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CHRONIC REJECTION: FAILURE OF IMMUNE REGULATION

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1. ABSTRACT

Current strategies for immunosuppression following organ transplantation focus on the prevention of acute rejection. As new generations of immunosuppressants have been developed, acute rejection rates have diminished markedly. The new challenge, then, is to prevent the devastating complications of chronic rejection, which have remained largely unchanged over the decades. The process of chronic rejection is a complex one, and it is likely that most, if not all, components of the immune system play some role in the long-term, smoldering failure of organs following transplantation. Through a better understanding of their individual contributions as well as interactions, new strategies may be developed to overcome this problem. We present here an overview of the major immune components thought to be involved in chronic rejection.

2. INTRODUCTION

Organ transplantation has revolutionized the care of patients with end-stage organ dysfunction. In 2002, 22,705 organ transplants were performed in the United States (1). Despite the impressive successes achieved in the field, transplantation is still plagued by the problems of organ shortage and rejection. The former has been addressed through public awareness campaigns, aggressive recruitment of potential donors, expansion of traditionally restrictive donor criteria, and advanced techniques to extend organ viability and maximize the number of recipients who may benefit from a single donor. Rejection, however, continues to be the "Achilles' heel" of organ transplantation. While acute rejection rates have decreased substantially over the last decade, chronic rejection has remained stable. For example, 10-year cadaveric kidney graft survival is only 35.8%, with lung graft survival a mere 16.8%. Heart graft survivals are somewhat better, and livers enjoy the best results (2). Further, while the acute rejection process has been quite well characterized, much still remains to be learned about the mechanisms of chronic rejection. Current immunosuppressive regimens are focused on the prevention of acute rejection, and have become increasingly effective over the years. However, as the fundamental basis of chronic rejection is less well understood, it is evident that current drugs do not effectively address this serious problem. Thus, chronic rejection may be seen as a failure of immune regulation. Only as we gain new insights into the molecular, humoral, and cellular components of chronic rejection will we be able to better tailor therapeutic strategies.

3. DEFINITION OF CHRONIC REJECTION

The phenomenon of late allograft dysfunction may result from both immunologic and non-immunologic injury. While non-immunologic contributors, such as organ preservation injury or recurrence of disease, play an important role in long-term outcomes, we will focus on the immunologic factors surrounding chronic rejection. While the manifestations of chronic rejection vary by organ, they all derive from a fundamental failure of current immunosuppressive agents to effectively control a

Table 1. Clinical and pathologic features of chronic rejection

Organ	Clinical Characteristics	Histologic Characteristics	Risk Factors	Incidence at 5 years
Kidney	Decline in renal function Proteinuria Hypertension	Interstitial fibrosis Tubular atrophy Occlusive vasculopathy Glomerulosclerosis	Acute rejection HLA mismatching Delayed graft function Hypertension Hyperlipidemia CMV infection	40-50%
Liver	Cholestasis	Absence of linterlobular spetal bile ducts in 50% or more portal tracts Obliterative arteritis	Age Acute rejection Autoimmune liver disease	< 5-10%
Heart	Left ventricular dysfunction Arteriosclerosis	Endarteritis obliterans in epicardial and smaller branch vessels Endothelialitis	Humoral rejection Hyperlipidemia Diabetes CMV infection	50%
Lungs	Cough Dyspnea on exertion Decreased FEV_1	Submucosal fibrosis Partial or total oblieteration of the bronchiolar lumen	Acute rejection HLA mismatching CMV infection	75-85%

persistent and lingering immune response. The cardinal features of chronic rejection common across organs include fibrosis, intimal thickening and luminal obliteration (Table 1). There is generally some cellular infiltrate, and increasing evidence for the presence of complement degradation products.

3.1. Chronic Renal Rejection

Chronic renal rejection is characterized by a progressive decline in renal function, persistent proteinuria, and hypertension (3). Renal biopsies are evaluated based on the Banff criteria (4). The category of "chronic/sclerosing allograft nephropathy" includes three grades of severity. Treatment of chronic rejection is difficult, with little benefit (and possible harm) derived from increasing immunosuppression (3). Instead, emphasis is placed on the correction of non-immunologic factors such as hypertension and hyperlipidemia. The decision to reinstitute dialysis follows the same criteria as for non-transplant patients, and retransplantation may be pursued following graft failure.

3.2. Chronic Hepatic Rejection

Unlike other solid organs, the liver seems relatively resistant to chronic rejection, with an incidence of less than 5% (3). It usually presents with a cholestatic clinical picture and pathology revealing an absence of interlobular septal bile ducts in 50% or more of portal tracts (3). Newer immunosuppressants may provide limited benefit in a small number of cases of chronic rejection, and retransplantation may be pursued in the case of severe hepatic dysfunction.

3.3. Chronic Cardiac Rejection

Chronic vascular rejection in cardiac transplantation is manifested primarily as severe coronary arteriopathy. Angiographic abnormalities may be detected in up to 50% of patients by five years after transplantation (3). These lesions are typically more severe and diffuse than those of naturally occurring atherosclerosis, and are characterized by endothelialitis and an infiltrate of T lymphocytes and macrophages within the vascular intima beneath the endothelium (3). Retransplantation may be pursued in the setting of severe coronary artery disease, but there are conflicting reports in the literature on its long-term success.

3.4. Chronic Pulmonary Rejection

Chronic allograft rejection manifests as obliterative bronchiolitis (OB) following pulmonary transplantation. OB ultimately develops in nearly all long-term survivors (3). Often, early OB is detected by asymptomatic reductions on routine pulmonary function testing. Progressive airway obstruction ensues in severe OB. Pathologic findings (Figure 1) include inflammation and fibrosis of the lamina propria leading to luminal occlusion (5). There are no effective treatment options for OB, and, as for other organs, retransplantation may be considered for end-stage pulmonary disease.

4. IMMUNE MECHANISMS OF CHRONIC REJECTION

The process of chronic rejection, as mentioned above, is influenced by both immune and non-immune factors. Within the immune category, numerous components have been shown to contribute, including elements of the humoral and cellular immune systems as well as cytokines and other circulating proteins. While there is certainly a complex interplay between these various components, it is helpful to look at each one separately in order to understand its relative impact in the process of chronic rejection.

4.1. Humoral Immunity

The two major categories of antibodies that may play a role in the rejection process are anti-HLA antibodies, and those directed against non-HLA determinants (such as endothelial and epithelial cell markers).

4.1.1. Anti-HLA Antibodies

Anti-HLA antibodies may be present prior to transplantation in sensitized individuals. This is due to prior exposure to HLA antigens, either through prior organ transplantation, blood transfusions, or pregnancy. It has

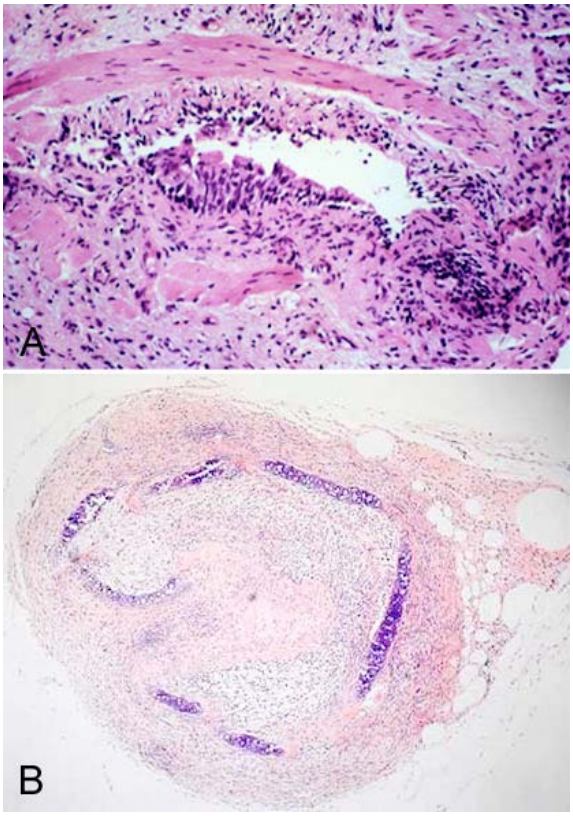

Figure 1. H&E sections demonstrating the pathologic findings of fibrosis and luminal obliteration from obliterative bronchiolitis (OB) following (a) human lung transplantation and (b) experimental murine heterotopic tracheal transplantation.

been well documented that preexisting anti-HLA antibodies lead to an unfavorable outcome following transplantation (6-8). Successful application of pretransplantation crossmatching has virtually eliminated the phenomenon of hyperacute rejection. There is growing evidence, however, that antibodies may play a role in the process of chronic rejection. Experimentally, we have shown that the development of antibodies precedes the development of OB in a murine model of obstructive airway disease. Following transplantation across a single mismatch (HLA-A2-transgenic), detectable antibodies were seen at 5 days, with full development coinciding with the development of characteristic luminal obliteration and chronic rejection (9). We have also observed fibroblast proliferation in response to anti-HLA class I-activated airway epithelial cells, suggesting that antibodies may also play a direct role in the development of chronic changes of rejection (unpublished results). In the clinical setting, the development of anti-HLA class I antibodies and HLA-A locus mismatches are independent predictors of chronic rejection following lung transplantation (10). A recent report has shown a correlation between the de novo development of anti-HLA class II antibodies and OB following lung transplantation (11). A report compiling data from five transplant centers detected anti-HLA antibodies in 96% of patients bearing rejected kidney grafts (12). Prospective evaluation of kidney transplant recipients revealed that 100% of patients with chronic rejection had anti-HLA antibodies; 14 patients developed these antibodies de novo. Only 27% of patients with functioning grafts developed antibodies (13). While it is not possible from studies such as these to infer causality, the strong correlation between anti-HLA antibody presence and rejection suggests that antibodies may play a role. There is, however, other compelling evidence for the end effects of circulating antibodies. Upon binding to tissues, antibodies may activate the complement cascade, leading to both direct cytotoxic effects on target cells as well as the recruitment of other members of the immune system. By assessing for the presence of complement split products, it is possible to detect the impact of antibodies in the rejection process. Mauiyyedi, *et al.* (14) examined tissues from 84 patients with and without histologic evidence of chronic rejection. Sixty-one percent of patients with chronic rejection demonstrated evidence of C4d staining in the peritubular capillaries, compared with only 2% of controls. C4d staining was found in a background of anti-donor HLA antibodies in 88% of patients with chronic rejection. This correlation between complement activation and rejection suggests a role for continued activation of the complement cascade by antibody developed following transplantation. This group has used the presence of C4d deposition as an indication to treat patients for chronic rejection (14).

4.1.2. Non-HLA Antibodies

While antibodies to mismatched HLA antigens are the best studied, there is some evidence for the pathologic nature of non-HLA antibodies. As the site of first interaction between donor and recipient, the endothelium may play an important stimulator role in the humoral component of rejection. Damaged vascular endothelium following renal transplant supports the notion of these cells as targets (15). Antibodies to vascular endothelium have been found in the absence of anti-HLA antibodies (16). Previous studies from our laboratory detected antibodies reactive to kidney cells in up to 78% of eluates prepared from chronically rejected renal allografts, indicating that antibodies are deposited in those kidneys undergoing chronic rejection (17). In a study of lung transplant patients with and without chronic rejection, we noted antibodies directed against airway epithelial cells (AEC) in 5 of 16 patients (31%) with OB as compared with 0 of 11 patients with stable graft function. Analysis of the effect of these antibodies on AEC cell lines demonstrated their ability to trigger intracellular calcium influx, tyrosine phosphorylation, proliferation, and up-regulation of transforming growth factor-beta and heparin-binding epidermal growth factor mRNA transcription (18). Non-HLA antibodies have also been found in the renal (19, 20) and cardiac transplant settings (21).

4.2. Cellular Immunity

Since hyperacute rejection has been essentially eliminated as an important cause of early graft loss, the majority of attention has been focused on cellular immunity and its involvement in the rejection process. The critical importance of T lymphocytes is demonstrated by the proliferation of new drugs designed to reduce or eliminate the effects of this immune subset.

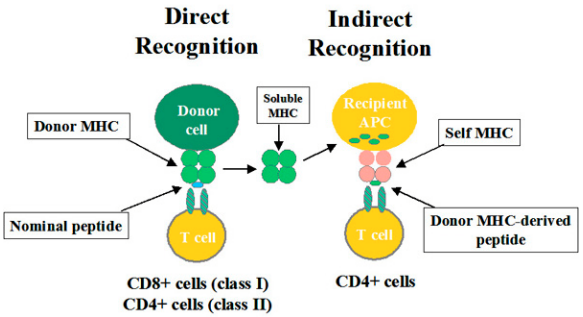

Figure 2. Schematic representation of the processes of direct and indirect antigen recognition by recipient T cells.

4.2.1. Direct antigen presentation and $CD8^+$ T cells

Alloreactive T cells may directly recognize donor MHC molecules presenting donor-derived peptides (Figure 2). These alloreactive T cells that recognize donor antigen presenting cells (APC) are present in much higher numbers than self-MHC-restricted T cells (22). Classically, this pathway has been considered to dominate the early immune response. Donor dendritic cells may migrate out of the graft to regional lymph nodes and thus present antigens to the recipient's T cells in a direct fashion. As the graft is subsequently repopulated by recipient APC, the direct pathway likely becomes less important to the process of chronic rejection (23). $CD8^+$ cytotoxic T cells certainly play an important role in the development of acute rejection. Their role in the process of chronic rejection is less well understood, although a growing body of evidence suggests that they may have a dramatic impact there as well. In this regard, the absence of CD8+ T cells in MHC class I-knockout recipients prevents the development of chronic rejection histology in a mouse aortic allograft model (24). Using MHC class I and II mismatched pairs in a rodent model of cardiac allograft vasculopathy (CAV), Fischbein, *et al.* (25) provide support for the importance of $CD8^+$ T cells. They noted a temporal relationship between $CD8^+$ T cells recruitment and CAV development; the presence of activated graft-infiltrating $CD8^+$ T cells; the production of IFN-gamma by graft-infiltrating $CD8^+$ T cells; and a marked decline in intimal lesion development in the absence of $CD8^+$ T cells. The presence of IFN-gamma secretion supports a functional role for the graft-infiltrating lymphocytes, through both direct atherosclerotic effects as well as the recruitment and activation of bystander cells (25). The effects of $CD8^+$ T cells on the destruction of arterial medial smooth muscle cells have been recently demonstrated (26). Depletion of $CD8^+$ cells limited medial destruction, but did not suppress it completely, supporting the conclusion that chronic allograft changes are a multifactorial process. In related studies, through the use of specific knockout mice, we have demonstrated that $CD8^+$ cells are sufficient to induce OB changes in a murine model of tracheal transplantation. Additionally, $CD4^+$ knockout mice developed pathologic changes at a much slower rate, suggesting that the kinetics and mechanisms of rejection differ for $CD8^+$ and $CD4^+$ T cells (27).

4.2.2. Indirect antigen presentation

While not as important early on, indirect antigen presentation (Figure 2) becomes a major component of immune pathology leading to chronic rejection. Experimentally, immunization with donor class I peptides prior to transplantation led to accelerated rejection and chronic allograft vasculopathy in a swine model of cardiac transplantation (28). Using an HLA-A2-transgenic murine model of OB in which recipient T cells are incapable of direct recognition, we have demonstrated the development of chronic rejection through indirect recognition of a single MHC mismatch (9). Baker, *et al.* (29) have examined indirect allorecognition in patients with longstanding living related renal allografts, finding higher frequencies of T cells with indirect anti-donor specificity in patients undergoing chronic allograft nephropathy. Following lung transplantation, we have observed that patients with OB display a dose-dependent proliferative alloreactivity response against both donor HLA class I- and class II-derived allopeptides (30). Indirect alloantigen recognition during the development of chronic rejection supports the notion that $CD4^+$ T cells are a major component in the pathogenesis of this disease. We have found that $CD4^+$ T cells alone are sufficient to produce OB with normal kinetics in mice lacking $CD8^+$ T cells (27). Further, using the HLA-A2-transgenic model, we have shown rejection of murine cardiac allografts by $CD4^+$ T cells primed against this mismatched antigen (31). Inhibition of $CD4^+$ T cell activation through the use of anti-CD28 antibodies has been shown to prevent chronic rejection of rat renal allografts (32). Transfection with CTLA4Ig in an adenovirus vector also inhibited chronic rejection of rat trachea, again through costimulatory blockade of T cells (33). Other investigations into the importance of T cell costimulation have been carried out with CD40-CD40L blockade (34). Circulating T cells have a wide variety of T cell receptors (TCR), and it is this repertoire that allows the immune system to respond to the multitude of infectious challenges. Once activated, however, an oligoclonal expansion may ensue which allows the immune system to focus on a particular pathogen. With such expansion may come refinement of the TCR and improved binding. There is evidence that such a repertoire focusing may also occur during the process of rejection. In renal transplant patients with acute rejection superimposed on chronic rejection, a highly altered T cell repertoire has been found, suggesting that a restricted set of antigens are presented and recognized in this immune destructive phenomenon (35). Following cardiac transplantation, Slachata, *et al.* (22) demonstrated oligoclonal populations of T cells infiltrating the coronary arteries of patients with graft arteriosclerosis. These expanded clones were found at different sites, suggesting systemic expansion of the T cell clones. Such studies provided support for the importance of indirect antigen recognition in the process of chronic rejection.

4.2.3. Regulatory T cells

As the concept of regulatory or suppressor cells has regained popularity, a number of investigators have looked for them in the transplant setting. A number of phenotypic markers for regulatory cells have been identified, including CD25, CD28, and $CD4^-/CD8^-$ (double negative T cells). In transplantation models, donor-specific $CD4^+CD25^+$ regulatory populations have been identified in mice with long-term surviving cardiac and pancreatic isl*et al*lografts (36). In patients with long-term functioning

kidney transplants, the number of $CD28^-CD4^+$ T cells increased in direct correlation with the length of graft survival. These cells presented a restricted T cell receptor repertoire, unlike the $CD28^+$ cells from the same patients, and demonstrated increased expression of TGF-beta and IFN-gamma genes (37). $CD28^-CD8^+$ may also play a role in suppression of rejection, as these cells have been found in the circulation of heart transplant recipients free of rejection (38). Thus, the end-result of rejection may represent a sensitive balance between effector and regulatory cells (39).

4.3. Soluble mediators

Cytokines and other soluble mediators appear to play an important role in the pathology of chronic rejection. They may transduce messages between cells of the immune system as well as have direct effects on transplanted cells. In the experimental setting, we have shown that neutralization of tumor necrosis factor (TNF) prevents the development of OB in a single MHC mismatch murine tracheal allograft model (5). TNF blockade may prevent lymphocyte homing by reducing the expression of important chemokines in the inflammatory milieu. Blockade of IL-1 reduced, but did not completely prevent, the development of OB, while neutralization of interferon-gamma had no effect (5). In the clinical setting, IL-4 expression is increased during rejection episodes following liver transplantation. The presence of high levels of IL-4 in the native livers of cholestatic patients with primary biliary cirrhosis supports the hypothesis that this cytokine may play an important role in bile duct injury that characterizes chronic rejection (40). Interestingly, IL-4 expression is low during kidney and heart rejection episodes, suggesting that differential cytokine profiles may be organ-specific (40). Following lung transplantation, IL-6 and interferon-gamma may play an important role, as gene polymorphisms leading to high expression of these cytokines has been demonstrated in patients with OB (41). Other studies point to the role of TGF-beta in the pathogenesis of lung fibrosis following transplantation (42). The relative contribution of cytokine panels under the Th1/Th2 paradigm has also received consideration. An appealing hypothesis posits that Th1-type cytokines (IL-2, IFN-gamma) may be the more important mediators of acute rejection (43). According to this theory, immunosuppression preferentially targets Th1 cells and skews the immune response to a Th2 profile. Cytokines such as IL-4, IL-5, IL-10, and IL-13 may then predispose to chronic rejection through the upregulation of alloantibodies and growth factors. A number of studies support the observation that Th2 cytokines are preferentially expressed in chronically rejecting grafts (43). However, a conflicting report suggests that Th2 cells may actually regulate and decrease rejection induced by Th1 subsets (44). Like cytokines, chemokines also play an important role in leukocyte recruitment. Cardiac transplantation in mice lacking the chemokine receptor CCR1 show significantly delayed or even absent acute and chronic rejection and addition of low doses of cyclosporin or monoclonal antibody therapy against $CD4^+$ cells markedly enhances the effects of chemokine absence (45). Matrix metalloproteinases (MMP) may also play a role in the rejection process. These extracellular matrix proteases are likely involved in tissue remodeling, important to chronic rejection. Elevated levels of circulating MMP-3 and MMP-2 have been identified in the serum of patients with chronic transplant nephropathy following renal transplantation (46). Elevated MMP-9 levels can be found in the sputum of posttransplant lung patients, and are significantly elevated in those patients undergoing chronic rejection as compared to those with stable function (47). Support for the remodeling concept can be seen from models of OB and chronic vascular rejection where luminal narrowing results from the uncontrolled proliferation and migration of recipient mesenchymal cells into the graft (48).

5. SUMMARY AND PERSPECTIVE

Progress in understanding the interaction of the immune system with transplanted organs has move through the phases of hyperacute humoral rejection to acute rejection. A considerable effort has been focused on designing new immunotherapies to prevent the ravages of acute rejection. As rates of early organ loss have fallen, chronic rejection has become a larger focus of research efforts. As mentioned above, the rates of late organ loss are quite high for most organs, and have changed little over the years. A more thorough understanding of the mechanisms behind chronic rejection will allow us to design therapeutic strategies specifically targeted to this unique entity. Clearly, the process of chronic rejection is a synergy of humoral and cellular immune components and soluble immune mediators, as well as non-immunologic factors. As organ donation rates plateau, it is even more imperative that we extend the usable life of each organ transplanted, and only through understanding the process of chronic rejection and improving immune regulation in the late phase will this goal be achieved.

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