Transpl Int (2003) 16: 137–145
DOI 10.1007/s00147-003-0554-x

REVIEW ARTICLE

Simone A. Joosten
Cees van Kooten
Leendert C. Paul

Pathogenesis of chronic allograft rejection

Received: 8 November 2002
Revised: 15 January 2003
Accepted: 23 January 2003
Published online: 25 February 2003

S.A. Joosten (✉) · C. Kooten
L.C. Paul
Department of Nephrology,
C3P, Leiden University Medical Centre,
Albinusdreef 2, 2333 ZA, Leiden,
The Netherlands
E-mail: s.a.joosten@lumc.nl
Tel.: +31-71-5263964
Fax: +31-71-5248118

Abstract Chronic allograft nephropathy (CAN) is, besides death of the recipient with graft function, the most common cause of renal transplant loss. It is characterized by loss of function and replacement of tissue by fibrotic material. The pathogenesis is not clear, but seems to be multifactorial and involves events both early and late after transplantation. Alloantigen-dependent mechanisms seem to be crucial for the development of chronic rejection (CR). Although modern immunosuppressive drugs have reduced the number and severity of acute rejection episodes, their effects on CR are less obvious. In this review we discuss the role of direct and indirect antigen presentation in the development of CR, and we will focus on the production of antibodies directed against HLA and non-HLA antigens on the graft and their influence on CR.

Keywords Chronic rejection · Antibodies · Tissue-specific · Transplantation · Indirect presentation

Chronic rejection

Chronic allograft nephropathy (CAN) is, besides death of the recipient with graft function, the most common cause of renal transplant loss [1]. It accounts for 50%–80% of graft failures in surviving patients and responds poorly to treatment with increased amounts of steroids or to antibody treatment [1]. CAN occurs at various time points after transplantation (Tx), generally after the first 6 months, but it can occur years after Tx and is characterized by a slow decline in function of the organ. Recent data show that over the past years, the survival of kidney grafts has improved both in the short and long term, indicating that there is some progress in prevention of CAN [2]. Histologically, it is characterized by vasculopathy, glomerulopathy, tubular atrophy and interstitial fibrosis. The vascular lesions show intimal thickening, smooth muscle cell proliferation and multilayering of peri-tubular capillary basement membranes [1]. Various alloantigen-dependent and alloantigen-independent factors result in a final common pathway of excessive repair, resulting in replacement of the original tissues by fibrotic tissue and in loss of function. One has to establish the diagnosis of CAN by taking into account other causes of diminished graft function, including recurrent or de novo renal diseases. CAN is a descriptive term, referring to the histological lesions that can be found in renal allografts with deteriorated function. One of the causes of CAN is chronic rejection (CR), which is the antigen-dependent immune process leading to the lesions observed in CAN.

Allotransplantation of organs results in activation of the immune system by alloantigen-dependent processes. Recognition of foreign antigens on cells of the graft results in activation of specific immune responses. However, alloantigen-independent processes, including donor brain death and ischemia-reperfusion, also induce or amplify the immune response via the production of reactive oxygen species, pro-inflammatory mediators and growth factors, increased expression of adhesion

molecules, and upregulation of both HLA class-I and class-II molecules [3, 4, 5, 6]. In rat transplantation models, grafts from brain-dead donors are lost at a higher rate than those from living donors [3]. Brain death causes upregulation of selectins on the endothelium and thereby to leukocyte adhesion [7]. The upregulation of major histocompatibility complex (MHC) class I-and class-II antigens after ischemia-reperfusion injury is mediated by interferon (IFN)-γ, which is induced by interleukin (IL)-12 and IL-18 production in response to tissue injury [8]. Subsequently, the endothelium is activated, and upregulation of co-stimulatory molecules facilitating T-cell interactions [3, 4, 5]. Tubular epithelial cells can go into apoptosis as a result of increased amounts of free radicals [9, 10]. Together, this results in increased recruitment of inflammatory cells and inflammatory processes, independent of alloantigens [11, 12]. Thus, these alloantigen-independent processes result in upregulation of molecules involved in alloantigen-dependent processes and can, therefore, be considered as progression factors for CAN.

In this review we focus on alloantigen-dependent processes in CR and the mediators involved. The contribution of direct and indirect antigen presentation in the induction of antibodies reactive with both HLA and non-HLA antigens will be described in greater detail.

Mediators of chronic rejection

Transplantation models have shown that syngeneic transplants remain free of CR lesions during the timespan in which allogeneic grafts develop CR. The lesions observed in syngeneic transplants resemble the lesions of ageing. This implies that the transplantation procedure by itself is not the sole factor inducing CR, although it can result in activation of some of the inflammatory mediators. However, syngeneic renal transplantation does result in increased recruitment of professional antigen-presenting cells [12]. Thus, CR is primarily alloantigen dependent and is influenced by early injury and ongoing host allo-responses [13].

Additional evidence comes from rat kidney re-transplantation models, in which allogeneic grafts were re-transplanted into syngeneic recipients at various time points after transplantation. A single, acute rejection episode was completely reversible and did not progress to CR if the kidneys were re-transplanted in the early stages [14, 15]. Late after transplantation, re-transplantation does not result in reversal of the lesions, suggesting that later in time alloantigen-independent factors become increasingly important [14].

Various factors and cells play a role in the pathogenesis of CR, but the mechanism by which it develops is not yet clear. In experimental transplantation models, different cell types have been investigated to determine their respective contributions to disease development. In the initial phases of the transplantation procedure, during ischemia-reperfusion injury, $CD4^+$ T cells are essential for the development of inflammatory responses [16]. Furthermore NUDE mice, that lack functional T cells, or recombinase-activating gene (RAG-1) deficient mice (RAG deficiency resulting in lack of functional B and T cells due to lack of immunoglobulin 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 CR, whereas mice that received $CD8^+$ T cells did not develop these lesions [17]. $CD8^+$ T cells and B cells seem to be the effector mechanisms, but they are dependent on the $CD4^+$ T-cell responses in this model. However, in another mouse heart transplantation model, $CD8^+$ T cells have been said to play a causative role in the induction of CR [18]. Recently, NK cells have been implicated in playing a role in cardiac allograft vasculopathy in the absence of a specific immune response, using SCID and RAG-deficient mice [19].

The role of antibodies in chronic transplant rejection has been studied in greater detail in various animal models. In a mouse cardiac allograft model, which used SCID mice (lacking B-cell and T-cell responses) as recipients, almost no lesions developed [20, 21]. Injection of anti-donor antibodies into the SCID recipient resulted in development of obstructive coronary lesions [22]. In another model, which used IGH knockout mice (lacking functional B cells) as recipients of cardiac allografts in the presence of neutralizing anti-CD4 antibodies, no chronic rejection was found, in contrast to wild-type animals treated with anti-CD4 antibodies [23]. Furthermore, an aortic allograft model with RAG-2-deficient recipient mice has shown that donor-specific cellular and humoral responses are required for both the initiation and perpetuation of CR [24]. Altogether, these data imply that humoral responses are important in chronic rejection.

Binding of antibodies directed against HLA or non-HLA antigens in the graft can result in activation of the complement system, leading to formation of the membrane-attack complex and lysis of the target cells. Alternatively, or in addition, Fc receptors on the surface of hematopoietic cells can interact with antibody–antigen complexes, resulting in stimulation or inhibition of cellular responses. This may lead to a variety of biological effects, including activation of cell-mediated killing, induction of mediator release, uptake, removal and destruction of antibody-coated particles, and regulation of immunity. On the other hand, antibody binding to HLA antigens can also exert its effects on the target cells. Binding to endothelial or smooth muscle cells can lead to increased expression of growth factors and their receptors, and increased proliferation resulting in transplant arteriosclerosis [25, 26].

Allo-immune responses

After organ transplantation the immune system of the recipient encounters various foreign antigens in the graft. All donor antigens that are different from those of the recipient may evoke an immune response if they are correctly presented to the immune system of the recipient. Individuals are not actually tolerant to self-proteins: they are tolerant to the small subset of peptide fragments from the self-proteins that can bind to any of their own HLA molecules [27]. Presentation of these peptides to T cells can thus result in an immune response. In clinical transplantation, donor and recipient are HLA antigen-matched as closely as possible, as increased HLA mismatching increases the immunogenicity of the graft, stimulating host allo-responses [13]. Furthermore, the sharing of HLA class-I antigens is associated with improved graft survival [28]. These data implicate the importance of antigen presentation in the pathogenesis of CR.

Direct vs indirect antigen presentation

After transplantation of organ allografts, there are two pathways of antigen presentation. Firstly, direct presentation of donor alloantigens by donor-derived antigen-presenting cells (APCs) to recipient T cells take place. This will mainly result in activation of type-1 responses and thus activation of $CD8^+$ cytotoxic T cells. Secondly, donor alloantigens can be presented after uptake by infiltrating recipient APC to recipient T cells, the indirect presentation route. Direct presentation is thought to be mainly involved in acute rejection [13], whereas indirect presentation is suggested to be important for chronic rejection (Fig. 1) [13, 29, 30, 31, 32, 33, 34]. Experimental data that support a role for indirect antigen presentation in CR include the observations that in a rat renal transplantation model, depletion of donor APCs from the graft does not influence the susceptibility to CR [35]. In addition, transplantation of allografts into immunocompromised recipients and re-transplantation in immunocompetent recipients results in CR, in the absence of acute rejection [35]. Immunization of rats or miniature swine with MHC class-I or class-II peptides before cardiac transplantation promotes transplant vasculopathy, supporting a role for indirect antigen presentation in the development of CR (Fig. 1) [34, 36]. In patients with CAN, higher frequencies of $CD4^+$ T cells activated by donor antigens in an indirect manner have been found [37]. Since it has been suggested that indirect presentation results in production of alloantibodies, and it is correlated with CR, antibodies are very likely to play a role in CR.

Induction of an immune response after recognition of antigens requires a second or co-stimulatory signal between the APC and the T cell. These co-stimulatory

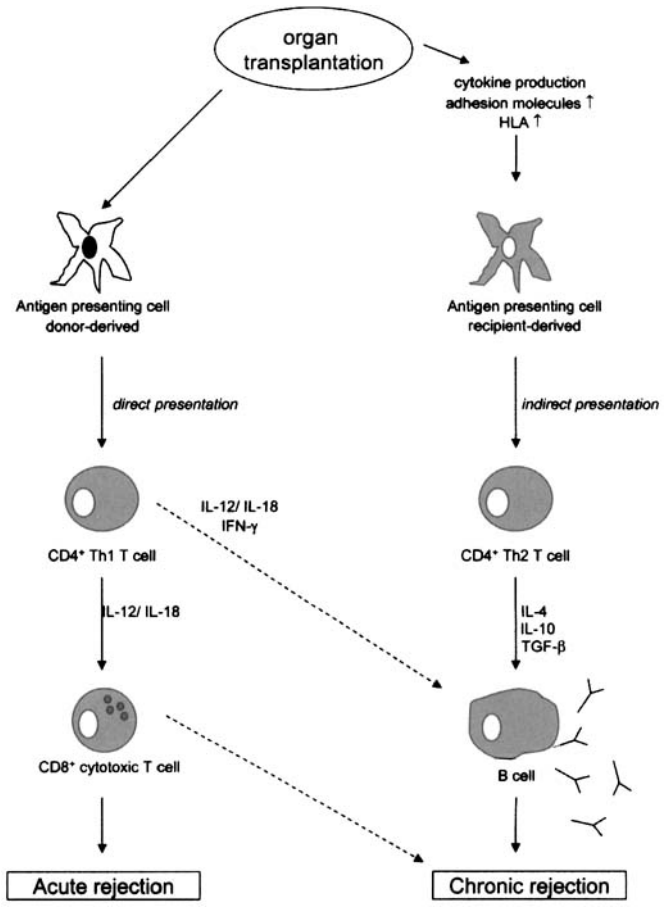

Fig. 1 Organ transplantation results in activation of both direct and indirect antigen presentation pathways leading to acute or chronic rejection

signals are essential for the induction of immune responses and thus for transplant rejection [38]. Co-stimulation blockade has been used in various allograft models, involving different co-stimulatory pathways. One of the co-stimulatory pathways frequently studied involved the CD28-B7 molecules. CD28 on T cells binds the B7 molecules on APCs after ligation of the T-cell receptor with antigens presented by APC. Injection of a single dose of anti-CD28 at the time of transplantation prevents the development of histological and functional characteristics of CR [39, 40]. In addition, blockade of T-cell activation with CTLA4-Ig, also inhibiting B7-CD28 interactions, prevents the development of renal ischemia damage and improves long-term graft survival [41, 42, 43]. This suggests that T-cell co-stimulation is important for the early induction of CR.

Another important co-stimulatory pathway involved in CR is the CD40–CD154 (CD40 ligand) pathway [44]. In various animal models this pathway has been blocked, by use of an anti-CD154 antibody. In mice aortic transplantation using CD40 knockout mice or anti-CD154 antibodies and anti-CD8 depleting

antibodies, the development of transplant arteriosclerosis is delayed, but it does not prevent the progression [45, 46]. In these mice there was an increased production of donor-specific IgG1 antibodies and an increase in IL-4 mRNA. The production of IL-4 seemed to be crucial for the development of transplant arteriosclerosis, indicating that Th2 responses are involved in this process [45, 46]. However, the effects of CD40–CD154 co-stimulation blockade are different in mice with impaired Th1 or Th2 responses. This indicates that the type of cytokines present in the graft also determines the efficacy of co-stimulation blockade [47]. In renal transplantation in non-human primates, acute rejection is prevented with anti-CD154 antibodies. In these animals antibodies that are reactive with donor antigens can still be formed, although the contribution of these antibodies to the development of CR is uncertain [48]. Further evidence for a crucial role of CD40–CD154 in CR is the induction of CR with an activating CD40 antibody in the absence of $CD4^+$ T cells [49]. Although blockade of CD40–CD154 interactions seem promising, recent data suggest that long-term blockade of CD40–CD154 is not sufficient to prevent the development of CR [50].

T-helper subsets

Antigen presentation via the indirect pathway mainly results in the activation of Th2 $CD4^+$ T lymphocytes [13, 33, 34]. Activation of Th2 $CD4^+$ T lymphocytes results in the production of cytokines, growth factors and alloantibody production by B cells (Fig. 1). Polarization of $CD4^+$ T cells into either Th1 or Th2 types is influenced by various factors, including the nature and strength of the antigenic stimuli, the type of antigen-presenting cells, the compartment where the response takes place, and most importantly, the cytokine milieu [33, 51]. Recently, it has been shown that the nature of the transplanted organ determines whether direct or indirect responses are evoked [52]. In the direct presentation, T cells encounter APCs that present high levels of allopeptides, resulting in differentiation into Th1 cells. In the indirect presentation, on the other hand, only limited amounts of allopeptides are presented to the T cells, resulting in Th2 differentiation [53]. Differences in strength of T-cell receptor signalling affect several factors involved in the regulation of IL-4 transcription. If sufficient IL-4 is present, Th2 responses are dominant over Th1 responses, thus IL-4 is a key-regulator in this process [54]. Grafts with CR express mainly Th2 cytokines, including transforming growth factor-β (TGF-β) and IL-4, IL-5 and IL-10. Th1 cytokines have also been found, including IL-2 and IFN-γ, suggesting that Th1 responses can also be involved [51, 55, 56, 57, 58, 59, 60]. The absence of IFN-γ resulted in less-severe vascular lesions in cardiac allografts [61], although severe necrosis developed [62]. This supports a role for (locally produced) Th1 cytokines in CR.

Th2 cytokines, especially IL-4, are potent inducers of antibody production by B cells. Th2 cytokines are involved in the regulation of many processes, including ECM production and degradation, vascular smooth muscle cell proliferation and migration into the intima, and alloantibody production [33, 63]. Type-2 responses play a critical role in the regulation of matrix metalloproteinases (MMPs) that are involved in degradation of ECM molecules, resulting in the release of growth factors and cytokines and generation of chemotactic peptides. IL-4, IL-10, and TGF-β, typical type-2 cytokines, inhibit the synthesis of MMP by smooth muscle cells and macrophages and stimulate the synthesis of inhibitors of MMP [64]. The action of both TGF-β and IL-4 can result directly in upregulation of matrix production [65, 66]. Together, the increased production of ECM induced by TGF-β and the decreased degradation result in accumulation of ECM in the graft.

Antibodies

Since alloantibodies seem to be major players in CR, we will describe the various antibody responses in detail. Antibodies are suspected of playing a role in CR of the kidney, liver, heart, lung, and cornea (Table 1) [67, 68, 69, 70, 71, 74, 75, 87]. In acute rejection of renal allografts, antibodies have been shown to be involved if vascular lesions are present. Antibodies are mainly reactive with class-I and class-II HLA antigens [72]. Long cold-ischemia times during transplantation result in increased immunogenicity; recipients of kidneys with prolonged cold-ischemia times have significantly more anti-HLA class-I antibodies after transplantation [73]. Deposition of immunoglobulins in the graft is transient and hard to detect. Since antibody binding results in deposition of complement, and the complement split product C4d is more stable, this can be used as a marker for humoral rejection. Early humoral rejection (within

Table 1 Antibodies described in clinical and experimental organ transplantation

Organ	Target	References
Kidney	HLA	[71,75, 76,77, 82]
	Perlecan/collagen	[78]
	Mesangial cells	[94,96]
	Endothelial cells	[85,86, 87,88]
Heart	HLA	[75,80, 81]
	Endothelial cells/vimentin	[90,91, 92,103]
	Cardiac myosin	[89]
Liver	HLA	[21,75]
	Tissue antigens	[67,93]
Lung	HLA	[75,78, 79]
Cornea	HLA	[68,75, 83]

the first 6 months of transplantation) has a strong impact on allograft survival [70]. Furthermore, in late allograft biopsies, C4d deposits were detected in 34% of the biopsies. In kidneys that already have advanced lesions of CR, glomerular basement membrane lesions, or allograft arteriopathy, C4d depositions have been found in approximately 60% of biopsies [74]. The high percentage of C4d positivity implies a role for antibodies in the pathogenesis of these lesions. The presence of C4d in biopsies correlated well with anti-donor HLA antibodies; 88% of patients with C4d deposits had antibodies in their circulation [70, 74].

Antibodies can be reactive with HLA, endothelial cell antigens or tissue-specific antigens (Table 1) [69]. Antibodies reactive with HLA can be detected easily by various methods, including complement-dependent cytotoxicity, flow cytometry and ELISA. The number of reports on tissue-specific antigens is rather limited, presumably because not all antigens are identified and, therefore, are hard to detect. 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 [75]. Renal transplant recipients with anti-HLA antibodies were five to six times more likely to develop CR [71, 76, 77]. Lung transplant recipients with antibodies have a two to four times higher risk of developing CR of their lung allografts; this is called bronchiolitis obliterans [75, 78, 79]. Heart transplant recipients with positive flow-cytometry cross-matching have increased rejection and graft-loss rates [80, 81]. 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 [75]. The presence of pre-transplant antibodies against both HLA class-I and class-II antigens is most detrimental to graft survival, whereas the presence of only antibodies against class-I or class-II antigens does not affect renal graft survival [75, 82]. In experimental cornea transplantation, antibodies are capable of inducing damage to the cornea in a complement-dependent manner [75, 83]. In clinical liver transplant rejection, anti-HLA antibodies seem to be less important [21, 75].

The majority (77%) of acute and chronic rejection episodes occurs in the absence of the circulating anti-HLA antibodies measured at the time of rejection [84]. At the time of rejection, antibodies might be not detectable in the circulation due to their binding to the inflamed tissue. However the de novo appearance of anti-donor antibodies after transplantation is frequently (84%) associated with acute or chronic rejection.

In addition to anti-HLA antibodies, antibodies against endothelial cells have been found in renal transplant recipients before and after transplantation (Table 1) [72, 85, 86]. In a group of patients with at least one failed graft, 14% had endothelial cell-specific antibodies, compared with 3% for recipients with stable graft function [87]. Endothelial cell activation induced by antibodies could also contribute to increased extravasation of allo-immune immunocompetent cells to facilitate rejection.

In rat cardiac allograft models, antibodies against both MHC and non-MHC antigens on endothelial cells are produced, but only antibodies that are reactive with MHC antigens are capable of activating complement [88]. It is suggested that these antibodies contribute to transplant vasculopathy via complement-mediated cytotoxicity [88]. Furthermore, in experimental cardiac transplantation, B-cell responses to cardiac myosin, a molecule specific for cardiac tissue, have been found [89]. In clinical cardiac transplantation, antibodies against endothelial cells are also present. One of the antigens that is recognized is the protein filament vimentin (Table 1). Antibodies against vimentin are independent predictors of transplant-associated coronary artery disease during cardiac transplantation [90, 91, 92].

Antibodies reactive with tissue antigens were detected in 71% of patients with chronically rejecting liver allografts [67]. Similar antibodies have also been found in experimental liver transplantation, the antibodies seem to recognize molecules that are not MHC [93]. The contribution of these antibodies to the pathogenesis of CR is not yet clear.

Antibodies to non-HLA, non-endothelial cell antigens have been described in animal models of chronic

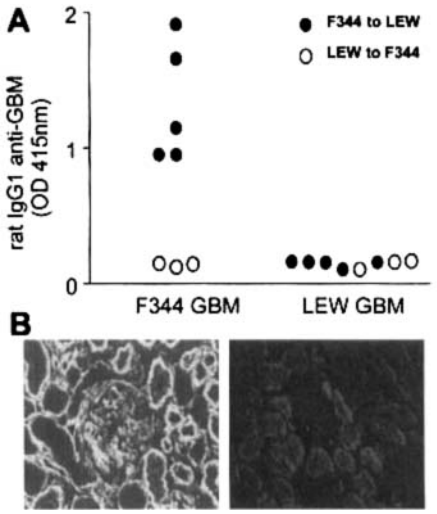

Fig. 2 In the F344-to-LEW rat model of CR we observed IgG1 antibodies reactive with F344 but not LEW GBM isolates in serum of LEW recipients with rejecting F344 allografts. **A** LEW recipients of long-term surviving F344 allografts do not develop antibodies. In the rejecting F344 kidneys IgG1 deposits were found that were not present in long-term surviving LEW allografts **B** *Left picture:* F344 allograft at 60 days post-transplant; *right picture:* LEW allograft at day 60. The antigens were identified as the heparan sulphate proteoglycan perlecan and the α1 chain of collagen type VI in association with the α5 chain of collagen type IV

renal allograft rejection (Table 1). In the F344-to-LEW rat model of chronic renal allograft rejection, various antibody responses against donor antigens have been described [94, 95, 96]. Antibodies are present in LEW recipients of F344 grafts, but not in F344 recipients of LEW grafts or in recipients of syngeneic grafts (Fig. 2). Antibodies are reactive with renal basement membranes, mainly the glomerular basement membrane and mesangial cells (Fig. 2) [94, 96]. We recently identified the nature of the antigens involved as the basement membrane components perlecan (a heparan-sulphate proteoglycan) and the α1 chain of collagen type VI in association with the α5 chain of collagen type IV [95]. Both antigens are found in the glomerular basement membrane (GBM) and can be synthesized by mesangial and endothelial cells of the glomerulus. Alterations of basement membrane charge or composition can directly influence glomerular permselectivity. This has been shown after injection of antibodies reactive with the heparan-sulphate side chain in rats that resulted in proteinuria and basement membrane alterations [97, 98]. In all rats that were investigated, the antibodies reactive with GBM preparations were only of the IgG1 isotype [95], supporting a Th2 response.

In summary, we think that antibodies reactive with both HLA and non-HLA antigens formed after Tx are relevant for the pathogenesis of CR. In particular, antibodies reactive with tissue-specific antigens are of great interest because they can cause direct damage to transplanted tissue.

Immunosuppressive drugs

Modern immunosuppressive agents, including calcineurin inhibitors, have decreased the incidence of acute rejection episodes. These drugs mainly inhibit type-1 responses and facilitate type-2 responses, therefore modern immunosuppression seems to contribute to immune deviation to Th2 responses [20, 33]. This might be further enhanced by type-2 cytokines, such as IL-4, IL-5, IL-10, and IL-13 that are produced by activated macrophages, NK, $CD8^+$ T cells in the graft. Th2 cytokines and growth factors activate B cells to produce alloantibodies and activate endothelial and smooth muscle cells to produce growth factors that contribute to interstitial fibrosis, ECM deposition and vascular neointimal hyperplasia [33]. Th2 responses seem to be more fibrogenic than Th1 responses.

Mycophenolate mofetil (MMF) reduces the risk of chronic renal allograft failure [99], independently of decreasing acute rejection episodes. It inhibits the de novo synthesis of purines, crucial to cell cycling of T and B cells. It thus blocks clonal expansion of B and T cells, preventing antibody production and the generation of cytotoxic T cells, as well as other effector T cells [100]. In contrast to other immunosuppressing drugs, MMF also inhibits antibody production by B cells. There seems to be a trend towards better graft survival at 3 years post-transplantation in kidney graft recipients treated with MMF [74, 101, 100, 102]. In cardiac transplantation, patients that were treated with MMF produced fewer de novo anti-vimentin antibodies than patients treated with azathioprine [103]. These data suggest that treatment with MMF prolongs renal graft survival due to decreased alloantibody production.

Conclusions

The pathogenesis of CR is complicated and multifactorial. Recent data suggest that indirect presentation of alloantigens is important for the induction of CR. Indirect presentation seems to favour type-2 responses and might, therefore, promote the production of alloantibodies. Alloantibodies can be reactive with HLA antigens or with tissue-specific antigens, and result in damage to the grafts. Future treatment protocols should be directed more towards inhibition of indirect presentation and B cell activation to prevent CR.

References

1. Paul LC (1995) Chronic renal transplant loss. Kidney Int 47:1491–1499
2. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D (2000) Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 342:605–612
3. Pratschke J, Wilhelm MJ, Laskowski I, Kusaka M, Beato F, Tullius SG, Neuhaus P, Hancock WW, Tilney NL (2001) Influence of donor brain death on chronic rejection of renal transplants in rats. J Am Soc Nephrol 12:2474–2481
4. Stoica SC, Goddard M, Large SR (2002) The endothelium in clinical cardiac transplantation. Ann Thorac Surg 73:1002–1008
5. Szabo A, Heemann U (1998) Ischemia reperfusion injury and chronic allograft rejection. Transplant Proc 30:4281–4284
6. Takada M, Nadeau KC, Hancock WW, Mackenzie HS, Shaw GD, Waaga AM, Chandraker A, Sayegh MH, Tilney NL (1998) Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Transplantation 65 1533–1542
7. Gasser M, Waaga AM, Kist-Van Holte JE, Lenhard SM, Laskowski I, Shaw GD, Hancock WW, Tilney NL (2002) Normalization of brain death-induced injury to rat renal allografts by recombinant soluble P-selectin glycoprotein ligand. J Am Soc Nephrol 13:1937–1945
8. Daemen MA, Van't Veer C, Wolfs TG, Buurman WA (1999) Ischemia/reperfusion-induced IFN-gamma up-regulation: involvement of IL-12 and IL-18. J Immunol 162:5506–5510

9. Chien CT, Lee PH, Chen CF, Ma MC, Lai MK, Hsu SM (2001) De novo demonstration and co-localization of free-radical production and apoptosis formation in rat kidney subjected to ischemia/reperfusion. J Am Soc Nephrol 12:973–982
10. Daemen MA, De Vries B, Buurman WA (2002) Apoptosis and inflammation in renal reperfusion injury. Transplantation 73:1693–1700
11. Dragun D, Hoff U, Park JK, Qun Y, Schneider W, Luft FC, Haller H (2000) Ischemia-reperfusion injury in renal transplantation is independent of the immunologic background. Kidney Int 58:2166–2177
12. Penfield JG, Wang Y, Li S, Kielar MA, Sicher SC, Jeyarajah DR, Lu CY(1999) Transplant surgery injury recruits recipient MHC class II-positive leukocytes into the kidney. Kidney Int 56:1759–1769
13. Waaga AM, Gasser M, Laskowski I, Tilney NL (2000) Mechanisms of chronic rejection. Curr Opin Immunol 12:517–521
14. Tullius SG, Hancock WW, Heemann U, Azuma H, Tilney NL (1994) Reversibility of chronic renal allograft rejection. Critical effect of time after transplantation suggests both host immune dependent and independent phases of progressive injury. Transplantation 58:93–99
15. Tullius SG, Nieminen M, Bechstein WO, Jonas S, Steinmuller T, Qun Y, Pratschke J, Graser E, Sinha P, Volk HD, Neuhaus P, Tilney NL (1998) Contribution of early acute rejection episodes to chronic rejection in a rat kidney retransplantation model. Kidney Int 53:465–472
16. Zwacka RM, Zhang Y, Halldorson J, Schlossberg H, Dudus L, Engelhardt JF (1997) CD4(+) T-lymphocytes mediate ischemia/reperfusion-induced inflammatory responses in mouse liver. J Clin Invest 100:279–289
17. Ensminger SM, Spriewald BM, Witzke O, Pajaro OE, Yacoub MH, Morris PJ, Rose ML, Wood KJ (2002) Indirect allorecognition can play an important role in the development of transplant arteriosclerosis. Transplantation 73:279–286
18. Fischbein MP, Yun J, Laks H, Irie Y, Fishbein MC, Bonavida B, Ardehali A (2002) Role of CD8+ lymphocytes in chronic rejection of transplanted hearts. J Thorac Cardiovasc Surg 123:803–809
19. Russell PS, Chase CM, Sykes M, Ito H, Shaffer J, Colvin RB (2001) Tolerance, mixed chimerism, and chronic transplant arteriopathy. J Immunol 167:5731–5740
20. Cramer DV, Shirwan H (1998) The importance of humoral immune responses in chronic rejection. Transplant Rev 12:166–176
21. Donaldson PT, Williams R (1997) Cross-matching in liver transplantation. Transplantation 63:789–794
22. Russell PS, Chase CM, Winn HJ, Colvin RB (1994) Coronary atherosclerosis in transplanted mouse hearts. II. Importance of humoral immunity. J Immunol 152:5135–5141
23. Hancock WW, Buelow R, Sayegh MH, Turka LA (1998) Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. Nat Med 4:1392–1396
24. Sun H, Woodward JE, Subbotin VM, Kuddus R, Logar AJ, Schaefer AT, Aitouche A, Rao AS (2002) Use of recombinase activation gene-2 deficient mice to ascertain the role of cellular and humoral immune responses in the development of chronic rejection. J Heart Lung Transplant 21:738–750
25. Bian H, Harris PE, Reed EF (1998) Ligation of HLA class I molecules on smooth muscle cells with anti-HLA antibodies induces tyrosine phosphorylation, fibroblast growth factor receptor expression and cell proliferation. Int Immunol 10:1315–1323
26. Harris PE, Bian H, Reed EF (1997) Induction of high affinity fibroblast growth factor receptor expression and proliferation in human endothelial cells by anti-HLA antibodies: a possible mechanism for transplant atherosclerosis. J Immunol 159:5697–5704
27. Orosz C (2002) Graft rejection: mechanism. Encyclopedia of Life Sciences
28. Sijpkens YW, Doxiadis II, De Fijter JW, Mallat MJ, Van Es LA, De Lange P, Zwinderman AH, Westendorp RG, Van Kemenade FJ, Bruijn JA, Claas FH, Paul LC (1999) Sharing cross-reactive groups of MHC class I improves long-term graft survival. Kidney Int 56:1920–1927
29. Allan JS, Madsen JC (2002) Recent advances in the immunology of chronic rejection. Curr Opin Nephrol Hypertens 11:315–321
30. Bradley JA, Mowat AM, Bolton EM (1992) Processed MHC class I alloantigen as the stimulus for CD4+ T-cell dependent antibody-mediated graft rejection. Immunol Today 13:434–438
31. Game DS, Lechler RI (2002) Pathways of allorecognition: implications for transplantation tolerance. Transpl Immunol 10:101–108
32. Sayegh MH (1999) Why do we reject a graft? Role of indirect allorecognition in graft rejection. Kidney Int 56:1967–1979
33. Shirwan H (1999) Chronic allograft rejection. Do the Th2 cells preferentially induced by indirect alloantigen recognition play a dominant role? Transplantation 68:715–726
34. Womer KL, Stone JR, Murphy B, Chandraker A, Sayegh MH (2001) Indirect allorecognition of donor class I and II major histocompatibility complex peptides promotes the development of transplant vasculopathy. J Am Soc Nephrol 12:2500–2506
35. Lechler RI, Batchelor JR (1982) Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J Exp Med 155:31–41
36. Lee RS, Yamada K, Houser SL, Womer KL, Maloney ME, Rose HS, Sayegh MH, Madsen JC (2001) Indirect recognition of allopeptides promotes the development of cardiac allograft vasculopathy. Proc Natl Acad Sci U S A 98:3276–3281
37. Baker RJ, Hernandez-Fuentes MP, Brookes PA, Chaudhry AN, Cook HT, echler RI (2001) Loss of direct and maintenance of indirect alloresponses in renal allograft recipients: implications for the pathogenesis of chronic allograft nephropathy. J Immunol 167:7199–7206
38. Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. N Engl J Med 338:1813–1821
39. Dong VM, Yuan X, Coito AJ, Waaga AM, Sayegh MH, Chandraker A (2002) Mechanisms of targeting CD28 by a signaling monoclonal antibody in acute and chronic allograft rejection. Transplantation 73:1310–1317
40. Laskowski IA, Pratschke J, Wilhelm MJ, Dong VM, Beato F, Taal M, Gasser M, Hancock WW, Sayegh MH, Tilney NL (2002) Anti-CD28 monoclonal antibody therapy prevents chronic rejection of renal allografts in rats. J Am Soc Nephrol 13:519–527
41. Azuma H, Chandraker A, Nadeau K, Hancock WW, Carpenter CB, Tilney NL, Sayegh MH (1996) Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection. Proc Natl Acad Sci U S A 93:12439–12444
42. Chandraker A, Azuma H, Nadeau K, Carpenter CB, Tilney NL, Hancock WW, Sayegh MH (1998) Late blockade of T cell costimulation interrupts progression of experimental chronic allograft rejection. J Clin Invest 101:2309–2318
43. Takada M, Chandraker A, Nadeau KC, Sayegh MH, Tilney NL (1997) The role of the B7 costimulatory pathway in experimental cold ischemia/reperfusion injury. J Clin Invest 100:1199–1203

44. Yamada AA, Sayegh MH (2002) The CD154–CD40 costimulatory pathway in transplantation. Transplantation 73:36–39
45. Ensminger SM, Spriewald BM, Sorensen HV, Witzke O, Flashman EG, Bushell A, Morris PJ, Rose ML, Rahemtulla A, Wood KJ (2001) Critical role for IL-4 in the development of transplant arteriosclerosis in the absence of CD40–CD154 costimulation. J Immunol 167:532–541
46. Ensminger SM, Spriewald BM, Witzke O, Morrison K, van Maurik A, Morris PJ, Rose ML, Wood KJ (2000) Intragraft interleukin-4 mRNA expression after short-term CD154 blockade may trigger delayed development of transplant arteriosclerosis in the absence of CD8+ T cells. Transplantation 70:955–963
47. Kishimoto K, Dong VM, Issazadeh S, Fedoseyeva EV, Waaga AM, Yamada A, Sho M, Benichou G, Auchincloss H Jr, Grusby MJ, Khoury SJ, Sayegh MH (2000) The role of CD154–CD40 versus CD28–B7 costimulatory pathways in regulating allogeneic Th1 and Th2 responses in vivo. J Clin Invest 106:63–72
48. Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Buchanan K, Fechner JH Jr, Germond RL, Kampen RL, Patterson NB, Swanson SJ, Tadaki DK, TenHoor CN, White L, Knechtle SJ, Harlan DM (1999) Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med 5:686–693
49. Fischbein MP, Ardehali A, Yun J, Schoenberger S, Laks H, Irie Y, Dempsey P, Cheng G, Fishbein MC, Bonavida B (2000) CD40 signaling replaces CD4+ lymphocytes and its blocking prevents chronic rejection of heart transplants. J Immunol 165:7316–7322
50. Guillot C, Guillonneau C, Mathieu P, Gerdes CA, Menoret S, Braudeau C, Tesson L, Renaudin K, Castro MG, Lowenstein PR, Anegon I (2002) Prolonged blockade of CD40–CD40 ligand interactions by gene transfer of CD40Ig results in long-term heart allograft survival and donor-specific hyporesponsiveness, but does not prevent chronic rejection. J Immunol 168:1600–1609
51. Le Moine A, Goldman M, Abramowicz D (2002) Multiple pathways to allograft rejection. Transplantation 73:1373–1381
52. Illigens B, Yamada A, Fedoseyeva E, Anosova N, Boisgerault F, Valujskikh A, Heeger P, Sayegh M, Boehm B, Benichou G (2002) The relative contribution of direct and indirect antigen recognition pathways to the alloresponse and graft rejection depends upon the nature of the transplant. Hum Immunol 63:912
53. Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K (1995) Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. J Exp Med 182:1591–1596
54. Leitenberg D, Bottomly K (1999) Regulation of naive T cell differentiation by varying the potency of TCR signal transduction. Semin Immunol 11:283–292
55. Hamar P, Szabo A, Muller V, Heemann U (2001) Involvement of interleukin-2 and growth factors in chronic kidney allograft rejection in rats. Transplant Proc 33:2160–2162
56. Hayashi M, Martinez OM, Garcia-Kennedy R, So S, Esquivel CO, Krams SM (1995) Expression of cytokines and immune mediators during chronic liver allograft rejection. Transplantation 60:1533–1538
57. Le Moine A, Flamand V, Demoor FX, Noel JC, Surquin M, Kiss R, Nahori MA, Pretolani M, Goldman M, Abramowicz D (1999) Critical roles for IL-4, IL-5, and eosinophils in chronic skin allograft rejection. J Clin Invest 103:1659–1667
58. Oliveira G, Xavier P, Murphy B, Neto S, Mendes A, Sayegh MH, Guerra LE (1998) Cytokine analysis of human renal allograft aspiration biopsy culture supernatants predicts acute rejection. Nephrol Dial Transplant 13:417–422
59. Piccotti JR, Li K, Chan SY, Eichwald EJ, Bishop DK (1999) Cytokine regulation of chronic cardiac allograft rejection: evidence against a role for Th1 in the disease process. Transplantation 67:1548–1555
60. Suthanthiran M (1997) Molecular analyses of human renal allografts: differential intragraft gene expression during rejection. Kidney Int [Suppl] 58:15–21
61. Raisanen-Sokolowski A, Glysing-Jensen T, Koglin J, Russell ME (1998) Reduced transplant arteriosclerosis in murine cardiac allografts placed in interferon-gamma knockout recipients. Am J Pathol 152:359–365
62. Halloran PF, Miller LW, Urmson J, Ramassar V, Zhu LF, Kneteman NM, Solez K, Afrouzian M (2001) IFN-gamma alters the pathology of graft rejection: protection from early necrosis. J Immunol 166:7072–7081
63. Lu CY, Penfield JG, Kielar ML, Vazquez MA, Jeyarajah DR (1999) Hypothesis: is renal allograft rejection initiated by the response to injury sustained during the transplant process? Kidney Int 55:2157–2168
64. Vaillant B, Chiaramonte MG, Cheever AW, Soloway PD, Wynn TA (2001) Regulation of hepatic fibrosis and extracellular matrix genes by the Th response: new insight into the role of tissue inhibitors of matrix metalloproteinases. J Immunol 167:7017–7026
65. Postlethwaite AE, Holness MA, Katai H, Raghow R (1992) Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. J Clin Invest 90:1479–1485
66. Ruger BM, Erb KJ, He Y, Lane JM, Davis PF, Hasan Q (2000) Interleukin-4 transgenic mice develop glomerulosclerosis independent of immunoglobulin deposition. Eur J Immunol 30:2698–2703
67. Dubel L, Farges O, Johanet C, Sebagh M, Bismuth H (1998) High incidence of antitissue antibodies in patients experiencing chronic liver allograft rejection. Transplantation 65:1072–1075
68. Hutchinson IV, Alam Y, Ayliffe WR (1995) The humoral response to an allograft. Eye 9:155–160
69. Kouwenhoven EA, IJzermans JN, De Bruin RW (2000) Etiology and pathophysiology of chronic transplant dysfunction. Transpl Int 13:385–401
70. Lederer SR, Kluth-Pepper B, Schneeberger H, Albert E, Land W, Feucht HE (2001) Impact of humoral alloreactivity early after transplantation on the long-term survival of renal allografts. Kidney Int 59:334–341
71. Sumitran-Holgersson S (2001) HLA-specific alloantibodies and renal graft outcome. Nephrol Dial Transplant 16:897–904
72. Bohmig GA, Exner M, Watschinger B, Regele H (2002) Acute humoral renal allograft rejection. Curr Opin Urol 12:95–99
73. Bryan CF, Luger AM, Martinez J, Muruve N, Nelson PW, Pierce GE, Ross G, Shield CF, III, Warady BA, Aeder MI, Helling TS (2001) Cold ischemia time: an independent predictor of increased HLA class I antibody production after rejection of a primary cadaveric renal allograft. Transplantation 71:875–879
74. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AB, Schneeberger EE, Colvin RB (2001) Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 12:574–582
75. McKenna RM, Takemoto SK, Terasaki PI (2000) Anti-HLA antibodies after solid organ transplantation. Transplantation 69:319–326

76. Abe M, Kawai T, Futatsuyama K, Tanabe K, Fuchinoue S, Teraoka S, Toma H, Ota K (1997) Postoperative production of anti-donor antibody and chronic rejection in renal transplantation. Transplantation 63:1616–1619
77. Piazza A, Poggi E, Borrelli L, Servetti S, Monaco PI, Buonomo O, Valeri M, Torlone N, Adorno D, Casciani CU (2001) Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: posttransplant analysis using flow cytometric techniques. Transplantation 71:1106–1112
78. Jaramillo A, Smith MA, Phelan D, Sundaresan S, Trulock EP, Lynch JP, Cooper JD, Patterson GA, Mohanakumar T (1999) Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation. Transplantation 67:1155–1161
79. Palmer SM, Davis RD, Hadjiliadis D, Hertz MI, Howell DN, Ward FE, Savik K, Reinsmoen NL (2002) Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation 74:799–804
80. McCarthy JF, Cook DJ, Massad MG, Sano Y, O'Malley KJ, Ratliff NR, Stewart RW, Smedira NG, Starling RC, Young JB, McCarthy PM (1998) Vascular rejection post heart transplantation is associated with positive flow cytometric cross-matching. Eur J Cardiothorac Surg 14:197–200
81. Tambur AR, Bray RA, Takemoto SK, Mancini M, Costanzo MR, Kobashigawa JA, D'Amico CL, Kanter KR, Berg A, Vega JD, Smith AL, Roggero AL, Ortegel JW, Wilmoth-Hosey L, Cecka JM, Gebel HM (2000) Flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. Transplantation 70:1055–1059
82. Susal C, Opelz G (2002) Kidney graft failure and presensitization against HLA class I and class II antigens. Transplantation 73:1269–1273
83. Hegde S, Mellon JK, Hargrave SL, Niederkorn JY (2002) Effect of alloantibodies on corneal allograft survival. Invest Ophthalmol Vis Sci 43:1012–1018
84. Supon P, Constantino D, Hao P, Cagle L, Hahn A, Conti DJ, Freed BM (2001) Prevalence of donor-specific anti-HLA antibodies during episodes of renal allograft rejection. Transplantation 71:577–580
85. Ball B, Mousson C, Ratignier C, Guignier F, Glotz D, Rifle G (2000) Antibodies to vascular endothelial cells in chronic rejection of renal allografts. Transplant Proc 32:353–354
86. Sumitran S (2000) Clinical importance of HLA-specific and non-HLA-specific antibodies in allogeneic kidney transplantation. Adv Nephrol Necker Hosp 30:29–39
87. Perrey C, Brenchley PE, Johnson RW, Martin S (1998) An association between antibodies specific for endothelial cells and renal transplant failure. Transpl Immunol 6:101–106
88. Derhaag JG, Duijvestijn AM, Damoiseaux JG, Van Breda Vriesman P (2000) Effects of antibody reactivity to major histocompatibility complex (MHC) and non-MHC alloantigens on graft endothelial cells in heart allograft rejection. Transplantation 69:1899–1906
89. Fedoseyeva EV, Zhang F, Orr PL, Levin D, Buncke HJ, Benichou G (1999) De novo autoimmunity to cardiac myosin after heart transplantation and its contribution to the rejection process. J Immunol 162:6836–6842
90. Jurcevic S, Ainsworth ME, Pomerance A, Smith JD, Robinson DR, Dunn MJ, Yacoub MH, Rose ML (2001) Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. Transplantation 71:886–892
91. Jurcevic S, Dunn MJ, Crisp S, Busing K, Rinaldi M, Pellegrini C, Yacoub MH, Vigano M, Banner NL, Rose ML (1998) A new enzyme-linked immunosorbent assay to measure anti-endothelial antibodies after cardiac transplantation demonstrates greater inhibition of antibody formation by tacrolimus compared with cyclosporine. Transplantation 65:1197–1202
92. Rose ML (2000) Role of antibodies in transplant-associated cardiac allograft vasculopathy. Z Kardiol 89 [Suppl 9]:11–15
93. Dubel L, Farges O, Sato Y, Bismuth H (1998) Development of anti-tissue antibodies in the rat liver transplant model. Transplantation 65:1135–1137
94. De Heer E, Davidoff A, Van der Wal A, Van Geest M, Paul LC (1994) Chronic renal allograft rejection in the rat. Transplantation-induced antibodies against basement membrane antigens. Lab Invest 70:494–502
95. Joosten SA, Van Dixhoorn MGA, Borrias MC, Benediktsson H, Van Veelen PA, van Kooten C, Paul LC (2002) Antibody response against perlecan and collagen types IV and VI in chronic renal allograft rejection in the rat. Am J Pathol 160:1301–1310
96. Paul LC, Muralidharan J, Muzaffar SA, Manting EH, Valentin JF, De Heer E, Kashgarian M (1998) Antibodies against mesangial cells and their secretory products in chronic renal allograft rejection in the rat. Am J Pathol 152:1209–1223
97. Miettinen A, Stow JL, Mentone S, Farquhar MG (1986) Antibodies to basement membrane heparan sulfate proteoglycans bind to the laminae rarae of the glomerular basement membrane (GBM) and induce subepithelial GBM thickening. J Exp Med 163:1064–1084
98. Van den Born J, Van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden JH (1992) A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. Kidney Int 41:115–123
99. Meier-Kriesche H, Ojo AO, Arndorfer JA, Magee JC, Cibrik DM, Leichtman AB, Campbell DA, Kaplan B (2001) Mycophenolate mofetil decreases the risk for chronic renal allograft failure. Transplant Proc 33:1005–1006
100. Mele TS, Halloran PF (2000) The use of mycophenolate mofetil in transplant recipients. Immunopharmacology 47:215–245
101. Meier-Kriesche HU, Ojo AO, Leichtman AB, Magee JC, Rudich SM, Hanson JA, Cibrik DM, Kaplan B (2001) Interaction of mycophenolate mofetil and HLA matching on renal allograft survival. Transplantation 71:398–401
102. Ojo AO, Meier-Kriesche HU, Hanson JA, Leichtman AB, Cibrik D, Magee JC, Wolfe RA, Agodoa LY, Kaplan B (2000) Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. Transplantation 69:2405–2409
103. Rose ML, Smith J, Dureau G, Keogh A, Kobashigawa J (2002) Mycophenolate mofetil decreases antibody production after cardiac transplantation. J Heart Lung Transplant 21:282–285