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Role of antibodies to self-antigens in chronic allograft rejection: potential mechanism and therapeutic implications

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Abstract

Significant progress has been made in preventing acute allograft rejection following solid organ transplantation resulting in improved allograft survival. However, long term function still remains disappointing primarily due to chronic allograft rejection. Alloimmune responses primarily defined by the development of antibodies (Abs) to donor mismatched major histocompatibility antigens during the post-transplantation period have been strongly correlated to the development of chronic rejection. In addition, recent studies have demonstrated an important role for autoimmunity including the development of Abs to organ specific self-antigens in the pathogenesis of chronic allograft rejection. Based on this, a new paradigm has evolved indicating a possible cross-talk between the alloimmune responses and autoimmunity leading to chronic rejection. In this review, we will discuss the emerging concept for the role of cellular and humoral immune responses to self-antigens in the immunopathogenesis of chronic allograft rejection which has the potential to develop new strategies for the prevention and/or treatment of chronic rejection.

Keywords

Alloimmunity; Autoimmunity; Chronic Rejection; Th17; regulatory T-cells and Organ transplantation

1. Introduction

Organ transplantation remains a treatment option for several end-stage diseases resulting in organ dysfunction. Due to the limited availability of the donors, the demand for organs which can be transplanted have increased and many patients continue to wait on the transplant list for a long time resulting in high morbidity and mortality. Chronic rejection following transplantation remains the most important menace for long term function of the transplanted organs which often necessitate re-transplantation with markedly reduced allograft function and increases the burden to find suitable donors for transplantation. Despite advances in understanding of donor and recipient physiology, operative technique,

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and immunosuppressive pharmacology, chronic rejection continues to be a major hurdle for long term function of the allograft. Regardless of transplanted organ, chronic rejection is characterized by fibrosis of the hollow structures within the graft resulting in loss of function which may develop anywhere from months to years post-operatively. Chronic rejection is initiated by a host-anti-graft-immune response with both antigen-dependent and antigen-independent factors leading to fibroproliferative changes affecting graft function [1].

1.2 Cellular and humoral immune responses following solid organ transplantation

The primary targets of the recipient immune response against the allograft are the mismatched donor major (MHC) histocompatibility antigens present on the allogeneic tissue. Immune recognition of mismatched donor histocompatibility antigens results in activation of both cellular and humoral immune mechanisms which leads to allograft rejection [2, 3]. Inflammation and tissue remodeling promoted by alloimmune mechanisms may also facilitate the induction of autoimmune responses against self-antigens [4]. Allorecognition can occur through two unique but not mutually exclusive pathways: the direct and indirect pathways of antigen presentation. The direct pathway involves recognition of intact donor MHC molecules on the cell surface, usually by antigen presenting cells (APC). Both CD8⁺ and CD4⁺ T cells can directly recognize donor MHC molecules, MHC class I and II respectively. In contrast, the indirect pathway involves presentation of processed donor antigens by recipient APC to recipient T cells. A 'semi-direct' pathway has also been recently described which involves recipient APCs that acquire donor MHC through cell-to-cell contact and activate host T-cell responses which contributes to chronic rejection [5].

While the direct pathway is more important for acute allograft rejection, the indirect pathway is thought to play a dominant role in chronic allograft rejection [6, 7]. Experiments have demonstrated that inhibition of acute rejection by depleting passenger APC significantly delays, but does not prevent development of chronic rejection [8–10]. It has been demonstrated that the frequency of direct alloreactive T cells exceeds indirect alloreactive T cells in the early post-transplant period [11]. The frequency of direct alloreactive T cells declines with time following transplantation while the continuous influx of the processed donor antigens by the recipient APC through the indirect pathways increases the frequency of indirect alloreactive T cells that are themselves more resistant to currently used immunosuppression [12, 13].

Humoral immune responses against mismatched donor histocompatibility antigens (HLA) in the pathogenesis of chronic rejection are well established [14]. Studies from our group, as well as others, have shown that development of immune responses to both mismatched MHC and self-antigens during the post-transplant period may also contribute to chronic rejection [15–17]. Development of antibodies (Abs) to tissue specific antigens has been strongly associated with allograft rejection. For example: K-alpha-1 tubulin (K α 1T) and collagen V (ColV) following lung, cardiac myosin and vimentin has been detected following cardiac, Col III following liver, and angiotensin II type 1 receptor and Col IV following kidney transplantation. However, their functional significance has not been fully characterized [15, 18–21]. It is also not clear what roles the alloimmune responses play in the development of immune responses to self-antigens. In this review, we will detail recent findings for the two distinct yet interdependent immune processes which contribute to the immunopathogenesis of chronic rejection.

1.3 Lung Transplantation

Chronic rejection after lung transplantation is characterized by obliteration of terminal airways and is clinically diagnosed based upon the reduced forced expiratory volume in one

second and is termed as Bronchiolitis Obliterans Syndrome (BOS) [22]. The development of BOS post lung transplantation is multi-factorial and involves both cell-mediated and humoral immunity [23].

A high frequency of indirect alloreactive CD4⁺ T cells against donor MHC class I and II molecules has been detected in a human lung allograft recipient years after transplantation and are associated with BOS [24]. Intrapulmonary lymphoid tissue has also been implicated in the pathogenesis of chronic rejection as it may serve as a reservoir for effector memory T cells and high endothelial venules which can contribute to the local immune responses in small airways leading to BOS [25].

Animal studies have identified ColV reactive T cells in rat lung allografts undergoing rejection [26]. These ColV specific T cells derived from rat lung allografts can mediate rejection of isografts when adoptively transferred without affecting native lung [27]. Studies from our lab have demonstrated a high frequency of ColV reactive T cells in human lung allograft recipients [28]. We have also demonstrated that development of BOS was associated with expansion of IFN- γ producing ColV specific Th-1 cells with a concurrent reduction in IL-10 secreting T cells [28]. A longitudinal study in lung transplant patients have determined an association between the development of ColV specific IL-17 responses with onset of BOS [29]. ColV-specific responses in BOS patients were dependent on both CD4⁺ T cells and monocytes and required IL-17, TNF- α , and IL-1 β [30]. Further, in experimental model of obliterative airway disease (OAD), adoptive transfer of lymph node cells expressing high levels of IL-17 and IL-23 gene transcripts from ColV-sensitized mice have been shown to induce obliterative lesions in the lung isograft [26].

Several studies have demonstrated that development of Abs to mismatched donor-HLA is associated with the development of chronic rejection [26, 31]. Based on studies by us [29] and others [32] the presence of 'shed' donor HLA antigens in the bronchoalveolar lavage fluids following lung transplant, provide the substrate for antigen presentation to T helper cells and induction of alloimmunity. These T helper cells, which are engaged in indirect recognition, can produce lymphokines required for the growth and maturation of allo-Ab producing B cells. Studies from our laboratory have also demonstrated that anti-HLA can activate human airway epithelial cells (AEC) resulting in the production of several fibrogenic growth factors which can play an important role in the pathogenesis of chronic rejection [15]. In addition to Abs to mismatched donor HLA, recent studies strongly suggest an important role for autoimmunity in the pathogenesis of allograft rejection [15]. Our laboratory and others have shown a strong correlation between the development of Abs to a self protein, K α 1T, as well as ColV and the development of BOS following human lung transplantation [15, 33].

Since lung transplant recipients often developed Abs to HLA months before any clinical evidence for BOS, our center instituted Ab directed therapy following detection of donor specific antigens (DSA) even though the patients were clinically stable [34]. Result from this preliminary report demonstrated improvement in long term function without development of BOS by this early intervention. Further analysis of this group of patients who cleared DSA but developed BOS demonstrated high levels of Abs to lung associated self-antigens K α 1T and ColV indicating a pathogenic role for Abs to self-antigens in the development of BOS. In addition, patients who cleared both DSA and Abs to self-antigens did not develop BOS during the study period. Recently, we analyzed patients waiting for lung transplantation for preexisting Abs to lung associated self-antigens. This study was aimed to determine whether pre-existing Abs to self-antigens are present in patients waiting for lung transplantation and the role of pre-existing Abs to self-antigens in the development of primary graft dysfunction (PGD) as well as chronic rejection following lung

transplantation. It is generally accepted that PGD immediately post-transplant period increases risk for development of BOS [35]. In this cohort, we analyzed 142 adult lung transplant recipients for pre-transplant Abs to self-antigens, ColV and K α 1T and those with Abs to self-antigens had increased risk of PGD (odds 3.09, 95% CI 1.2 – 8.1, $p=0.02$) compared to those without. Ab+ patients demonstrated high levels of pro-inflammatory cytokines IL-1 (2.1 fold increase), IL-2 (3.0), IL-12 (2.5), IL-15 (3.0) and chemokines IP-10 (3.9) and MCP-1 (3.1, $p<0.01$ for all). On 5-yr follow-up, patients without Abs showed greater freedom from development of HLA-Abs compared to those with Abs (class I: 67% versus 38%, $p=0.001$; class II: 71% Vs 41%, $p<0.001$). Patients with pre-transplant Abs were found to have an independent relative risk of 2.3 (95% CI 1.7 – 4.5, $p=0.009$) for developing BOS.

One of the target non-HLA antigens that have received considerable attention recently in the field of solid organs transplantation is the MHC class I-related chain A (MICA). MICA, a glycoprotein expressed on cellular membrane, indicates cellular stress and triggers a range of immune effector mechanisms, including cellular cytotoxicity and cytokine secretion [36, 37]. MICA, plays an important role in initiating an immune response by binding to the immune-receptor NKG2D and stimulates cell lysis pathways hence provide co-stimulatory signal for the activation of NK (natural killer), CD8⁺ T and $\gamma\delta$ T cells following transplantation [38]. Recent reports suggest that development of Abs to MICA following solid organ transplantation is an independent risk factor for the development of chronic rejection [39]. During donor procurement and lung transplantation, the AECs undergo mechanical stress which causes damage to epithelial integrity. Similarly, anti-HLA Abs developed following transplantation can also injure epithelial cells, leading to the expression of otherwise cryptic self-antigens such as K α 1T and ColV and cell surface expression of MICA antigens.

Development of Abs either to MICA alone or MICA and HLA together significantly correlated ($p<0.01$) with development of BOS. Kinetic analysis in the post-lung transplant period revealed that development of anti-HLA Abs (7.6 ± 4.7 months) preceded the development of anti-MICA Abs (10.0 ± 3.5 months). Abs to MICA developed approximately 6 months after lung transplantation and peak titers were present at the time of clinical diagnosis of BOS (16.3 ± 2.7 months). The development of Abs to HLA and MICA was strongly associated with the development of BOS thereby suggesting a role for MICA as an independent risk factor for development of BOS and synergistic effect with HLA Abs [40]. However, due to polymorphic nature of the MICA, further studies are warranted to identify the Ab specificity present in the serum from BOS positive lung transplant recipients to establish the donor specificity. Cumulatively, immune responses to self-antigens K α 1T and ColV, and stress induced antigens like MICA may contribute to the pathogenesis of chronic rejection following human lung transplantation.

1.4 Cardiac transplantation

Chronic rejection of cardiac allografts is manifested by the clinical entity cardiac allograft vasculopathy (CAV): an accelerated form of occlusive coronary disease affecting both intramural and epicardial coronary arteries and veins; 5-year incidence of CAV is 30–40% [41]. Animal studies have supported a critical role of alloimmune responses in the development of chronic rejection in cardiac allografts and clinical experience has identified the number of acute rejection episodes and donor HLA mismatching as independent risk factors for CAV after heart transplant (HTx) [42].

Cell mediated immunity has also been implicated in the pathogenesis of CAV as histologic examination of perivascular infiltrates often reveals a predominance of immune cells including T cells (CD4⁺, CD8⁺), NK cells, macrophages and dendritic cells. In a study

using a T bet^{-/-} murine model of CAV, Th17 cells mediated a pro-inflammatory response in the absence of a Th1 response leading to CAV [43]. T-cell-mediated autoreactivity against cardiac myosin has been shown to develop and persist in the absence of an alloimmune response, indicating that response to myosin, a self-antigen, is associated with the pathogenesis of CAV [31, 44].

Humoral immunity has also been implicated in the pathogenesis of CAV. The presence of circulating Abs specific to mismatched donor HLA and the deposition of complement product C4d within the graft have been associated with poor allograft outcome. Studies utilizing syngeneic heart transplant using animal models have shown that chronic rejection can be induced even in the absence of an alloimmune response [42, 45]. Studies have also indicated that Abs against vimentin, a cytoskeleton protein, are an independent predictor of atherosclerosis following cardiac transplantation and can accelerate the course of CAV [31, 46]. Our studies using serial sera from patients with acute Ab mediated rejection (AMR) in the early post-heart transplant period (<12 months) and CAV in the late post-transplant period (>12 months) demonstrated that the presence of DSA in AMR and CAV is significantly associated with development of Abs to cardiac self-antigens myosin and vimentin [47]. Furthermore, the induction of high CD4⁺Th specific to cardiac self-antigens that predominantly secrete IL-5 and IL-17 suggest that alloimmune responses to donor HLA may play a significant role in the development of Abs to self-antigens leading to AMR and CAV, respectively.

Humoral immune responses to mismatched donor HLA and MICA have been reported to contribute to immunopathogenesis of AMR in the early period and CAV in the late part after heart transplant [48]. We analyzed the development of both DSA and MICA Abs in 95 post-heart transplant recipients (43 patients in the early period (<12 months) and 52 patients in the late period (>12 months)). Development of DSA and anti-MICA (AMR+, 63–75%, AMR–, 11%, $p=0.009$) was significantly associated with AMR. Serial monitoring revealed DSA (2.7 ± 1.4 months) preceded development of anti-MICA (6.5 ± 2.1 months) in recipients diagnosed with AMR at 8.3 ± 2.5 months post-heart transplant. Similarly, development of DSA and anti-MICA (CAV+, 67–75 %, CAV–, 13%, $p=0.004$) was significantly associated with CAV. AMR+DSA+, and CAV+DSA+ patients demonstrated increased anti-MICA levels compared with respective DSA-patients ($p=0.01$). Abs to HLA are associated with and precede development of anti-MICA in AMR and CAV. Therefore, these studies support that anti-MICA can be used as non-invasive markers for monitoring AMR and CAV following cardiac transplantation [48]. Furthermore, anti-MICA precedes the clinical diagnosis of AMR and can be used as non-invasive markers for post-transplantation monitoring of AMR and CAV in cardiac transplant recipients. It is of interest to note that studies by Zhang et al [49] demonstrated that among 72/168 recipients, 5 of 19 (25%) AMR+ve patients had Abs MICA compared to 1 of 53 (2%) patients without AMR ($p<0.01$) Two patients with AMR and 10 patients without AMR also developed Abs to MICA not present in the donor. Antibodies to MICA that were not donor specific were not associated with AMR ($p=0.99$). Interestingly, seven patients developed Abs against their own MICA. One of these, seven patients developed AMR. Taken together, results from this report demonstrated that Abs to MICA correlate with the development of AMR [49]. However, further studies along these lines are warranted to confirm the role of donor versus recipient specific Abs to MICA in inducing allograft rejection.

1.5 Kidney transplantation

Chronic allograft nephropathy (CAN) is a major cause of late graft loss in renal transplant recipients. The histopathologic signs of CAN are interstitial fibrosis, tubular atrophy, glomerulopathy and vasculopathy. It is thought to account for approximately 40% of graft loss at 10 years [50]. Work with both animal models and clinical studies have implicated

both cell-mediated and humoral arms of alloimmunity contribute to its development [51]. CD4⁺ alloreactive T cells responding to donor derived peptides bound to recipient MHC class II have been correlated with CAN.

Increased levels of pre-transplant anti-HLA and *de novo* post-transplant donor specific Abs has also been associated with CAN. However, Abs developed *de novo* and directed at the donor mismatched HLA are not always detectable in the circulation of patients undergoing chronic rejection, questioning the significance of Abs to HLA in the pathogenesis of chronic rejection. Clinical experience with refractory vascular allograft rejection in the absence of detectable anti-HLA demonstrated the presence of Abs directed to non-HLA molecules. One such Ab was directed to two epitopes of the second extracellular loop of the angiotensin II type 1 (AT1) receptor [52] and has been suggested to identify those at risk for refractory allograft rejection. Other Ab targets for kidney allograft rejection include perlecan and Col types IV and VI as well as glomerular basement membrane protein Agrin [53, 54]. Recent studies have also suggested a role for anti-vimentin in the development of CAN [55].

Transplant glomerulopathy (TG) has been demonstrated in approximately 20% patients by 5 years following transplantation [53]. These patients develop proteinuria, hypertension and declining graft function. Studies with clinical and animal models suggest that TG arises as a consequence of persistent endothelial cell (EC) injury by the humoral arm of the immune system [53]. The TG associated acute rejection, not only occurs due to the presence of HLA Abs but is also associated with additional risk when Abs to cryptic self-antigens such as AT1, Col IV, fibronectin, MICA and agrin are developed following transplantation. Studies by Joosten et al, using biopsy proven TG patients (n=19) has shown that anti-glomerular basement membrane specific Abs developed against heparan sulphate proteoglycan agrin which correlated with development of TG following transplantation [56]. In a small cohort of 26 biopsy proven TG patients, we have identified the development of Abs to kidney specific antigens Col IV and fibronectin in addition to DSA (unpublished finding). Taken together, the above mentioned studies strongly suggest an important role of autoimmunity in TG following kidney transplantation.

1.6 Liver transplantation

Chronic rejection after liver transplantation is manifested as fibrous tissue replacement in the allograft. Fibrogenesis is a complex, dynamic process mediated by necro-inflammation and activation of hepatic stellate cells under the influence of virally induced immunomodulation. Cell-mediated and humoral immunity are both implicated in the progression of fibrosis after liver transplant [57, 58]. Hepatitis C virus (HCV) recurrence with accelerated fibrosis following orthotopic liver transplantation (OLT) is a universal phenomenon in HCV infected recipients [59]. We have previously evaluated mechanisms contributing to HCV induced allograft fibrosis/cirrhosis. We have investigated HCV-specific CD4⁺Th17 cells and their induction in OLT recipients with recurrence utilizing 51 HCV+ OLT recipients [60]. Recipients with recurrent HCV induced allograft inflammation and fibrosis demonstrated a significant increase in frequency of HCV specific CD4⁺Th17 cells. Increased pro-inflammatory mediators (IL-17, IL-1 β , IL-6, IL-8 and MCP-1) levels were identified. OLT recipients with allograft inflammation and fibrosis/cirrhosis demonstrated increased frequency of Tregs that inhibited HCV specific CD4⁺Th1 but not Th17 cells. This suggests that recurrent HCV infection in OLT recipients induces an inflammatory milieu characterized by increased IL-6, IL-1 β and decreased IFN- γ which facilitates induction of HCV specific CD4⁺Th17 cells. These cells are resistant to suppression by Tregs and may mediate an inflammatory cascade leading to cirrhosis in OLT recipients following HCV recurrence [60].

HCV infection and its recurrence after OLT are associated with the remodeling of extracellular matrix (ECM) components (particularly Col), which leads to fibrosis. In our recent studies we determined that development of Abs to self-antigen Col in HCV-infected patients correlates with the fibrosis stage and the peripheral cytokine response [61]. The HCV subjects (n = 70) were stratified as follows: (1) a non-OLT group without fibrosis (Scheuer stages 0–2), (2) a non-OLT group with fibrosis (Scheuer stages 3–4), (3) a post-OLT group without fibrosis (Scheuer stages 0–2), and (4) a post-OLT group with fibrosis (Scheuer stages 3–4). There were increased levels of Abs to Col II, Col IV, ColV, and vimentin in the non-OLT fibrosis group and in the post-OLT fibrosis group in comparison with the non-fibrosis groups. The non-OLT and post-OLT fibrosis groups demonstrated significantly higher T helper 2 (Th2) and T helper 17 (Th17) cytokine levels and lower T helper 1 cytokine levels in comparison with the no-fibrosis groups. Our results demonstrate that in HCV-infected patients, the levels of Abs to ECM positively correlate with liver fibrosis, which is associated with a predominantly Th2 and Th17 cytokine profile [61]. This strongly suggests that chronic HCV infections lead to the development of an immune response to self-antigens which may play a role in liver fibrosis in OLT HCV patients. This will further provide basis for conducting prospective, longitudinal studies to validate the idea that serum measurements of anti-Col titers can serve as early, noninvasive markers of fibrosis progression in HCV patients.

1.7 Pathophysiologic effect of auto-Abs

The phenomenon of chronic rejection after solid organ transplantation is likely the result of a multi-factorial interplay of various effector arms of alloimmunity. Studies in the arena of heart, lung, liver, and kidney transplantation have identified putative mechanisms that contribute towards development of chronic rejection. In these instances, an emerging theme is that inflammation and subsequent tissue remodeling attendant to the post-transplant state exposes cryptic self-antigens or their determinants that, along with a favorable cytokine milieu, allows for loss of peripheral tolerance and the activation of cell-mediated immunity towards development of *de novo* immune responses to self-antigens. As discussed above, there is convincing evidence for both alloimmunity as well as autoimmunity to self-antigens in the development of chronic rejection. We will now discuss the possible role of an alloimmunity in the development and perpetuation of autoimmune responses post-transplant which we propose to be an important mediator of chronic rejection of the allograft.

Abs to HLA have been shown to activate endothelial and smooth muscle cells upon specific binding to HLA class I molecules expressed on the cell surface [54]. Recent studies from Elaine Reed's laboratory demonstrate that surface ligation of anti-HLA class I induce an intracellular signal through mammalian target of rapamycin (mTOR) promoting EC proliferation [62]. Furthermore, *RNAi* based knockdown of mTOR inhibited class I-mediated phosphorylation of proteins downstream of mTOR complex 1 and mTOR complex 2. These results support the role of anti-HLA Abs in the process of transplant vasculopathy and suggest that exposure of the graft endothelium to anti-HLA Abs may promote proliferation through the mTOR pathway [62]. We demonstrated that when AECs were incubated with anti-MHC class I, they underwent proliferation with the secretion of pro-fibrogenic growth factors [63]. In addition, we also demonstrated that administration of anti-MHC class I in a murine model of heterotopic tracheal transplantation in immune deficient RAG knockout mice, there was increases in growth factors and pro-apoptotic genes as well as pro-inflammatory cytokines resulting in OAD [64]. Further, classical complement activation is an important step in the alloimmune mediated graft injury [65]. Evidence supporting a critical role for complement in kidney [66] and heart transplantation [67] include local deposition of complement split products (e.g., C4d) along allograft capillaries which is significantly correlated with inferior allograft outcome.

We previously demonstrated that Abs to K α 1T, an epithelial cell surface gap junction protein correlate with development of BOS and also causes upregulation of pro-fibrotic growth factors [15]. We have earlier demonstrated that lipid rafts play a critical role in the surface ligation of Abs to K α 1T to its specific receptor (K α 1T) of the AEC surface [68]. Recently, we have determined the molecular basis for fibrogenesis mediated by ligation of K α 1T expressed on AECs by its specific Abs [69]. Normal human bronchial epithelial (NHBE) cells upon ligation of K α 1T with specific Abs caused upregulation of pro-fibrotic growth factors VEGF, HGF and TGF- β . Furthermore, incubation of NHBE with K α 1T Abs increased expression of hypoxia inducible factor (HIF-1 α). The K α 1T Ab-mediated growth factor expression is dependent on HIF-1 α as inhibition of HIF-1 α returned fibrotic growth factor expression to basal levels. Thus, demonstrating that HIF-1 α -mediated upregulation of fibrogenic growth factors induced by ligation of K α 1T Abs is critical for development of fibrosis leading to chronic rejection of lung allograft [70]. Further, although polymorphisms have been reported in self-antigens including for ColV [71], their relevance to the development of cellular immune responses and Abs resulting in rejection following transplantation is yet to be determined. Taken together, these series of studies from our laboratory and others strongly suggest a direct pro-fibrotic pathologic role of the Abs to self-antigens playing a critical role in the development of chronic rejection.

1.8 Putative mechanisms for the Treg vs Th17 imbalance in chronic rejection

Immunological mechanisms leading to the development of immune responses to self-antigens following solid-organ transplantation has not been fully understood. Our recent studies have determined that a shift in the immunodominant epitopes of ColV skews the T-cell mediated immune responses towards Th17 phenotype [72]. This 'constrictive epitope shift' of ColV is likely induced by the activation of matrix metalloproteinase enzymes, which has the potential to 'uncover' cryptic epitopes of self-antigens. Further, following transplantation there is attenuation of self-antigen specific IL-10 responses which can result in the loss of peripheral tolerance mediated by Tregs which can result in the induction of autoimmunity leading to the development of chronic lung allograft rejection [72].

Studies from our laboratory, as well as others, strongly support the notion that IL-17 mediated immune responses to self-antigens is a key player in the development of BOS following lung transplantation [72–75]. Th17 cells have been demonstrated to contribute to the development of mucosal autoimmunity in both animal models and humans [75]. IL-17 is a pro-inflammatory and pro-fibrotic cytokine that can facilitate the process of chronic rejection. Using a murine model of OAD we demonstrated that anti-MHC administration lead to immune responses to self-antigens resulting in OAD and more significant is our finding that neutralization of IL-17 leads to complete prevention of OAD [33]. Furthermore, depletion of CD4⁺ Foxp3⁺ T reg in this murine OAD model resulted in attenuation of IL-17 mediated OAD lesions (unpublished data). Therefore, Treg mediated suppression of IL-17 may play an important role in controlling immune responses to self-antigens and breakdown of this peripheral regulation can result in the development of immune responses to self-antigens leading to chronic rejection.

Tregs are known to inhibit both autoreactive as well as alloreactive effector T cells [76, 77]. Several reports, including data from our laboratory, have shown that loss of Tregs is associated with the development of chronic rejection [78–80]. Further, an imbalance in the self-antigens specific Treg vs Th17 responses have been determined to be the central cause for development of autoimmune responses [81]. However, in the context of potent immunosuppression following transplantation such as with calcineurin inhibitors which abrogate Treg proliferation, there is a greater chance for loss of Tregs leading to breakdown of peripheral tolerance to self-antigens [82] resulting in autoimmune responses to tissue specific self-antigens which can result in allograft rejection. Thus, therapeutic strategies

utilizing Treg sparing immunosuppressants may benefit towards maintaining the Treg population and therefore improving the long term survival and function of the transplanted organs [83].

2. Conclusion

Alloimmune responses and the development of *de novo* autoimmunity to self-antigens appear to be the principal player in the development of chronic rejection after solid organ transplantation [84]. Tissue inflammation and remodeling provides the substrate for the activation of both cell-mediated and humoral effector arms. This activation of the alloimmune response in part leading to activation of Th-17 cells leading to IL-17 secretion and autoimmunity including production of antibodies to tissue restricted self antigens. IL-17 has been shown to facilitate the activation of fibrogenic pathways and we propose that this can lead to the development of chronic rejection following solid organ transplantation. Future work in the field is needed to identify the mechanistic role for auto-Abs in the development of rejection and their clinical utility as a biomarker for chronic rejection. The IL-17 and development of autoimmunity in chronic rejection also represents new pathways to focus to expand the immunosuppressive armamentarium. Since most of the current results strongly suggest that alloimmune response to mismatched HLA antigen as evidenced by circulating Abs to donor HLA precedes the development of Abs to self-antigens, it is logical to intervene as early after detection of Abs to HLA to prevent the development of Abs to self-antigens and therefore prevent or delay the onset of chronic rejection. Studies in lung transplant recipients are currently underway to test this hypothesis by administering IVIG and Rituxan as early as circulating Abs to donor specific HLA were identified following lung transplantation. Viral infections also represent another preventable risk factor for reducing the incidence of chronic rejection. Therefore, aggressive surveillance for infection along with prophylactic medication in the post-transplant period may also help to prevent Treg suppression by viruses [78] that can lead to chronic rejection. Treg sparing immunosuppressive agents should also be of value in preventing the development of immune responses to self-antigens and thus preventing chronic rejection. Agents which will suppress IL-17 and pro-fibrogenic HIF-1 α [85, 86] which are already in anti-cancer therapy, should be of value in attenuating autoimmune responses and therefore reducing chronic rejection.

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Abbreviations used

Abs	antibodies
AEC	airway epithelial cells
AMR	antibody mediated rejection
APC	antigen presenting cells
AT1	angiotensin II type 1
BOS	bronchiolitis obliterans syndrome
CAN	chronic allograft nephropathy
CAV	cardiac allograft vasculopathy

ColV	Collagen V
DSA	donor specific antigens
EC	endothelial cell
ECM	extracellular matrix
HCV	hepatitis C virus
HIF-1α	hypoxia inducible factor
HLA	histocompatibility antigens
Kα1T	Kalpha 1 Tubulin
MHC	major histocompatibility complex
MICA	MHC class I related chain A
mTOR	mammalian target of rapamycin
NHBE	normal human bronchial epithelial
NK	natural killer
OAD	obliterative airway disease
OLT	orthotopic liver transplant
PGD	primary graft dysfunction
TG	transplant glomerulopathy
Th	t helper
Tregs	regulatory T cells

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