The role of autoimmunity in obliterative bronchiolitis after lung transplantation

Daniel J. Weber¹,² and David S. Wilkes¹,³

¹Center for Immunobiology, ²Department of Surgery, ³Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

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Weber DJ, Wilkes DS. The role of autoimmunity in obliterative bronchiolitis after lung transplantation. Am J Physiol Lung Cell Mol Physiol 304: L307–L311, 2013. First published December 21, 2012; doi:10.1152/ajplung.00378.2012.—First performed in the 1960s with long-term successes achieved in the 1980s, lung transplantation remains the only definitive treatment option for end-stage lung disease. Chronic lung rejection, pathologically classified as obliterative bronchiolitis (OB) with its clinical correlate referred to as bronchiolitis obliterans syndrome, is the limiting factor than keeps 5-yr survival rates for lung transplant significantly worse than for other solid organ transplants. Initially, OB was largely attributed to immune responses to donor antigens, alloimmunity. However, more recent work has demonstrated the role of autoimmunity in the process of lung transplant rejection. IL-17 and autoantigens such as collagen type V and K-α1 tubulin have been implicated in the development of chronic rejection. Ultimately, this translational review discusses the role that autoimmunity plays in the development of OB and lung transplant rejection and then discusses options for therapeutic intervention.

liver transplantation; autoimmunity; obliterative bronchiolitis

THE FIRST ATTEMPTED LUNG TRANSPLANT was performed on a prisoner in 1963, who died eighteen days later. It was not until the mid-1980s that any long-term success was able to be achieved (2). Since that time, a great deal of effort has led to advances in understanding of lung transplant immunology and rejection. Today, lung transplantation is the definite treatment option for end-stage lung disease with well over one thousand transplants performed annually (33). However, the 5-yr survival rates for lung transplant recipients remain around 50%, which is the worst of all solid organ transplants (38). These survival rates are attributed to the prevalence of chronic rejection or bronchiolitis obliterans syndrome (BOS). The histopathological correlate is obliterative bronchiolitis (OB), which is characterized by small airway epithelial disruption and progressive fibrosis, which obliterates these smaller airways (40).

Although the pathophysiology that leads to the development of OB has not been fully elucidated, research has demonstrated that there is an immune response against donor antigens, which plays a key role in this process. This role for alloimmunity has long been established because of work with a heterotopic tracheal transplant model requiring alloresponses for development of BOS. Additionally, early studies showed that low immunosuppression led to earlier onset of BOS, suggesting that alloimmunity plays a key role in chronic rejection (37).

More recent studies, however, have begun to demonstrate the role of autoimmunity in the development of BOS (24, 39). Further investigations offer the possibility of therapeutic options to help mitigate the development of OB. This review will discuss the role of autoimmunity in BOS with particular attention to the work that has already been done as well as options for further exploration.

Cellular and Humoral Autoimmunity

Autoimmunity can be defined as an immune response to a self-antigen. It can be cellular or humoral in nature and can be either innate or acquired (Fig. 1). Although there are several well-known systemic autoimmune diseases, such as rheumatoid arthritis and lupus, autoimmunity is commonly classified as a spectrum depending on pathogenesis. Many of these diseases are thought to occur as a result of the loss of immunogenic tolerance, defined as the ability to react to antigens and ignore self-antigens.

Historically, research has focused on the cellular aspects of the alloimmune response after transplantation. However, the role of transplant-induced humoral allo- and autoimmunity is being investigated as well. Research has shown that antibodies to self-antigens contribute to the development of OB and chronic rejection. Peripheral blood mononuclear cells taken from lung transplant recipients were found to be reactive against self-antigens, demonstrating the presence of T-cell-mediated autoimmunity (10).

The mechanisms by which humoral autoimmunity induces rejection have not been fully discovered. Unlike other organs, the lung is able to mount immune responses to induce graft dysfunction without need for secondary lymphoid tissues (19). This unique feature is one reason why there is not a clear histopathological definition for humoral responses in transplanted lungs. The presence of inducible bronchus-associated lymphoid tissue (iBALT) provides an environment by which antigens and lymphocytes can induce both humoral and cellular immunity (36). These factors highlight the role that auto-
immunity plays in the development of lung transplant rejection.

Autoantibodies, Self-Antigens, and Complement

Type V collagen \([\text{col}(V)]\) is a minor fibrillar collagen that has been found in the lung, skin, and placenta. It plays a role in fibrillogensis for type I collagen, and mutations of \(\text{col}(V)\) have been identified in Ehlers-Danlos syndrome (15). It is expressed by small airway epithelial cells and located in the perivascular and peribronchial tissue of the lung, locations of rejection activity. The interstitial remodeling that occurs with transplantation has been shown to enhance exposure of \(\text{col}(V)\) (47). Its expression in lung isografts has been found to be increased with ischemia reperfusion injury, and studies have demonstrated that matrix metalloproteases, induced by ischemia reperfusion injury, are able to cleave collagen, exposing \(\text{col}(V)\) and releasing its antigen fragments after lung transplantation (25, 45, 47). Although not detected at high levels in normal lung tissue, studies have shown elevated \(\text{col}(V)\) levels in OB lungs after transplantation (10).

Oral tolerance as a means of mitigating rejection with regards to cellular immunity has also been investigated. Studies from our laboratory using a rat model have demonstrated that \(\text{col}(V)\)-induced oral tolerance prevents acute and chronic rejection of minor histoincompatible lung allografts (45, 46). Further work showed that \(\text{col}(V)\) plus low-dose cyclosporine downregulated the rejection of fully major histocompatibility complex (MHC)-incompatible lung grafts, suggesting that \(\text{col}(V)\)-induced oral tolerance plus low doses of calcineurin inhibitors could offer an effective therapeutic approach to lung transplantation (44). The role of oral tolerance in terms of humoral immune responses still needs further elucidation.

Like \(\text{col}(V)\), \(\text{K-}\alpha1\text{tubulin}\) is also expressed on airway epithelial cells and has also been shown to play a role in autoimmunity-mediated development of OB and chronic rejection. Autoantibodies to \(\text{K-}\alpha1\text{tubulin}\) binding to airway epithelial cells have been shown to increase transcription factors related to development of OB, especially the increased expression of fibrotic growth factors (21). Additional work in mice demonstrated that autoimmune responses to \(\text{col}(V)\) and \(\text{K-}\alpha1\text{tubulin}\) were induced by administration of alloantibodies against class I MHC antigens (19). As will be discussed later, inhibition of IL-17 did reduce the autoimmune response. This shows an important relation between alloimmunity, autoimmunity to self-antigens such as \(\text{K-}\alpha1\text{tubulin}\), and the role of IL-17.

Although it has been shown that autoantibodies can cause cell death without the complement cascade, the autoimmune response frequently involves complement activation (9). When transferring \(\text{col}(V)\) immune serum from immune animals into isograft recipients, it was discovered that \(\text{col}(V)\) immune serum was able to induce complement-dependent cytotoxicity on epithelial cells (26).

More recent work by our group examining the role of complement activation in OB demonstrated that complement regulatory protein expression is downregulated in an IL-17-dependent manner. This leads to complement activation and production of C3a, which was indeed found to be elevated (Suzuki H, Lasbury ME, Fan L, Vittal R, Mickler EA, Benson
biochemical mediators that contribute to rejection of the lung allograft. These include complement factors, granzyme B, perforin, nitric oxide, and macrophages. Among 48 patients with IPF, more than 80% were found to have circulating autoantibodies (1). These results suggest that, even before transplantation, the preexisting conditions in the recipient are contributing to the development of autoimmunity.

T regulatory cells (Tregs), are a subset of T cells, and their role in maintaining tolerance to self-antigens is well established. These cells have been shown to prevent autoimmune diseases and downregulate antibody responses against alloan-tigens (18). Among lung transplant recipients, decreased or defective levels are correlated with OB/BOS (8, 34). When looking at T cells reactive to col(V) among 10 transplant recipients, CD4+ T cells producing IL-10 were absent in those patients with BOS (4). Although these cells lacked the CD25+ and foxp3+ characteristics of Tregs, this study did demonstrate a key relationship between Tregs and these IL-10-producing cells. More recently, depletion of Treg cells in mice demonstrated increased IL-17-mediated injury in response to the self-antigens col(V) and K-α1 tubulin (20). Combined, these results propose a key role for IL-17 in the development of autoimmunity. However, recent work has highlighted that not all Th17 cells are pathogenic and that IL-23 is required for the potential to induce autoimmune disease (30). Additionally, the connection between IL-17 and autoimmunity in human studies needs to be further investigated.

**Inflammation, T Regulatory Cells, and Viruses**

Research has shown the inflammation can both induce and worsen autoimmunity. (32) However, the presence of inflammation by itself does not induce autoimmunity. The prevailing notion is that inflammation and cellular damage can expose self-antigens to the circulation. This induces an autoimmune response from self-reactive T cells. The degree of inflammation and the responses generated combined with multiple other factors likely determine whether or not autoimmunity is initiated or enhanced. In the context of lung transplantation, pre-existing inflammation as well as inflammation after transplant may both contribute to the development of autoimmunity.

Before transplantation, patients with end-stage lung disease have been exposed to some degree of inflammation depending on the etiology of their lung disease. Cigarette smoking has been linked to the development of multiple autoimmune disorders (13). Additionally, Arson et al. (1) examined how smoking and asthma alter the immune system and found increased levels of proinflammatory mediators among these patients (1). In addition to smoking and asthma, autoimmunity has been discovered in idiopathic pulmonary fibrosis (IPF). Among 48 patients with IPF, more than 80% were found to have circulating autoantibodies (17). These results suggest that, even before transplantation, the preexisting conditions in the recipient are contributing to the development of autoimmunity.

**Th17 Cells, IL-17, and Autoimmunity**

Originally characterized by differentiation into Th1 and Th2 cells, it is now known that T cell differentiation is more complex. Defined by their ability to produce the cytokines IL-17 as well as IL-21 and IL-22, Th17 cells are a subset of T helper cells distinct from Th1 and Th2 cells (41). First described in 1983, IL-17a is the first member of the IL-17 family that is comprised of six isoforms. Produced by T lymphocytes, these cells promote neutrophil growth and activation in the lungs, joint space, central nervous system, and intensities. IL-17a and IL-17f specifically have been shown to play an important role in host defense and autoimmunity (29).

When Verleden et al. (43) looked at biopsies and BAL specimens from lung transplant recipients undergoing acute rejection, higher IL-17 were correlated with increased neutrophils and lymphocytes, demonstrating the potential role of IL-17 in acute rejection. Additionally, this group then demonstrated that higher IL-17 mRNA and protein levels in BALs from transplant recipients were associated with the development of BOS (43). Another mechanism by which IL-17 may contribute to rejection was postulated with IL-17 inducing iBALT, which, as has been discussed earlier, may contribute to autoimmune reaction in allograft lungs (36). Finally, work from our laboratory in a mouse model demonstrated that neutralizing IL-17 prevented the development of OB and slowed the onset of acute rejection (16). These studies offer multiple roles by which IL-17 may mediate immune responses and rejection.

IL-17 has also been implicated in the development of immune responses to self-antigens. Autoantibodies to col(V) from lung transplant recipients were IL-17 dependent and associated with the development of OB after transplantation (10). Interestingly, the adoptive transfer of lymph node cells reactive against col(V) from immunized donors into isograft recipients induced OB without an alloimmune response. IL-17 has been found to contribute to the autoimmune response to K-α1 tubulin as well. Among mice who were administered antibodies to donor MHC class I antigens, inhibition of IL-17 resulted in decreased levels of autoantibodies to col(V) and K-α1 tubulin (20). Combined, these results propose a key role for IL-17 in the development of autoimmunity. However, recent work has highlighted that not all Th17 cells are pathogenic and that IL-23 is required for the potential to induce autoimmune disease (30). Additionally, the connection between IL-17 and autoimmunity in human studies needs to be further investigated.

**Types of Antibodies**

In terms of the types of antibodies involved in autoimmune reactions, IgG has been the primary isotype identified. When looking at human lung allograft recipients, the anti-col(V) antibodies were all of the IgG type without evidence of any IgMs (10). Among lung transplant recipients who did and did not develop primary graft dysfunction (PGD), autoantibody and gene expression profiles were compared, looking at proteins aside from col(V) and K-α1 tubulin. The results showed that the transplant recipients developing a wide array of autoantibody-specific profiles could be used to identify those patients who developed PGD, and these autoantibodies were of the IgG and IgM isotypes (23). The role of these IgM autoantibodies and whether or not IgM plays a role in chronic rejection from lung transplantation are yet to be determined.
It is well established that environmental exposures are important to the development of autoimmunity (13). Research has shown that respiratory viral infections are a key risk factor for development of OB/BOS. In particular, the presence of community-acquired respiratory viral infections among a large cohort of lung transplant recipients was found to be associated with a higher likelihood of BOS and death (28). The specific viruses implicated with OB were respiratory syncytial virus, parainfluenza, influenza, and adenovirus. Further work showed that viral respiratory infections were associated with Treg cell death (5). Among humans, decreased levels of Tregs were associated with increased autoantibodies to col(I), col(V), and K-α1 tubulin. Additionally, mice infected with respiratory viruses were found to induce Treg apoptosis (5). This suggests that viruses may play a role in the development of chronic rejection by inhibiting Tregs, thereby allowing an uninhibited autoimmune response.

Clinical Manifestations of Autoimmunity After Transplantation: PGD and OB

PGD is a condition that arises within 72 h of transplantation. Clinically, it is characterized by hypoxemia and diffuse infiltrates on imaging. Pathologically, diffuse alveolar damage is noted in addition to noncardiogenic pulmonary edema (31). It accounts for more than 50% of deaths in the early postoperative period, and those who do survive have increased long-term mortality (11, 12). Although its etiology and mechanisms are not clearly understood, airway epithelium has been found to be the target of PGD, similar to the mechanism of injury in OB. The ischemia reperfusion injury that takes place at the time of transplantation likely plays a key role in the development of PGD.

A retrospective cohort of 334 adult lung transplant recipients demonstrated that PGD increases the risk of developing OB independent of other factors, and the severity of PGD correlates with a higher likelihood of OB (14). Further work among 127 transplant recipients has shown that PGD increases serum levels of proinflammatory mediators as well as alloreactive T cells and alloantibodies (6). These reports highlight the role that PGD plays in the development of alloimmune responses to the allograft. As has already been discussed, alloimmunity can interact with and induce autoimmunity, contributing to chronic rejection.

Recent work has also demonstrated a link between both cellular and humoral autoimmunity and the development of PGD. Among 142 lung transplant recipients, those who developed PGD without identifiable antibodies were found to have elevated soluble C4d levels in BAL specimens, raising the possibility of preformed autoantibodies contributing to complement activation and PGD (7). In a rat model, PGD developed after antibodies against col(V) were transferred followed by isograft lung transplantation. Samples from the isografts demonstrated complement as well as the presence of autoantibodies (25). This suggests that, upon transplantation, these preformed antibodies contributed to allograft dysfunction.

Rituximab, trade-name Rituxan, is an anti-CD20 monoclonal antibody against the CD-20 protein located on pre-B and mature B lymphocytes (35). Hachem et al. (22) implemented a clinical trial among lung transplant recipients with self-antibodies, and positive donor-specific antibodies were treated with Rituxan. Fifty-four patients were treated with Rituxan, and those who cleared their self-antibodies were less likely to go on to develop OB (22). These interesting findings highlight the important role of preventing chronic rejection based on our understanding of autoimmunity and offer insights into therapeutic options such as B cell depletion.

Conclusions

Autoimmunity and its role in the development of OB and chronic rejection in lung transplant recipients is an exciting development and remains an under-investigated field. This translational review has sought to discuss the complex role autoimmunity plays in the development of OB or chronic allograft rejection as well as present the various research that has contributed to this emerging field. Ultimately, more work will need to be done to understand how self-antigen exposure occurs and the complex allo- and autoimmune responses that lead to the development of rejection. A variety of therapeutic options, some already being investigated, exist to mitigate the onset and progression of chronic rejection in this setting.

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DISCLOSURES

David S. Wilkes is the cofounder and chief scientific author of ImmuneWorks, which is developing novel forms of treatments for immunologically mediated lung diseases. The author has also received income as a consultant for ImmuneWorks.

AUTHOR CONTRIBUTIONS

Author contributions: D.J.W. and D.S.W. conception and design of research; D.J.W. and D.S.W. interpreted results of experiments; D.J.W. and D.S.W. prepared figures; D.J.W. and D.S.W. drafted manuscript; D.J.W. and D.S.W. edited and revised manuscript; D.J.W. and D.S.W. approved final version of manuscript.

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