Lung transplantation is a therapeutic option for patients with end-stage pulmonary disorders. Unfortunately, due to post-lung transplant complications, both infectious and noninfectious, it is only a treatment and not a cure. Importantly, despite induction combined with triple or quadruple maintenance immunosuppressive therapy, chronic lung rejection, in the form of obliterative bronchiolitis or its clinical correlate bronchiolitis obliterans syndrome (BOS), continues to be highly prevalent and is the major limitation to long-term survival. In this review we evaluate the presentation, diagnosis, histopathology, pathologic mechanisms, risk factors, and prevention/treatment options for BOS. A better understanding of the risk factors and how it relates to the pathologic mechanisms of chronic lung allograft rejection should lead to better pharmacologic targets to prevent/treat this syndrome without increasing the recipient’s risk for infections.

**Keywords:** lung transplant; chronic allograft rejection; acute allograft rejection; obliterative bronchiolitis; bronchiolitis obliterans syndrome

Chronic rejection is the leading cause of morbidity and late mortality (>1 year) after lung or heart-lung transplantation (1). Obliterative bronchiolitis (OB) or bronchiolitis obliterans syndrome (BOS), the clinical correlate of OB, is a manifestation of chronic lung allograft rejection (2). The cardinal clinical feature of BOS is a reduction in forced expiratory volume in 1 second (FEV1), which does not respond to bronchodilators (3–5). Progressive dyspnea, often accompanied by cough, is the predominant symptom (3, 5). Lung biopsies (transbronchial or surgical) are not essential to diagnose BOS, but may be useful to exclude alternative diagnoses (5). The clinical course of BOS is highly variable, with a median survival after onset of 3 to 4 years, with a wide range (0–9.4 yr) (3, 6, 7).

**DIAGNOSIS OF BOS**

The diagnosis of BOS is defined by physiologic criteria: that is, a sustained (>3 wk) decline in expiratory flow rates (i.e., an obstructive defect), provided that alternative causes of pulmonary dysfunction (e.g., anastomotic stricture/complications, infection, acute cellular rejection [AR], recurrent or progressive native disease) have been excluded (4). The salient physiologic parameters include FEV1 and mid-expiratory flow rates (FEF25–75) (4) (Table 1). Baseline FEV1 and FEF25–75 were defined as the average of the two highest measurements without the use of a bronchodilator at least 3 weeks apart after lung transplantation (LT) (4). Patients without evidence for airflow obstruction are categorized as BOS 0 (i.e., FEV1 > 80% of baseline); while progressive stages of BOS (1, 3) reflect worsening degrees of airflow obstruction (i.e., BOS stage 1: FEV1 66–80% of baseline) (Table 1) (4). In the updated 2002 classification, stage BOS 0-p (potential BOS) was added to detect early change in lung function and was defined as a FEV1 81 to 90% of baseline and/or FEF25–75 less than or equal to 75% of baseline (Table 1) (4). However, optimal parameters to define airflow obstruction, especially with regard to BOS 0-p, have not been validated. Changes in FEV1 have been most often used, but are less sensitive than changes in FEF25–75 in detecting early airflow obstruction (4, 8). However, FEF25–75 displays wide intrasubject variability, particularly in recipients with single lung transplants (SLTs) (4, 8). Two studies have examined the validity of BOS 0-p as a predictor of future BOS (9, 10). A retrospective study involving over 200 heart-lung or bilateral lung transplant (BLT) recipients found that BOS stage 0-p by FEV1 criteria was superior to FEF25–75 in predicting the progression to BOS stage 1 within 1 year (positive predictive value [PPV] = 79%, negative predictive value [NPV] = 82% with 57% of lung transplant recipients [LTRs] with stage 0-p [by FEV1] progressing to stage 1 at 1 year) (9). The second study examined 197 SLT recipients and reported similar performance characteristics (10). Collectively, these studies suggest that FEV1 criteria for BOS 0-p, but not FEF25–75, is at best a reasonable predictor for future BOS.

**HISTOLOGIC FEATURES OF LUNG ALLOGRAFT REJECTION**

A grading system for lung acute rejection was adopted by the International Society for Heart and Lung Transplantation (ISHLT) in 1990 and was modified in 1996 and again in 2007 (11). Acute cellular rejection (ACR), based on perivascular and interstitial infiltrates, is graded from A0 (none) to A4 (severe) (11). Small airways inflammation (i.e., lymphocytic bronchitis/bronchiolitis) is now graded as follows: Grade B0 (none), B1R (low grade); B2R (high grade); BX (ungradeable) (11). Obliterative bronchiolitis (the histologic hallmark of chronic rejection) is described as present (C1) or absent (C0), irrespective of the presence of inflammatory activity (11). Chronic vascular rejection may also be present and associated with fibrointimal thickening of pulmonary arteries and veins, and has many features that are similar to transplant coronary artery disease (11, 12). Acute antibody-mediated (humoral) rejection may occur in allosensitized LTRs (13, 14), but the incidence and significance of this lesion has not been fully elucidated (11). In this chapter, we focus on chronic lung allograft rejection. Acute rejection is discussed in detail in a separate chapter in these proceedings.

**HISTOLOGIC FEATURES OF CHRONIC LUNG ALLOGRAFT REJECTION**

The histologic hallmark of chronic rejection is OB, an inflammatory/fibrotic process affecting the small noncartilaginous airways or bronchioles (11, 15). The initial process is lymphocytic infiltrates of the submucosa (i.e., lymphocytic bronchitis or...
bronchiolitis [LB]); this is 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 (4, 5, 11) (Figures 1a and 1b). Polypoid intraluminal granulation tissue may lead to subtotal or total obliteration of airway lumens (11). Advanced OB can include a spectrum from partial to completely acellular fibrotic obliteration where only a scar remains of airway lumen (“vanishing airways disease”) (4, 5, 11) (Figures 1c and 1d). This small airway obstruction can lead to the accumulation of foamy macrophages as well as mucostasis in the distal airspaces (11). Fibrointimal changes involving pulmonary arteries and veins are also seen but have been overshadowed by the airway lesions (4, 5, 11, 12). Due to the patchy nature of OB, the diagnosis is often missed by transbronchial lung biopsies (TBBs) (4). Serial (step) sections and trichrome and elastic tissue stains facilitate identification of damaged or obliterated airways (4) (Figures 1e and 1f). The pathologic term “obliterative bronchiolitis” or bronchiolitis obliterans should be reserved for histologic specimens showing dense fibrosis within the small airways (4). The presence of LB or intraluminal granulation tissue is not sufficient to diagnose OB (4).

**INCIDENCE OF BOS**

Although BOS is rare within the first year after LT, the cumulative incidence of BOS ranges from 43 to 80% within 5 years of transplantation (2, 5, 6, 16, 17). Data from the ISHLT Registry comprising over 10,000 LTRs followed from April 1994 through June 2006 noted that 25% had developed BOS by 2.5 years and 50% by 5.6 years (17).

**TABLE 1. BRONCHIOITIS OBLITERANS SYNDROME CLASSIFICATION SYSTEM**

<table>
<thead>
<tr>
<th>1993 Classification*</th>
<th>2003 Classification†</th>
</tr>
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<tbody>
<tr>
<td>BOS 0 FEV&lt;sub&gt;1&lt;/sub&gt; &gt; 80% of baseline</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; &gt; 90% of baseline and FEV&lt;sub&gt;25-75&lt;/sub&gt; &gt; 75% of baseline</td>
</tr>
<tr>
<td>BOS 0 p FEV&lt;sub&gt;1&lt;/sub&gt; 81–90% of baseline and/or FEV&lt;sub&gt;25-75&lt;/sub&gt; &lt; 75% of baseline</td>
<td></td>
</tr>
<tr>
<td>BOS 1 FEV&lt;sub&gt;1&lt;/sub&gt; 66–80% of baseline</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; 66–80% of baseline</td>
</tr>
<tr>
<td>BOS 2 FEV&lt;sub&gt;1&lt;/sub&gt; 51–65% of baseline</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; 51–65% of baseline</td>
</tr>
<tr>
<td>BOS 3 FEV&lt;sub&gt;1&lt;/sub&gt; ≤ 50% of baseline</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; ≤ 50% of baseline</td>
</tr>
</tbody>
</table>

* Modified from ISHLT Guidelines (176).
† Modified from ISHLT Guidelines (3).

**Definition of abbreviation:** BOS = bronchiolitis obliterans syndrome.

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**Figure 1.** Representative histopathology of bronchiolitis obliterans. (a) Early lesion with chronic inflammation present beneath epithelium in wall of small airway (hemtoxylin and eosin [H&E] stain, ×200). (b) Completely obliterated bronchiole in which both inflammation and fibrosis are present (H&E stain, ×40). (c and d) Scar (S) next to an artery (A), that on higher magnification shows a strip of smooth muscle (arrow), indicating that this was once an airway (H&E stain, c ×40, d ×200). (e) Completely obliterated bronchiole (B) next to a normal artery (A) (trichrome/elastic stain ×40). (f) A bronchiole (B) with mild luminal fibrosis (arrow) next to an artery (A) with mild graft arteriopathy (arrow) (trichrome/elastic stain ×100).
CLINICAL PRESENTATION

The time to onset and clinical course of BOS is highly variable (3, 4, 6, 7, 16, 18). 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 (7, 18, 19) (Figure 2). British investigators described 204 LTRs with BOS; 56% exhibited a sudden drop in FEV$_1$ (acute onset), whereas 18% followed a smooth linear decline (chronic onset) (18). Median post-BOS survival was 29 months in the acute onset group versus 58 months in the chronic onset group. Lama and coworkers analyzed 111 LTRs with BOS; the steepest decline in FEV$_1$ was in 58 months in the chronic onset group. Median steep declines over the next 18 months (7). In that cohort, the time the first 6 months after BOS onset, followed by progressively less steep declines over the next 18 months (7). In that cohort, the time to onset of BOS (early versus late) and rapidity of fall in FEV$_1$ over time predicted long-term outcome (7). A fall in FEV$_1$ greater than 20% in the 6 months preceding BOS (termed “rapid onset”) was associated with a worse prognosis (i.e., a shorter time to BOS onset, a lower FEV$_1$% predicted at BOS onset; a steeper decline in the first 6 mo after onset of BOS, and a lower FEV$_1$% predicted at 2 yr after onset of BOS) (7). Furthermore, among patients developing BOS within 2 years of LT (“early onset”), FEV$_1$% predicted remained lower than that of patients with “late onset” during follow-up (7). Danish investigators found that worsening BOS grade (1 to 2 or 2 to 3) was associated with threefold increase in mortality, irrespective of whether BOS developed early or late (6). Survival at 5 years after diagnosis of BOS ranges from 26 to 43% (19, 20). Data from the ISHLT registry (adults) noted that BOS accounted for fewer than 5% of deaths within the first year after LT, but accounted for 27.6% of deaths between less than 1 and 3 years, 30.0% between less than 3 and 5 years, and 26.5% after 5 years (17).

Respiratory infections may play an important role in the onset of BOS and its progression (19). As the disease progresses, airway colonization/infections with Pseudomonas aeruginosa and Aspergillus spp. is common (18, 19). In addition to its impact on long-term survival, BOS causes significant morbidity (19), impairs quality of life (21), and increases costs (22). Health-related quality of life (HRQL), accounting for physical, mental, and social well-being, and not merely absence of disease, has been studied in LTRs with BOS. One study included 140 LTRs with a maximum follow-up of 10 years, assessed the physical dimension by the Nottingham Health Profiles Energy and Mobility Scales and the psychological dimension by the Zung depression and STAI anxiety scores (23). The presence of BOS was a significant predictor of poor energy and mobility as well as increased anxiety and depression. A smaller prospective study followed 58 LTRs for 5 years or more and demonstrated that the development of BOS at or beyond stage 2 was a significant predictor of a lower score on the St George’s Respiratory Questionnaire, reduced time free of clinical complications/adverse illnesses, yet surprisingly did not significantly reduce 6-minute walk distance, as compared with those that did not develop BOS (24). This study demonstrates that moderate to severe BOS impairs HRQL, but does not necessarily prevent LTRs from walking independently and pursuing an autonomous life. This finding may be a function of emphasis on home exercise in this single center.

MECHANISMS INVOLVED IN THE DEVELOPMENT OF BOS

The pathogenesis of BOS is complex and involves both alloimmune and nonalloimmune mechanisms that act alone or in combination (19, 25). Alloimmune-independent factors, such as airway injury from primary graft dysfunction (PGD), allograft infections, airway ischemia, and gastroesophageal reflux (GER), likely contribute to the development of BOS via up-regulation of an inflammatory milieu that initiates/directs a alloimmune response (3). Alloimmune-dependent factors such as AR and LB are known to be associated with BOS (3).

Rejection is a recipient (host) response to a foreign antigen (i.e., the newly transplanted allograft). The presentation of alloantigen by major histocompatibility complex (MHC)/HLA molecules is integral to the process of allograft rejection. In the direct pathway, host T cells recognize intact allo-MHC molecules on the surface of the donor antigen-presenting cells (APCs). In the indirect pathway, T cells recognize processed alloantigen (donor MHC molecules shed from the graft) presented as peptides by recipient-APCs (host-APCs). Either pathway is capable of stimulating an intense inflammatory/immune response. Despite immunosuppressive therapy, the continuum of allograft injury from acute to chronic rejection, in the form of inflammation, alloimmunity, and fibroproliferation, continues to be a problem.

CYTOKINES AND BOS

Critical to airway wound repair is a delicate balance between pro- and antiinflammatory cytokines. Changes in this balance can influence allograft airway remodeling. The specific mechanisms that lead to the fibroobliteration of allograft airways during BOS may involve the interactions between Type 1, 2, and 17 cells/immune responses. The Type 1 immune response is mainly associated with cell-mediated immunity and is identified by the production of interleukin-2 (IL-2), IL-12, γ-interferon (IFN-γ), and lymphotoxin, which drives a cytotoxic T-lymphocyte (CTL) and delayed type hypersensitivity (DTH) response. The Type 2 immune response is identified by the production of IL-4, IL-5, and IL-13 and promotes mucosal, allergic, and humoral immunity. The Type 17 immune response is identified by the production of IL-17 and IL-23 and is associated with autoimmunity.

The nature of the “allo-antigen” and the pattern of cytokines released into the microenvironment are considered the most important factors dictating whether the immune response is directed toward a Type 1, 2, or 17 response. However, due to the intensity of allograft rejection, total inhibition of this injury may depend on the downregulation of all three types (1, 2, and 17) of immune responses.

Figure 2. Changes in pulmonary function over time in two representative patients with bronchiolitis obliterans syndrome (BOS). The FEV$_1$, as a percentage of the post-transplant baseline value is plotted against time after the last baseline measurement. The solid line represents a patient with gradual onset BOS. The dashed line represents a patient with rapid onset BOS.
IFN-γ is considered the prototypical Type 1 immune response cytokine and has been associated with rejection. Human studies have demonstrated that elevated expression of IFN-γ from bronchoalveolar lavage fluid (BALF) was associated with acute and refractory lung allograft rejection, both risk factors for BOS (26, 27). However, animal models of rejection have demonstrated a few unanticipated results (i.e., IFN-γ−/− recipients demonstrated accelerated acute rejection and decreased chronic transplantation coronary artery rejection) (28). Moreover, a study involving human LTRs demonstrated that low levels of IL-12 in BALF were able to predict the development of BOS (29). Collectively, these studies suggest a dichotomous role for the Type 1 immune response, in part, promoting acute rejection, yet able to limit chronic allograft rejection. Thus, it appears that not only is the type of response or cytokine profile important (Type 1, 2, or 17) but the timing of that response is also important in the development of acute and chronic rejection.

While many studies suggest that rejection is strictly a Type 1 immune response, recent evidence indicates that Type 2 responses also promote rejection, especially chronic rejection (30). IL-13 in human BALF has been associated with BOS (31, 32). Importantly, in vivo depletion of IL-13 led to a reduction in the fibroobliteration of airway allografts (31, 32). This supports the “concept” that acute rejection is, in part, a Type 1 immune response (i.e., the immune system is trying to eliminate the alloantigen) while chronic rejection is, in part, a Type 2 response (i.e., the immune system cannot eradicate the alloantigen and defaults to fibrosis, trying to “wall it off”).

PGD, infections, and alloimmune-mediated injury to the lung allograft may reveal previously unexposed self-antigens to the recipient’s immune system, potentially activating autoimmune responses. Interestingly, animal models of lung rejection have been associated with autoimmunity to a native protein, minor type V collagen [col (V)]. Col (V) is intercalated within type I collagen, a major collagen in the lung. In fact, col (V) is considered to be a sequestered antigen in the normal lung and is located in the perivascular and peribronchiolar connective tissue (i.e., the same sites of rejection activity). Importantly, PGD after LT allows for antigenic fragments of col (V) to be released locally (33). Yoshida and associates demonstrated that col (V)–reactive T cells developed within lung allografts, when adoptively transferred, cause a “rejection-like” histopathology in an isograft lung, but not in the naive lung. This phenomenon was associated with the up-regulation of IL-17 transcripts (34). Translational human studies have demonstrated that cell-mediated immunity to col (V) is associated with increased incidence and severity of BOS (35, 36). Similarly, autoimmunity to epithelial antigen K- al tubulin has been shown to potentially be involved in chronic lung allograft rejection (37).

CHEMOKINES AND BOS

Chemokines and their interaction with specific cell receptors are essential components of Type 1, 2, and 17 immune responses via recruitment of specific leukocyte subpopulations. With regard to chemokines and lung allograft rejection, RANTES/CCL5 and its interaction with CCR1 and CCR5, CXCR3/ligand interactions, CCR2/CCL2 interactions, CXCR2/ligand interactions have all been shown to be important in acute and chronic lung allograft rejection (albeit through different nonredundant mechanisms) (38–41). For example, the CXCR2/ligand biological axis was important in vascular remodeling independent of neutrophil recruitment, CXCR3/ligand biological axis was critical for T cells and natural killer (NK) cell recruitment, and CCR2/CCL2 biological axis was pivotal in recruiting mononuclear phagocytes without having any effect on lymphocytes or NK cells, all of which demonstrate distinct mechanisms by which a specific receptor/chemokine biological axis is involved in the pathogenesis of BOS (38–41).

GROWTH FACTORS AND BOS

Epithelial injury and cytokine responses acting upon epithelial cells, myofibroblasts/fibroblasts (FB), and potential precursor cells from both the donor and recipient contribute to allograft airway fibroplasia (42–44). During the fibroproliferative phase of OB, several growth factors secreted by epithelial cells, FB, and inflammatory cells (e.g., platelet-derived growth factor [PDGF], basic fibroblast growth factor [BFGF] [45], transforming growth factor-β [TGF-β], hepatic growth factor [HGF] [46], and insulin-like growth factor-1 [IGF-1] [47]) contribute to epithelial damage/inflammation and the fibrogenesis that is involved in BOS (1, 48).

HUMORAL FACTORS AND BOS

While humoral rejection is classically described in hyperacute lung allograft rejection due to preformed donor antibodies before LT, there have been descriptions of allo-specific antibodies that can develop de novo after LT, which have been associated with acute lung injury/diffuse alveolar damage (ALI/ADAD), AR, LB, BOS, and increased mortality (49–51). These HLA antibodies have been shown to damage allograft airway epithelium and endothelium, and lead to the up-regulation of cytokines that have been associated with AR and BOS (48, 52). However, histopathologic evidence of antibody-mediated rejection (AMR) has been somewhat elusive. Markers for complement proteins C1q, C3, C4d, C5b-9, and immunoglobulins IgG and IgM, which can be useful with other organs, are generally not helpful in lung transplantation (53, 54). Future research on the role of B/plasma cells in the development of AMR and BOS is desperately needed.

ENDOTHELIUM/ANGIOGENESIS, EPITHELIAL INJURY, AND BOS

Injury to the allograft bronchial epithelial cells and subepithelial structures is a central component that leads to excessive fibroproliferation and aberrant tissue repair in OB (3, 55). This is also supported by studies demonstrating that ciliated bronchial epithelial cells express class II HLA antigens, which are up-regulated during chronic rejection (3). Just as important is the damage to airway microvasculature that has been demonstrated in human and animal models of OB (39, 56, 57). Similarly, these allograft airways were found to have increased angiogenic activity (39). Taken together, this suggests that allograft airway microvascular injury causes local ischemia that contributes to loss of epithelial integrity and the up-regulation of proinflammatory cytokines/chemokines that recruit injurious inflammatory cells. Furthermore, local ischemia will stimulate hypoxia inducible factors (i.e., HIF-1α), which stimulates angiogenesis, a requirement to support chronic inflammation/fibroobliteration.

INNATE IMMUNITY, REGULATORY CELLS, AND BOS

Innate immunity relies on cell surface receptors that bind pathogen-associated molecular pathways (PAMPs), which are conserved for bacteria, fungi, parasites, and viruses and are called Toll-like receptors (TLRs). TLRs are present on mononuclear phagocytes, dendritic cells (DCs), polymorphonuclear leukocytes (PMNs), epithelial cells, and recently been shown to be present on T cells. TLRs upon interacting with pathogens immediately signal inflammatory events, up-regulate MHC ex-
pression/antigen presentation, and can promote adaptive immunity. With regard to lung transplantation, LTRs with heterozygosity for TLR4 Asp299Gly or the Thr399Ile polymorphisms (i.e., hypo-responsive to the pulmonary effects of the TLR4 ligand lipopolysaccharide) demonstrated a reduction in severity, frequency, and a delayed onset of AR, as well as a trend toward a lower incidence of BOS and death (58, 59).

Regulatory T cells (classically thought of as CD4+CD25+high and FOXP3+) can occur naturally or be induced. A retrospective study of LTRs demonstrated a higher proportion of CD4+CD25+ high cells in LTRs without as compared with those with BOS, suggesting that these regulatory T cells may be able to tone down an alloimmune response and prevent rejection (60).

Overall, there are multiple mechanisms involved in the development of BOS after LT (Figure 3), and only future studies will determine the hierarchy of these pathways. Only then will we be able to develop more specific strategies that can prevent/treat BOS without increasing the risk of infections and malignancies.

**RISK FACTORS FOR BOS**

Several studies cited ACR as the most important risk factor for BOS (2, 4, 5, 16, 20, 61, 62). The relationship between ACR and BOS is not intuitive, as the former involves blood vessels and the later obliterates airways. Indeed, a recent study suggested that the intensity of LB was a more important risk factor for BOS (63). Additional co-factors for BOS include cytomegalovirus (CMV) (62) and non-CMV respiratory infections (64, 65), injury to the allograft or airways (20, 66), PGD (67–69), HLA mismatching (19), and organizing pneumonia (70).

**Alloimmune-dependent Risk Factors and BOS**

**Acute cellular rejection (ACR).** Numerous studies implicated ACR as the dominant risk factor for BOS (2, 4, 20, 61, 62). Multiple (71) or severe episodes of ACR (5) or late-onset ACR (72, 73) increase the risk of BOS. Higher acute perivascular rejection scores (A) and higher B airway scores were risk factors for development of BOS (61). Importantly, even single episodes

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**Figure 3.** Overall pathogenesis of BOS. (A) Allogeneic injury causes the release of cytokines (e.g., IL-13 and IL-17) and growth factors (e.g., PDGF, HGF, TGF-β, IGF-1) from the allograft airway. (B) These cytokines and growth factors cause fibroblast proliferation/matrix deposition. (C) Both the cytokines and growth factors drive the expression of CC chemokines (CCL2 and CCL5) and CXC chemokines (CXCL9, CXCL10, CXCL11), which recruit mononuclear cells to areas of allo-injury. (D) These mononuclear cells express specific chemokine receptors and are phenotypically distinct (e.g., Type 1 immune response mononuclear cells express CCR5 and CXCR3, while Type 2 immune response mononuclear cells express CCR2). (E) Persistent expression of cytokines/growth factors during allogeneic injury is, in part, due to the recruitment of these distinct populations of mononuclear cell by virtue of their chemokine receptors. These mononuclear cells in turn release cytokines/growth factors creating a positive feedback loop (increased cytokines/growth factors → chemokines → recruiting more mononuclear cells expressing specific chemokine receptors and expressing more cytokines and growth factors). (F) ELR+ CXC chemokines (e.g., CXCL8) released during allo-injury causes angiogenesis/vascular remodeling, a requirement to support chronic inflammation/fibroplasia. (G) Ultimately, there is fibroobliteration/BOS.
of ACR (all grades) increase the risk for BOS (9, 71, 74, 75). ACR (≥ grade 1) develops in 77 to 92% of LTRs (74, 76); ≥ grade 2 ACR develops in over 50% (71, 77). The incidence of ACR episodes ≥ A2 decreases with increasing time from transplantation (e.g., 43% at 2 wk to only 4% at 2 yr) (74). However, many patients with ACR do not develop BOS, and some patients with BOS have never experienced ACR (19). Thus, additional factors are operative.

Lymphocytic bronchitis/bronchiolitis (LB). Lymphocytic bronchitis/bronchiolitis is likely a precursor of BOS (3, 11, 61). In a retrospective analysis of 341 LTRs, severity of LB (i.e., highest B grade) was the most significant risk factor for development of BOS (even in the absence of ACR) (63). Multivariate analysis showed that risk factors for death included BOS and LB (highest B grade). Interestingly, ACR was a risk factor for BOS in univariate analysis, confirming many other studies, but was not an independent risk factor for either BOS or death by multivariate analyses (63).

Organizing pneumonia. Organizing pneumonia (OP) among LTRs may represent a form of AR but also may be associated with infection (78, 79). In one review, 10/17 LTRs (59%) with OP eventually developed BOS (70). Other investigators noted that OP on transbronchial biopsies (TBBs) was associated with an increased risk of BOS (HR of 1.75) (16). Exudative bronchiolitis (defined by CT criteria) in LTRs was also associated with an increased risk of BOS (80).

Human leukocyte antigen (HLA) mismatches. The development of anti-HLA class I and II antibodies after LT is associated with BOS (19, 51). HLA-specific antibodies were associated with increased risk of persistent/recurrent ACR, LB, and BOS (49, 50). However, the influence of HLA mismatch on the development of BOS is controversial. Some centers noted associations between BOS and mismatches at the A locus (73, 81), two DR mismatches (82), or total mismatches at the A-, B-, or DR-loci (16, 81). Data from the ISHLT Registry found that greater than or equal to 4 HLA mismatches was associated with worse survival (17). In a large series comprising 3,549 LTRs, HLA mismatches at the HLA-A and HLA-DR loci predicted 1-year mortality; total HLA mismatches predicted mortality at 1, 3, and 5 years (83). However, the effect of each covariate was small. Mismatches at the HLA-A (but not HLA-B) locus were associated with ACR but not BOS (83). Importantly, three or more HLA mismatches were present in 95.4% of LTRs (83). Thus, strategies to provide allografts with two or fewer mismatches are not currently feasible.

NONALLOIMMUNE-DEPENDENT RISK FACTORS AND BOS

Nonalloimmune acute lung injury enhances MHC Class II expression, proinflammatory cytokines, and development of HLA class II alloantibodies and may increase allograft rejection (36). Although disparate results have been noted, risk factors for BOS in some studies have included PGD (20, 67, 84), repetitive multiple injury airway disease (85), increased donor age (84), and receipt of organs from donors who died of traumatic brain injury (86). Some investigators found that ischemic time did not increase the risk of BOS (5, 63). Most studies found that recipient age and blood group matching were not associated with OB/BOS (5). Although sex does not increase the risk of BOS (5), female sex has been associated with worse survival after LT (87) and a steeper rate of decline in FEV1% predicted after BOS onset (7). Further, sex mismatch (donor–recipient) influences BOS and other outcomes. In a single-center study of 98 LTRs, sex mismatch was associated with improved survival, whereas BOS developed earlier in male donor/female recipient pairs (88). Data from the ISHLT Registry (> 9,600 LTRs) found that 90-day mortality was higher in female donor/male recipient pairs compared with male/ male or female/female (89).

ROLE OF INFECTIONS IN BOS

Chronic low-grade infection may amplify inflammatory responses and increase the risk of BOS (90–92). CMV shares nucleic acid sequence homology with MHC class I and HLA-DR antigens (93). CMV infection results in increased epithelial cell HLA I and II expression (94) and up-regulates proinflammatory cytokines and allogeneic responses (93, 95). CMV disease is an independent risk factor for BOS in some (16, 73, 94), but not all (20, 61, 93, 96, 97) studies of LTRs (95). The disparate results may reflect different strategies to treat or prevent CMV infections (96, 98). One study found that combined treatment with CMV-IgG and ganciclovir reduced BOS in high-risk patients (99). However, in a cohort of 44 CMV-seropositive LTRs, prophylaxis with CMV-IgG alone did not affect the incidence of CMV disease, ACR, BOS, or 1-year survival (100). With the use of more aggressive preventive strategies against CMV, serious CMV infections have declined considerably among LTRs (101). Oral vagancirol or ganciclovir prophylaxis reduced the incidence of CMV infections and BOS and improved survival (102).

Lower respiratory tract infections due to community-acquired respiratory viruses (CARV) (64, 65, 103), *Chlamydia pneumoniae* (104), and human herpes virus-6 (105) may increase the risk of ACR and BOS. Treatment of infections due to CARV (106) theoretically may reduce the incidence of CMV disease, but data are limited. Lower airway colonization with *Pseudomonas aeruginosa* was associated with increased risk of BOS (107, 108), higher BOS stage (108), and less freedom from BOS days (107). The mechanism may reflect influx of neutrophils to the airways (109).

ROLE OF PGD IN BOS

PGD is an acute lung injury that results predominately from ischemia-reperfusion injury. This injury is initiated/enhanced from donor brain death, ventilator induced lung injury, aspiration events, and cold ischemia. Studies have demonstrated that LTRs with PGD grade 3 have higher short- and long-term mortality (67–69). In addition, when excluding LTRs who died in the first year after LT, those who developed PGD had a significantly worse long-term survival than those without PGD, suggesting that PGD continues to hinder survival beyond the early postoperative recovery period (69). In a recent retrospective single-center study, LTRs who developed PGD had an increased risk of BOS independent of AR, LB, and CARV infections (67). This study also demonstrated that there was a direct relationship between severity of PGD and risk of BOS. Collectively these studies suggest that nonspecific allograft injury (i.e., PGD) may predispose the allograft to BOS in the future.

GASTROESOPHAGEAL REFLUX IN BOS

Gastroesophageal reflex (GER) is common in patients with end-stage lung disease (110) and may be exacerbated after LT (19, 111). Importantly, GER can cause allograft injury (19) and appears to be a risk factor for BOS. The mechanisms by which GER induces BOS are not clear, but lung denervation, impaired cough reflex, abnormal mucociliary clearance, and delayed gastric emptying may be involved (5). The presence of bile acids and pepsin in BAL samples from LTRs suggests that aspiration may elicit airway injury (110, 112). Aggressive treatment of GER is recommended to reduce the chance for airway injury/inflamm-
mation. However, proton pump inhibitors reduced acid reflux but did not affect nonacid reflux (e.g., pepsin or bile) (112). Pepsin or bile in BALF may be risk factors for BOS (112). In retrospective studies, early fundoplication (within 3 mo of LT) was associated with greater freedom from BOS (113, 114) and improved lung function (115) in LTRs with GER.

STRATEGIES TO DETECT EARLY ACR AND PREVENT BOS

Bronchoscopy with Transbronchial Lung Biopsies

Transbronchial lung biopsies (TBBs) have an important role in assessing the presence or absence of ACR and infections (76, 116, 117). Because of the patchy nature of OB, and small sample size, the yield of TBBs in detecting OB is low (< 20%). The role of routine surveillance bronchoscopies to detect asymptomatic ACR is controversial (118), but even minimal (A1) ACR studies seem to be a risk factor for BOS (71, 75, 119). Identifying histologic evidence for ACR triggers intensification of immunosuppressive therapy to reverse ACR and prevent late allograft damage (76). Hopkins and colleagues reviewed 1,235 TBBs performed in 230 LTRs over 5 years (76). Histologic features of ACR, LB, or infection were found in 18.9% of surveillance and 86.4% of clinically indicated TBBs (76). The yield of surveillance TBB between 4 and 12 months after LT was only 6.1% for ACR and 1.1% for CMV infections (76). In a subsequent study by that group, A1 lesions were detected in 128/284 (69%) LTRs, but were associated with symptoms in only 9% (71). Among surveillance TBBs, A1 progressed to higher grade (A2 or A3) in 25% of patients; LB developed in 15.7% (71). Among the clinically indicated TBBs showing A1 lesions, only 8% progressed to higher A grade within 3 months. This likely reflects the fact that all asymptomatic patients with A1 were treated with oral pulse steroids. Importantly, BOS developed earlier among patients exhibiting multiple episodes of A1 (71). Another retrospective study of 228 adult LTRs found that A1 rejection was a risk factor for BOS stage 1, 2, and 3 by both univariate and multivariate analysis (75). Interestingly, a small subgroup analysis showed that treated A1 rejection (n = 14) was associated with a reduction in the development of BOS stage 1 as compared with untreated A1 (n = 34), yet this treatment effect was not found for BOS stage 2 or 3 (75). In another study by these investigators, a single episode of A1, similar to a single episode of A2 rejection, was found to be a risk factor for BOS but not death (119). Two early studies followed 41 untreated asymptomatic patients with ACR (A1 or A2) (120, 121). Histologic grade or pulmonary function worsened in 19 (48%). These various studies support treating even asymptomatic A1 lesions with augmented immunosuppression; however, the benefit of this practice requires confirmation in randomized trials.

TBBs are often repeated 3 to 5 weeks after histologic evidence of ACR to assure resolution of the inflammatory process. However, this practice is controversial. Abouyoun and coworkers analyzed 173 follow-up TBBs performed in 99 patients with A2 ACR over a 5-year period (116). Despite aggressive treatment for rejection, A2 lesions persisted in 45 of 173 (26%). New B2 lesions were seen in 11 of 45 (24%) with persistent A2 rejection. Overall survival and freedom from BOS were similar in patients with or without persistent inflammation on repeat TBB.

BAL Findings

BAL neutrophilia is characteristic of BOS (109, 122, 123). However, BAL cell profiles evolve over time after LT. During the first 3 months after LT, elevated neutrophils are common, likely representing the cellular response to ischemia-reperfusion allograft injury (118). With increasing time after LT, the CD4/CD8 falls (118). Lymphocytic alveolitis with a decreased CD4/CD8 ratio is suggestive of ACR, viral pneumonia, or BOS. A combined lymphocytosis and neutrophilia without evidence of infection suggests BOS (118). In BOS, elevated BAL neutrophils, myeloperoxidase (MPO), ECP (eosinophilic cation protein), and IL-8 have been noted (124, 125). Overall BAL cell counts, cytokine levels, and other protein alterations have not been found to be sensitive or specific enough to be used to diagnose or predict BOS. Future studies are encouraged for furthering our understating of pathogenesis of this unresponsive syndrome.

High-resolution Computed Tomographic Scans

High-resolution computed tomographic (HRCT) scans may reveal myriad abnormalities with BOS, including hyperlucency (air-trapping), a mosaic pattern of attenuation, thickening of septal lines, bronchiectasis, or tree-in-bud (126). Expiratory CT may reveal air-trapping that is not evident on inspiratory scans (126, 127). The extent of air-trapping may correlate with BOS severity (127). Notably, the HRCT is complimentary to bronchoscopy to help differentiate other causes of a declining FEV1 such as anastomotic abnormalities, infection/rejection, and recurrence or progression of native disease as opposed to BOS.

Other Diagnostic Studies for BOS

Several surrogate markers to detect or predict development of BOS are presently being studied and validated. For instance, the slope of the alveolar plateau for helium (SHE) during a single breath washout (reflecting an increase in the heterogeneity of ventilation distribution in peripheral Airways) predicted the development of BOS (8, 123, 128). Similarly, exhaled nitric oxide (ENO) and exhaled carbon monoxide (ECO), both of which indirectly reflect increased airway inflammation, also seems to be a reasonable method to predict BOS. A recent study using all three (SHE, ENO, and ECO), measures in a longitudinal study involving 65 LTRs demonstrated operating characteristics for BOS-O-p and stage 1 with a sensitivity (86, 94%), specificity (61, 61%), PPV (44, 42%), and NPV (93, 98%) (129). While these tests are noninvasive and can be repeated multiple times, this combination is expensive, logistically difficult, and not widely available. Other potential measures include: exhaled breath condensate (130), methacholine challenge (131), induced sputum (132), inflammatory markers in BAL fluid (109), and hyperpolarized 3He magnetic resonance imaging (133). However, the predictive value of these techniques in large, longitudinal, prospective studies has not been established.

TREATMENT OF BOS

Prevention and treatment of BOS remains disappointing (19, 25). Given the link between ACR and BOS, augmentation of immunosuppression or changing immunosuppressive medications within therapeutic classes has been the mainstay of therapy for refractory ACR and BOS (3, 19, 25). However, this approach is of unproven value (25). In nonrandomized studies, anecdotal responses were cited with diverse strategies (e.g., switching from azathioprine [AZA] to mycophenolate mofetil [MMF]; conversion from cyclosporine [CsA] to tacrolimus [TRL]; addition or substitution of sirolimus for calcineurin inhibitor [CNI]; methotrexate; cyclophosphamide; inhaled cyclosporine; inhaled corticosteroids; polyclonal and monoclonal cytolytic agents [e.g., OKT3 antibody, antilymphocyte globulin [ALG], antilymphocyte globulin [ATG]]; IL-2 receptor antagonists [e.g., daclizumab, basiliximab]; total lymphoid irradiation; extracorporeal photopheresis) (5, 25, 134). However, the benefit of these various therapeutic modalities is not convincing. Interpretation of pub-
lished studies is clouded by small sample sizes, heterogeneous patient populations, concomitant use of other immunosuppressive or immunomodulatory agents, and lack of suitable controls (25). In several 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. Investigators at the University of Michigan noted that the greatest rate of decline in FEV1 after the onset of BOS occurs within the first 6 months, with the slope of decline much less thereafter (7).

**Antimetabolites**

Rates of ACR were lower with immunosuppressive regimens employing MMF compared with AZA in retrospective studies (25), but in a prospective randomized, open-label trial involving 22 sites found similar rates of ACR, BOS, and survival at 3 years with these agents (135). Anecdotal reports cited slower rates of decline in FEV1 after conversion to MMF from AZA as rescue therapy for ACR or treatment of BOS (136, 137). However, objective improvement was not observed. We believe MMF as compared with AZA is of doubtful value for patients with established BOS.

**Calcineurin Inhibitors**

Tacrolimus appears to have a slight advantage over CsA as maintenance immunosuppression for LTRs. Two open-label randomized trials compared TRL with CsA in LTRs (138, 139). In one study, the incidence of ACR trended lower in the TRL group (P = 0.07); BOS developed in 22% in the TRL group compared with 36% with CsA (P = 0.025) (138). The second prospective trial found fewer ACR episodes in the TRL cohort (139). A randomized trial of 90 adult LTRs cited a lower burden of cumulative ACR and LB as well as a trend to a greater freedom from BOS stage 0 (P = 0.1) and BOS stage 1 (P = 0.09) in the TRL cohort (140). Among LTRs with established BOS, switching from CsA to TRL was associated with less steep rates of decline in FEV1, but improvement was rarely observed (141–143). Conversely, a prospective, open-label, two-center randomized trial comparing CsA with TRL in 74 LTRs demonstrated no difference in freedom of AR, incidence of AR per 100 patient-days, or survival rates at 6 and 12 months (144). In all of the above-mentioned trials, CsA levels were measured using the predose blood concentration (C0), which does not correlate well with drug exposure as measured by area under the curve (AUC). Recent studies involving other sold organ transplantations have demonstrated that 2-hour post-dose concentrations (C2) are more effective methods of optimizing CsA immunosuppression. Future randomized multicenter studies are required to determine if there is a difference between TRL and C2 optimized CsA dosing for the prevention of AR/LB and BOS in LTRs.

**Cytolytic Therapy and IL-2 Receptor Antagonists**

Antilymphocyte and antithymocyte preparations deplete T cells via complement, cell-mediated antibody–dependent cytosis, and through opsonisation and phagocytosis. In addition, they can have prolonged effects on T cell function through non-depletive mechanisms (e.g., effects on antigen-presenting cells and B cells). One of the first studies involving cytolytic therapy used ATG induction in a single-center, randomized, prospective, partially blinded study involving 44 LTRs (145). Twenty-two received rabbit ATG (RATG; local preparation) 1.5 mg/kg/day for 3 days with conventional immunosuppression (CsA, AZA, steroids), as compared with 22 patients who received conventional immunosuppressive therapy alone. The primary endpoint was met; RATG yielded a significant reduction in AR (P < 0.0005). Freedom from BOS at 4 years was best in the IL-2RA group (NI, 67%; IL-2RA, 69%; ATG, 58) (P = 0.04). Four-year graft survival was also best in the IL-2RA group (NI, 57%; IL-2RA, 64%; ATG, 60%) (P = 0.0067). Multivariate analysis demonstrated that LTRs treated with an IL-2RA had a significant survival advantage compared with no induction therapy, independent of other risk factors (relative risk, 0.82; 95% confidence interval, 0.71–0.95). These data suggest that induction therapy increases the risk of infection, yet decreases rejection, and specifically that IL-2RA induction may improve freedom from BOS and long-term survival. However, while the large sample size allows for the detection of small differences in outcomes, this study suffered from problems inherent with all registry data, and unintended selection biases may skew results. For instance, BOS data were not available in more than 30% of recipients within this cohort. The most important message may be that prospective, multicenter studies are needed to determine if and which induction therapy helps LTRs.
Sirolimus

Sirolimus (rapamycin) and related compounds (e.g., everolimus) are structural analogs of tacrolimus and bind to the same intracellular target (3, 157). Conversion from calcineurin inhibitor (CNI) to sirolimus (Sir) (158) or the addition of Sir to CNI was associated with stabilization or improvement of lung function in some BOS patients in small nonrandomized studies (159). However, adverse effects (particularly renal insufficiency) may be significant with the combination of Sir and CNI (159). In a multicenter, randomized, double-blind trial, 223 LTRs who were free of BOS received maintenance immunosuppression (CsA and corticosteroids) with either everolimus or AZA (157). Efficacy failure (i.e., drop in FEV\textsubscript{1} > 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, efficacy failure was similar between groups. Interestingly, the incidence of treated ACR was significantly reduced at both 12 and 24 months in the everolimus cohort. While 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 (157). Importantly, approximately 25%, 40%, and 60% of the LTR could not tolerate everolimus at 1, 12, and 24 months; respectively. The increased incidence of renal dysfunction raises the possibility that everolimus may have increased CsA levels, an effect which may not have been appreciated since Cs\textsubscript{0} and not Cs\textsubscript{2} levels were used in this trial. Notably, deleterious effects of these antiproliferative agents on healing of the bronchial anastomoses after LT has also been hypothesized, but this is controversial (160).

Alemtuzumab (Campath 1H)

Alemtuzumab (Campath 1H; Genzyme, Cambridge, MA), a humanized anti-CD52 antibody that depletes CD4 lymphocytes, has been used as induction therapy for LTRs (161, 162) or treatment of refractory ACR or BOS (163). In a nonrandomized trial, induction therapy with alemtuzumab was associated with greater freedom from rejection compared with ATG or daclizumab (161). In a small cohort (n = 22) of patients with refractory ACR or BOS, treatment with alemtuzumab reduced histologic rejection scores, whereas ATG did not (163). Among 10 patients with established BOS, FEV\textsubscript{1} increased in 4 and BOS histologic grade improved, but mean FEV\textsubscript{1} did not change (163). The role of alemtuzumab in the treatment or prevention of BOS awaits randomized controlled trials.

Azithromycin

Macrolides display immunomodulatory effects and have been beneficial in some bronchiolar or pulmonary disorders. Azithromycin has been used, with anecdotal successes, in LTRs with BOS (164–169). In the sentinel study, five of six LTRs with BOS improved their FEV\textsubscript{1} (by a mean of 17%) (164). In a subsequent prospective study, 14 LTRs with BOS were treated with azithromycin (in addition to conventional immunosuppression) for 3 months (167). Overall, mean FEV\textsubscript{1} increased by 13% and BAL neutrophils and IL-8 levels decreased. Importantly, FEV\textsubscript{1} increased more than 10% above baseline in six patients (43%). By contrast, Shiritei and coworkers cited stable FEV\textsubscript{1} (mean duration follow-up 10 mo) among 11 LTRs with BOS treated with azithromycin in an open-label trial (9 were colonized with P. aeruginosa) (166). Although no patient improved, azithromycin may have slowed the rate of disease progression. Gottlieb and colleagues treated 81 adult LTRs that had BOS with azithromycin (250 mg thrice weekly); FEV\textsubscript{1} improved in 24/81 (30%) by 6 months; 22 had improved by 3 months (165). The disease progressed in 33 patients (40%). Responders exhibited higher pretreatment BAL neutrophilia. A rapid pretreatment decline in FEV\textsubscript{1} and the use of mammalian target of rapamycin inhibitors predicted disease progression. These data are encouraging. However, given the limited number of patients in these various studies, and the lack of placebo-controlled trials, the long-term impact of azithromycin is uncertain.

Aerosolized CsA

The topical delivery of cyclosporine to the airway in addition conventional systemic immunosuppression has potential theoretical benefits. A randomized, single-center, double-blind, placebo-controlled trial of aerosolized CsA in LTRs demonstrated potential efficacy for the prevention BOS (170). Specifically, 58 LTRs were randomized to either aerosolized CsA (n = 28) or aerosolized placebo (n = 30) for 2 years after LT. While the primary endpoint (rate of histologic AR) was not met, a post hoc analysis demonstrated both improved survival and a reduction in BOS. A future study powered for survival and BOS is required before the efficacy of this drug can be established.

Infusion of Donor Bone Marrow

Mixed chimerism (i.e., incorporation of both donor and recipients cells) may be a method to achieve tolerance to allografts. In animal models, infusion of donor bone marrow (BM), simultaneous with or before LT, achieved mixed chimerism and tolerance. Furthermore, mixed chimerism of bronchial and alveolar epithelial cells was noted in explanted lungs from human LTRs (171), suggesting that recipient-derived cells (possibly multipotent BM precursors) contribute to pulmonary regeneration (43). In one study in humans, 26 LTRs receiving infusion of donor BM in combination with LT were compared with 13 patients receiving LT alone (172). Among patients surviving more than 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.

Retransplantation

Retransplantation has been performed for LTRs with BOS, with lower survival rates than initial transplants (173, 174). The incidence of BOS was higher with retransplantation compared with initial transplant procedures in some (174), but not all (175) studies. In light of limited availability of donor lungs, the role of retransplantation remains controversial.

CONCLUSIONS

Lung transplantation is a therapeutic option for endstage lung diseases. Unfortunately, due to post-transplant complications including BOS, it is only a treatment and not a cure. BOS is a chronic inflammatory condition that is initiated by various combinations of alloimmune, nonalloimmune (PGD, infections, GER, etc.), and possibly autoimmune injuries to the allograft airway. Dysregulated attempts at injury repair lead to a cascade of events that ultimately results in airway fibroobliteration and BOS. While nonspecific immunosuppression seems to allow lung allograft accommodation for some, most LTRs experience various degrees of late allograft dysfunction in the form of BOS. Unfortunately, there is currently no proven therapy for the prevention or treatment of BOS. Future advances in the understanding of acute lung injury/PGD, infection, GER, AR, and LB will lead to novel targets of the alloimmune response that may
lead to a decreased incidence of BOS and improved survival among LTRs.

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References


