Tremendous progress has been made in the field of transplantation with significant improvement in preventing acute rejection that has resulted in improved graft survival. However long term graft survival still remains limited due to chronic allograft rejection. Chronic rejection is a major impediment for long-term graft function and its incidence is almost universal especially following human lung transplantation. Alloimmune responses to mismatched major histocompatibility antigens (MHC) have been implicated as the most important process leading to rejection. However there is increasing evidence for cross talk between the immune responses to infection, peri-operative inflammation and autoimmunity with allo-immune responses in the transplanted organ leading to chronic rejection. The role of autoimmunity in the development of chronic rejection has been an exciting avenue with significant potential to develop novel strategies in the future to prevent chronic allograft rejection. In this review, we will discuss studies and the emerging concept of the role of autoimmunity and the cross talk between alloimmune and autoimmune responses in the development of chronic allograft rejection, with special emphasis on chronic rejection following human lung transplantation – clinically diagnosed as Bronchiolitis Obliterans Syndrome (BOS). We will provide an important role for Th17 cells, regulatory T cells (Tregs) and immune responses to self-antigens expressed in lung parenchyma in the immunopathogenesis of BOS.

Key words: lung transplantation, allograft rejection, alloimmunity, autoimmunity, bronchiolitis obliterans syndrome (BOS)
1. Introduction

It has been almost 57 years since the first successful solid organ transplantation was performed (1). Advances in immunosuppressive strategies were critical to the success of organ transplantation and this led to a significant increase in both short and long term survival up to the late 1990s (2). However, more recent studies have shown that despite a continuing decrease in acute rejection, the long-term graft survival remains relatively unchanged in the last decade (3). Chronic allograft rejection continues to remain the most important cause of long-term graft failure and current immunosuppressive regimens have not demonstrated significant effect on reducing its incidence. Chronic rejection is characterized, as an immunologically mediated mechanism during which there is tissue inflammation and remodeling that eventually leads to fibrotic changes in the allograft tissue. This process which primarily affects the vessels and other tubular structures in the graft, is the characteristic feature seen in chronic allograft nephropathy in kidney, cardiac allograft vasculopathy in heart and bronchiolitis obliterans syndrome (BOS) in lung allografts.

Chronic rejection is hypothesized to have multiple etiologies and risk factors. These include:

1. **Cellular immune responses**: These are mediated by both CD4 and CD8 T-cells that can recognize donor MHC class I and II antigens by themselves (direct recognition) as well as by recipient antigen presenting cells (APCs) that present the processed donor antigens (indirect recognition). This cellular response is clinically manifested as acute rejection that results in a cellular infiltration of the graft tissue and vessels. Even single episodes of acute rejection can lead to chronic rejection long term, as it is postulated that these episodes of rejection can alter and augment the inflammatory milieu in the graft and thus lead to chronic rejection.

2. **Humoral immune responses**: Besides cellular responses, our laboratory as well as others have demonstrated a role for de novo as well as pre-transplant antibodies (Abs) to donor mismatched HLA class I and II in the development of chronic rejection (4-6). These Abs can bind to epithelial and endothelial cells on target tissue and lead to activation and release of pro-inflammatory and pro-fibrotic cytokine milieu that facilitates development of chronic rejection.

3. **Infections**: The immunosuppressed state in the post-transplant period leaves the recipient vulnerable to various bacterial and viral infections. Respiratory viral infections in the case of lung transplants and cytomegalovirus (CMV) infection in lung, cardiac, and renal transplantation are known to increase the risk of long term graft dysfunction due to chronic rejection. This is attributed to changes in the inflammatory milieu in the graft and also to changes in the other immune cells in the host including a suppression of regulatory T cells (Tregs). These mechanisms leading to the development of chronic rejection will be discussed later.

4. **Autoimmunity**: This is the most exciting and the recent avenue of focus of several laboratories including ours, in delineating the pathogenesis of chronic rejection. Cellular as well as Abs mediated autoimmunity to various self-antigens including Collagen (Col), k-alpha-1 tubulin (K!T), myosin, vimentin, heat shock proteins, angiotensin II type 1 receptor, major histocompatibility class I chain related peptide A (MICA) have been demonstrated to play a role in chronic rejection in the setting of lung, heart and kidney transplantation.

5. **Post-transplant inflammation**: Besides the important risk factors and etiologies listed above, other non-specific inflammatory factors have been shown to contribute towards development of chronic rejection. Inflammatory changes in the allograft can contribute to enhancing allo- and auto-immune responses. Ischemia reperfusion injury in the immediate post-transplant period is an important cause for inflammation in the grafts. In the setting of lung transplantation – factors such as gastro-esophageal reflux disease, etc., are also shown to contribute to allograft inflammation and development of chronic rejection.

Chronic rejection is currently considered to be multifactorial in etiology with a significant contribution of various risk factors as depicted in Figure 1. However, the most prevailing hypothesis is that early alloimmune responses lead to the immunologic graft injury that facilitate the development of autoimmune responses to self-antigens that further perpetuate the chronic inflammatory process. In this review, we will discuss the role of alloimmune responses that facilitate the development of an immune response to self-antigens that leads to chronic allograft rejection.

2. BOS

BOS is the long-term chronic rejection sequel following human lung transplantation. It is a clinical syndrome diagnosed as a progressive and persistent decline in post-transplant forced expiratory volume after other causes including anastomotic strictures of the airways are excluded. Histopathologically it is characterized as obliterative bronchiolitis (OB) that mainly affects the distal airways and results in
chronic fibrosis. Several studies indicate that up to 80% of all lung allografts are affected by BOS within five years post-transplant. Thus BOS is a highly important and relevant issue and shall be the primary focus of this review.

2.1 Animal models of obliterative airway disease (OAD)

The utility of animal models of lung transplantation and OAD has greatly facilitated the investigations of the pathogenesis of chronic rejection (7). Marck and Prop introduced the model of orthotropic left lung transplantation (8-10) which was of immense importance, since MHC mismatched transplantation could be performed and this procedure closely resembled the clinical setting of human lung transplantation in comparison to other models of heterotopic tracheal transplantation. The major limitations to this model are that it is a technically demanding and time consuming experimental procedure. Besides this the OAD lesions have not been entirely reproducible and the lack of easily available knock out or transgenic strains in rats limits the full extent of its utility. Krupnick et al. standardized a murine model of lung transplantation, but this model in their hands did not develop OAD lesions (11). Wilkes and Burlingham's group have more recently adapted this murine lung transplantation model across minor histocompatibility mismatches (C57BL/10 mice to C57BL/6 mice) that demonstrated OAD lesions in 44% percent of the transplanted mice (12).

Rodent lung transplantation, in general, has been technically challenging and most primary investigations by various laboratories, including ours, utilized the tracheal transplant model in mice to study the pathogenesis of OAD. In our lab, we developed a murine model of OAD wherein intra-tracheal administration of anti-MHC class I on days 1, 2, 3, 6 and weekly thereafter resulted in OAD lesions within 15 to 30 days (13). This model has been of immense significance to understand the role of alloimmunity as well as the cross talk between allo- and autoimmunity in the pathogenesis of OB that is discussed below.

2.2 Alloimmunity in BOS

Our laboratory has demonstrated using prospective serial analysis that Abs against mismatched donor HLA can be detected about 20 months prior to the development of BOS (14). These Abs bind to epithelial and endothelial cells in the allograft and lead to their activation and proliferation and secretion of pro-fibrogenic growth factors including platelet derived growth factor, heparin binding epidermal growth factor, insulin like growth factor-1, granulocyte monocyte colony stimulating factor, basic fibroblast growth factor and transforming growth factor-beta (TGF-β) (15, 16).

We proposed that this pro-fibrotic inflammatory milieu facilitates the development of chronic rejection and therefore de novo developed Abs are one of the causes that can mediate chronic rejection.

2.3 Autoimmunity in BOS

Studies in animal models of lung transplantation as well as following human lung transplantation have provided supporting evidence for the role of immune responses to self-antigens in the development of chronic rejection (BOS) (17-19). Peri-operative inflammatory processes including ischemia reperfusion injury can lead to the exposure and expression of cryptic self-antigens to the immune system. The role of immunity to Col V in this context has been well investigated. In the lung, Col V is expressed in the peribronchial and perivascular connective tis-
2.4 Alloimmunity: role in induction of de novo autoimmunity

As discussed above, it is established that both alloimmunity as well as autoimmunity to self-antigens play a significantly role in the development of chronic rejection. We shall further elaborate on the possible role of an alloimmunity in the development and perpetuation of this de novo autoimmune response post-transplant that eventually leads to chronic rejection of the allograft.

When airway epithelial cells were stimulated with anti-MHC class I Abs, they were found to undergo proliferation with the secretion of pro-fibrogenic growth factors (16). In addition, we also found that in a murine model of heterotopic tracheal transplantation following administration of anti-MHC class I in immune deficient RAG knockout mice, there was an increase in growth factors and pro-apoptotic genes as well as pro-inflammatory cytokine resulting in OAD (27). This led to the hypothesis that such a pro-inflammatory cytokine milieu could facilitate the development and immune reactivity to self-antigens due to exposure of cryptic antigens or their determinants as well as lowering the threshold for the activation of T cells and this allowing for the proliferation of self reactive T cells.

Using the murine model of administration of anti-MHC class I intratracheally that leads to the development of OAD, we were able to further establish the role of alloimmunity in the development of autoimmunity (13). In this model, following administration of anti-MHC class I, there was development of autoimmunity that lead to cellular infiltration, epithelial hyperplasia as well deposition of collagen and fibro-proliferation along the bronchioles and vessels. The mice that were administered isotype control Abs, non-specific MHC Abs or anti-keratin Abs did not demonstrate any lesions. More importantly, anti-MHC induced the development of Abs to Col V and K\textsubscript{1T}. This humoral response was concomitant with a cellular response to Col V and K\textsubscript{1T} that resulted in an increase in auto-reactive T cells that secreted IFN-\gamma, IL4 and IL-17. It is of interest to note that in this model, the role of IL-17 in this autoimmune response was better characterized, since administration of neutralizing Abs to IL-17 not only abrogated obstructive airway lesions, but also significantly reduced both the humoral as well as cellular response to self-antigens.

In a more recent study we serially followed lung transplant recipients and monitored them for the development of donor specific antibodies (DSA), Abs to self-antigens (Col V, K\textsubscript{1T}) and the development of BOS (18). It was found that in patients who develop BOS, DSA was detectable on an average 3 months following transplantation, which was then followed by the development of Abs to self-antigens within about 60 days following detection of DSA (18). It was also found that in patients in whom DSA was undetectable, Abs to self-antigens continued to persist.

Fedoseyeva et al have also demonstrated the concept of alloimmunity being essential to the development of autoimmunity in a murine heart transplant model (28). They found that mice that received an allograft across MHC barrier developed T cell responses to cardiac
myosin, however, mice that received a syngeneic cardiac graft or those that were recipients of an allogeneic skin graft, did not demonstrate such an immune response to myosin. They also indicated that this sensitization to myosin results in the rejection of cardiac allografts. Besides animal models in a recent prospective serial study on heart transplant recipients we found that DSA developed within 2.8 months of cardiac transplantation which was followed by the development of Abs to myosin and vimentin at 4 to 6 months post-transplant (29). This preceded the diagnosis of rejection detected at 8 months of transplantation.

Thus these reports strongly support that alloimmune responses precede autoimmune responses which leads to the pathogenesis of chronic rejection. They also suggest that monitoring for the development of DSA as well as immunity to self-antigens can be important biomarkers to predict the development of chronic rejection following transplantation. In early preliminary studies in lung transplant recipients who developed DSA, we have found that those who cleared DSA as well as Abs to self-antigens following Ab directed treatment with rituximab and intravenous immunoglobulin demonstrated greater freedom from development of BOS as compared to those that did not clear Abs (T. Mohanakumar et al, unpublished data). This suggests the possibility of therapeutic intervention to prevent chronic rejection by monitoring and early detection of DSA and/or auto-Abs.

2.5 Cross talk between alloimmunity and autoimmunity

Thus far, we have primarily focused on post-transplant alloimmunity that leads to development of autoimmunity. However, pre-existing auto-Abs in patients with end stage lung disease and hepatitis C related liver disease before transplantation may also play a part in augmenting alloimmunity and perpetuation of immune responses to self-antigens leading to chronic rejection. (6, 30). In patients with end stage lung disease or hepatitis C related liver disease, there is inflammatory tissue damage, that can result in the exposure of self-antigens to the immune system, and thus the presence of a favorable inflammatory milieu lead to the development of autoimmunity. In a cohort of lung transplant recipients, we found that patients with pre-existing Abs to K\text{u}1 T or Col V had a significantly increased risk for the development of primary graft dysfunction (PGD) following transplantation (relative risk 3.1, p=0.02) (6). Occurrence of PGD is significantly associated with an increased risk of BOS (31). PGD was itself associated with increased inflammatory cytokines including IL-2, IFN-\gamma, IL-12, IL-1\beta as well as the development of Abs and CD4+ responses to mismatched HLA class II and thus leading to BOS (32). This suggests that pre-existing autoimmunity to self-antigens may lead to the development of PGD, which leads to allo-immune responses, and BOS.

2.6 Regulatory T-cells in the development of de novo autoimmunity

Regulatory T-cells characterized by CD4+ CD25+Foxp3+ expression have been shown to play an important role in maintaining tolerance toward self-antigens, and it has been suggested that a reduction in either their number or function can potentiate autoimmune responses (33). Several reports including from our laboratory have shown that loss of Tregs is associated with the development of chronic rejection (34-36). These Tregs have also been shown to promote the development of IL-10 secreting T-cells that were protective against both development of autoimmunity as well as the development of chronic rejection (23, 24). In the post-transplant period, immunosuppression can significantly affect T-cell kinetics. Current immunosuppressive drugs such as cyclosporine and tacrolimus can have a profound effect in T-cell suppression. This is beneficial in preventing acute rejection, but also results in the decrease in various immune cell regulatory cytokines including IL-2 that is essential for the development of Tregs (33). Thus these immunosuppressive drugs albeit prevent acute rejection, may potentially lead to development of autoimmunity due to suppression of Tregs. Viral infections represent another major etiology for the development of chronic rejection following organ transplantation (34, 37, 38). In a study in lung transplant recipients, we found that respiratory viral infections were a significant risk factor for a decline in Treg numbers in the post-transplant period. This viral induced decline in Tregs resulted in an increased prevalence of Abs to Col V (7% vs. 0%) and K\text{u}1 T (31% vs. 12%) when compared to those without viral infection and Treg decline (34). In a murine model, we demonstrated that murine respiratory virus (Sendai virus) infected airway epithelial cells had increased expression of Fas-ligand (FasL) (34). Co-culturing Tregs with these FasL+ epithelial cells resulted in increased apoptosis of T-cells compared to those co-cultured with normal epithelial cells, thus demonstrating that viral infection can lead to apoptosis of Tregs. These indicate that a decrease in Tregs brought about by immunosuppression and/or viral infections in the post-transplant period results in the development of an autoimmune response.
due to the loss of peripheral tolerance to self-antigens.

2.7 Role of Th17 cells

As discussed above, cellular immune responses to self-antigens are key players in the development of BOS. These responses are predominantly mediated by IL-17 secreting Th17 cells (39). Th17 cells that are involved in mucosal immunity contribute to the development of autoimmunity in both animal models and humans. IL-17 is a pro-inflammatory and pro-fibrotic cytokine and thus can facilitate the process of chronic rejection. Experiments discussed above using intratracheal anti-MHC administration leading to immune responses to self-antigens have shown that the neutralization of IL-17 can abrogate the development of OAD.

Cytokines that have been postulated to influence the differentiation of T-cells into Th17 cells include IL-1β, IL-6, IL-23, TGF-β and IL-21. These cytokines especially IL-1β, IL-6 and TGF-β have been shown to be increased in lung transplant recipients. IL-6 in particular can also direct Tregs to express IL-17 in an antigen specific manner, thus linking the adaptive and the innate immune response to self-antigens. Thus neutralization of IL-17 as well as IL-6 is an important direction towards future therapy in the prevention of chronic rejection.

3. Evidence for autoimmunity in other organs

In the heart, chronic rejection is characterized by cardiac allograft vasculopathy (CAV) is characterized by coronary disease that leads to accelerated occlusion of both intramural and epicardial coronary vessels. Both humoral and T-cell mediated responses to the self-antigen myosin have been shown to play a role in the pathogenesis of CAV (28, 29, 40, 41). Besides myosin, several laboratories including ours, have found evidence for immunity to vimentin as well as major histocompatibility complex class I related chain A (MICA) in the pathogenesis of both acute Ab mediated rejection as well as chronic rejection following cardiac transplantation (29, 40, 42). There was an increase in CD4 T cells that secreted IFN-γ, IL-5 and IL-17 which were specific cardiac antigens myosin and vimentin in patients with cardiac allograft vasculopathy providing evidence for the role of cellular immunity against cell antigens in the development of chronic rejection following cardiac transplantation (29).

In kidney transplantation Abs to angiotensin-II type 1 receptor (AT1R) and heat shock proteins have been implicated in the pathogenesis of rejection (43). Our early preliminary results have indicated that patients with chronic allograft nephropathy have significantly increased Abs to AT1R (unpublished data, T. Mohanakumar et al). In pancreas transplantation immune responses to islet cells (44) as well as other pancreas specific antigens have been demonstrated in rejection. More interesting is the setting of simultaneous kidney-pancreas where although originating from the same donor, in some patients only one of the organs demonstrates rejection. Our early studies have led us to hypothesize that in these patients immune responses to organ specific antigens may play an important role in rejection, i.e. responses only to kidney specific antigens in those who reject the kidney alone and responses to pancreatic antigens alone in those who reject the pancreas.

4. Future therapeutic implications

Evidence presented above gives important insight toward future therapy post-transplant to prevent and treat chronic rejection. The role for allo- and auto-Abs has been strongly suggested since depletion of these Abs by plasmapheresis both pre-transplant as well as post-transplant have been shown to significantly decrease the incidence of chronic rejection. Similarly administration of IVIG that has been successfully used in treating various autoimmune diseases has been shown to decrease the incidence of Ab mediated rejection and possibly chronic rejection. Viral infections represent the most preventable risk factor with regard to chronic rejection and active and aggressive surveillance for infection along with prophylactic medication in the post-transplant period may help prevent Treg suppression that leads to chronic rejection. Besides this, Treg sparing immunosuppressive agents such as Tacrolimus, Rapamycin etc. may be utilized. Chronic rejection currently is untreatable – however, since neutralization of IL-17 and IL-6 has been shown to effectively alleviate OAD in murine models – this may be another potential target towards the treatment of chronic rejection.

5. Conclusions

With significant improvements in short-term survival of allografts, chronic rejection is increasingly recognized in organ transplantation. Studies on chronic rejection are growing everyday, providing us with a better understanding of its complex pathogenesis. Alloimmunity and resulting immune responses to self-antigens during the post-transplant period is emerging to be one of the most important
risk factor for the development of chronic allograft rejection. Both these immune responses seem to perpetuate each other with alloimmune responses triggering autoimmune responses which further enhance alloimmune responses, resulting in constant tissue inflammation and repair that eventually results in a pro-inflammatory and pro-fibrogenic state with cellular infiltration and deposition of fibrous tissue that is hallmark of the chronic rejection process. Besides this there is emerging evidence for the role of Th17 cell that contributes to allo and autoimmunity as well as fibrosis. Understanding the role of Tregs in the development of de novo autoimmunity and chronic rejection may potentially evolve into strategies to prevent Treg decline and preserve their function by preventing viral infection or using newer Treg sparing immunosuppressive agents.

**Abbreviations**

Abs antibodies  
APC antigen presenting cell  
AT1R angiotensin-II type 1 receptor  
BOS bronchiolitis obliterans syndrome  
Col collagen  
CMV cytomegalovirus  
DSA donor specific antibodies  
Ka1T K-alpha 1 tubulin  
MHC major histocompatibility antigens  
MICA major histocompatibility class I chain related peptide A  
OAD obliterative airway disease  
OB obliterative bronchiolitis  
PGD primary graft dysfunction

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