Chronic renal allograft rejection: Pathophysiologic considerations

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Chronic rejection is currently the most prevalent cause of renal transplant failure. Clinically, chronic rejection presents by chronic transplant dysfunction, characterized by a slow loss of function, often in combination with proteinuria and hypertension. The histopathology is not specific in most cases but transplant glomerulopathy and multilayering of the peritubular capillaries are highly characteristic. Several risk factors have been identified such as young recipient age, black race, presensitization, histoincompatability, and acute rejection episodes, especially vascular rejection episodes and rejections that occur late after transplantation. Chronic rejection develops in grafts that undergo intermittent or persistent damage from cellular and humoral responses resulting from indirect recognition of alloantigens. Progression factors such as advanced donor age, renal dysfunction, hypertension, proteinuria, hyperlipidemia, and smoking accelerate deterioration of renal function. At the tissue level, senescence conditioned by ischemia/reperfusion (I/R) may contribute to the development of chronic allograft nephropathy (CAN). The most effective option to prevent renal failure from chronic rejection is to avoid graft injury from both immune and nonimmune mechanism together with nonnephrotoxic maintenance immunosuppression.

With time, the short- and long-term results of kidney transplantation have improved [1] but despite these improvements a substantial portion of grafts develop progressive dysfunction and fail within a decade, even with the use of immunosuppressive drugs in doses sufficient to prevent acute rejection [2]. The decline in function is often associated with hypertension and an increase in urinary protein loss, elements of a clinical syndrome that has been called chronic allograft dysfunction. Kidney graft

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biopsies of these patients show fibrointimal thickening of arteries, interstitial fibrosis and tubular atrophy, lesions characteristic for chronic allograft nephropathy (CAN). CAN may be the result of chronic calcineurin nephrotoxicity, chronic rejection, recurrent or de novo glomerulonephritis, and a variety of other entities such as renal artery stenosis, nephrosclerosis, or BK virus nephropathy. The term "chronic" has been debated because it has to do with time, histopathology, and mechanism of injury [3]. However, in most patients with chronic rejection all three meanings are applicable. Although graft biopsies are helpful to differentiate the underlying conditions, chronic calcineurin nephrotoxicity remains difficult to distinguish from chronic rejection, especially since both conditions often coexist in the same specimen. De novo fibrointimal thickening of arteries, transplant glomerulopathy, capillaropathy, and absence of other causes of other causes of CAN favor the histologic diagnosis of chronic rejection [4]. Immunologic risk factors are an underused diagnostic tool for true chronic rejection. They should be distinguished from nonimmunologic factors which merely act as progression factors responsible for accelerated loss in renal function in the presence of chronic rejection. In this overview the features and risk factors of chronic rejection are reviewed and hypotheses regarding its pathophysiology are highlighted. Subsequently we describe the progression factors and tissue responses to injury. Finally, we discuss prevention and therapeutic strategies that should improve the prognosis of many transplant patients.

CLINICAL AND HISTOPATHOLOGIC FEATURES

Chronic transplant dysfunction as a consequence of chronic rejection is characterized by a relatively slow but variable rate of decline in renal function after the initial 3 posttransplant months. The declining renal function is often found in combination with proteinuria and aggravation or de novo hypertension [5, 6]. Linear regression analysis of the reciprocal of the serum creatinine concentration over time showed progressive loss of function in more than 80% of patients with histologic proof of CAN. Twenty percent to 28% of patients with CAN have more

than 0.5 g proteinuria/24 hours compared with 6% to 8% of patients who do not have this condition [7]. The diagnostic value of posttransplant hypertension is very limited because of its high prevalence.

The histopathology of chronic rejection is characterized by CAN (i.e., fibrous intimal thickening of arteries, glomerulosclerosis, interstitial fibrosis and tubular atrophy) [8, 9]. CAN is the result of cumulative damage to the kidney and is common at 10 years after transplantation being present in over 50% of patients [10]. In the background of CAN kidneys with chronic rejection frequently show transplant vasculopathy or glomerulopathy. Transplant vasculopathy is characterized by fibrointimal thickening of arteries, breaks in the elastic layer, and vessel wall infiltration with inflammatory cells. The intimal thickening is thought to result from the migration of (myo)fibroblast followed by local proliferation and deposition of extracellular matrix proteins. Originally it was thought that the intimal fibromyoblasts were derived from muscle cells of the media of the affected vessel or the adjacent media but evidence has been presented that those cells are from recipient origin and derived from circulating precursor cells [11]. Not only large arteries are affected, also the small peritubular capillaries can show basement membrane layering as a marker of transplant capillaropathy [4]. Although this lesion is not specific, more than seven layers of basement membranes seems specific for chronic rejection and is found in 38% of CAN specimens [4]. Finally, the glomerular lesions in transplant biopsies are variable and include wrinkling and collapse of the glomerular tuft, glomerular hypertrophy, mesangial matrix expansion, and focal glomerulosclerosis. Transplant glomerulopathy is a lesion characterized by enlargement of the glomeruli with swelling of the endothelial and mesangial cells, mesangiolysis, infiltration of the glomeruli with mononuclear cells, mesangial matrix expansion, and splitting of the glomerular basement membrane (GBM) with a subendothelial deposition of electron lucent material [12]. Transplant glomerulopathy can be discriminated from recurrent or de novo membranoproliferative glomerulonephritis (MPGN) using electron and immunofluorescence microscopy. In transplant glomerulopathy the deposits are electron-lucent and in MPGN electron-dense deposits were present. Furthermore, patients with transplant glomerulopathy show IgM with a greater intensity than C3, whereas MPGN patients showed a greater intensity of C3 [13]. The presence of transplant glomerulopathy in a renal biopsy is associated with accelerated graft loss [14].

Since 1991, there have been four international meetings in Banff, Canada, to standardize renal transplant pathology interpretations and reporting to establish objective and reproducible end points for clinical trials of new antirejection agents and to guide therapy [15–17]. Although the Banff scheme has focused mainly on the

Table 1. Risk and progression factors of chronic rejection

Risk factors	Progression factors
Young recipient age	Cadaveric donor
Sensitization pretransplantation	Old donor age
Sensitization posttransplantation	Recipient smoking
Histoincompatibility	Renal insufficiency
Therapy noncompliance	Proteinuria
Acute vascular rejection	Hypertension
Late acute rejection	Hyperlipidemia
	Overweight
	Drug nephrotoxicity

classification of acute rejection based on routine light microscopy readings, the more recent versions of the scheme deal with chronic allograft nephropathy, recognizing that tubulointerstitial changes are most accurately sampled and appear to have prognostic significance [9, 18]. Early damage in the first posttransplant months occurs predominantly in the tubulointerstitial area and accumulation of this damage with the injury from rejection episodes in combination with the irreversibility of these insults determine outcome [19]. The grading of severity of chronic rejection focused initially on interstitial fibrosis and tubular atrophy but recently also on chronic glomerular and vascular changes [17].

RISK FACTORS OF CHRONIC REJECTION

Acute rejection episodes

The most important risk factor of chronic rejection is previous acute rejection episodes (Table 1). The estimated half-life for cadaveric transplants is shorter in patients who had acute rejection episode than those who did not, 6.6 years versus 12.5 years [20]. In the recent era, the average yearly reduction in the relative hazard of graft failure after 1 year was 4.2% for all recipients, 6.3% for those who did not have acute rejection episodes but only 0.4% for those who had an acute rejection episode [1]. Not all acute rejection episodes lead to chronic rejection as type, severity, number, and timing of rejection determine outcome. Acute vascular rejection is an adverse prognostic feature compared with tubulointerstitial rejection [21, 22]. In patients receiving tacrolimus-based immunosuppression, vascular rejection was the most important predictor of medium-term graft loss.

Acute rejection episodes followed by partial loss of graft function exert a more detrimental effect on long-term outcome than acute rejections with complete functional recovery [23, 24]. Recipients with repeated acute rejection episodes have lower graft survival rates than those with no or only one acute rejection episode [25, 26]. Finally, timing of the first acute rejection episode has an impact on the long-term outcome. Acute rejection episodes within the first 3 months may have no effect on chronic rejection, whereas acute rejections

occurring after 2 to 6 months confer the greatest risk [7]. We found more contrast in prognosis when the interval to the last acute rejection episode was used [22]. Ten-year graft survival rates censored for causes of graft loss other than chronic rejection were 94%, 86%, and 45% for patients without an acute rejection episode, with early acute rejection episodes, and with late acute rejection episodes, respectively [27]. Apart from clinical acute rejection episodes, patients may have subclinical rejection that causes ongoing immunologic injury leading to chronic rejection [26].

Sensitization

Antibodies against human leukocyte antigens (HLA) antigens elicited by pregnancies, blood transfusions, or failed transplants are determined by testing the serum against a panel of HLA-typed leucocytes. Due to a decrease in blood transfusions since the introduction of erythropoietin, there is a substantial decrease in mean value of panel-reactive antibodies (PRA) [1]. Despite a negative cross-match at time of transplantation, sensitized recipients have an increased risk of chronic rejection [28]. Especially, sensitization against both HLA class I and class II results result in an increased rejection of HLA mismatched grafts [29, 30]. De novo anti-HLA antibodies posttransplantation has also been correlated with chronic rejection [31]. More specifically, posttransplant antibodies could be detected in 24% to 100% of the patients and predate renal dysfunction and graft loss from chronic rejection [32]. Therefore, the presence of anti-HLA antibodies, both before and after transplantation, is associated with chronic rejection.

HLA matching

Major histocompatibility complex (MHC) molecules of the graft are the principal targets of the immune response posttransplantation. The clinical benefits of HLA matching on graft survival as appreciated in large registries persists in the recent era despite new immunosuppressive drugs [33–36]. HLA-matched grafts have an estimated half-life of 12.4 years, as compared with 8.6 years for HLA-mismatched grafts [35]. MHC class I antigens share immunogenic epitopes, which have been assigned to one or more cross-reactive groups (CREG). In the United Network of Organ Sharing (UNOS) database the risk of chronic rejection is 62% higher in CREG-mismatched patients compared with those receiving a HLA- and CREG-matched kidney [28]. CREG matching is associated with a reduced frequency of late acute rejection episodes and improved graft function at 2 years [27]. In our well-matched cohort of renal transplants mismatches were not reciprocal to shares due to an increased homozygosity of donors. Sharing less CREG was correlated with inferior long-term graft survival [22].

Delayed graft function

Brain death and ischemia/reperfusion (I/R) may culminate clinically in delayed graft function and trigger an inflammatory cascade with up-regulation of cytokines, adhesion, and HLA-DR molecules [37]. This "injury" response increases the graft immunogenicity leading to more early acute rejection episodes. Delayed graft function, mostly defined as requirement of dialysis during the first week after transplantation, is associated with a small increased risk of chronic rejection in the UNOS database of almost 89000 cadaveric donor transplant recipients [28]. In single center studies the risk of delayed graft function on long-term outcome depends on the presence of acute rejection episodes and the requirement of a minimum follow-up time [38]. Fully recovered delayed graft function without acute rejection episodes may not necessarily be detrimental for long-term graft survival. In our center, delayed graft function is one of the risk factors of acute rejection and suboptimal function at one year, but not independently associated with an increased rate of graft loss from chronic rejection [39].

Recipient age and race

Young age is associated with a relatively high state of immune responsiveness to alloantigens, as documented by a more frequent production of lymphocytotoxic antibodies in response to blood transfusions [40]. Young individuals are also more likely to forget to take immunosuppressive medication [41]. In single center studies, young recipient age appears to be predictive of chronic rejection and graft loss censored for patient death with a functioning graft [7].

Graft survival in blacks is poor as illustrated by a current projected half-life of 7.2 years compared with 13.3 years in whites [1]. Acute rejection episodes occur more common in blacks than in white recipients, a finding that is mainly caused due to differences in immunologic responsiveness [42]. In several single center studies black race is a risk factor of chronic rejection [43, 44].

Inadequate immunosuppression

Low dose, low levels of the drug, and variable oral bioavailability of cyclosporine in the early posttransplant period have been reported to correlate with higher rates of chronic rejection [45, 46]. Noncompliance with immunosuppressive treatment occurs in about a fourth of recipients, as assessed by interview, and is associated with lower graft survival at 5 years after transplantation [47].

PATHOPHYSIOLOGY OF CHRONIC REJECTION

The majority of risk factors identified are related to the activation status of the immune system and thereby recognition of foreign antigens of the graft by the

recipient. The extent of the alloresponse is a balance between the immunogenicity of the graft, recipient responsiveness, and the level of immunosuppression. Recognition of the foreign graft–derived antigens can evoke an immune response of the recipient resulting in rejection of the graft. However, the degree of histoincompatibility between donor and recipient determines the immunogenicity of the grafts as HLA-mismatched grafts fare worse as compared to matched grafts [34]. T cells can recognize the foreign HLA antigens either via direct or indirect pathways. In the direct presentation pathway donor-derived antigens are presented on donor-derived professional antigen presenting cells to recipient T cells. This mainly results in the activation of CD8$^+$ T cells and thus T-cell effector functions. Alternatively, indirect presentation is mediated by uptake of donor antigens by infiltrating recipient-derived antigen presenting cells. Indirect presentation might result in activation of B-cell responses and thus production of alloantibodies. Indirect antigen presentation has been suggested to play an important role in the induction of chronic rejection [48, 49].

Cellular responses

T cells can express either CD4$^+$ or CD8$^+$, having helper or cytotoxic T-cell characteristics. The CD4$^+$ helper T cells are thought to be important for initiation of graft rejection [50]. Depletion of T cells using antithymocyte globulin, antibodies direct against CD3 or the interleukin-2 (IL-2) receptor α (IL-2Rα) chain are frequently used as treatment for acute rejection episodes [51]. Furthermore, blockade of various costimulation pathways have been used in order to find therapeutic strategies to prevent both acute and chronic rejection.

If the antigen-MHC complex is recognized by naive T cells, the dendritic cells can activate the T cell by providing the proper costimulation of CD28 on the T cell with B7.1 (CD80) or B7.2 (CD86) on the antigen presenting cells [52]. In addition, costimulation via CD40 on the DC and CD40L (CD154) on the T cells enhances the response. The presence of certain cytokines in the environment determine the type of T helper cell (Th) that develops, Th1 cells secrete IL-2, interferon-γ (IFN-γ), and tumor necrosis factor β (TNF-β), whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 [53]. Th1 responses favor cell-mediated immune response and Th2 cells potentiate humoral immune responses.

Humoral responses

Currently, humoral responses are thought to be involved in the development of chronic rejection. Humoral responses are induced after binding of antigen to the B-cell receptor on immature B cells and subsequent activation of several intracellular signaling pathways and internalization of the antigen. The activated signaling pathways determine the fate of the B cell [54]. The internalized antigen is processed and presented as a peptide in the context of MHC class II, interaction of this MHC complex with the appropriate T-cell receptor in the presence of CD40-CD40L and B7h-ICOS (inducible costimulator) costimulation results in activation of the B-cell and antibody production. Costimulation by CD40-CD40L and B7h-ICOS are crucial for generation of humoral responses [54, 55].

Blockade of B7h-ICOS [56], CD40-CD40L [57], and B7-CD28 [52, 58] costimulation have been used as therapeutic strategies to prevent acute and chronic rejection in experimental models. Most attention has been paid to the effects of costimulation blockade in acute rejection models and the analysis focused on cellular responses. A limited number of reports present the effect of costimulation blockade on chronic rejection and even less on humoral responses. Various reports have suggested that costimulation via CD40-CD40L is not required for the development of chronic rejection in specific models, in these models alloantibody responses seem less important. In nonhuman primates blockade of CD40L did not affect the production of antidonor antibodies [57]. In contrast, blockade of B7h-ICOS costimulation demonstrated a key role for this pathway in chronic rejection, in addition decreased antidonor alloantibodies have been found upon ICOS blockade [56]. On the other hand, late treatment of the recipients with CTLA4-Ig to block CD28-B7 interactions also attenuated antibody responses. Both CTLA4-Ig and blocking, anti-CD28 monoclonal antibodies prevent the development of chronic rejection [58]. In conclusion, both CD28-B7 and ICOS-B7h seem to be involved in the pathogenesis of chronic rejection, and both are involved in antibody production, supporting a role for humoral responses in chronic rejection.

Experimental models have frequently been used to study the relative contributions of different cell types to the development of chronic rejection. Various deficient or knockout mice that lack components of the immune system were used as recipients of mismatched allografts. Nude mice, that lack functional T cells, or RAG-1–deficient mice (recombinase activating gene deficiency resulting in lack of functional B and T cells due to lack of Ig or T-cell receptor rearrangements) have been used as recipients of mismatched aortic allografts in the presence of CD4$^+$ or CD8$^+$ T cells. Mice that received CD4$^+$ T cells developed vascular lesions similar to chronic rejection, whereas mice that received CD8$^+$ T cells did not develop these lesions [59]. CD8$^+$ T cells and B cells seem to be the effector cells, but they are dependent on the CD4$^+$ T-cell responses. The role of antibodies in chronic transplant rejection has been studied in more detail using similar animal models. In a mice cardiac allograft model, using SCID mice (lack B- and T-cell responses) as

recipients almost no lesions developed [60]. Injection of antidonor antibodies into the SCID recipient resulted in development of obstructive coronary lesions. In another model, using IGH knockout mice (lack functional B cells) as recipients of cardiac allografts in the presence of neutralizing anti-CD4 antibodies, no chronic rejection was present in contrast to wild-type animals treated with anti-CD4 antibodies [61]. Furthermore, in an aortic allograft model with RAG-2–deficient recipient mice it has been shown that donor-specific cellular and humoral responses are required for both the initiation and perpetuation of chronic rejection [60]. Altogether these data implicate that humoral responses are important in chronic rejection.

C4d and chronic rejection

In recent years most attention has been paid clinically to C4d deposits in tissue as in situ marker for humoral rejection. C4d is one of the degradation products of complement component C4, that remains covalently linked to the tissue after activation. C4d is thought to be more stably deposited as compared to IgG and therefore frequently used as a marker for humoral rejection. In renal allograft biopsies with chronic rejection C4d deposits have been found in the peritubular capillaries of 34% of late allograft biopsies [62]. The percentage of C4d-positive biopsies is even higher if only biopsies with chronic rejection were included (61%) and in the subgroup of patients with transplant glomerulopathy (53%) [62]. The presence of C4d deposits in the allograft biopsies seems to be an independent predictor of kidney graft dysfunction [63]. C4d deposits in peritubular capillaries of biopsies taken within the first 6 months posttransplantation were associated with inferior graft survival at 1 year. This risk was reduced if the treatment of the recipient with mycophenolate mofetil was started 2 to 4 hours before transplantation [64]. Recently, not only C4d deposits in the peritubular capillaries were studies but specific antibodies became available that allowed the study of glomerular C4d deposits in paraffin-embedded material [65]. Glomerular C4d deposits have been found in about 16% of renal allograft biopsies [65]. In biopsies with transplant glomerulopathy the majority of patients have glomerular C4d deposits [66].

Antibodies against HLA antigens

The high percentage of C4d-positive biopsies with CAN supports a role for humoral immune responses in the pathogenesis of chronic rejection. Humoral responses can be directed against HLA or non-HLA antigens of the graft. Most attention has been paid to antibodies directed against donor HLA antigens. The presence of C4d in biopsies correlated well with anti-donor HLA antibod-

ies, 88% of patients with C4d deposits had antibodies in their circulation [67].

After kidney transplantation anti-HLA antibodies have been found in 12–60% of recipients. Anti-HLA antibodies have also been found in recipients of heart, lung, liver and cornea transplants [29]. Renal transplant recipients with anti-HLA antibodies were 5–6 times more likely to develop CR and lose their grafts [32]. De novo formation of antibodies after transplantation is correlated with the poorest graft outcome, although the presence of antibodies does not necessarily cause immediate graft loss [29, 68]. The presence of pretransplant antibodies against both HLA class I and II antigens is most detrimental to graft survival, whereas the presence of only antibodies against class I or II antigens does not affect renal graft survival [30]. The majority (77%) of acute and chronic rejection episodes occur in the absence of circulating anti-HLA antibodies measured at the time of rejection [69]. However, almost all patients with chronic rejection have had circulating antibodies against HLA antigens, but not necessarily donor HLA antigens [31]. At the time of rejection, antibodies might be not detectable in the circulation due to binding to the inflamed tissue [70], underscoring the need for multiple measurements in time.

Antibodies against non-HLA antigens

Since not all patients with chronic rejection have circulation antibodies directed against donor-HLA antigens and the majority of biopsies showed C4d deposits, antibodies against other, non-HLA antigens might be involved. Antibodies against non-HLA antigens can be reactive with endothelial cells but also with other nonendothelial cell antigens. In clinical transplantation little is known about the production of antibodies reactive with non-HLA antigens upon kidney transplantation. Antibodies reactive with a nonclassic HLA class I molecule on microvascular endothelial cells in the kidney have been identified in patients with irreversible rejection [71]. Furthermore, nonspecified antibodies binding to endothelial cells have been found in renal transplant recipients [72]. Antiendothelial cell antibodies were more frequently found in patients with at least one failed graft as compared to stable renal transplant recipients. Not only kidney transplant recipients produce antibodies against non-HLA antigens these can also be found in recipients of cardiac or liver grafts [60].

In experimental models for chronic renal allograft rejection antibodies directed against glomerular antigens have been described [60, 73]. In these rats antibodies reactive with mesangial cells and GBM antigens were found. The antigens involved were identified and include the heparan sulfate proteoglycan perlecan and the $\alpha1$ chain of collagen type VI in association with the $\alpha5$ chain of collagen type IV [73]. Alterations in especially the

heparan sulfate proteoglycans in the GBM can result in the development of GBM lesions and the induction of proteinuria [74]. This suggests that these antibodies are involved in the development of the lesions found in patients with transplant glomerulopathy. In fact, in patients with transplant glomerulopathy glomerular C4d deposits were found [66] and these patients have circulating antibodies reactive with GBM antigens [75]. Antibody responses were only detected in patients with TGP but not in patients without glomerular abnormalities. The presence of antibodies correlated with the number of rejection episodes. Detailed characterization of the antibody responses in these patients revealed that the GBM-heparan sulfate proteoglycan agrin was recognized [75]. Antibodies reactive with the renal tubular basement membranes have previously been described in patients with chronic rejection [76]. This suggests that antibodies reactive with renal basement membranes play a role in the development of chronic rejection. However, large-scale studies will be necessary to determine the specificity and predictive value of these antibodies.

PROGRESSION FACTORS

Renal function

Beyond certain time points progression of chronic transplant dysfunction is largely dependent on nonimmune factors (Table 1). Loss of renal mass with subsequent glomerular hypertension and proteinuria and further loss of nephrons play a role as a progression factor that controls the rate of decline to end-stage renal failure [77]. The importance of this type of injury is illustrated by a lower graft survival rate of transplants that come from female, black, very young, or very old donors compared with transplants from donors supposed to be endowed with a larger nephron mass [78]. However, other studies could not confirm an effect on graft outcome of donor kidney size or the ratio of donor versus recipient body surface area as surrogate marker of renal mass [79].

The relation between renal dysfunction and subsequent chronic rejection or graft failure has been reported in several ways. The relation between renal dysfunction at this time point and late failure might be confounded by other risk factors like donor age and previous acute rejection episodes [22]. Analysis of the course of renal function is another way to assess the relationship between renal dysfunction and graft failure. A negative slope of glomerular filtration rate between 6 and 12 months is significantly associated with the occurrence of chronic rejection after 12 months [80]. Recently, changes in allograft function were systematically investigated. The best predictor of failure, a 30% decline in inverse creatinine, was superior to baseline function and independent of other risk factors of chronic rejection [6].

Donor age

Increasing donor age is associated with atherosclerosis, glomerulosclerosis, tubular atrophy, and interstitial fibrosis and is associated with decreased long-term graft function [81]. In addition to increased graft loss, the use of older donors also results in increased cardiovascular events and thereby decreases patient survival [82]. In single center studies, old donor age is an independent risk factor of chronic rejection [44]. Kidneys from donors older than 55 years have an increased risk of chronic rejection in the UNOS database, but also of nonrejection failure [28]. These findings are ascribed to the reduced renal mass, leading to glomerular hypertension, or more recently to accelerated senescence [83]. Furthermore, it has been suggested that the higher rate of acute rejection episodes in kidneys from older donors reflects increased immunogenicity [84]. With the reduction of acute rejection episodes and progression of transplant care, the impact of donor age on outcome has been attenuated [85].

Donor source

The higher graft survival of living donor kidneys compared with cadaveric kidneys is often used to illustrate the importance of early injury. Recipients of unrelated living donors have better long-term survival than recipients from cadaveric donors with better degrees of HLA matching [86]. However, differences in graft survival are evident only in recipients undergoing acute rejection episodes [87, 88]. In a group of 588 recipients (326 cadaveric and 260 living) treated for acute rejection episodes a 10-year censored graft survival of 45% was recorded compared to 91% in recipients without acute rejection episode. Graft loss from chronic rejection occurred in 30% of cadaveric and 16% of living donors [87]. These data indicate that the benefit of living related transplantation results from the fact that a living related graft progresses from acute to chronic rejection at a slower rate than a cadaveric graft and that the higher rate of survival is attributed to the fact that kidneys from living donors are uniformly healthy [88].

Hypertension

Graft survival is inferior in hypertensive patients but the relation has been shown to be confounded by renal function [89]. Both high systolic and diastolic blood pressures at 1 year posttransplant are significant predictors of long-term graft survival [90]. The rate of deterioration of graft function is associated with diastolic blood pressure. Blood pressure after acute rejection episode correlates with graft survival, in contrast to patients without acute rejection episodes [91]. Hypertension may promote atherosclerosis within renal blood vessels or glomerular

hypertension, which can increase glomerular permeability and consequently enhance protein trafficking.

Proteinuria

Proteinuria at 1 year posttransplantation is an important risk factor for chronic rejection [7, 92]. Transplant patients with persistent proteinuria of more than 2 g per day have a high risk of subsequent deterioration of renal function. Patients on cyclosporine and persistent proteinuria of greater than 1 g/day as a result of chronic rejection have a compromised 5-year graft survival [93]. Resorption of excessive amounts of protein by proximal tubular epithelial cells leads to release of inflammatory mediators from tubular cells and subsequent interstitial injury.

Hyperlipidemia

Hyperlipidemia is a common problem as elevated cholesterol levels are present in 70% to 80% and hypertryglyceridemia in 30% to 40% of transplant patients [94]. Hypertryglyceridemia is correlated with graft dysfunction in some studies [94]. Hypercholesterolemia posttransplantation is also associated with graft dysfunction or death-censored graft loss [95]. Hypercholesterolemia is an independent risk factor for kidney graft loss from chronic rejection in male patients with previous acute rejection episode [96]. Outcome may be adversely affected through the accumulation of oxidized low-density lipoprotein (LDL) in the renal interstitium and the development of fibrosis [97].

Smoking

Smoking is a risk factor for renal outcome as documented in several studies [98]. A recent report revealed that 24% of transplant recipients smoke cigarettes at time of transplantation, of which 90% continues this habit after transplantation. Smokers had a relative risk on death-censored graft loss of 2.3, which was independent of acute rejection episodes [99]. Chronic cigarette smoking reduces renal plasma flow, probably by increasing the synthesis of the vasoconstrictor endothelin and by reducing the generation of the vasodilatory endothelial nitric oxide.

TISSUE RESPONSE TO INJURY

Donor brain death and I/R injury

On the long-term, the survival of renal allografts derived from brain death donors was less compared to living donors [100]. In clinical transplantations, a substantial amount of the kidney grafts are derived from brain death donors underscoring the need to understand this phenomenon. Brain death results in activation of various inflammatory mediators in renal tissue, resulting in an influx of mononuclear cells [101]. In these kidneys more damage is present than in living donors and brain death results in an up-regulation of selectins on the endothelium, leading to increased leukocyte adhesion and thus increased inflammation [102].

Not only donor brain death results in increased inflammation, also prolonged ischemia times in transplantation of cadaveric organs contributes to decreased function of these grafts compared to living (related) donation. Prolonged ischemia leads to the increased occurrence of delayed graft function, which again can be a predictor for worse long-term graft survival [39]. Reperfusion with oxygenated blood is important in restoring the substrates for oxidative metabolism but this can result in the production of free oxygen radicals and thus oxidative stress [103]. This triggers endothelial cells and leukocytes and results in the up-regulation of adhesion molecules and cytokine production contributing to renal damage and inflammation [103].

The damage of renal I/R injury is independent of the immunologic background [104] although the immune system becomes activated during this process. During ischemia, IL-12 and IL-18 are up-regulated in response to damage, resulting in the up-regulation of INF-γ and subsequently in increased expression of MHC class I and class II antigens [105]. The endothelium becomes activated and costimulatory molecules are up-regulated facilitating T cell interactions possibly leading to rejection [100].

Acute rejection episodes

Acute rejection episodes can be important in the progression to chronic rejection. In a rat kidney retransplantation model, early retransplantation in syngeneic recipients prevented progression to chronic rejection, but later retransplantation resulted in progression to chronic rejection in the absence of ongoing immune responses [106]. This suggests that not all factors in the process of chronic rejection are dependent on alloantigen-mediated immune responses. In clinical transplantation, persistent or repeated subclinical acute rejection seemed to contribute to the development of CAN [26]. So persistent immune activation contributes to the ongoing process of deterioration of graft function.

Acute rejection episodes are most detrimental if the organs are derived from donors over 50 years of age [84]. Graft survival of older donor kidneys is lower compared to organs from younger donors, this effect is more pronounced if the grafts that experienced one or more acute rejection episodes [84].

Donor age

Donor age is one of the most important progression factors for the development of chronic rejection [107].

The effect of acute rejection episodes on older donor kidneys suggests that these kidneys have more difficulties to cope with the repair of damage. In addition, the increased occurrence of delayed graft function in older donor kidneys [108] also suggests that the graft has more problems in overcoming I/R damage.

CELLULAR AGING IN AGING AND SENESCENCE

Cells in senescence, induced by telomere shortening or other unknown stimuli, become arrested in the G_1 phase of the cell cycle and are resistant to apoptotic and other external signals. The cells remain metabolically active and are called to be in replicative or cellular senescence [109]. Cells in senescence show alterations in shape, and expression of extracellular matrix metalloproteinases and cytoskeletal collagens [110]. Cells in senescence have several characteristics, including shortened telomeres, increased expression of specific tumor suppressor genes, and increased activity of senescence associated β-galactosidase (SA β-gal) [111, 112]. The cell cycle inhibitors p16 and p21 are frequently studied in relation to cellular senescence and both are thought to be involved in the G_1 arrest observed [113].

Senescence and aging

The lesions observed in aging kidneys are very similar to the lesions observed in kidneys with CAN; therefore, it was proposed that the underlying process might be similar [83]. In the aging kidney telomere shortening was predominantly found in the cortex and much less in the medulla [114]. In addition, p16 expression in the kidney correlates with age [115].

In an animal study using rats of different ages, no shortening of telomeres was observed during aging. However, the expression of p16 increased and SA β-gal and lipofuscin (an aging pigment) accumulated in the tubular epithelial cells, thus indicating that shortening of telomeres is not required for the induction of the senescent phenotype [116, 117]. However, if specifically measured, the percentage of short telomeres was significantly increase with ageing compared to young organs in all organs except the brain [117]. The finding of normal length telomeres in kidneys that have phenotypic characteristics of senescence suggests that shortening of telomeres is not essential. However, it could still be that other changes in telomere structure are present. The cell cycle regulator p21 is also described in tubular epithelial cells of aging rats [118], supporting the development of senescent tubular epithelial cells in aging.

Senescence in renal transplantation

In human renal allograft biopsies with CAN an increased expression of p21 was found. P21 protein was present in glomerular, tubular, and interstitial cells, but only the expression in tubular epithelial cells correlated with the number of acute rejection episodes [119]. Furthermore, in biopsies with CAN, they also found increased expression of p16 and p27, another member of the Kip family [120]. The tubular expression of p16 and p27 in normal kidneys was dependent on age. SA β-gal staining of kidneys with CAN was associated with the severity of CAN [121]. In addition the age of the donor was a major determinant in the occurrence of replicative senescence [121].

We used the Fisher (F344) to Lewis (Lew) rat model of chronic renal allograft rejection to determine telomere shortening, p21 and p16 expression and SA β-gal accumulation [122]. Telomere length analysis of both F344 to Lew and Lew to F344 renal allografts revealed shortening of telomeres. More detailed analysis revealed shortening of telomeres after 45 minutes of warm ischemia. This supports the hypothesis that oxidative stress is responsible for telomere shortening [123, 124]. Furthermore, we found a transiently increased expression of p21 at day 7, and p16 accumulation in tubular epithelial cells starting at day 7 both in rats with or without chronic rejection. However, SA β-gal expression was exclusively observed in kidneys with chronic rejection.

Based on the data of Melk et al [116] and our results [122] we conclude that telomere shortening is not required and not sufficient to induce replicative senescence in rat kidneys. More important, these papers together show that senescence is present both in ageing rat kidneys and in kidneys allografts with chronic rejection, which supports the hypothesis that chronic rejection is a representation of accelerated aging [83]. However, both in aging rats and in grafts with chronic rejection the characteristics of senescence were only found in the tubulointerstitial compartment. Therefore, we believe that senescence is most important in the development of the tubulointerstitial lesions, including tubular atrophy, but that additional mechanisms are required for the development of specific glomerular and vascular lesions that are observed in grafts with chronic rejection.

PREVENTION AND TREATMENT
Prevention

Because of the lack of effective treatment, efforts should be made to prevent chronic rejection. Measures are directed to the risk factors of chronic rejection, including sensitization, histoincompatibility, acute rejection episodes, and insufficient immunosuppression. Allocation strategies should primarily aim for HLA-matched transplants that have an established superior long-term outcome compared to HLA-mismatched grafts [34, 125]. In the case of mismatches, functional matching should aim for the selection of donors with HLA molecules

nonstimulatory to both the cellular and humoral immune system of the recipient [126]. In this way, sensitization due to a transplant could be prevented and facilitate future transplants in the case of graft loss. Besides optimal immunosuppression, prevention of premature graft failure requires a multifactorial approach aiming at early and tight control of blood pressure, proteinuria, lipids, glucose, weight, and smoking [127, 128].

Monitoring

Protocol biopsies and immune monitoring of both the cellular and humoral response are potential tools to detect subclinical rejection activity beyond the early phase after transplantation. Protocol biopsies and treatment of subclinical acute rejection episodes with corticosteroids may lead to better outcome [26]. Gene expression profiling of acute rejection biopsies showed increased expression of immunoglobulins supporting a role for antibodies in the pathogenesis of acute and chronic rejection [129]. The enzyme-linked immunosorbent spot assay (ELISPOT) of peripheral blood lymphocyte reactivity to HLA peptides or donor-stimulator cells might be a useful method of measuring indirect alloreactivity [130]. Early detection of in situ C4d deposition and circulating donor-specific antibodies may lead to timely specific strategies for humoral rejection [131].

Immunosuppressive treatment

The introduction of cyclosporine Neoral, tacrolimus, and mycophenolate mofetil in the 1990s has been associated with a reduction in the incidence of acute rejection episodes during the first year after transplantation [132, 133]. Unfortunately, longer follow-up of these agents did not reveal much effect on long-term graft survival or the prevalence of chronic rejection [24, 132]. Although some papers suggest a slightly improved long-term graft survival [1], improved graft survival by the prevention of late acute rejection episodes has been observed in patients who stay on mycophenolate for a prolonged period of time [134, 135]. Rapamycine (sirolimus) may have the ability to reduce the rates of chronic rejection by further reduction of the incidence of acute rejection episodes and inhibition of smooth muscle cell proliferation [136]. Manifestations of chronic rejection are also inhibited by the novel antiproliferative macrolide everolimus (a sirolimus derivative) in preclinical models [137]. Furthermore, the use of sirolimus-eluting stents in coronary arteries supports the vasculoprotective effects of sirolimus [138].

Calcineurin inhibitor nephrotoxicity induced by both cyclosporine A and tacrolimus still results in loss of graft function [139]. Rapamycin and mycophenolate mofetil are immunosuppressive drugs that are not nephrotoxic [139]. Prevention of calcineurin inhibitor nephrotoxicity by using decreased dosing or switching to nonnephrotoxic

immunosuppressive drugs might contribute to increase long-term graft survival [140]. In addition everolimus seems promising as immunosuppressive therapy in patients with calcineurin inhibitor nephrotoxicity [139, 141].

There is no established treatment for chronic rejection, mainly because of the presence of irreversible damage at time of diagnosis. Nevertheless, in early phases of the disease or in those patients in whom inadequate immunosuppression is the precipitating cause, a change in the immunosuppressive regimen may stabilize or even reverse part of the renal dysfunction. However, randomized trials regarding the treatment of chronic rejection have not been reported. If there is evidence of coexisting acute rejection episode, a beneficial response of a trial with methylprednisolone has been observed [142]. In some recipients on cyclosporine (Neoral), conversion to tacrolimus resulted in sustained improvement of renal function [143]. Adding mycophenolate mofetil to maintenance immunosuppression provided no clear benefit in a small retrospective study [144]. Recently, a large multicenter randomized trial was conducted to study the effect of mycophenolate mofetil in comparison to azathioprine in cadaveric kidney transplantation [145]. In this study there was no difference in the occurrence of rejection episodes and since the costs for mycophenolate were 15 times higher they concluded that azathioprine should be the first choice [145]. In contrast, others have reported that treatment with mycophenolate mofetil reduced the risk of chronic renal allograft failure [146]. Biopsy-proven CAN at 1 year posttransplantation was decreased in patients treated with mycophenolate mofetil compared to azathioprine treatment [147] and graft survival at 3 years posttransplantation seemed better if mycophenolate mofetil was used [36]. Furthermore, reduction and possible withdrawal of calcineurin inhibitors with either the addition or continuation of mycophenolate mofetil slowed the rate of loss of renal function in patients with CAN [148]. Reduction of antidonor antibody synthesis by the combination of mycophenolate and tacrolimus is a novel promising approach for the treatment of humoral chronic rejection [149].

Nonimmune interventions

Nonimmunologic measures to halt or retard progression of chronic rejections have focused on aggressive control of blood pressure, proteinuria, and hyperlipidemia. Treatment of hypertension reduces progression to renal failure in native kidney diseases but this effect has not yet been proven in renal transplantation. In patients on calcineurin inhibitors dose reduction or withdrawal may improve blood pressure [148]. Calcium entry blockers, beta blockers and angiotensin-converting enzyme (ACE) inhibitors have similar antihypertensive efficacy after renal transplantation and are often used in combination to

achieve adequate control. Significant reduction of proteinuria has been reported as a beneficial effect of ACE inhibitors and angiotensin II receptor antagonists in clinical transplantation [150, 151]. These drugs have the potential to prevent the progression of chronic failure. In a small group of transplant recipients the slope of the curve of inverse serum creatinine and time decreased when they were subjected to a low-protein diet of 0.6 g/kg [152]. It is not yet clear whether treatment of hyperlipidemia slows the progression of chronic transplant dysfunction, but in the presence of concomitant risk factors of cardiovascular disease an increasing number of patients are being treated with statins [153].

CONCLUSION

Chronic rejection remains an important problem upon renal transplantation, not at least since its pathogenesis is still not completely understood and thus treatment is difficult. Chronic rejection seems to be an accumulation of damage obtained in both the early posttransplant period and later in time. In addition, the renal quality to start with is an important factor and injury by repeated (sub)clinical rejection episodes seems a major contributor. Risk factor analysis revealed that besides acute rejection episodes, recipient age, race, sensitization, HLA matching, pretransplantation injury, and immunosuppression determine outcome. Graft survival is furthermore limited by progression factors, including renal dysfunction, donor age, donor source, hypertension, proteinuria, hyperlipidemia, and smoking. In the pathophysiology immune responses appear crucial, especially humoral responses against both HLA and non-HLA, tissue-specific antigens seem involved. Moreover, the tubulointerstitial lesions present during CAN might be a consequence of accelerated aging. In order to find appropriate therapeutic strategies for prevention and treatment of chronic rejection more detailed insights in the pathogenesis are indispensable.

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