Humoral Immunity and the Development of Obliterative Bronchiolitis after Lung Transplantation

Is There a Link?

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Lung transplantation is considered the definitive treatment for many end-stage lung diseases. However, the lung is rejected more commonly than other solid organ allografts. Obliterative bronchiolitis (OB) is the leading cause of chronic allograft dysfunction, and the key reason why the 5-year survival of lung transplant recipients is only 50%. The pathophysiology of OB is incompletely understood. Although a clear role for the immune response to donor antigens has been observed (also known as anti-human leukocyte antigens), evidence is emerging about the role of autoimmunity to self-antigens. This review highlights the current understanding of humoral immunity in the development of OB after lung transplantation.

Keywords: antibodies; lung transplant; obliterative bronchiolitis; rejection

Lung transplantation remains the only treatment option for many end-stage lung diseases. However, the survival of lung allografts is limited by chronic allograft rejection, a pathological condition referred to as obliterative bronchiolitis (OB), with an incidence of 50% within 3 years after transplantation. In fact, the lung is rejected more commonly than any other solid allografts, and the 5-year survival of lung transplant recipients is approximately 50% (1).

OB is a pathological entity presenting in two forms. In most cases, it is characterized by extensive peribronchiolar connective tissue deposition, the loss of bronchiolar epithelium, and progressive scarring that ultimately obliterates the small airways. Although some debate remains, proliferative forms of bronchiolitis obliterans have also been considered part of the spectrum in chronic allograft dysfunction. These lesions differ from classic OB in that their histopathology may reveal proliferating fibrous plugs of granulation tissue that protrude into and may ultimately obliterate small airways (2, 3). Because certain lung pathologies may reflect varied pathogenic mechanisms, it remains unclear whether differential immune pathways, either cellular (T cell-mediated) or humoral (antibody-mediated), account for these two forms of OB.

The diagnosis of OB via transbronchial biopsy is difficult because of its patchy nature in the transplanted lung. Therefore, its clinical correlate, bronchiolitis obliterans syndrome (BOS),

CLINICAL RELEVANCE

This Red in Translation article describes the current, state-of-the-art understanding of the role, if any, for humoral immunity in obliterative bronchiolitis after lung transplantation.

defined by a sustained decline of 20 to 50% in forced expiratory volume in 1 second (FEV₁) relative to the maximum posttransplant, has become the standard clinical marker of OB/BOS (4).

The pathophysiology of OB is incompletely understood. Several risk factors have been associated with the development of OB, including acute rejection, human leukocyte antigen (HLA) mismatches, HLA antibodies, lymphocytic bronchiolitis, viral infection, gastroesophageal reflux, and primary graft dysfunction (PGD) (5). Although a clear role exists for immune responses in the form of anti-donor antigen (anti-HLA) antibody formation, referred to as alloimmunity, a role has also emerged for immune responses to self-antigens (autoimmunity) in the development of OB (6–8). We will review our current understanding and recent advances in the study of humoral immunity, along with the implications of humoral immunity in the development of OB in lung transplant recipients.

INITIATION OF THE IMMUNE RESPONSE IN LUNG ALLOGRAFTS

Like other solid organ allografts, the primary targets of the recipient immune response involve the donor-derived major histocompatibility complex (MHC) antigens present in allogeneic tissue. Donor antigen can be presented to recipient T cells through two unique, but not exclusive, pathways, namely, direct or indirect. The direct pathway involves the presentation of donor-derived MHC antigens expressed in donor antigen-presenting cells (APCs) to recipient T cells. In the indirect pathway, recipient APCs present peptides derived from donor MHC molecules to recipient T cells (9, 10). T-helper cells, activated through either pathway, will stimulate antigen-specific B cells, resulting in antibody formation against donor HLA antigens. This process is common among all solid organ allografts.

The immune response to alloantigens and autoantigens is believed to occur in the regional lymph nodes, with the trafficking of effector cells/molecules back to the allograft, where they mediate pathology. However, the lung is able to mount its own immune responses *in situ*, without secondary lymphoid tissues (11, 12). This is attributable to the development of inducible bronchus-associated lymphoid tissue (iBALT), which contains a full complement of antigen-presenting cells (i.e., B and T cells) that are able to induce local humoral and cellular

(Received in original form September 6, 2012 and in final form October 18, 2012)

This work was supported by National Institute of Health grants R01HL067177, HL096845, and National Institute of Allergy and Infectious Disease (NIAID) P01AI084853 (D.S.W.).

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Am J Respir Cell Mol Biol Vol 48, Iss. 2, pp 145–149, Feb 2013

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Originally Published in Press as DOI: 10.1165/rcmb.2012-0349RT on October 18, 2012

Internet address: www.atsjournals.org

immunity (13, 14). As such, the lung may function as a “lymph node with alveoli” (15). In addition, many immune cells in the lung are phenotypically and functionally distinct, relative to those associated with other solid organs. Specifically, the function and subtypes of dendritic cells, key initiators of immune responses, and T cells are unique (16). In general, these reports suggest that the types of cellular and humoral immune responses that occur in other organ allografts may not be applicable to the lung.

HUMORAL AUTOIMMUNITY AND ITS ROLE IN OBLITERATIVE BRONCHIOLITIS

A significant body of evidence has associated the development of chronic allograft rejection with the presence of anti-HLA antibodies in the lung (8, 17–19). Specifically, therapy that decreased anti-HLA antibodies was associated with a decreased risk for OB (8, 20). However, anti-HLA immunity was not been detected in a proportion of lung-transplant recipients who developed OB (8, 21). This observation led to identifying and further linking the presence of antibodies to self-antigens (autoimmunity) to the development of OB. For example, autoimmune responses after lung transplantation were initially reported by Ende and colleagues, who demonstrated that anti-lung tissue antibodies developed after human lung transplantation (22). Pretransplant anti-epithelial cell antibodies (non-HLA antibodies) were associated with graft failure in lung allograft recipients (23). More recently, Hagedorn and colleagues demonstrated the correlation of donor lung–derived transcripts and the formation of autoantibodies, both before and after transplant in patients with PGD and OB (24, 25). The transcripts represented proteins involved in apoptosis and cell metabolism. Using a different approach, Magro and colleagues reported a variety of circulating anti-endothelial antibodies in lung transplant recipients (26–28). These reports span more than three decades, and suggest that the presence of autoantibodies after lung transplantation may occur in multiple settings, and may be detected by varying techniques. However, the pathogenesis of antibody production and the ability of these antibodies to induce *in vivo* cytotoxicity were not described in these studies.

Studies from our laboratory determined that human lung transplant recipients developed antibodies to Type V collagen, or col(V), a minor lung collagen that is intercalated within the helices of Type I collagen. Col(V) is a heterotrimer helix consisting of two chains of $\alpha 1$ and one chain of $\alpha 2$ (29). The majority of col(V) occurs within the lung interstitium and is not exposed to the immune system. However, it is also expressed by epithelial cells (30). Early events related to ischemia and reperfusion injury after lung transplantation result in interstitial remodeling, partly attributable to the activation of matrix metalloproteases able to cleave collagenous molecules, thereby exposing col(V) (31). Indeed, interstitial col(V) is readily detected within 4 hours after lung transplantation, and remains detectable for more than 30 days after transplantation in preclinical models.

These early studies contained the notable finding that these antibodies did not detect collagen Types I, II, III, IV, or VI. Extending these studies into an orthotopic rat lung transplant model, we reported that rat lung allografts transplanted into minor histocompatibility antigen–mismatched recipients induced anti-col(V)–specific T and B cells after transplantation (6). Examination of human lung allograft recipients revealed the presence of anti-col(V) CD4⁺ T cell–mediated immunity in peripheral blood mononuclear cells, and this finding was strongly correlated with the onset of OB/BOS after transplantation (32). Moreover, the adoptive transfer of these cells to lung isograft recipients induced OB in the transplanted lung, despite the absence of any alloimmunity (32).

Hachem and colleagues (8) and Fukami and colleagues (33) reported that human lung transplant recipients developed an antibody response to K- $\alpha 1$ tubulin, as well as to col(V), and that the presence of these antibodies was associated with OB/BOS in clinical transplantation. Furthermore, using *in vitro* models, additional experiments revealed that the treatment of airway epithelial cells with K- $\alpha 1$ tubulin–specific antibodies resulted in an increased expression of fibrogenic growth factors, providing further evidence for the involvement of K- $\alpha 1$ tubulin autoimmunity in the development of OB (34). Similar to col(V), K- $\alpha 1$ tubulin is also expressed in airway epithelial cells, and appears to be a prominent target for the immune response after lung transplantation, particularly in the pathogenesis of OB. These data again highlight the role of the airway epithelium as a key target in the pathogenesis of OB.

OB is part of the spectrum of chronic allograft dysfunction, but early events after transplant also affect mortality. PGD, an entity characterized by varying degrees of noncardiogenic pulmonary edema and impaired systemic oxygenation occurring within 72 hours after transplantation, is the leading cause of early mortality in lung transplant recipients (35, 36) and a major risk factor for OB (1). A potential immune basis for PGD has been debated for several years. In a rat model, we previously demonstrated that autoimmunity to col(V), in the absence of alloimmunity, can induce lung pathology consistent with PGD. In a cohort of patients, we also demonstrated higher concentrations of serum anti-col(V) total IgG at 6, 24, 48, and 72 hours after transplantation in a group with PGD, compared with non-PGD allograft recipients (31). The presence of autoimmunity immediately after lung transplantation in this group of patients is most likely representative of developing autoimmunity before lung transplantation, secondary to underlying lung disease. These observations are consistent with recent studies showing that interstitial lung diseases (among which idiopathic pulmonary fibrosis makes up the vast majority) pose a major risk factor for PGD (37). Reports from a number of investigators, including our group, have shown the presence of autoimmunity in the pathophysiology of idiopathic pulmonary fibrosis (38, 39). Because PGD is a risk factor for OB/BOS (1), the autoimmune status of the recipient before transplantation could greatly affect chronic allograft dysfunction after lung transplantation. Therefore, humoral autoimmunity seems to be a putative converging final pathway in the development of OB/BOS.

Despite evidence regarding the immune response to self-antigens and the implications of this response in the development of OB/BOS, the mechanism of autoantibody induction remains unclear. Furthermore, the immunodominant humoral epitopes of col(V) are just beginning to be characterized. Using sequential analyses of the serum from 12 lung transplant recipients before and after the development of OB/BOS, Tiriveedhi and colleagues showed an initial development of anti-col(V) antibodies to the $\alpha 1$ and $\alpha 2$ chains of col(V), and upon the diagnosis of OB/BOS, the antibodies were restricted to $\alpha 1$ chains. These data suggest a shift in immunodominant epitopes of col(V) before the development of OB/BOS. This shift coincides with a decrease in serum concentrations of IL-10, and an increase in serum IFN- γ and IL-17, suggesting a T-cell phenotype change favoring the development of OB/BOS. Furthermore, bronchoalveolar lavage (BAL) analysis of the same cohort of lung transplant recipients was remarkable for the up-regulation of matrix metalloprotease–2 (MMP2) and MMP9 before the shift in immunodominant epitopes of col(V) (40). This finding suggests that the activation of MMPs after lung transplant could result in the differential cleavage and exposure of cryptic col(V) epitopes (40, 41). Indeed, inhibiting MMPs in a model of ischemia–reperfusion injury resulted in lower concentrations of col(V)

fragments in the transplanted lung (31). In addition, an influx of APCs to the lung in the presence of inflammation could lead to lowering the activation threshold of the T cell, and priming autoreactive T cells that could provide help for autoreactive B cells (7, 42).

IS THERE ANY LINK BETWEEN ALLOIMMUNITY AND AUTOIMMUNITY?

The identification of alloimmunity and autoimmunity in allograft recipients, with compelling evidence associating alloimmunity and autoimmunity with the development of OB/BOS, has raised the question of whether these are linked events, or are they parallel phenomena?

Fukami and colleagues showed in a murine model that the intrapulmonary instillation of anti-MHC Class I antibodies induced OB-like pathology in recipient lungs, and these same mice developed antibodies to col(V) and K- α 1 tubulin (33). Furthermore, in lung transplant recipients, donor-specific antibodies (alloimmunity) were detectable on average 3 months after transplantation and before the development of antibody to self-antigens (autoimmunity) (43). In the same study, the clearance of alloimmunity using the preemptive antibody-directed therapy of patients who developed alloimmunity was associated with an increased rate of clearance for antibodies to self-antigens. Interestingly, the clearance of antibodies to self-antigens was associated with a lower risk of OB/BOS, and this effect was independent of reduced anti-donor antibodies (8). These data strongly suggest a putative role of humoral autoimmunity in the development of OB/BOS. Such observations have led to the hypothesis that alloimmunity after transplantation may occur early, and may result in unmasking cryptic antigens attributable to inflammation and the subsequent development of autoimmunity. Although this evidence is compelling, studies continue to show that not all patients who develop autoimmunity demonstrate previous or coincident robust alloimmune processes. This suggests that although alloimmunity can result in autoimmunity after lung transplantation, it is not the only pathway for the development of autoimmunity (8, 21).

INDUCTION OF HUMORAL AUTOIMMUNITY: A BALANCE BETWEEN TH-17 AND T-REGULATORY CELLS

Since the introduction of the Th1 and Th2 model by Mosmann and colleagues, it has been widely recognized that not all immune responses fit easily into these designations (44). In organ transplantation, Th1 cells, namely, those that produce primarily IFN- γ , were associated with rejection, whereas Th2 cells, which are major sources of IL-4 and IL-10, were considered to be protective (45). However, multiple studies using mice genetically deficient in Th1 or Th2 cytokines revealed that rejection could still occur, although the tempo of the alloimmune response may vary (45). Over the past two decades, the paradigm of Th1/Th2 has been expanded to include the Th17 subset, T-regulatory cells, T-follicular helper cells, and other T-helper cells (46–48).

Th17 cells, defined by their secretion of IL-17, are not only important for host defense, but also play a role in the pathogenesis of autoimmunity and allotransplantation (47–49). Secreted primarily by CD4⁺ T cells, many other cellular sources have been reported for IL-17, and include $\gamma\delta$ T cells, natural killer (NK) cells, CD8⁺ T cells, NK T cells, and neutrophils (49, 50).

TH17, IL-17, AND THE DEVELOPMENT OF OB IN LUNG TRANSPLANTATION

Th17-associated cytokines, in particular IL-17, have been linked to the development of acute and chronic rejection after lung transplantation in both animal models and humans (31–33, 51). The

BAL fluid of lung transplant patients with BO/BOS has been shown to contain increased concentrations of IL-6 and IL-1 β , which are two cytokines known to play key roles in the induction of IL-17 production (52). Similarly, that same study detected increased transcripts for TGF- β , a known inducer of IL-17, as well as IL-17 and IL-23 (52). Vanaudenaerde and colleagues found that increased IL-17 in BAL fluid was associated with an increase in BAL lymphocytes and neutrophils during acute rejection in transbronchial biopsies from lung transplant patients (53). The concentrations of IL-17 also correlated with increased severity of rejection (53). In addition, nontransplant studies have reported that IL-17 is fibrogenic in the lung (54). This finding is notable because OB is a fibrotic disease, which implies that IL-17 may play key roles in the fibrogenesis that is characteristic of OB. A recent study revealed that IL-17 plays a key role in the induction of inducible bronchus-associated lymphoid tissue (13), and iBALT is readily detected in lung allografts (55). Because iBALT could be the source of local immune responses in the transplanted lung, it is interesting to speculate that another role for IL-17 in rejection responses could be attributable to the induction of iBALT.

Extending these observations to a murine model of OB (56), our group has demonstrated that neutralizing IL-17 abrogated the onset of OB and down-regulated the severity of acute rejection (56). The finding of a causal link to IL-17 and OB may not be limited to chronic allograft dysfunction. For example, Sharma and colleagues reported that murine lung ischemia reperfusion injury, which may reproduce clinical PGD, was dependent on IL-17 production derived from NK T cells (50).

Similar to other autoimmune diseases, Th17 cells have been implicated in the autoimmune response linked to the pathogenesis of OB. Our group previously showed IL-17-dependent anti-col(V) CD4⁺ T cell-mediated immunity in peripheral blood mononuclear cells in human lung allograft recipients (32). These data are consistent with a previous report showing that col(V)-reactive lymphocytes that develop from immunizing rats with col(V) are of the Th17 type (57), and that the adoptive transfer of these cells to lung isograft recipients induced OB in the transplanted lung, despite the absence of any alloimmunity (32). Furthermore, in a murine model, Fukami and colleagues induced OB-like pathology in recipient lungs, using an intrapulmonary instillation of anti-MHC Class I antibodies. These same mice developed antibodies to col(V) and K- α 1 tubulin (33). Moreover, the data showed that neutralizing IL-17 abrogated the OB-like pathology, including airway fibrosis and the production of autoantibodies (33). Recently, the same group showed freedom of OB is more likely in a group of lung transplant recipients with autoantibodies to col(V) and K- α 1 tubulin, if responding to antibody-directed therapy evident by clearance of autoantibodies. Interestingly, those patients who cleared their autoantibodies after treatment also produced significantly lower serum concentrations of IL-17 and IL-1 β (8). These data strongly suggest that IL-17 plays key roles not only in autoantibody production, but also in the autoantibody-induced fibrogenesis that may culminate in OB.

CONCLUSIONS AND FUTURE DIRECTIONS

Despite a marked decrease in lung allograft acute rejection through advancements in donor selection, surgical techniques, immunosuppressive therapy, and optimized postoperative care, long-term lung allograft survival remains very low because of the development of OB/BOS. The pathophysiology of OB is incompletely understood. We have presented the evidence supporting the role of humoral immunity, and more importantly the emerging role of humoral autoimmunity, in the development of OB. Despite an increased understanding of humoral immunity, the

histopathology of autoantibody-mediated rejection remains unclear. Furthermore, OB is not a monomorphic pathology but a spectrum of histopathological findings, ranging from extensive peribronchiolar connective tissue deposition and the progressive scarring that ultimately obliterates the small airways, to proliferative forms presenting as proliferating fibrous plugs of granulation tissue that protrude into and may ultimately obliterate the small airways (2, 3). To the best of our knowledge, the role of humoral immunity and autoimmunity in the differentiation of these two forms has not been investigated. Accordingly, robust biomarkers indicative of humoral autoimmunity are needed to determine the exact link of these processes to OB/BOS after lung transplantation.

Author disclosures are available with the text of this article at www.atsjournals.org.

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