Bronchiolitis Obliterans Syndrome: The Achilles’ Heel of Lung Transplantation

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Abstract

Lung transplantation is a therapeutic option for patients with end-stage pulmonary disorders. Unfortunately, chronic lung allograft dysfunction (CLAD), most commonly manifest as bronchiolitis obliterans syndrome (BOS), continues to be highly prevalent and is the major limitation to long-term survival. The pathogenesis of BOS is complex and involves alloimmune and nonalloimmune pathways. Clinically, BOS manifests as airway obstruction and dyspnea that are classically progressive and ultimately fatal; however, the course is highly variable, and distinguishable phenotypes may exist. There are few controlled studies assessing treatment efficacy, but only a minority of patients respond to current treatment modalities. Ultimately, preventive strategies may prove more effective at prolonging survival after lung transplantation, but their remains considerable debate and little data regarding the best strategies to prevent BOS. A better understanding of the risk factors and their relationship to the pathological mechanisms of chronic lung allograft rejection should lead to better pharmacological targets to prevent or treat this syndrome.

Keywords
- lung transplantation
- chronic rejection
- bronchiolitis obliterans syndrome

Lung transplantation (LT) is a treatment option for select patients with end-stage pulmonary or pulmonary-vascular disease. For the majority of recipients, the procedure is intended to alleviate symptoms, improve quality of life, and improve survival as compared with expectations without LT. Unfortunately, graft failure and mortality rates after LT exceed most other solid organ transplants. According to the most recent report from the International Society for Heart and Lung Transplantation (ISHLT) registry, the median survival after LT is now 5.9 years, improved from 5.3 years among those transplanted between 1996 and 2003 and from 3.9 years among those transplanted before 1996. The improvement in survival is mainly because of better operative and perioperative outcomes, whereas mortality rates after the first year posttransplant remain essentially unchanged. The main limitation to better long-term survival after LT remains bronchiolitis obliterans syndrome (BOS), which is the most common form of chronic lung allograft dysfunction (CLAD). Other less common manifestations of CLAD include restrictive allograft syndrome (RAS) and neutrophilic reversible allograft dysfunction (NRAD). These phenotypes are discussed in detail elsewhere in this issue. BOS is the leading cause of death after the first year posttransplant, and, according to the latest ISHLT report, 48% and 76% of patients develop BOS by 5 and 10 years after transplantation, respectively. In addition to its impact on long-term survival, BOS causes significant morbidity, impairs quality of life, and increases costs. This article provides an overview of the diagnosis, pathogenesis, and treatment of BOS.
Diagnosis of BOS

The diagnosis of BOS is defined by a sustained (≥3 weeks) decline in the forced expiratory volume in the first second of expiration (FEV₁) provided alternative causes of pulmonary dysfunction (e.g., anastomotic stenosis/complications, infection, acute rejection, and recurrent or progressive native disease) have been excluded. The baseline FEV₁ is defined as the average of the two highest posttransplant measurements, without the use of a bronchodilator, at least 3 weeks apart. A decline in FEV₁ from baseline of 20% or more is defined as BOS. Progressive stages of BOS (stages 1 through 3) reflect worsening degrees of airflow obstruction (Table 1).

In the 2001 updated definition and classification of BOS, stage BOS 0-p (potential BOS) was added to detect early change in lung function and was defined as an FEV₁ 81 to 90% of baseline and/or forced expiratory flow (FEF) 25 to 75% (measurement of midexpiratory flow rates) less than or equal to 75% of baseline. Studies have examined the validity of BOS 0-p as a predictor of future BOS in bilateral and single-lung transplant recipients. Each study reported similar performance characteristics with the FEF 25 to 75% criterion for BOS 0-p performing poorly, whereas the FEV₁ criterion was a modest predictor of BOS. Still, the positive predictive value of BOS 0-p (by FEV₁) for progression to BOS within 1 year was less than 60%. Studies have also shown that the FEV₁ criterion was a modest predictor of BOS.

The histological hallmark of BOS is obliterative bronchiolitis (OB) (Fig. 1). OB is an inflammatory/fibrotic process affecting the small noncartilagenous airways (membranous and respiratory bronchioles) characterized by subepithelial fibrosis causing partial or complete luminal occlusion. The fibroobliteration may be concentric or eccentric and is often associated with atrophy of the smooth muscle and destruction of elastica of the airway wall. The presence of lymphocytic bronchiolitis or intraluminal granulation tissue is not sufficient to diagnose OB. Distinctions between subtotal and total obliteration and between active versus inactive lesions (presence or absence of inflammation) have been abandoned in the recent revisions of the nomenclature. Trichrome and elastic tissue stains may facilitate identification of damaged or obliterated airways.

Due to its patchy nature, transbronchial biopsy (TBBx) is an insensitive method for detecting OB, and the clinical use of BOs with its functional grading (to be described) is the preferred means for diagnosis and monitoring. Mucostasis and/or foamy histiocytes in the distal air spaces are commonly associated with OB and may be seen on TBBx. However, the pathological term obliterative bronchiolitis should be reserved for histological specimens showing dense fibrosis within the small airways. Fibrointimal thickening and mononuclear inflammation of pulmonary arteries and veins, similar to what is seen in chronic allograft vasculopathy of transplanted hearts, may also be present with chronic rejection, but appreciation of this pathology generally requires open biopsy or autopsy and is not generally amenable to TBBx.

Table 1 Bronchiolitis obliterans syndrome classification system

<table>
<thead>
<tr>
<th>1993 Classification</th>
<th>2001 Classification</th>
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<tr>
<td>BOS 0</td>
<td>FEV₁ &gt; 80% of baseline</td>
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<tr>
<td>BOS 1</td>
<td>FEV₁ 66–80% of baseline</td>
</tr>
<tr>
<td>BOS 2</td>
<td>FEV₁ 51–65% of baseline</td>
</tr>
<tr>
<td>BOS 3</td>
<td>FEV₁ &lt; 50% of baseline</td>
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<tr>
<td>BOS 0-p</td>
<td>FEV₁ 81–90% of baseline and/or FEF₂₅₋₇₅ ≤ 75% of baseline</td>
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Source: Modified from International Society for Heart and Lung Transplantation diagnostic criteria.
BOS is not usually diagnosed before 6 months and is most common between ~1.5 and 4 years posttransplant.\textsuperscript{11} Like the time to onset, the subsequent clinical course of BOS is highly variable.\textsuperscript{5,12–16} The course may be insidious, with a gradual decline in lung function over months to years, or abrupt, with severe decline in lung function over a few weeks.\textsuperscript{2,15,16} In one study of 111 lung transplant recipients with BOS, the steepest variable.

The time to onset of BOS and rapidity of fall in FEV\textsubscript{1} were related to outcome.\textsuperscript{16} For example, early-onset BOS (within 2 years of transplant) was associated with lower FEV\textsubscript{1} than late-onset BOS (after 2 years). Similarly, rapid-onset BOS (FEV\textsubscript{1} decline > 20% in the 6 months preceding BOS) was associated with greater dysfunction of the lung allograft (i.e., a lower FEV\textsubscript{1} predicted at BOS onset; a steeper decline in the first 6 months after onset of BOS, and a lower FEV\textsubscript{1} predicted at 2 years after onset of BOS).\textsuperscript{16} In another study, the median survival after BOS diagnosis was 2.5 years with only 26% surviving 5 years.\textsuperscript{11} Not surprisingly, early-onset BOS and high-grade-onset BOS (grade 2 or 3) predicted worse survival following the diagnosis of BOS.\textsuperscript{11}

Mechanisms of BOS Pathogenesis

The pathogenesis of BOS is complex and is driven by both alloimmune and nonalloimmune mechanisms that may act alone or in combination. Histological evaluation of allograft airways suggests that the pathogenesis first involves lymphocytic infiltrates of the submucosa (i.e., lymphocytic bronchiolitis), followed by epithelial cell injury, necrosis, and ulcerations of the mucosa. The associated inflammatory reaction in the airway lumen results in recruitment/proliferation of fibroblasts/myofibroblasts.\textsuperscript{5,8,17} Epithelial mesenchymal transition (EMT) may play a role in the fibroproliferative process, but this remains controversial.\textsuperscript{18,19} Ultimately, intraluminal polypoid granulation tissue leads to subtotal or total obliteration of airway lumens.\textsuperscript{8}

Cytokines, Growth Factors, and Chemokines

Critical to airway wound repair is a delicate balance between type 1, 2, 17, and regulatory T (Treg) immune responses. Disruption of this balance may lead to fibro-obliteration of allograft airways and BOS. The type 1 immune response is mainly associated with a cytotoxic T lymphocyte (CTL) and delayed type hypersensitivity (DTH) response. The type 1 immune response is characterized by the production of interleukin (IL)-2, IL-12, γ-interferon (IFN-γ), and lymphotoxin. Classically, type 1 cytokines have been associated with acute cellular rejection, as well as BOS in some but not all studies.\textsuperscript{20–26} The type 2 immune response is characterized by the production of IL-4, IL-5, and IL-13, which promote mucosal, allergic, and humoral immunity. Although a type 2 immune profile has favored the acquisition of tolerance in some animal models, there is increasing evidence implicating a role for type 2 responses in rejection, especially chronic rejection.\textsuperscript{27–29}

A twist to the classic type 1/2 immune response paradigm was seen in a study using a cardiac allograft rejection model: rodents lacking a type 1 immune response skewed toward a type 17 response.\textsuperscript{30} A type 17 immune response is characterized by the production of IL-17 and IL-23 and is associated with autoimmunity. Interestingly, recent studies suggest that allograft dysfunction can be associated with immunity against self-antigens (e.g., col [V] and K-α1 tubulin) that may become unveiled at the time of organ harvest, implantation, ischemia-reperfusion, acute rejection, and infections.\textsuperscript{31–37} Thus increasing attention is being paid to the role of type 17 immune response in the pathogenesis of allo/autoimmunity in LT. Autoimmunity mediated by col (V)-specific T(H)17 cells predisposes patients to the development of BOS.\textsuperscript{37} Furthermore, type 17 skewing cytokines—transforming growth factor-β (TGF-β), IL-1β, IL-6, and IL-23—and the type 17 effector cytokine IL-17 were elevated in the bronchoalveolar lavage fluid (BALF) of patients with BOS.\textsuperscript{38} Moreover, in a murine model of transplant obliteratorive airway disease, neutralization of IL-6 led to a reduction in T(H)-17 cells, which was associated with a dramatic reduction in allograft airway obliteration.\textsuperscript{39} Importantly, the role of IL-17 during lung allograft injury has been confirmed in multiple models of lung alloreactivity.\textsuperscript{36,40} Thus an understanding of the complex interactions between cytokine networks will be critical for designing therapeutic strategies that can abrogate allograft rejection and induce donor-specific tolerance.

With regard to allo/autoimmunity, the balance between effector type immune responses (type 1, 2, 17) and regulatory (Treg) type immune responses may dictate outcomes of allograft accommodation or rejection. Treg cells are CD4+T helper cells characterized by constitutively high expression of the transcription factor FoxP3 and have the ability to “tone-down” effector responses. Interestingly, some but not all studies demonstrate that Tregs are a biomarker of clinical outcomes. In renal transplant recipients there was no significant correlation between intragraft FoxP3+ cells with severity of graft rejection or renal function at 1 or 2 years.\textsuperscript{41} Similarly, another study found that a transient increase in FoxP3+Tregs within the graft does occur during rejection but does not correlate with clinical outcomes\textsuperscript{42} at 3 or 12 months. Conversely, an analysis on peripheral blood FoxP-3 mRNA expression by qPCR (polymerase chain reaction) demonstrated that low expression was associated with more chronic renal allograft rejection.\textsuperscript{43} In LT, flow cytometry studies performed on BALF cells demonstrated that CD4 + FoxP3+ “Tregs” could distinguish stable lung transplant recipients from those that go on to develop BOS.\textsuperscript{44} In another study with similar methodologies, phenotypically distinct Tregs (i.e., CD3 + CD4 + CD25hiFoxP3 + CCR7 + ) were key in determining which patients would have long-term graft stability.\textsuperscript{45} Collectively, these studies suggest that successful prevention of BOS post-LT may depend on the downregulation of all three effector immune responses (type 1, 2, and 17) while preserving or augmenting the regulatory immune response.
The proliferation of epithelial cells, myofibroblasts/fibroblasts, smooth muscle, and mesenchymal precursor cells from both the donor and the recipient may all contribute to the development of BOS. Platelet-derived growth factor (PDGF) is elevated in the BALF of patients with BOS, and its inhibition in animal models reduced myofibroblast proliferation, smooth muscle proliferation, and obliterative changes. TGF-β, hepatocyte growth factor (HGF), and insulin-like growth factor-1 (IGF-1) can contribute to fibroproliferation and have each been implicated in the development of BOS.

Chemokines and their interaction with specific cell receptors are essential components of inflammatory and immune responses via recruitment of specific leukocyte subpopulations. In the lung allograft, regulated on activation, normal T cell expressed and secreted (RANTES)/CCL5 and its interaction with CCR1 and CCR5, as well as CXCR3–CXCL9 biology, proved to be important in acute lung allograft rejection. The same is true with neutrophils. Similarly, CXCR3-ligand, CCR2-CCL2, and CXCR2-ligand interactions have all been shown to be important in chronic lung allograft rejection; albeit, through different nonredundant mechanisms. Recently, IL-8/CXCL8, a CXCR2 ligand, has received a great deal of attention in the lung transplant literature. CXCL8 is elevated in the BALF of patients with BOS, and this elevation may precede the development of BOS. Furthermore, CXCL8 is elevated during pseudomonal infections and gastroesophageal reflux (GER) and may be a part of the mechanistic link between these events and BOS.

Importantly, CXCL8 is a potent attractor of neutrophils, and BALF neutrophilia has been proposed as a marker for the development of BOS as a potent attractor of neutrophils, and BALF neutrophilia has been proposed as a marker for the development of BOS. Not surprisingly, numerous studies have also implicated acute cellular rejection (ACR) as the most important risk factor for BOS. The nomenclature for ACR in the lung, adopted by the ISHLT in 1990, modified in 1996 and again in 2007, is based upon TBBx and provides separate A and B grades. A-grade ACR describes the presence and extent of perivascular inflammatory cell infiltrates. With increasing severity, these infiltrates extend into the interstitium and alveolar spaces. A-grades range from A0 (no rejection) to A4 (severe rejection). Multiple episodes, high grade, or late-onset A-grade ACR predicts a greater risk of BOS. Importantly, even a single episode of any A-grade ACR increases the risk for BOS. Because A-grade ACR is characterized by a perivascular lymphocytic infiltrate, the mechanism linking it to small airways obliteration is not well understood.

Alloimmune Reactivity

The immune response to allogeneic tissues is mediated by major histocompatibility complex (MHC) molecules. In humans, class I MHC molecules are known as human leukocyte antigens (HLA) A, B, and C and are constitutively expressed on most nucleated cells. Class II molecules are known as HLA DR, DP, and DQ and are constitutively expressed only by bone marrow–derived antigen-presenting cells. These molecules play a critical role in the immune system via the presentation of peptides in a form that can be recognized by T cells. Allograft rejection is achieved through cytotoxicity caused by CD4+ or CD8+ T cells that are recognizing donor MHC molecules through the direct or indirect pathway, memory T-cell–mediated delayed-type hypersensitivity, and via complement activation or antibody-dependent cytotoxicity of allograft cells opsonized by allogeneic antibodies.

Classically, BOS is considered to be the end-stage consequence of alloimmune-mediated injury to the lung allograft. Observations that support this assertion include the following: an increasing number of HLA mismatches between donor and recipient is associated with increased risk of BOS. T cells from lung transplant recipients with BOS are sensitized to donor antigens presented via the indirect route, and patients with BOS have an oligoclonal CD4+ T-cell expansion not present in patients without BOS.

Fig. 3 Perivascular lymphoid infiltrate with rare eosinophils, consistent with mild acute cellular rejection (hematoxylin and eosin stain; original magnification ×200).

Fig. 4 Circumferential lymphoid infiltration around small bronchiole with frequent eosinophils, consistent with high-grade small airway inflammation (grade B2R) (hematoxylin and eosin stain; original magnification ×400).
understood. However, B-grade ACR, also known as lymphocytic bronchiolitis (LB), describes the presence and extent of peri-airway lymphocyte infiltration. The ISHLT has recommended comment on the presence and severity of LB for grading ACR since 1996 (Fig. 4). Multiple studies have linked LB with the development of BOS, but the severity of LB was the most significant predictor of BOS in one large study. Interestingly, A-grade ACR was a risk factor for BOS in univariate analysis, confirming many other studies, but was not an independent risk factor for BOS in multivariable analyses adjusting for LB. Importantly, ISHLT guidelines call for rigorous exclusion of infection before ascribing the features of LB to rejection, a difficulty that often hampers the interpretation of LB.

Humoral Immunity

Antibody-mediated rejection (AMR) is a recognized clinical entity in renal and heart transplantation and may be a major cause of late graft loss, especially in renal transplantation. However, conclusive evidence for the existence of AMR after LT and its role in the pathogenesis of BOS is lacking. In the latest revision (2007) of the ISHLT Lung Rejection Study Group iteration of the nomenclature of lung rejection, there was no consensus reached on the histological hallmarks of AMR in the lung. The group therefore urged caution in the diagnosis of AMR until there is further evidence.

The ability of B cells to recognize alloantigen is not controversial. B cells recognize antigen via their B-cell receptor, internalize and process the antigen to peptide epitopes, and present it in the context of self-MHC to T cells (i.e., indirect pathway). T cells then stimulate B-cell differentiation and antibody class switching. Stimulated B cells become either plasmablasts (i.e., secrete low-affinity antibodies) or activated B cells. Activated B cells, with the help of other mononuclear cells, further proliferate, hypermutate, and undergo affinity maturation resulting in their becoming either plasma cells (PCs) or memory B cells. PCs secrete high-affinity antibodies, whereas memory B cells undergo secondary stimulation, proliferation, and differentiation into PCs when reexposed to alloantigen or other stimuli (e.g., infections). Therefore, the presence of anti-HLA antibodies is undoubtedly a marker of indirect allore cognition. However, the controversy lies in whether or not a specific pathology in the lung allograft is directly attributable to donor-specific alloantibodies (DSA).

The strongest evidence for the concept of AMR is hyperacute rejection, clinically manifested as primary graft failure occurring very early after transplantation in the setting of preformed antibodies to donor HLA antigens or endothelial cells. Although rare, hyperacute rejection is well described after lung transplantation. Features of hyperacute rejection include fibrin thrombi in alveolar septa, fibrinoid necrosis of alveolar septal walls, and hemorrhage. These histopathologic features likely represent the severest form of AMR in the lung, but histopathological criteria for AMR outside of the hyperacute rejection clinical scenario remain to be determined.

Among the criteria for a humoral response proposed by the National Conference to Assess AMR in Solid Organ Transplantation, foremost is the detection of circulating DSA. Several early studies demonstrated that an increasing pretransplant panel reactive antibody (PRA) test is associated with increasing mortality, especially in the first 30 days posttransplant (HR 2.6). Considered together with the hyperacute rejection scenario, these studies suggest that alloantibodies might compound the allograft injury initiated by ischemia reperfusion. Other studies have investigated the impact of incident humoral responses after LT. Infiltration of B cells in the human lung allograft during ACR was associated with refractoriness to augmented immunosuppression, and several other studies have also correlated the development of anti-HLA antibodies with steroid-refractory ACR. Likewise, the development of antibodies specific to HLA predicts the development of BOS. Interestingly, the administration of alloantibody is capable of causing airway obliteration in a murine model, demonstrating that an alloantibody can induce airway injury. Collectively, these studies suggest a possible role of alloantibodies in the pathogenesis of acute and chronic allograft rejection.

According to the National Conference to Assess AMR in Solid Organ Transplantation, any degree of humoral reaction greater than a latent humoral response requires the demonstration of C4d deposition in the allograft. But there may be problems extending this proposed criterion to LT because positive C4d staining in a lung allograft may lack the specificity seen in other solid-organ allografts. For instance, in a cohort of 33 lung transplant recipients, C3d (positive in 20) and C4d (positive in 11) staining was associated with primary graft dysfunction (PGD) and airway infection, but not with ACR or chronic rejection, or with presumed morphological features of AMR (necrotizing septal capillary injury or the presence of intracapillary macrophages). Another study demonstrated variable nonspecific C4d staining without any consistent pattern among lung transplant cases grouped according to the presence of acute and/or chronic rejection. Interestingly, half of nontransplant constrictive bronchiolitis and diffuse alveolar damage (DAD) controls also had positive C4d staining. Recently, Yousem and Zeevi examined characteristics of 17 biopsies from patients with ACR and DSA compared with 26 biopsies from patients with ACR and no anti-HLA antibodies. In this study, C4d staining was more common in the group with DSA, but it did not reliably separate the two groups. Likewise, we have recently shown that C3d and C4d staining showed no correlation with each other, the presence of DSA, or histopathologic findings. Collectively, these studies demonstrate that C4d and C3d are not specific enough to distinguish AMR from other lung pathologies.

Possibly, the combination of DSA with a characteristic histopathology and the appropriate clinical scenario will improve our ability to detect AMR in the lung allograft. More work is required to define AMR histopathology in the lung, but capillary inflammation and injury are likely to be key. In the recent study by Yousem and Zeevi, capillaritis was the only histopathologic feature that separated groups with and without anti-HLA antibodies, although it was seen in only a minority of cases. Along these lines, we have also shown that capillary inflammation, defined as capillary neutrophil infiltration with at least two back-to-back
intracapillary neutrophils (Fig. 5), or DAD, in the absence of infection, was significantly associated with DSA (69 vs. 24%). Although more work is required, these studies suggest that the finding of capillary inflammation, in combination with DSA and the right clinical scenario, may be a useful tool for identifying AMR in the lung.

Autoimmunity

Immunologic response to cryptic self-antigens and/or their determinants has recently been shown to possibly contribute to the pathogenesis of chronic rejection in many solid organ transplants. Thus any injurious process to the donor organ may lead to the unveiling of intercalated self-antigens and/or their determinant initiating an immune response that has been coined “autoimmunity” posttransplantation. This response is due to a combination of cellular and antibody-mediated injuries and has been described in cardiac transplantation (e.g., autoimmune response to myosin and vimentin), renal transplantation, autoimmune response to angiotensin type I receptor antibodies, col (IV), and MHC class I chain-related peptide A and LT (e.g., autoimmune response to col (V) and K-α1 tubulin).

Human LT studies have demonstrated an association between responses to self-antigens and BOS. Mechanistic studies involving humans and animals suggest that any inflammatory change in the lung allograft (e.g., PGD, ACR, and/or infection) allows cryptic self-antigens to be exposed to the immune system causing a sensitization that can then lead to immune-mediated allograft injury and eventual lung allograft dysfunction.

Col (V) is ubiquitously expressed in perivascular/bronchial connective tissues where it is incorporated within collagen I fibrils that protect it from immunological responses. Following LT col (V) fibrils can be detected in BALF in a rat model system. Additionally, animals can develop a T-cell immune response to col (V) characterized by IFN-γ expression that is associated with rejection. Furthermore, the transfer of col (V)-specific T cells to rats with lung isografts develops perivascular/bronchial mononuclear cell infiltration (e.g., areas full of exposed col (V) due to ischemia-reperfusion) mimicking the pathology of ACR and LB. This suggests that a lymphocyte-specific response to self-antigens can cause rejection. Moreover, oral tolerance to col (V) in a non–fully mismatched rat transplant model was protective of lung allograft rejection. Translational human studies have also shown that human lung transplant recipients with detectable col (V) specific immune responses in patients before (likely from there underlying lung disease) or after transplantation are possibly at risk for the development of PGD and BOS.

Studies have also found that their may be a pathogenic role for an autoimmune response to K-α1 tubulin in the development of chronic rejection. K-α1 tubulin is one of six isotypes of α-tubulin, is expressed in the gap junction of airway epithelial and endothelial cells, and is a component of cellular microtubules, making it important in microtubule formation, GTP binding, and cellular movement. Antibodies to K-α1 tubulin correlate with the development of chronic rejection. Mechanistically, these antibodies can bind to epithelial cells and stimulate profibrotic growth-factor signals. Other studies demonstrate that the responses to col (V) and K-α1 tubulin are both important in allograft injury, and these recipient responses can lead to a skewed immune response (e.g., high type 1/17 and low type 2) favoring the development of BOS.

Both allo- and autoimmunity occur together and may have the potential to drive one another in the immunopathogenesis of BOS. Mice treated with anti-MHC class 1 antibodies developed de novo antibodies to self-antigens col (V) and K-α1 tubulin. Likewise, in human lung transplant recipients, there is a strong correlation between the development of donor-specific anti-HLA antibodies and antibodies to self-antigens col (V) and K-α1 tubulin, with the development of DSA preceding antibodies to self-antigens. Conversely, pretransplant antibodies to self-antigens were associated with the posttransplant development of DSA, as well as with increased risk of PGD and BOS. Importantly, recipients with both DSA and antibodies to self-antigens who cleared both antibody types after treatment (rituximab and/or intravenous immunoglobulin) were at lower risk of developing BOS than those who cleared only the DSA but had persistent antibodies to self-antigens. Collectively these studies demonstrate cross-talk between auto- and alloimmune responses that may perpetuate lung allograft injury, ultimately leading to BOS.

Innate Immunity

A growing body of literature supports an association between BOS and ostensibly nonalloimmune responses to local injury and foreign antigens unrelated to donor-specific MHC. Theoretically, any insult, including infection, aspiration, and ischemia-reperfusion injury can lead to the propagation of “danger signals” that activate professional antigen-presenting cells (e.g., dendritic cells) via Toll-like receptors (TLRs), leading to optimized antigen presentation to alloreactive T cells. In animal models, TLR engagement has been found to hinder the induction of transplant tolerance. Local innate immune activation through lipopolysaccharide has been shown to induce alloimmune lung injury via TLR4 activation. Polymorphisms in TLR2, TLR4, TLR9, the lipopolysaccharide

Fig. 5 Diffuse and back-to-back capillary neutrophils (hematoxylin and eosin stain; original magnification ×600).
receptor CD14,123 as well as altered levels of mannose-binding lectin in transplant recipients124–126 have all been associated with BOS. This supports the hypothesis that innate immunity appears to be an important cofactor linking alloimmune-independent mechanisms of lung injury to accelerated alloimmune responses and BOS.

Primary graft dysfunction (PGD) is a form of acute lung injury that arises within the first 72 hours of transplantation, resulting from multiple pathological mechanisms inherent to the process of transplantation, including physiological changes in the donor following brain death, explantation, cold ischemia, and reperfusion within the recipient.127 The innate immune response fundamental to PGD and other forms of ischemia-reperfusion injury involves activation of TLR signaling,128–130 increased expression of proinflammatory cytokines,131–133 and recruitment of recipient lymphocytes and antigen-presenting cells,134,135 and has been associated with enhanced expression of major histocompatibility class (MHC) II antigens.136,137 Although PGD is a well-described major factor in early mortality following lung transplantation, there is increasing evidence that PGD also contributes to late morbidity and mortality.138,139 In two single-center studies, PGD was found to be an independent risk factor for the subsequent development of BOS.140,141 Polymorphisms in pentraxin-3, a key mediator of innate immunity, were associated with PGD, suggesting that variations in recipient innate immunity may affect the incidence of PGD and the subsequent risk of BOS.142,143

Following transplantation, recipients may be at increased risk of developing GER and/or aspiration of gastric fluids due to delayed gastric emptying, lung denervation, impaired cough reflex, and abnormal mucociliary clearance.144,145 In a rat model of LT, histological findings consistent with OB have been reproduced by instillation of gastric fluid into allografts.146 Two centers have found that the presence of bile acid in BALF from transplant recipients was associated with BOS, whereas a more recent study found that the presence of bile acid in lavage specimens from patients who already have BOS is associated with a more rapid decline in lung function and an increased rate of mortality.147–149 GER confirmed by pH probe testing has also been associated with an increased rate of acute rejection, as well as reduced plateaus in lung function following LT.150–152 High levels of bile acids within the allografts of patients with GER have been associated with lower surfactant collectin proteins and surfactant phospholipids, all components of innate immunity.153 Treatment of GER is recommended posttransplant; however, proton pump inhibitors may not affect non-acid reflux, and in the previously noted rat model, pH neutralization of the instilled gastric fluid had no impact on the subsequent induction of OB.148,154 In transplant recipients with known reflux, retrospective studies have found that more aggressive treatment with early gastric fundoplication may be associated with greater freedom from BOS, and improved lung function.152,155

Multiple infectious processes have been linked to the development of BOS. The best evidence for this is in patients who develop cytomegalovirus (CMV) pneumonitis.14,76,95,156–161 CMV infection increases epithelial expression of donor HLA in transplant recipients,95 and upregulates proinflammatory cytokine expression.162 The virus has also been found to share nucleic acid sequence homology with specific HLA antigens.159 Two nonrandomized trials have found that pharmacological prophylaxis against CMV leads to both a reduced rate of CMV as well as BOS.163,164 Similarly, associations between BOS and community-acquired respiratory viruses (CARVs),165–168 human herpesvirus-6,169 and Chlamydia pneumonia170 infection have been described in retrospective single-center series. Our group recently found that CXCR3 chemoattractants are upregulated during CARV infection, and elevated expression of these chemokines among infected patients is associated with chronic allograft dysfunction, suggesting a potential mechanistic link between nonalloimmune responses to these acute infections and the subsequent development of alloreactivity.171

Low-grade chronic infections may also be important risk factors for BOS. Lung transplant recipients, especially those transplanted for cystic fibrosis, commonly develop lower airway colonization with Pseudomonas aeruginosa and/or Aspergillus species. In two retrospective studies, pseudomonal colonization was associated with increased risk and higher stage of BOS,172–174 and levels of antipseudomonal antibodies in BAL among colonized transplant recipients have been associated with local innate immune responses.175 Similar findings were reported with Aspergillus colonization. In a time-dependent analysis, Aspergillus colonization was a risk factor for the development of BOS, independent of ACR.176 Furthermore, those with new or persistent Aspergillus colonization after the development of BOS had a greater risk of progression to severe BOS (stage 3) or death.176

Treatment of BOS

Treatment options for BOS generally remain disappointing. Historically, uncontrolled studies have cited treatment responses with diverse strategies, but interpretation is often clouded by small sample sizes and lack of suitable controls. Frequently in these studies, favorable responses were defined as “stabilization” or reduction in the rate of decline of FEV1; improvement was rarely documented. Importantly, “stabilization” may reflect the natural history of the disease.16 Anecdotal improvements in FEV1 are also reported. However, it is interesting that lung biopsies from patients with BOS typically have varying degrees of ACR.177 Thus responses to therapy may be due to resolving ACR rather than BOS.

Changes in Maintenance Immunosuppression

Uncontrolled studies have cited slower rates of FEV1 decline after conversion to mycophenolate mofetil (MMF) from azathioprine (AZA),178,179 or to tacrolimus from cyclosporine.180–182 Controlled data confirming benefit are not available, and the positive findings in these studies probably reflect the natural history of BOS. We believe that conversion from AZA to MMF or cyclosporine to tacrolimus is unlikely to benefit most patients with BOS.
Cytolytic Therapy
Antilymphocyte and antithymocyte preparations deplete T cells and can have prolonged effects on T-cell function through nondepletive mechanisms (e.g., effects on antigen-presenting cells and B cells). Salvage treatment for BOS with cytolytic therapies has been reported to slow the decline of FEV₁. Alemtuzumab (Campath 1H, Genzyme, Cambridge, MA) is a humanized CD52 directed cytolytic antibody that results in a rapid and sustained (6 months or longer) depletion of lymphocytes. In a small cohort \( n = 10 \) of patients with BOS, FEV₁ improved in four patients and remained stable in an additional three patients, but overall the FEV₁ for the group was unchanged 6 months after treatment. Importantly, infectious complications following alemtuzumab treatment were common (73%), limiting our enthusiasm for its use in BOS. No controlled data exist for alemtuzumab or other cytolytics for the treatment of BOS at this time.

Azithromycin
Gerhardt et al first reported the results of a small pilot study using add-on azithromycin (250 mg three times a week) for BOS in 2003. In this study, five of six patients had a significant improvement in lung function over a short follow-up period. This sparked intense interest in azithromycin as an immune-modulating agent with relatively few side effects. Most, but not all, subsequent studies have also suggested that a subset of patients with BOS do respond to treatment with azithromycin. Response may be predicted by pretreatment BALF neutrophilia. Some have proposed that this group of patients represents a distinct phenotype of CLAD, termed neutrophilic reversible allograft dysfunction (NRAD), although this diagnosis has not yet been formally recognized. Recently, the results of a small, single-center, randomized trial of azithromycin for the treatment of BOS were published in abstract form. In this study, azithromycin treatment was associated with improved FEV₁ at 12 weeks, relative to placebo. At the press time for this review, the final peer-reviewed publication is not yet available. However, as the drug is relatively inexpensive, has few side effects, and there is little else to offer, a trial of therapy seems indicated for any patient who develops BOS.

Extracorporeal Photopheresis
Extracorporeal photopheresis (ECP) involves the removal of a fraction of the patient’s blood and the isolation of leukocytes, which are then exposed to ultraviolet light in the presence of 8-methoxypsoralen. This forms covalent bonds to DNA pyrimidine bases, cell-surface molecules, and cytoplasmic components in exposed cells. ECP is a safe and effective treatment of cutaneous T-cell lymphoma. It also has been used successfully to treat graft versus host disease (GVHD) in hematopoietic stem cell transplant recipients and for the prevention and treatment of acute cellular rejection in heart transplant recipients. ECP therapy involves multiple treatment cycles (ECP on 2 consecutive days) at regular intervals for a total of 3 to 12 months. The mechanisms of action are not fully understood. However, studies suggest that ECP results in leukocyte apoptosis and induction of regulatory T cells.

There are no controlled studies of ECP in lung transplant recipients. Several observational studies have shown that the rate of decline in FEV₁ is reduced after initiation of ECP for the majority of patients, whereas a minority experience improved lung function. In the largest published series including 56 lung transplant recipients with BOS, 25% had an increase in their FEV₁. In another recent study including 51 patients, 30% had at least an initial improvement in lung function, and 18% had a sustained improvement in FEV₁ 12 months after starting ECP. Early BOS, defined as onset within 3 years of transplant, was associated with a greater likelihood of response in one study but not in the other, where early BOS was defined as onset within 2 years of transplant. ECP is generally well tolerated without an appreciable increased risk of infections, but it is relatively expensive. Unfortunately, the evidence for benefit with ECP for BOS is insufficient to support a recommendation at this time. However, appropriately controlled studies examining this question are welcomed.

Fundoplication
Given the relationship between GER and BOS, there may be a role for antireflux surgery in patients with GER who develop BOS. In a series of 43 lung transplant recipients who underwent fundoplication after transplantation, FEV₁ improved by an average of 24% by 6 months after antireflux surgery. Fifty percent of the 26 patients with BOS at the time of the antireflux surgery no longer met criteria for BOS after fundoplication. The potential benefit of fundoplication in patients with GER and BOS needs to be weighed against the risks of surgery in patients with obstructive lung disease.

Retransplantation
Retransplantation has been performed for lung transplant recipients with BOS, with lower survival rates than with initial transplants. However, survival after retransplantation for BOS is better than survival after retransplant for early (within 30 days of transplant) causes of graft failure. The incidence of BOS after retransplant is higher than after initial transplant (HR 2.0 [1.4–3.0]). In light of limited availability of donor lungs, the role of retransplantation for BOS remains controversial.

Strategies for the Prevention of BOS
By the time BOS is diagnosed, it may be too late for treatments to reverse the airways pathology for the majority of patients. Therefore, strategies aimed at the prevention of BOS are most likely to favorably impact long-term morbidity and mortality outcomes after LT.

Induction Therapy
According to the latest report of the ISHLT registries, the overall percentage of lung transplant recipients treated with induction therapy in 2010 declined to 51%, down from more than 60% in the 3 years prior. However, over the past decade there...
has been an overall increase in the use of IL-2 receptor antagonists (e.g., daclizumab or basiliximab) and alemtuzumab. Interestingly, the use of polyclonal antilymphocyte globulin/antithymocyte globulin (ALG/ATG) induction has been falling over this same time period. In this same report, any induction therapy was associated with a significantly better overall survival. However, these analyses were not adjusted for the propensity to receive induction regimens and thus may be confounded by center, diagnosis, and other recipient variables. Furthermore, the large sample size in this comparison permits statistical significance for a small difference that may not be clinically meaningful.

There is a limited clinical trial experience examining the effectiveness of different induction therapies. In one small randomized trial, induction with rabbit ATG yielded a significant reduction in ACR > 2 compared with no induction. However, in a study by the same group that included longer-term follow-up, there was no difference in freedom of ACR > 1, and there was no difference in freedom of BOS, infection, malignancy, or survival. In other studies of lung transplant recipients, the incidence of ACR was lower with daclizumab than with ATG in some, but not all. Studies. The incidence of BOS was lower with daclizumab in one study and similar to ATG in two studies in a nonrandomized trial, induction therapy with alemtuzumab was associated with greater freedom from rejection compared with ATG or daclizumab. Recently, the same group has published their longer-term follow-up and the findings continue to appear favorable: alemtuzumab was associated with improved survival as well as a greater 5-year freedom from BOS. There was no difference in the incidence of PTLD, and although rates of infection were not described, alemtuzumab-treated patients were not more likely to die of infection than patients treated with other or no induction. However, in the absence of randomized, controlled trial data, concerns remain about the risks of infection and malignancy after alemtuzumab induction. The most important message may be that prospective, multicenter studies are needed to determine if and which induction therapy is beneficial in LT.

Maintenance Immunosuppression

ISHLT registry data suggest that rates of ACR are lower with immunosuppressive regimens employing mycophenolate mofetil (MMF) compared with azathioprine (AZA). However, a randomized, open-label trial involving 22 sites found similar rates of ACR, BOS, and survival at 3 years with these agents. ACR rates are also lower with immunosuppressive regimens employing tacrolimus as compared with cyclosporine in the most recent report of the ISHLT registry, and prospective trials appear to confirm the slight advantage for tacrolimus. In one randomized trial, the incidence of ACR trended lower (p = 0.07), and the incidence of BOS was lower (p = 0.025) in the tacrolimus group. A second trial found fewer ACR episodes in the tacrolimus cohort. A third trial also reported a lower burden of ACR (both A and B grades), as well as a trend to a greater freedom from BOS (p = 0.09) in the tacrolimus group. In the most recent and largest randomized trial, tacrolimus was associated with a lower cumulative incidence of BOS despite no difference in the rates of ACR. Importantly, in each of these trials cyclosporine dosing relied on blood trough concentrations (C0) for some or all patients receiving cyclosporine. Studies have demonstrated that 2-hour postdrug concentrations (C2) are a more accurate measure of drug exposure than C0 levels. Therefore, it is possible that C2 optimized cyclosporine dosing would perform better and be more comparable to tacrolimus for the prevention of ACR and BOS in lung transplant recipients.

Sirolimus and Everolimus

Sirolimus (rapamycin) and related compounds (e.g., everolimus) bind to the same intracellular target as tacrolimus, FK binding protein. However, thereafter their activity involves modulation of the activity of the mammalian target of rapamycin (mTOR), which in turn inhibits IL-2-mediated signal transduction, thus blocking the activation and proliferation of T and B cells. In a multicenter, randomized, double-blind trial, 223 lung transplant recipients who were free of BOS received maintenance immunosuppression consisting of cyclosporine and corticosteroids together with either everolimus or AZA. Efficacy failure (i.e., drop in FEV1 > 15%, graft loss, death, or loss to follow-up) at 12 months was less frequent (22%) among patients receiving everolimus compared with AZA (34%). However, by 24 months, freedom from BOS was similar between groups. Interestingly, the incidence of treated ACR was significantly reduced at both 12 and 24 months in the everolimus cohort. Although everolimus is a promising therapy, a potential serious concern raised in this study was the increased rate of adverse reactions in the everolimus-treated patients, including bacterial infections, fungal infections, and elevated serum creatinine. Theoretically these antiproliferative agents could have deleterious effects on healing of the bronchial anastomoses following LT and should probably be avoided in the early posttransplant period.

Surveillance Bronchoscopy with Transbronchial Biopsy

Given the relationship between the severity and recurrence of ACR with the development of BOS, a surveillance protocol aimed at the early diagnosis and treatment of ACR has been advocated by some as a strategy for the prevention of BOS. TBBx is the principal diagnostic modality for the assessment of ACR in the lung allograft, but the sensitivity of this procedure is dependent upon the number of samples taken. In one study, 18 samples per bronchoscopy were required to achieve 95% confidence of finding rejection. Most programs report practices of obtaining far fewer biopsies. The specificity of ACR histopathology is also of some concern: the reported interobserver agreement of ACR grading is moderate at best, even between experienced pathologists.

Irrespective of these concerns, studies have demonstrated that surveillance bronchoscopy protocols can detect asymptomatic acute rejection. In one study of 1,235 TBBx in 230 lung transplant recipients, 836 (67.7%) were performed for surveillance. ACr was diagnosed in 18.9% of surveillance procedures, and 86.4% of clinically indicated TBBx. However, the yield of surveillance TBBx to diagnose ACR between 4 and
12 months decreased to 6.1%. Therefore, the utility of surveillance TBBx beyond 4 to 6 months is a matter of debate.

Still others advocate for no routine surveillance after LT. In fact, there is no current evidence that demonstrates surveillance protocols including TBBx have any impact on BOS or survival after LT. Valentine et al reported a small multicenter trial comparing surveillance with clinically indicated TBBx and BAL. The clinically indicated group \( n = 23 \) underwent fewer TBBx/BAL than the surveillance group \( n = 24 \) (84 vs 156, respectively). In the surveillance group, 54 TBBx/BAL procedures were defined as true surveillance procedures, and no episode of ACR was diagnosed by a true surveillance procedure. In this study, there were no differences in freedom from BOS or survival between groups receiving either clinically indicated or surveillance bronchoscopy, but the small size of the study was vastly underpowered to answer this question conclusively.

There remains no consensus on the best practice, but most programs report some version of a surveillance bronchoscopy protocol after LT. In a 2004 survey of lung transplant centers, 69% of responding programs performed surveillance TBBx, and 60% of responding programs performed surveillance BAL procedures were defined as true surveillance procedures, and no episode of ACR was diagnosed by a true surveillance procedure. In this study, there were no differences in freedom from BOS or survival between groups receiving either clinically indicated or surveillance bronchoscopy, but the small size of the study was vastly underpowered to answer this question conclusively.

Hematopoietic Cell Transplant

Full or mixed-chimerism has long been recognized for the potential to facilitate allograft tolerance. Patients who have undergone myeloablative conditioning and human lymphocyte antigen (HLA)-matched bone marrow transplantation for a hematologic-oncology disorder, who later receive a renal transplant from the same donor, have not required immunosuppression, confirming that chimerism can lead to tolerance. Similarly, a recipient of simultaneous renal and hematopoietic-cell transplant treated with a conditioning regimen of total lymphoid irradiation and ATG developed a persistent mixed chimerism with no rejection or GVHD following discontinuation of immunosuppressive drugs. Likewise, a pretransplant nonmyeloablative conditioning regimen (anti-CD2 antibody, cyclophosphamide, thymic irradiation, and rituximab) resulted in four of five patients with stable immunosuppression-free long-term kidney graft survival. Another recent small series also demonstrated successful graft acceptance using a different conditioning strategy (e.g., total body irradiation, 200 Gy, fludarabine, cyclophosphamide, and administration of a special population of bone marrow–derived cells termed facilitator cells along with donor hematopoietic stem cells) in non–HLA matched kidney transplant pairs. This regimen resulted in a durable mixed chimerism for five of eight recipients without GVHD and allowed weaning from all immunosuppression by 1 year after transplant.

Unfortunately, even if effective, the sporadic and unpredictable timing of cadaveric donors for LT make the implementation of pretransplant conditioning logistically impossible at this time. Furthermore, the risks of infections with conditioning regimens required to induce chimerism may outweigh the benefits for LT. Lung transplant recipients are uniquely susceptible to posttransplant infections for a variety of reasons, including chronic pretransplant immunosuppression, pretransplant colonization, PGD, prolonged mechanical ventilation, and disrupted cough reflex. Even nonmyeloablative conditioning regimens can lead to prolonged neutropenia/lymphopenia and thus a high risk of serious infections.

Protocols for the simple infusion of donor bone marrow (BM), without conditioning, simultaneous or prior to transplant, would be preferable in LT. In a human study, 26 lung transplant recipients receiving infusion of donor BM (without conditioning) in combination with LT were compared with 13 patients receiving LT alone. Chimerism was detectable in more than half of the recipients 1 year posttransplant and in none of the control recipients tested. Among patients surviving >4 months, OB developed in 1 of 22 BM and 4 of 12 control patients \( (p = 0.04) \). Patient survival and freedom from ACR were similar between groups. This technique has promise, but additional studies are required to determine the efficacy, safety, and role of this procedure in humans.

Conclusion

BOS is the dominant factor as to why long-term outcomes after LT remain disappointing. Although alloimmune pathways have a clear role in the pathogenesis of chronic rejection, nonspecific causes of airway injury also appear to promote the development of BOS. Airway injury can accelerate alloimmune responses via innate immune pathways and/or may expose antigens that activate autoimmune responses that lead to BOS independent of alloimmunity. Although nonspecific immunosuppression seems to allow lung allograft accommodation for some, most lung transplant recipients eventually experience late allograft dysfunction in the form of BOS. Unfortunately, there is currently no proven therapy for the prevention or treatment of BOS. Advances in our knowledge of risk factors and pathogenesis should lead to novel strategies for the prevention/treatment of BOS and improvements in long-term outcomes after LT.

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