strategies to prevent the background lesions.

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Fig. 1. Schematic representation of factors influencing kidney graft function and the lesions observed in kidneys with chronic allograft nephropathy, including transplant vasculopathy (A), transplant glomerulopathy (B), and tubular atrophy and interstitial fibrosis (C), F344 to LEW renal transplantation at days 30 to 60 (original magnification ×200).


