The role of autoimmunity in obliterative bronchiolitis after lung transplantation

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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.

lung 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 [col(V)] is a minor fibrillar collagen that has been found in the lung, skin, and placenta. It plays a role in fibrillogenesis for type I collagen, and mutations of 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 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 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 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 col(V)-induced oral tolerance prevents acute and chronic rejection of minor histoincompatible lung allografts (45, 46). Further work showed that col(V) plus low-dose cyclosporine downregulated the rejection of fully major histocompatibility complex (MHC)-incompatible lung grafts, suggesting that 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 col(V), K-α1 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 K-α1 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 col(V) and K-α1 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 K-α1 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 col(V) immune serum from immune animals into isograft recipients, it was discovered that 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

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Fig. 1. Autoimmunity in lung transplantation. After transplantation, exposure of collagen type V [col(V)] and K-α1 tubulin triggers autoimmune responses, both humoral and cell mediated, which contribute to chronic rejection and obliterative bronchiolitis. APC, antigen presenting cell.
antibodies to donor MHC class I antigens, inhibition of IL-17
1 tubulin as well. Among mice who were administered
K-\( \alpha \)/H9251 has been found to contribute to the autoimmune response to
recipients induced OB without an alloimmune response. IL-17
reactive against col(V) from immunized donors into isograft
(10). Interestingly, the adoptive transfer of lymph node cells
from lung transplant recipients were IL-17 dependent and
autoimmune responses to self-antigens. Autoantibodies to col(V)
acute rejection (16). These studies offer multiple roles by which
IL-17 prevented the development of OB and slowed the onset of
rejection from lung transplantation are yet to be determined.

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 pro-
teins aside from col(V) and K-\( \alpha \)1 tubulin. The results showed
that the transplant recipients developing a wide array of au-
toantibody-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 autoan-
tibodies and whether or not IgM plays a role in chronic
rejection from lung transplantation are yet to be determined.

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 de-
scribed in 1983, IL-17a is the first member of the II-17 family
that is comprised of six isofoms. 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 autoimmune (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 neutro-
phils and lymphocytes, demonstrating the potential role of
IL-17 in acute rejection. Additionally, this group then demon-
strated that higher IL-17 mRNA and protein levels in BALs
from transplant recipients were associated with the develop-
ment 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 im-
mune 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
recipient induced OB without an alloimmune response. IL-17
has been found to contribute to the autoimmune response to
K-\( \alpha \)1 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-\( \alpha \)1 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 patho-
genic and that IL-23 is required for the potential to induce
autoimmune disease (30). Additionally, the connection be-
tween 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 inflam-
lation 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 inflamma-
ion and the responses generated combined with multiple other
factors likely determine whether or not autoimmunity is initi-
ated 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 dis-
orders (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.

T regulatory cells (Tregs), a subset of T cells, and their
role in maintaining tolerance to self-antigens is well estab-
lished. 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 demon-
strate 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-\( \alpha \)1 (42). These studies suggest
that recipients may be able to use these Treg cells to prevent
autoimmune responses from inducing OB/BOS and chronic
rejection.

In addition to Tregs, regulatory B cells (Bregs) may also
play a key role in the development of autoimmunity. Bregs
produce inhibitory cytokines such as IL-10 and TGF-\( \beta \) (27).
They serve as negative regulators of inflammation and auto-
immunity, which has been demonstrated in animal models.
Research has suggested that Bregs act earlier than Tregs and
likely interact with and trigger them. Additionally, Breg stim-
ulation has been shown to be an effective treatment modality
for several autoimmune disorders (3). However, the role
of Bregs in lung transplant rejection is presently unknown.

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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.

REFERENCES


