Published in final edited form as:
Am J Transplant. 2009 August ; 9(8): 1714–1718. doi:10.1111/j.1600-6143.2009.02690.x.

Immunobiology of Chronic Lung Allograft Dysfunction: New Insights from the Bench and Beyond

Rebecca A. Shilling[1] and **David S. Wilkes**[2]

[1]Section of Pulmonary and Critical Care Medicine, Department of Medicine and Committee on Immunology, University of Chicago, Chicago, IL, USA

[2]Departments of Medicine, Microbiology and Immunology, Center for Immunobiology, Indiana University School of Medicine; Indianapolis, IN, USA

Abstract

The first successful human lung transplants were performed in the 1980's. Since that time lung transplantation has been a therapeutic modality for end-stage pulmonary diseases. However, chronic rejection, known as obliterative bronchiolitis/bronchiolitis obliterans syndrome (BOS), is the key reason why the five year survival is only 50%, which is significantly worse than most other solid organ transplants. Recent studies have provided exciting advances that are beginning to be translated into findings in humans. This review will highlight the current advances in understanding the mechanisms of obliterative bronchiolitis/BOS in lung transplant recipients.

Keywords

lung transplantation; obliterative bronchiolitis; bronchiolitis obliterans syndrome; alloimmunity; autoimmunity; IL-17

Introduction

Pathologically, chronic rejection in the lung is known as obliterative bronchiolitis (OB), which is characterized by progressive fibrotic destruction of the small airways. This lesion is analogous to chronic vasculopathy in kidney and heart transplants (reviewed in (1)). Clinically, OB is diagnosed as bronchiolitis obliterans syndrome (BOS) which manifests as progressive decreases in expiratory air flow in the absence of other acute processes such as infection or acute rejection (1). OB/BOS can occur within the first year to several years after transplantation and has no effective cure. Due to the high rates of chronic rejection the five year survival of lung transplant recipients is 50% which is the worst of all solid organ transplants except small bowel transplants (2). While the pathophysiology is not well understood, recent studies are beginning to shed light on the underlying mechanisms of chronic lung allograft rejection.

Risk factors previously identified for the development of OB/BOS include acute rejection episodes, HLA mismatches and the development of HLA antibodies demonstrating an essential role for alloimmunity in chronic rejection (1). A complete understanding of how these processes might be linked to fibrous obliteration of the airways is not known, but several recent studies have provided some insight into the mechanisms involved. Bharat and colleagues serially evaluated serum from lung transplant recipients matched for

Address correspondence to : David S. Wilkes, M.D., Center for Immunobiology, Indiana University School of Medicine, 635 Barnhill Drive, Medical Science Building 224C, Indianapolis, Indiana 46202, Phone: 317-278-7020, Fax: 317-278-7030, dwilkes@iupui.edu.

demographic factors that had or had not developed BOS after transplantation (3). The authors found that subjects who developed BOS had evidence of a Th1-type inflammatory response at early time-points after transplantation with increased serum levels of the chemokines MCP-1 and IP-10 and the cytokines IL-1B, IL-12, IL-2 and IL-15. BOS subjects also had increased levels of class II alloantibodies and increased frequencies of IFNγ producing alloreactive T cells in the serum. These data suggest the early events post transplantation augment the alloimmune response and predispose to BOS.

Primary graft dysfunction predisposes to BOS

One of the most severe early events after lung transplantation is primary graft dysfunction (PGD) which has previously been associated with poor outcomes in lung transplant recipients (reviewed in (4)). PGD manifests as an acute lung injury defined by diffuse infiltrates on chest x-ray and abnormal oxygenation (4). Investigators have recently demonstrated that PGD increases the risk of the development of BOS independent of other risk factors, and the severity of PGD is directly associated with increased risk for BOS (5). Subjects with PGD post-transplantation were found to have increased serum levels of the cytokines, IFNγ, IL-12, IL-2 and IL-1β and the chemokines, MCP-1 and IP-10, as well as increased donor alloreactive T cells and alloantibodies (6). Taken together these studies suggest that severe ischemia/reperfusion (I/R) injury, as in PGD, augments the alloimmune response and has deleterious consequences for the graft in the long run. Therapies targeted at dampening the early inflammatory response may prevent or delay the development of OB.

The consequences of augmented alloimmunity after PGD and I/R injury are likely to be chronic damage to the epithelium and endothelium, which may initiate a cascade of events leading to airway fibrosis. Airway epithelium in response to alloimmune mediated injury can produce growth factors and pro-fibrotic factors implicated in fibrosis (reviewed in (2). Investigators have also demonstrated in a mouse tracheal transplant model that an alloresponse to the epithelium is sufficient to promote obliteration of tracheal allografts (7). However, in a recent mouse model of orthotopic lung transplantation despite severe vascular rejection in a fully allogeneic mismatched lung transplant, epithelial damage did not occur and fibrotic obliteration was not found (8). This could be due to the fact the current mouse model is a short term injury (28 days) which may be an insufficient period of time to observe epithelial injury that could result in OB. These data strongly argue for the epithelial response to injury as a key mediator of airway fibrosis. The data also suggest that while epithelial damage is critical for development of OB/BOS, ischemia and alloimmunity are not the only causative agents. In humans other insults, such as infections, acid reflux and even immunosuppression may be critical for sufficient epithelial damage to initiate a fibrotic response in the small airways (reviewed in (2).

The early events after transplantation may also have profound effects on vascular endothelium. Babu et al. found that tissue hypoxia after tracheal allograft transplantation correlated with increased allograft rejection and obliteration (9). Tissue hypoxia was attenuated by immunosuppression demonstrating the alloimmune response initiated the defects in vascular perfusion. However, if immunosuppression was delayed and the vascular endothelium was already damaged the graft could not be rescued. Injury to the tracheal allograft microvasculature led to ineffective revascularization, damage to the epithelium and subsequent fibrotic obliteration of the graft. It follows that if PGD promotes endothelial damage and initiates a severe alloimmune response over time, repair mechanisms in the lung may be inadequate to prevent OB. If this is true one might predict that PGD would be associated with earlier onset of BOS but none of the studies to date have addressed this hypothesis. Alternatively immunosuppression may not be able to overcome the alloimmune response in the face of ongoing endothelial damage. In other solid organs the endothelium is

an important mediator of the alloimmune response (10). While Babu and colleagues elegantly showed that endothelial damage may be critical in promoting OB, as discussed above in the orthotopic mouse lung transplant model, vascular rejection was not associated with epithelial damage and small airway obliteration (8,9). This result in murine lung transplant may have been due to the use of a specific donor-recipient combination that resulted in a very rapid rejection response, or possibly related to the altered pulmonary circulation relative to humans. For example the human lung is highly dependent on a low pressure pulmonary arterial circulation and a systemic bronchial circulation. While technically quite challenging, and not proven clinically, re-establishing bronchial arterial circulation, as suggested by some, may reduce ischemia and the predisposition to OB/BOS (9).

Innate immunity affects alloimmune response

Another challenge for lung transplantation, in addition to avoiding PGD, is the constant environmental and infectious exposures of the lung compared to other solid organs, such as the heart, liver and kidney. The exposure to the environment may uniquely predispose the lung to chronic injury. Non-alloimmune insults, infectious and non-infectious, have been associated with increased BOS and are activators of the innate immune response of the lung that may facilitate rejection (2). The innate immunity of the lung includes the neutrophils, dendritic cells, macrophages and structural epithelial and endothelial cells. These cells respond to foreign microbial or viral products using pathogen recognition receptors (PRRs), such as toll-like receptors (TLRs), NOD-like receptors (NLRs) and the RIG-like helicases (RLHs). PRRs may also be responsible for recognizing endogenous ligands, such as hyaluronan (HA) or heat shock proteins (HSP) that may be released after cellular damage during transplantation (11). Tesar and colleagues found that hyaluronan degradation products activated dendritic cells in vitro and primed alloimmunity (11). The presence of HA in human lung allograft samples correlated with BOS, linking endogenous TLR ligands to the development of airway fibrosis. Interestingly, human lung transplant patients with specific polymorphisms in TLR4 have a decreased sensitivity to LPS and a lower incidence of BOS (12). The role of the innate immune response in BOS is clearly emerging and may provide novel targets for therapeutics.

While the mechanisms by which PRRs promote BOS are not known, activation of the innate immune response has been shown to augment the alloimmune response. Kuo and colleagues have found that infection with Sendai virus at the time of transplantation was associated with more severe OB in a mouse model (13). In humans, a variety of viruses have been linked to OB/BOS and include cytomegalovirus and multiple community acquired respiratory virus infections (1). It stands to reason that limiting the innate immune response to infectious agents or other stimuli may prevent augmentation of the alloimmune response and protect against chronic rejection.

Autoimmunity promotes BOS

The most compelling recent work to shed light on the ongoing immune response to the graft is the development of autoimmunity. Autoimmunity is emerging as one of the most significant contributors to the development of OB/BOS and PGD in human lung transplantation. Type V collagen [col(V)] is located within the lung interstitium and expressed by airway epithelial cells and its expression is enhanced by ischemia reperfusion injury and interstitial remodeling (14). T cell reactivity to col(V) was also found to exacerbate acute rejection in rat allografts suggesting autoreactive T cells may promote graft failure (15). Col(V)-reactive $CD4^+$ T cells were associated with a nearly 10 fold increased risk for BOS in clinical lung transplantation, which was a greater risk than that associated

with acute rejection episodes, HLA mismatch, or anti-HLA antibodies (16). Cellular immune responses to col(V) were mediated by IL-17A, TNFα, and IL-1β, but not IFNγ (16). While dendritic cells are known to be key in initiating cellular immunity, col(V) reactivity was dependent on monocytes ($CD14^+$) (16). These data provide evidence of a new paradigm involving coordination between $CD4^+$ T cells and monocytes to produce an effector response and suggest autoreactive Th17 cells to be mediators of BOS.

The lung with a large number of monocytes and macrophages may be a unique environment for promoting Th17 cells. The newly described Th17 cells are involved in the maintenance of mucosal immunity in both the gut and lung and have been associated with the development of autoimmunity in animal models and humans (17). Why autoreactive T cells differentiate to Th17 or Th1 cells in vivo is not well understood but has to do with the cytokine milieu and activation status of the antigen presenting cells encountered by the T cells. Several cytokines have been found to promote development and maintenance of Th17 cells including IL-1β, TGFβ, IL-6, IL-23 and IL-21 (17). Vanaudenaerde and colleagues investigated the role of these known Th17 mediators in BOS and found that lung transplant recipients with BOS had increased BAL levels of IL-1β and IL-6 and increased mRNA for TGFβ, IL-17, IL-23 and IL-8 compared to controls (18). IL-8 the major chemokine for neutrophils has previously been associated with BOS but it has not been clear whether neutrophils were a marker of inflammation or a key mediator of OB (1) Since IL-17 is chemotactic for neutrophils, the current data suggest that the presence of neutrophils may be secondary to a Th17 mediated alloimmune or autoimmune response. A greater understanding of the innate immune response of the lung and the secretion of cytokines involved in differentiating T cells to Th17 may provide novel targets for the prevention of BOS.

Col(V) is not the only autoantigen that has been identified in lung transplant recipients. Goers et al. reported that antibodies to the epithelial specific protein, K-α1 tubulin, were present in a significant number of patients with BOS (19). Sera positive for anti-K-α1 tubulin antibodies induced pro-fibrotic growth factors from airway epithelial cell lines providing evidence that autoreactivity like alloimmunity may induce fibrosis. The same group has also found that an alloimmune response in the lung can promote the development of col(V) and K-α1 tubulin autoreactivity (20). Both autoreactive T cells and autoantibodies were induced by the administration of Class I MHC alloantibodies in the airways of mice. Interestingly, blocking IL-17 diminished the severity of the airway injury and fibrosis found (20). Thus, alloimmune mediated damage may lead to epitope spreading that results in an autoimmune response and the characteristic response appears to be IL-17 mediated. The macrolide, azithromycin, an important treatment for a subset of patients with BOS, has previously been found to suppress IL-17 induced IL-8 production from human smooth muscle cells providing further support for IL-17 playing a role in BOS (2). The data in lung transplantation have now come full circle to provide a basis for older studies associating BAL neutrophilia with BOS. Autoreactive Th17 cells may underlie the pathogenesis of BAL neutrophilia and promote BOS.

Interestingly, anti-col(V) autoreactivity may not be limited to post-transplantation. Recent studies have found that anti-col(V) cellular and antibody responses are associated with a higher incidence of PGD (21,22). Prior to transplantation, patients with idiopathic pulmonary fibrosis (IPF) were found to have a higher incidence of col(V) autoreactivity than non IPF patients (21). As in the study in patients with BOS, col(V)- induced responses were dependent on $CD4^+$ T cells and $CD14^+$ monocytes, as well as IL-17, IL-1β and TNFα. Transplanting lung isografts into rats previously immunized with col(V) induced a phenotype similar to humans with PGD with a marked mononuclear cell infiltrate and decreased oxygen saturation (21). Similarly anti-col(V) antibodies of the IgG2c subtype

induced a PGD-like phenotype in rat lung isografts suggesting humoral immune responses are also sufficient to worsen post-transplantation outcomes (22). This finding was consistent with data showing that the presence of systemic anti-col(V) antibodies pre-transplantation was strongly associated with severe PGD in humans (22). It will be interesting to determine if patients with pulmonary arterial hypertension (PAH), who have some of the highest rates of PGD, also have anti-col(V) antibodies prior to transplant and if anti-Col(V) reactivity correlates with PGD incidence (reviewed in (4)). However, it is possible that non-immunologic mechanisms play a role in the higher incidence of PGD in these patients. Nevertheless, the involvement of both cellular and humoral immune responses in PGD makes strategies aimed at inducing tolerance to col(V), as reported in rodents (reviewed in (15)) an attractive approach in humans prior to transplantation.

Tregs may be affected after lung transplantation

One possible hypothesis for why autoimmunity and alloimmunity cannot be as easily suppressed once initiated is that T regulatory cells (Tregs) are absent or dysfunctional after transplantation. A correlation between decreased Tregs and the incidence of BOS has been reported in lung transplantation recipients (23). With the identification of col (V) as a target of autoimmunity in lung transplantation a new strategy to promote Tregs may be through oral tolerance. Animal studies have found that oral tolerance to col(V) can be induced and is associated with the development of TGFβ-dependent Tregs (reviewed in (15)). Interestingly, Bharat et al. in 2006 found that T cell lines reactive to col(V) isolated from lung transplant recipients produced IL-10 and had a regulatory phenotype (24). These cell lines were capable of suppressing proliferation and IFNγ secretion from autoreactive T cells in a CTLA-4-dependent manner. Subjects who developed BOS had an associated decline in the frequency of IL-10 producing T cell clones (24). These data suggest lung transplant recipients may dampen the autoimmune response to col(V) through either natural Tregs or adaptive regulatory T cells. Accordingly, strategies to promote immune tolerance to col(V), alloantigens, or as yet to be identified antigens prior to lung transplantation may prevent not only PGD but the devastating complication of OB/BOS.

In addition to Tregs being decreased or suppressed post transplantation, inflammatory conditions such as infection, acute rejection or other insults may suppress the regulatory activity of Tregs. For example, an indirect relationship exists between Foxp3, a transcription factor that identifies many natural Tregs; and RORγt, a transcription factor expressed in Th17 cells. Both Foxp3 and RORγt are critically dependent on TGFβ for differentiation (reviewed in (17)). *In vitro*, IL-6 can redirect $Foxp3^+$ T cells to express IL-17 and investigators have reported that this transition may occur *in vivo* during an inflammatory response in experimental autoimmune encephalitis (17). Since IL-6 and IL-1β have previously been associated with BOS and are known mediators of Th17 development an intriguing hypothesis is that Tregs with autoreactive T cell receptors convert to pathogenic T cells in the presence of these cytokines (17,18). Further studies are required to investigate this hypothesis.

The primary function of the transplanted lung is gas exchange which must occur despite intense immune suppression and constant exposure to blood borne and inhaled antigens. In this context the lung could be considered a “lymph node with alveoli” that is highly susceptible to any perturbations in local immune homeostasis. Indeed, the lung is replete with immunocompetent cells within bronchus associated lymphoid tissue-BALT that are sufficient to mount local immune responses even in the absence of systemic secondary lymphoid tissues (25). Cells derived from the lymphoid lung tissues are able to interact with other immunologically active components of the lung such as extracellular matrix, and epithelial and endothelial cells, as well as cells of the innate immune system. Therefore, the

lung is immunologically unique compared to other solid organ allografts such as the kidney, heart, or liver. The lung is almost as difficult to transplant as the small bowel, which also has its own unique immunology with Peyer's patches. The insults the lung encounters during transplantation, such as non-immune mediators, I/R injury, infection, and acid reflux may induce chemokines which recruit lymphoid cells to the lung and promote the development of BALT. BALT in turn may be a site of continued antigen presentation and T and B cell proliferation, perpetuating the alloimmune response and providing an environment that may be prone to autoreactivity. Further, the lung may have distinct mechanisms of maintaining immunity while trying to avoid impacting gas exchange, but these mechanisms may be deleterious in the face of chronic immune modulation as in lung transplantation. Current studies are only beginning to unravel the intricacies of these immune networks in the lung and their impact on lung function. Substantive improvements in the survival of lung transplant recipients is likely to occur only after we are able to fully understand how the distinct interactions of immune and non-immune cells in the lung impact the immunobiology of the transplanted lung.

Acknowledgments

This work was supported by grants from the National Institute of Health K08 AI 059105 and the Louis Block Family Fund to R.A.S. and HL60797 and HL/Al67177 to D.S. Wilkes.

REFERENCES

1. McDyer JF. Human and murine obliterative bronchiolitis in transplant. Proc Am Thorac Soc. 2007; 4(1):37–43. [PubMed: 17202290]
2. Sato M, Keshavjee S. Bronchiolitis obliterans syndrome: alloimmune-dependent and -independent injury with aberrant tissue remodeling. Semin Thorac Cardiovasc Surg. 2008; 20(2):173–182. [PubMed: 18707652]
3. Bharat A, Narayanan K, Street T, Fields RC, Steward N, Aloush A, et al. Early posttransplant inflammation promotes the development of alloimmunity and chronic human lung allograft rejection. Transplantation. 2007; 83(2):150–158. [PubMed: 17264811]
4. Lee JC, Christie JD. Primary graft dysfunction. Proc Am Thorac Soc. 2009; 6(1):39–46. [PubMed: 19131529]
5. Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. Am J Respir Crit Care Med. 2007; 175(5):507–513. [PubMed: 17158279]
6. Bharat A, Kuo E, Steward N, Aloush A, Hachem R, Trulock EP, et al. Immunological link between primary graft dysfunction and chronic lung allograft rejection. Ann Thorac Surg. 2008; 86(1):189–195. discussion 196–197. [PubMed: 18573422]
7. Fernandez FG, Jaramillo A, Chen C, Liu DZ, Tung T, Patterson GA, et al. Airway epithelium is the primary target of allograft rejection in murine obliterative airway disease. Am J Transplant. 2004; 4(3):319–325. [PubMed: 14961983]
8. Okazaki M, Gelman AE, Tietjens JR, Ibricevic A, Kornfeld CG, Huang HJ, et al. Maintenance of airway epithelium in acutely rejected orthotopic vascularized mouse lung transplants. Am J Respir Cell Mol Biol. 2007; 37(6):625–630. [PubMed: 17717320]
9. Babu AN, Murakawa T, Thurman JM, Miller EJ, Henson PM, Zamora MR, et al. Microvascular destruction identifies murine allografts that cannot be rescued from airway fibrosis. J Clin Invest. 2007; 117(12):3774–3785. [PubMed: 18060031]
10. Valujskikh A, Lantz O, Celli S, Matzinger P, Heeger PS. Cross-primed CD8(+) T cells mediate graft rejection via a distinct effector pathway. Nat Immunol. 2002; 3(9):844–851. [PubMed: 12172545]
11. Tesar BM, Jiang D, Liang J, Palmer SM, Noble PW, Goldstein DR. The role of hyaluronan degradation products as innate alloimmune agonists. Am J Transplant. 2006; 6(11):2622–2635. [PubMed: 17049055]

12. Palmer SM, Burch LH, Trindade AJ, Davis RD, Herczyk WF, Reinsmoen NL, et al. Innate immunity influences long-term outcomes after human lung transplant. Am J Respir Crit Care Med. 2005; 171(7):780–785. [PubMed: 15640363]
13. Kuo E, Bharat A, Goers T, Chapman W, Yan L, Street T, et al. Respiratory viral infection in obliterative airway disease after orthotopic tracheal transplantation. Ann Thorac Surg. 2006; 82(3): 1043–1050. [PubMed: 16928532]
14. Yoshida S, Haque A, Mizobuchi T, Iwata T, Chiyo M, Webb TJ, et al. Anti-type V collagen lymphocytes that express IL-17 and IL-23 induce rejection pathology in fresh and well-healed lung transplants. Am J Transplant. 2006; 6(4):724–735. [PubMed: 16539629]
15. Sumpter TL, Wilkes DS. Role of autoimmunity in organ allograft rejection: a focus on immunity to type V collagen in the pathogenesis of lung transplant rejection. Am J Physiol Lung Cell Mol Physiol. 2004; 286(6):L1129–L1139. [PubMed: 15136293]
16. Burlingham WJ, Love RB, Jankowska-Gan E, Haynes LD, Xu Q, Bobadilla JL, et al. IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. J Clin Invest. 2007
17. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009
18. Vanaudenaerde BM, De Vleeschauwer SI, Vos R, Meyts I, Bullens DM, Reynders V, et al. The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation. Am J Transplant. 2008; 8(9):1911–1920. [PubMed: 18786233]
19. Goers TA, Ramachandran S, Aloush A, Trulock E, Patterson GA, Mohanakumar T. De novo production of K-alpha1 tubulin-specific antibodies: role in chronic lung allograft rejection. J Immunol. 2008; 180(7):4487–4494. [PubMed: 18354170]
20. Fukami N, Ramachandran S, Saini D, Walter M, Chapman W, Patterson GA, et al. Antibodies to MHC class I induce autoimmunity: role in the pathogenesis of chronic rejection. J Immunol. 2009; 182(1):309–318. [PubMed: 19109162]
21. Bobadilla JL, Love RB, Jankowska-Gan E, Xu Q, Haynes LD, Braun RK, et al. Th-17, monokines, collagen type V, and primary graft dysfunction in lung transplantation. Am J Respir Crit Care Med. 2008; 177(6):660–668. [PubMed: 18174545]
22. Iwata T, Philipovskiy A, Fisher AJ, Presson RG Jr, Chiyo M, Lee J, et al. Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. J Immunol. 2008; 181(8):5738–5747. [PubMed: 18832733]
23. Mamessier E, Lorec AM, Thomas P, Badier M, Magnan A, Reynaud-Gaubert M. T regulatory cells in stable posttransplant bronchiolitis obliterans syndrome. Transplantation. 2007; 84(7):908–916. [PubMed: 17984845]
24. Bharat A, Fields RC, Trulock EP, Patterson GA, Mohanakumar T. Induction of IL-10 suppressors in lung transplant patients by CD4+25+ regulatory T cells through CTLA-4 signaling. J Immunol. 2006; 177(8):5631–5638. [PubMed: 17015751]
25. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, Goodrich S, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nat Med. 2004; 10(9):927–934. [PubMed: 15311275]