Lymphocytic Airway Inflammation in Lung Allografts

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Lung transplant remains a key therapeutic option for patients with end stage lung disease but short- and long-term survival lag other solid organ transplants. Early ischemia-reperfusion injury in the form of primary graft dysfunction (PGD) and acute cellular rejection are risk factors for chronic lung allograft dysfunction (CLAD), a syndrome of airway and parenchymal fibrosis that is the major barrier to long term survival. An increasing body of research suggests lymphocytic airway inflammation plays a significant role in these important clinical syndromes. Cytotoxic T cells are observed in airway rejection, and transcriptional analysis of airways reveal common cytotoxic gene patterns across solid organ transplant rejection. Natural killer (NK) cells have also been implicated in the early allograft damage response to PGD, acute rejection, cytomegalovirus, and CLAD. This review will examine the roles of lymphocytic airway inflammation across the lifespan of the allograft, including: 1) The contribution of innate lymphocytes to PGD and the impact of PGD on the adaptive immune response. 2) Acute cellular rejection pathologies and the limitations in identifying airway inflammation by transbronchial biopsy. 3) Potentiators of airway inflammation and heterologous immunity, such as respiratory infections, aspiration, and the airway microbiome. 4) Airway contributions to CLAD pathogenesis, including epithelial to mesenchymal transition (EMT), club cell loss, and the evolution from constrictive bronchiolitis to parenchymal fibrosis. 5) Protective mechanisms of fibrosis involving regulatory T cells. In summary, this review will examine our current understanding of the complex interplay between the transplanted airway epithelium, lymphocytic airway infiltration, and rejection pathologies.

Keywords: lung allograft, lung allograft immunity, lung allograft inflammation, NK cell, T cell, inflammation, lymphocyte

INTRODUCTION

Since the first successful series of heart-lung and lung transplants in the 1980s, obliterative bronchiolitis has been recognized as the predominant pathologic finding of chronic lung allograft rejection. Both proliferative bronchiolitis, characterized by transmural fibroproliferative tissue or Masson bodies, and constrictive bronchiolitis, characterized by concentric subepithelial fibrosis, were observed in these early allografts, typically surrounded by lymphocytes (1). Chronic lung allograft dysfunction (CLAD) is the syndrome of lung function decline in transplant recipients that is the major barrier to long term survival following lung transplant and includes both obstructive and restrictive phenotypes (2, 3). The obstructive phenotype is termed Bronchiolitis Obliterans...
 Syndrome (BOS), because the predominant decline in one-
second forced expiratory volume (FEV1) is presumed to be 
secondary to obliterative bronchiolitis pathology (3).
Restrictive allograft syndrome (RAS) is pathologically 
associated with pleuro-parenchymal fibroelastosis (2). As was 
demonstrated in this original autopsy series, the pathologic 
hallmarks of BOS and RAS were frequently observed together 
(4, 5). Although, autopsy and explant studies typically reflect 
advanced or end stage lung disease which may limit conclusions 
drawn regarding disease processes marked by significant 
evolution. Further, advanced lung disease has significant tissue 
heterogeneity, rendering temporal conclusions involving focal or 
diffuse pathology challenging.

A similar syndrome of bronchiolitis obliterans is seen 
following allogeneic, but not autologous, stem cell transplant, 
suggesting that bronchiolitis obliterans results from an immune-
mediated process. Indeed, increasing numbers of donor-
recipient major histocompatibility complex (MHC) 
mismatches have been associated with risk of CLAD (6, 7).
Even a minor histocompatibility antigen mismatch encoded by a 
single amino acid can drive obliterative airway disease, a murine 
alog of bronchiolitis obliterans, via CD8+ T cell-mediated 
alloimmune responses (8). In the absence of MHC mismatch 
between the lung and immune system, obliterative bronchiolitis 
is associated with some unusual exposures. Identified as a result 
of environmental exposures among popcorn factory workers, the 
butter flavoring butane-2,3-dione (diacetyl) covalently binds 
arginine residues in the small airways, forming hapten that 
trigger lymphocytic inflammation as a precursor to obliterative 
bronchiolitis (9–11). Together, these findings implicate 
lymphocytic immune responses in the airways as central to 
CLAD pathogenesis, as this review will explicate.

INNATE AND ADAPTIVE LYMPHOCYTES 
IN THE LUNG

Among transplanted solid organs, lung and intestine allografts 
have continual exposure to microbes and non-infectious 
environmental stimuli, necessitating mucosal-associated 
lymphoid tissue. Accordingly, lung allografts are predisposed to 
lymphocytic inflammation. The lung is notable for a diverse 
resident lymphocytic cell population at rest, and is a site for 
lymphocyte trafficking from peripheral reservoirs during acute 
j Injury (12). As such, across the various lung transplant 
inflammatory syndromes, lymphocytes can play a variety of 
roles. Where possible, this review attempts to distinguish 
disease processes where it is known that lymphocytes directly 
mediate injuries from those where there may be non-
specific recruitment.

Innate lymphoid cells (ILCs) provide a first line of 
immunologic defense and are distinct from adaptive immune 
cells, discussed further below (Table 1). ILC activation is 
dependent upon integration of signals from cytokine 
stimulation, activating and inhibitory receptors, and 
physiological cues from their microenvironment (13, 14).

There are three major ILC subsets: ILC1s defend against 
viruses and some bacteria primarily through cytokines like 
IFN-γ (interferon-gamma) and TNF-α (tumor necrosis factor-
alpha). ILC2s classically respond to parasites and play important 
roles in allergic responses with cytokines like IL-4, IL-5, and IL-
13 (18); and ILC3s play important antibacterial roles though IL-
1β, IL-22, and IL-17. ILC1s and natural killer (NK) cells have 
overlapping roles and functions. Functionally, ILC1s are largely 
tissue-resident, while NK cells more commonly circulate and 
have greater cytotoxic function (12, 19). NK cells are a major 
source of IFN-γ in the lung and comprise up to 10% of the 
resident lymphocyte populations. NK cells mediate infectious 
and sterile lung diseases and have been implicated in both 
alloraft injury and tolerance through a variety of mechanisms 
(12). NK cell function is determined by the integration of 
multiple activating and inhibiting signals from a variety of 
somatically-encoded receptors (20). As such, their role in 
early mediating versus trafficking to sites of injury depends 
upon tissue contexts. For example, the role of NK cells during 
influenza infection is contested as some studies show NK 
 depletion in experimental models leads to worse outcomes; 
whereas, other studies show no differences in experimental 
lung injury (21–23).

T cells develop in the thymus, where T cell receptor (TCR) 
genes rearrange to generate a diverse array of receptors that are 
subsequently selected for low-level binding to self-antigens. 
Recognition of near-self antigens makes T cells adept at 
recognizing virally infected cells, but also explains how auto-
and alloimmune responses develop. In fact, 5-15% of circulating 
T cells will typically react to donor alloantigen, depending on 
HLA (human leukocyte antigen) mismatching and recipient 
immune status (24, 25). T cells are further subdivided based on 
function and cellular markers into 3 major groups: CD4+ T cells, 
CD8+ T cells, and γδ T cells. Helper CD4+ T cells primarily 
secrete cytokines to drive immune response and provide co-
stimulation to drive cytokotoxic CD8+ T cell and B cell humoral 
responses (26, 27). The types of cytokines produced by helper T 
cells lends to their subcategorization into Th1, Th2, Th17, and T 
regulatory subsets. There is some debate in the literature over the 
relative contributions of helper T subtypes, but there is evidence 
supporting a role for all four (15, 28, 29). Th1, Th2, and Th17 
phenotypes are analogous to ILC1, ILC2, and ILC3 subclasses 
and are mediated by similar transcription factors, Tbet, GATA3, 
and RORγ T, respectively (30–32). Like NK cells, CD8+ T cells 
have cytotoxic properties and secrete perforin and granzymes to 
lyse virally infected or malignant cells. Within this construct of 
in innate and adaptive lymphoid cells lies a multitude of pathways 
to mediate injury, either non-specifically or in a targeted fashion. 
Following transplantation, donor antigens can be presented on 
either donor or host antigen presenting cells, resulting in direct 
or indirect antigen presentation, respectively (33).

B cells and plasma cells comprise the final major category of 
lymphoid cells and are responsible for producing antibodies. As 
with T cell maturation, B cell diversity is determined by somatic 
recombination, although B cells undergo a subsequent 
optimization step, called somatic hypermutation to heighten
antigen specificity. B cells are activated by APCs (antigen presenting cell) and CD4+ T cells and contribute to acute and chronic allograft dysfunction through the process of antibody mediated rejection (AMR) (16, 17). As such B cells and plasma cells mediate allograft injury by directing effector cells to tissue deemed “non-self.”

While most lymphocyte populations amplify the cascade of responses that promote inflammation, there are a collection of T cell and B cell subsets which work to dampen this process. Regulatory immune cells help to limit the amount of collateral damage from the innate and adaptive immune systems. Regulatory T cells (Tregs) impair the expansion of conventional T lymphocytes, dampen T cell function, secrete immunosuppressive cytokines, adsorb proinflammatory cytokines, potentiate tolerogenic APCs, and create an environment to facilitate expansion of other Tregs (34). Preclinical studies in mouse models of solid organ transplant, have shown long-term graft acceptance by augmentation of Treg populations in transplant recipients (35, 36). Regulatory B cells (Bregs) are proposed to play a key role in homeostasis after lung transplant (37–39). Breg features may contribute to tolerance, making it possible to reduce immunosuppression (40). Finally, while NK cells do not have a specific regulatory subset, their actions may be curtailed via inhibitory surface receptor signaling. NK cells may also perform regulatory functions such as targeting pro-inflammatory cells, in certain contexts (41).

**LYMPHOCYTIC INFLAMMATION IN THE CONTEXT OF PRIMARY GRAFT DYSFUNCTION (PGD)**

Primary graft dysfunction (PGD) is a syndrome of acute lung dysfunction in the early transplant period. Clinically, it is defined as multi-lobar chest X-ray opacifications and a decreased ratio of arterial oxygen to inspired oxygen (PaO2/FiO2) within the first 72 hours post-transplant. PGD is graded from absent (grade 0) to severe (grade 3). Severe PGD accounts for 30% of mortality in the first 30 days after transplant, 50% of the mortality within the first year of transplant and has been associated with lower baseline lung function and risk of CLAD (42, 43). PGD is the clinical manifestation of the pathologic process of ischemia-reperfusion injury (IRI) (44). Accordingly, PGD risk is dependent on the severity of ischemic injury, including warm and cold ischemic time. Allograft ischemia is further potentiated by chronic hypoperfusion, as bronchial arteries are not typically re-anastomosed during transplant. Advancements in surgical technique and allograft handling have reduced rates and severity of ischemia through limited use of cardiopulmonary bypass, limiting intra-operative blood transfusions, and limiting fraction of inspired oxygen intraoperatively (45–49). PGD risk is also driven by non-ischemic mediators of graft injury, including recipient BMI, donor tobacco use, and operative transfusions as stated above (47). Such factors may contribute to PGD by potentiating inflammation.

IRI is primarily mediated by the innate immune system but can be further amplified through adaptive immune responses (Figure 1). Experimental and clinical data suggest a biphasic nature to this inflammatory process. The early phase of IRI is marked by oxidative stress, epithelial and endothelial dysfunction leading to further injury. Airway epithelial cells release chemokines and damage-associated molecular patterns (DAMPs) (50, 51), while endothelial cells upregulate adhesion markers (50, 52). Within murine models, oxidative stress measured via isoprostanes, was increased after IRI and could be mitigated by administration of azithromycin (53). These signals recruit and activate innate immune cells, including neutrophils and macrophages, and drive antigen presentation (54, 55). Accordingly, macrophage depletion is associated with reduced lung injury in murine models of PGD (56, 57). IL-17 and DAMPs promote neutrophil migration to the interstitial space. Neutrophils can amplify IRI through neutrophil extracellular traps (58, 59). CD1d-restricted NKT cells (natural killer T cell) have been shown to secrete IFN-γ and help recruitment of neutrophils to the site of injury, suggesting innate immune cells may play an important role as a major source of IFN-γ in the lung (60).

Innate and adaptive lymphocytes play a key role in bridging early and late IRI. In both mouse models of IRI and in human lung transplant recipients following PGD, NK cells are observed in and around airways (61). By contrast, in lung allograft biopsies taken peripherally (excluding airways) before implantation and immediately after reperfusion NK cell populations are decreased (62). This would suggest the airways as central sites of NK-cell mediated IRI. The NKG2D receptor on NK cells recognizes stress molecules that are absent or lowly expressed at baseline but rapidly increased in response to a variety of injurious stimuli (63). In mouse models of IRI, NKG2D receptor stress ligands were shown to be increased on pulmonary endothelial and epithelial cells (61). Further, blockade of the NKG2D receptor or genetic deletion of the receptor on NK cells alone, was enough to abrogate pulmonary injury in these mouse models. Although, it should be repeated that NK cells predominantly influence the
early phase of IRI, with other cell populations becoming more important as the initial wave of injury subsides. Consequently, renal models of IRI also show a similar role for NK cells in mediating renal tubule epithelial cell injury. This suggests that NK cells, from the moment of reperfusion, may be critical in translating epithelial cell stress during IRI to allograft damage.

These early reperfusion responses of NK cells may potentiate long-term outcomes by killing graft APCs. NK cell activity against APCs occurs in the setting of licensing mismatch (61). During NK cell development, NK cells express inhibitory receptors to host MHCI molecules to avoid self-cytotoxicity. During transplant with a mismatch in donor and recipient MHCI, this inhibitory signal is absent which releases NK cells for activation. For example, NK cells from an HLA-Bw4 positive recipient are licensed to Bw4 antigen and will kill APC lacking Bw4 antigens. This phenomenon plays a critical role in allogenic stem cell transplant, where graft versus host activity can prevent leukemia relapse (64). In a mouse skin transplant model of NK licensing mismatch, graft-derived APCs were largely destroyed by donor NK cells and skin allograft survival was improved via reduced antigen presentation to recipient lymphocytes (41, 65). A similar phenomenon has been observed in mouse lung transplant models, where NK cells could improve tolerance of an orthotopic lung allograft in a perforin dependent manner and in association with dendritic cell depletion (66). While this is predominantly animal model evidence there is some data pointing to HLA Bw4 mismatching that potentiates NK cell host-versus-graft activity has been linked to improved outcomes in two cohorts of lung transplant recipients (41).

Conventional lymphocytes are also implicated in driving the lung injury of IRI (67). IRI may potentiate HLA- or neo-antigen presentation and subsequent alloimmune responses (68, 69). While there is not a prominent influx of CD4+ T cells into the allograft during experimental IRI, depletion of CD4+ T cells attenuates injury. This suggests that CD4+ T cells have other roles than direct injury, such as recruitment of effector cells (70). Although, this also points towards CD4+ T cells being complimentary to other underlying disease processes. Within severe combined immunodeficient (SCID) mice a documented lack of lymphocytes caused decreased neutrophil invasion into ischemic lungs (71). A deeper look into this process shows that lymphocyte attraction of neutrophils occurs as early as during warm ischemia time (72, 73). Finally, IRI may also amplify anti-donor anti-MHC and anti-autoantigen antibody production (61).

**ACUTE CELLULAR REJECTION PATHOLOGIES AND THE SIGNIFICANCE OF AIRWAY INFLAMMATION**

Acute lung allograft rejection is mediated via two primary pathologies: acute cellular rejection and antibody mediated rejection. Some degree of acute cellular rejection (ACR) occurs
in up to 30% of all lung transplants within the first post-operative year (74). ACR is predominantly a T cell mediated process. Recipient-derived effector memory T cells infiltrate the allograft traversing vascular endothelium, proliferate and migrate to the airways, where they can persist as resident memory cells (75). The diagnosis of ACR is currently confirmed with transbronchial biopsies and quantified based on standardized histopathologic patterns (76, 77). Risk factors for ACR include the degree of human leukocyte antigen mismatching and genetically determined differences within the innate and adaptive immunologic responses of the recipient (78–80). A-grade rejection refers to a mononuclear perivascular infiltrate. B-grade rejection refers to lymphocytic bronchitis or small airway inflammation. Lymphocytic bronchiolitis after transplant is linked to worse CLAD-free survival (81). C-grade rejection refers to obliterative bronchiolitis on transbronchial biopsy. However, this finding is neither sensitive nor specific for CLAD. D-grade rejection denotes accelerated graft atherosclerosis, which is not typically seen on transbronchial biopsies. Finally, E-grade rejection is not a part of standard ISHLT criteria but refers to lymphocytic inflammation on endobronchial (large airway) biopsies (82).

While B-grade rejection is generally assessed on transbronchial biopsies, similar criteria can be used to grade airway inflammation on large airway endobronchial biopsies. In a single center study, diagnosis of E-grade rejection within the first year after transplant was associated with a subsequent 1.8-fold increased risk of CLAD or death. Interestingly, gene expression profiling of A-, B-, and E-grade rejection pathologies identified signatures of allograft rejection that are shared across solid organ transplant, suggesting that these histopathologic findings may share a common pathobiology (82).

Much of the effect seen in E-grade rejection was attributable to high-grade lymphocytic bronchitis (83). The presence of lymphocytic inflammation on transbronchial or endobronchial biopsies has been termed Lymphocytic Airway Disease (LAD). In a separate study, LAD was associated with a 1.6-fold increased risk of CLAD or death. Interestingly, this association was limited to the cohort not taking azithromycin for CLAD prophylaxis (84). The use of azithromycin has been suggested to improve lung function after development of BOS as well as improve overall survival, when used as rescue therapy (85–87). There is evidence in animal models that azithromycin may be linked to reduced production of IL-17 from Th17 cells (88). At our center, we observed a decreased incidence of lymphocytic bronchitis since the introduction of azithromycin for CLAD prophylaxis (89). However, data are mixed regarding the effectiveness of azithromycin on improving CLAD-free survival or overall survival when used prophylactically (90–92). Additionally, the mechanism whereby azithromycin reduces airway inflammation remains unclear. However, there is some evidence supporting multiple pathways via: the reduction in free radicals, suppression of vascular endothelial growth factor's (VEGF) effects on angiogenesis, and the reduction of gastroesophageal reflux owning to azithromycin's gut motility effects (53, 93, 94).

Young age is also associated with a higher rate of acute rejection within the first year after transplantation, perhaps owing to a stronger immune response or exposure to a diverse antigens as recipients age lends to less immunogenic responses (95). ACR in the pulmonary allograft is a serious complication that is both an acute cause of graft-dysfunction and inflammation-related morbidity, but also a major risk factor for the development of CLAD (96). Acute rejection contributes to some low risk of mortality, particularly in the first year after lung transplantation, representing approximately 3.3% of all deaths within the first 30 days (95).

Antibody-mediated rejection is rarer in the context of lung transplantation and occurs when de novo or pre-formed antibodies against donor antigens trigger cell injury via two primary pathways. In the classic pathway, complement-binding antibodies activate the complement cascade resulting in membrane attack complex formation and direct target cell death. However, injury may also occur when antibodies bound to target are non-specifically recognized by cells carrying Fc-receptors leading to a process termed antibody-dependent cell mediated cytotoxicity (ADCC). Irrespective of mechanism, the increased frequency of de novo donor-specific antibodies (DSA) is associated with increased risk of CLAD (97, 98). While antibodies against donor antigens are common and associated with CLAD, definitive acute AMR occurs in fewer than 5% of all lung transplant recipients (99, 100). The development of DSA depends on T follicular helper cell interactions with B cells, including CD28-dependent costimulation (101). Thus, DSA may be a marker for alloimmune activation as much as biological mediator. Neutrophils, macrophages, and NK cells have been implicated in ADCC. Though, NK cells are thought to be the primary effector cell in human ADCC as their Fc receptor, CD16, is activating-only. In contrast, CD32 and CD64 lead to a mix of activating and inhibiting signals. In support of this mechanism, CD16 polymorphisms that enhance ADCC are associated with increased CLAD risk (102, 103). Thus, the roles of lymphocytes and airway inflammation in AMR require further investigation.

There are two pathways of allorecognition implicated within ACR, the direct and indirect pathways. In the direct pathway, donor APCs migrate to secondary lymphoid tissue and present alloantigen directly to recipient T cells. In the indirect pathway, recipient APCs present alloantigen derived from dying donor APCs to T cells, either in the secondary lymphoid organs or in the allograft itself (104). ACR is suspected to reflect the direct pathway (105), and ACR is associated with increased in donor-specific CD8+, conventional CD4+, and regulatory T cell responses in the peripheral blood (24). Within other solid organ transplant models, recipients one year post-transplantation may demonstrate hypo-responsiveness to alloantigen via the direct pathway (105–107). This type of partial tolerance to donor MHC is inconsistently observed following lung transplantation and may depend on conventional or regulatory T cell immune senescence (108). Conversely, one year post-transplantation, recipients show...
hyper-responsiveness towards alloantigen via the indirect pathway, where “primed” T cells have been identified on bronchoalveolar lavage (BAL) (106, 107). Thus, repeated rejection could lead to CLAD via either pathway.

ACR has important limitations as a predictor of CLAD development. While both A- and B-grade rejection have been linked to CLAD (81), the association between A1-grade ACR and CLAD risk is inconsistent (83, 109, 110). This perhaps points to a common theme among several studies that although in the acute setting lymphocytic inflammation is a major contributor of injury long term outcomes likely are underpinned by a multitude of inflammatory mediators and effects.

ACR is typically heterogenous and sometimes a symptomatically silent process. There is poor interobserver reliability for ACR grading across sites, with a Cohen’s kappa value of 0.18 to 0.48 for A-grade rejection and 0.04 to 0.47 for B-grade (111, 112). Inadequate tissue sampling is an issue for both grades, but insufficient airway tissue for confident assessment of B-grade rejection has been reported in up to two-thirds of transbronchial biopsies (113). During incipient CLAD, with active decline in FEV1, there are no reliable histopathologic correlates on transbronchial biopsy (113). ACR diagnosis can depend upon institutional surveillance and biopsy protocols. Multiple studies have identified gene expression or BAL cell counts or cytology as better predictors of CLAD than ACR itself (114–117). For example, a gene signature of lymphocytic bronchitis assessed in small airway cytologic brushings identified cases of FEV1 decline that would go on to death or retransplant in the next two years, even when transbronchial biopsies showed no evidence of rejection (113). These inconsistencies suggest that these sampling and interpretation issues may be under appreciated on transbronchial biopsy and have led to an underappreciation of the importance of airway inflammation leading to CLAD. Gene expression-based diagnostics using BAL or airway brushes would sample a larger proportion of small airway tissue, may facilitate detection of airway inflammation, and could guide potential therapies to reduce CLAD progression (29, 115).

**POTENTIATORS OF LYMPHOCYTIC AIRWAY INFLAMMATION AND HETEROLOGOUS IMMUNITY**

Airway inflammation may be challenging to quantify but can yield insights into alloimmune responses and the risk for progression to CLAD. However, there are multiple drivers of airway inflammation outside of alloimmune responses that are relevant to long term lung transplant outcomes including air pollution, infections, and aspiration of gastric acid (Figure 2) (118).

Lung transplant recipient exposure to air pollution, as quantified by the concentration of particulate matter less than 10 micrometers in diameter (PM10), is associated with increased risk of airway inflammation on biopsy and in BAL in the 2–3 days following exposure (84). In a study including 13 centers in Europe, PM10 and proximity to roads were associated with worse CLAD-free survival (119). Interestingly, azithromycin appeared to mitigate this effect.

Infections may stimulate alloimmune responses and precipitate CLAD development directly and through increased ACR (120). Bacterial infections like Pseudomonas, as well as infections from fungi like Aspergillus may affect CLAD risk through impacts on inflammation, airway epithelial cells, and other constituents of the respiratory microbiome (121, 122). Lung transplant recipients are at particular risk for community-acquired respiratory virus (CARV) infections: respiratory syncytial virus (RSV), coronavirus, rhinovirus, influenza, and parainfluenza viruses (123). Several studies independently demonstrate that community respiratory virus infections convey an increased risk of CLAD development. When stratified between upper and lower viral respiratory tract infections there is an increased risk, almost 3-fold, for lower respiratory tract viral infections (124). Additionally, there appears to be a temporal component to the development of CLAD and onset of respiratory viral infection (RVI), where a recent infection confers a larger risk of CLAD development (125). CARV infection within the first year of transplant confers a risk to CLAD development several years thereafter (126). Early treatment of RSV infection decreased the incidence of new or progressive CLAD (127).

CARV infection may drive airway inflammation and subsequent CLAD through multiple mechanisms. In a rat model of lung transplantation, parainfluenza virus infection potentiated lymphocytic inflammation and oblitative airway disease in allogeneic lungs relative to syngeneic or uninfected lungs (128). Viruses are potent inducers of interferons and interferon-associated chemokines can recruit cytotoxic lymphocytes to airways. Specifically, in CARV-infected lung transplant recipients, higher concentrations of chemokine C-X-C motif ligand 10 (CXCL10) and C-C motif chemokine ligand 11 (CCL11) predicted FEV1 decline over the next 6 months (129). CARV infection can impair regulatory T cells and expose cryptic antigens leading to de novo anti-ColV and k-alpha1 tubulin antibodies that are associated with CLAD (130). Viral infections can also lead to the release of exosomes containing self-antigens that can trigger responses to self-antigens and CLAD pathology (131). Viral infections can potentiate donor-specific immune responses through heterologous immunity. For example, CD8+ T cells specific for Human cytomegalovirus (CMV) or Epstein-Barr virus (EBV) have been shown to cross react with donor alloantigen (132). NK cells can also mediate recall immune responses to CMV through the NKG2C receptor, and elevations in NKG2C+ NK cells in the BAL is a risk factor for CLAD (63).

CMV infection, within immunocompetent hosts, establishes immunity which controls infection even if the virus is reactivated (133). However, there is evidence to suggest CMV infection may cause life-threatening complications in organ transplant recipients and has been associated with more frequent acute and chronic rejection (134–136). CMV-reactive T cells can cause tissue damage by several mechanisms: (i) direct cytotoxic effect
on CMV infected (allograft) cells, (ii) indirect bystander activation and proinflammatory milieu formation, and (iii) heterologous (cross-reactive) allorecognition (137). The cross-reactivity of CMV-reactive effector T cells to HLA class I antigens is widely accepted and have been isolated from the peripheral blood of kidney transplant recipients (132, 138, 139).

Chronic exposure to gastric acid secondary gastroesophageal reflux disease (GERD) has also been shown to be associated with the development of CLAD (140). Gastric acid may directly trigger lymphocytic airway inflammation. For example, chronic exposure to gastric fluid in rodent lung transplant models is associated with ACR, peribronchial T cell infiltration, T cell-dependent cytokine release in BAL, and increased frequencies of obliterative bronchiolitis lesions (141, 142). Conversely, anti-reflux surgery is associated with decreased BAL lymphocytes and neutrophils (143). For these reasons, many centers will perform anti-reflux surgery for lung transplant recipients with uncontrolled GERD and risk of CLAD progression (143–145).

AIRWAY INFLAMMATION IN THE PATHOGENESIS OF CLAD

CLAD pathology may reflect a final common pathway of injury responses leading to airway remodeling and fibrosis. For example, neutrophils in BAL fluid are identified as a reversible CLAD risk factor. A syndrome >15% BAL neutrophils and ≥10% decreased in FEV1 that reverses with azithromycin treatment is termed azithromycin-responsive allograft dysfunction (ARAD), previously known as neutrophilic reversible allograft dysfunction (NRAD) (146). ARAD is closely linked with lymphocytic airway inflammation and may reflect a paradoxical IL-17-dependent production of IL-8 in airway epithelial cells exposed to tacrolimus that is reversed by azithromycin (87, 147). Nonetheless, while azithromycin prophylaxis can potently reduce airway inflammation, it has been inconsistently associated with CLAD prevention (91, 148). That lung transplant recipients continue to develop CLAD despite azithromycin prophylaxis suggests multiple pathways to CLAD.

Airway inflammation can induce and activate myofibroblasts. These cells deposit the extracellular proteins like collagen and fibronectin that constitute airway fibrosis (149). Myofibroblasts may derive from airway epithelial cells via epithelial to mesenchymal transition (EMT) as well as from pericytes via pericyte-mesenchymal transition (PMT) (150–152). Pathologic EMT can be triggered by lymphocyte activation and secretion of transforming growth factor-beta (TGF-β). Mouse models with knockout of TGF-β show protection from fibrosis and EMT (153–155). Growth factors such as VEGF and TGF-β also mediate interactions between the lung endothelium and pericytes and have been independently studied as drivers of fibrosis (156, 157). Myofibroblasts can also differentiate from donor-derived resident mesenchymal stem cells in response to Th2 lymphocytic inflammation (158).
CONCLUSIONS

CLAD is primarily a disease of airway or parenchymal fibrosis resulting from alloimmune responses and lymphocytic airway inflammation is likely to be a major driver of CLAD pathology. However, lymphocytic airway inflammation can be challenging to detect using standard of care histopathologic analysis on transbronchial biopsies. Transcriptional analysis of airway brushings biopsies, or BAL fluid may allow more reliably detection of pathogenic airway inflammation (29, 113, 121). Airway lymphocytes include ILCs, T cells, B cells, and NK cells, which have distinct roles in PGD, ACR, AMR, and CLAD. Together with acute peri-vascular rejection, antibody-mediated responses, ischemia-reperfusion injury, graft infections, and gastroesophageal reflux disease, airway inflammation appears to drive an inflammatory milieu leading to airway-centric fibrosis (6, 24, 81, 116, 166–169).

At the same time there are some limitations to the current data linking airway lymphocytes to rejection pathology. The observation of lymphocytes coincident with graft pathology does not imply lymphocytes are causal. These lymphocytes could be a consequence of injury, or actively counteracting pathology, such as with regulatory T and B cells (24). While there are some causal data from rodent lung transplant models, the models have limitations and may not always match human immunobiology (170). Additionally, lymphocytes are only a component of the immune cells contributing to lung injury, as neutrophils, monocytes, and other cells also play key roles.

Targeting immune suppression to airway lymphocytes is a promising strategy to prevent or delay CLAD. For example, a trial of inhaled cyclosporin showed encouraging results, even though it was terminated early for business reasons (171). The JAK-1 inhibitor itacitinib has shown promise as inhibitor of lymphocytic mucosal inflammation and is under investigation to address inflammation in the context of early CLAD (172). The use of azithromycin as prophylaxis for CLAD or as a rescue from BOS has been implemented by several institutions, as detailed previously with varying degrees of success (85–87, 90, 92). Also, an adenosine A2A receptor antagonist is under investigation to reduce invariant NKT cell mediated inflammation in PGD (173). Other strategies to dampen airway inflammation, such as regulatory T cell adoptive therapy and/or pretransplant allograft modification during ex vivo lung perfusion, have shown preclinical promise as adjuncts to traditional immune suppression (174). A fair portion of our understanding of allograft injury comes from in vitro, ex vivo, and animal models which are extremely important in studying the biology that informs our clinical pursuits. However, it is vital to continue to test these theories within robust and safe clinical trials.

AUTHOR CONTRIBUTIONS

All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Yousen SA, Burke CM, Bellingham ME. Pathologic Pulmonary Alterations in Long-Term Human Heart-Lung Transplantation. *Hum Pathol* (1985) 16 (9):911–23. doi: 10.1016/S0046-8177(85)80130-1
2. Glanville AR, Verleden GM, Todd JL, Benden C, Calabrese F, Gottlieb J, et al. Chronic Lung Allograft Dysfunction: Definition and Update of Restrictive Allograft Syndrome–A Consensus Report From the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* (2019) 38(5):483–92. doi: 10.1016/j.healun.2019.03.008
3. Verleden GM, Glanville AR, Lease ED, Fisher AJ, Calabrese F, Corris PA, et al. Chronic Lung Allograft Dysfunction: Definition, Diagnostic Criteria, and Approaches to Treatment–A Consensus Report From the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* (2019) 38(5):493–503. doi: 10.1016/j.healun.2019.03.009
4. Burke CM, Theodore J, Dawkins KD, Yousen SA, Blank N, Bellingham ME, et al. Post-Transplant Obliterative Bronchiolitis and Other Late Lung Sequelae in Human Heart-Lung Transplantation. *Chest* (1984) 86(6):824–9. doi: 10.1378/chest.86.6.824
5. Tazelaar HD, Yousen SA. The Pathology of Combined Heart-Lung Transplantation: An Autopsy Study. *Hum Pathol* (1988) 19(12):1403–16. doi: 10.1016/S0046-8177(88)80233-8
6. Belperio JA, Weigt SS, Fishbein MC, Lynch JP 3rd. Chronic Lung Allograft Rejection: Mechanisms and Therapy. *Proc Am Thorac Soc* (2009) 6(1):108–21. doi: 10.1513/pats.200807-073GO
7. Ditschkowski M, Elmaagacli AH, Koldchoff M, Gromke T, Trenschel R, Beelen DW, Bronchiolitis Obliterans After Allogeneic Hematopoietic SCT: Further Insight–New Perspectives? *Bone Marrow Transplant* (2013) 48 (9):1224–9. doi: 10.1038/bmt.2013.17
8. Higuchi T, Maruyama T, Jaramillo A, Mohanakumar T. Induction of Obliterative Airway Disease in Murine Tracheal Allografts by CD8+ CTLs Recognizing a Single Minor Histocompatibility Antigen. *J Immunol* (2005) 174(4):1871–8. doi: 10.4049/jimmunol.174.4.1871
9. Wallace KB, Veith GD. Safe Exposure Level for Diacetyl. *Int J Occup Environ Health* (2014) 20(1):4–5. doi: 10.1179/1077352513Z.000000000100
10. Greenland JR, Jones K, Singer JP. Bronchiolitis. In: VC Broaddus, et al, editors. *Murray & Nadel’s Textbook of Respiratory Medicine*. Philadelphia, PA: Philadelphia: Elsevier (2021). 994–1004.
Responses During Superinfection. Cell Rep Med (2020) 1(4):100055. doi: 10.1016/j.xcrm.2020.100055.

Calarco DR, Wang P, Cheng T, Hoover J, Singer JP, Torgerson D, et al. Distinct Genetic Deficiency Predicts Chronic Lung Allograft Dysfunction and Death. JCI Insight (2019) 4(22):e133083. doi: 10.1172/jci.insight.133083.

Hoover J, Mintz MA, Deiter F, Aminian E, Chen J, Hays SR, et al. Rapid Molecular Detection of Airway Pathogens in Lung Transplant Recipients. Transpl Infect Dis (2021) 23(4):e3579. doi: 10.1111/tid.13579.

Khalilah AP, Hachem R.R, Chakinala MM, Schechtman KB, Patterson GA, et al. Late-Acute Renal Allograft Rejection and Symptomless Chronic Rejection of Life. J Heart Lung Transplant (2016) 35(2):213–21. doi: 10.1016/j.healun.2015.08.012.

Winter JB, Gouw AS, Groen M, Waldevuur C, Prop J. Viral Respiratory Infections Aggravate Airway Damage Cause by Chronic Rejection in Rat Lung Allografts. Transplantation (1994) 57(4):318–22. doi: 10.1097/00007890-199402150-00018.

Magunsson J, Westin J, Andersson LM, Brittain-Long R, Riise GC. The Impact of Viral Respiratory Tract Infections on Long-Term Morbidity and Mortality Following Lung Transplantation: A Retrospective Cohort Study Using a Multiplex PCR Panel. Transplantation (2013) 95(2):383–8. doi: 10.1097/TP.0b013e318271d780.

Gottlieb J, Jamora MR, Hodges T, Musk AW, Sommerwerk U, Dilling D, et al. ALN-RSV01 for Prevention of Bronchiolitis Obliterans Syndrome After Respiratory Syncytial Virus Infection in Lung Transplant Recipients. J Heart Lung Transplant (2016) 35(2):213–21. doi: 10.1016/j.healun.2015.08.012.

Fisher CE, Preiksaitis CM, Lease ED, Edelman J, Kirby KA, Leisenring WM, et al. Symptomatic Respiratory Virus Infection and Chronic Lung Allograft Dysfunction. Clin Infect Dis (2016) 62(3):313–9. doi: 10.1093/cid/ciw871.

10.1016/j.clinthy.2012.08.016.

Chiu S, Fernandez R, Subramanian V, Sun H, DeCamp MM, Kreisel D, et al. Cytomegalovirus Infection in Apparently Immunocompetent Patients: A Case-Control Study. J Leukoc Biol (2014) 95(3):405–12. doi: 10.1111/jlb.2013.03.066.

Robertson AG, Krishnan A, Ward C, Pearson JP, Small T, Corris PA, et al. Anti-Reflux Surgery in Lung Transplant Recipients: Outcomes and Effects on Quality of Life. Eur Respir J (2012) 39(3):691–7. doi: 10.1183/09031936.00061181.

Abbassi-Ghadi N, Kumar S, Cheung B, McDermott A, Knaggs A, Zacharakis E, et al. Anti-Reflux Surgery for Lung Transplant Recipients in the Presence of Impedance-Detected Duodenogastric reflux and Bronchiolitis Obliterans Syndrome: A Study of Efficacy and Safety. J Heart Lung Transplant (2013) 32(6):588–95. doi: 10.1016/j.jhlt.2013.02.009.

Verleden SE, Vandemeulem E, Rutten D, Vos R, Vaneylen A, Dupont LJ, et al. Neutrophilic Reversible Allograft Dysfunction (NRAD) and Restrictive Allograft Syndrome (RAS). Semin Respir Crit Care Med (2013) 34(3):352–60. doi: 10.1055/s-0033-1348463.

Vanaudenarde BM, Wuys WA, Geudens N, Dupont LJ, Schoofs K, Smeets S, et al. Macrolides Inhibit IL17-Induced IL8 and 8-Isoprostane Release From Human Airway Smooth Muscle Cells. J Immunol (2017) 209(7):71–82. doi: 10.1182/jimmunol.1505259.

Gunasekaran M, Bansal S, Ravichandran D, Sharma M, Perincheri S, Rodriguez F, et al. Viral Respiratory Infection in Lung Transplantation induces Exosomes That Trigger Chronic Rejection. J Heart Lung Transplant (2020) 39(4):379–88. doi: 10.1016/j.healun.2019.12.009.

Heutink KM, Yong SL, Tonneijck L, Heuvel den van H, Weerd van der NC, Pant der van KA, et al. Virus-Specific CD8(+) T Cells Cross-React to Donor-Allotriogene Are Transiently Present in the Circulation of Kidney Transplant Recipients Infected With CMV and/or EBV. Am J Transplant (2016) 16(5):1480–91. doi: 10.1111/ajt.13618.

Rafailidis PI, Mourtzoukou EG, Varbobitis IC, Falagas ME. Severe Cytomegalovirus Infection in Apparently Immunocompetent Patients: A Systematic Review. Virol J (2008) 5:47. doi: 10.1186/1743-422X-5-47.

Klenerman P, Oxenius A. T Cell Responses to Cytomegalovirus. Nat Rev Immunol (2016) 16(6):367–77. doi: 10.1038/nri.2016.38.

Reinke P, Fietze E, Ode-Hakim S, Prosch S, Lippert J, Ewert R, Volk HD, et al. Late-Acute Renal Allograft Rejection and Symptomatic Cytomegalovirus Infection. Lancet (1994) 344(8939-8940):1737–8. doi: 10.1016/S0140-6736(94)92887-8.

Canelli F, Vento S. Infections and Solid Organ Transplant Rejection: A Cause-and-Effect Relationship? Lancet Infect Dis (2002) 2(9):539–49. doi: 10.1016/S1473-3099(02)00370-5.

Sharma S, Thomas PG. The Two Faces of Heterologous Immunity: Protection or Immunopathology. J Leukoc Biol (2014) 95(3):405–16. doi: 10.1189/jlb.0713386.

Gamadia LE, Remmerswaal EB, Surachino S, Lardy NM, Dillen Wertheim-van PM, Liev van RA, et al. Cross-Reactivity of Cytomegalovirus-Specific CD8(+) T Cells to All-Major Histocompatibility Complex Class I Molecules. Transplantation (2004) 77(12):1879–85. doi: 10.1097/01.TP.0000131158.

Stranavova L, Pelak O, Svaton M, Hruba P, Fronkova E, Slavcev A, et al. Heterologous Cytomegalovirus and Allo-Reactivity by Shared T Cell Receptor Repertoire in Kidney Transplantation. Front Immunol (2019) 10:2549. doi: 10.3389/fimmu.2019.02549.

Hathorn KE, Chan WW, Lo WK. Role of Gastroesophageal Reflux Disease in Lung Transplantation. World J Transplant (2017) 7(2):103–16. doi: 10.5500/ wjt.v7.i2.103.

Li B, Hartwig MG, Appel JZ, Bush EL, Balsara KR, Holzknecht ZE, et al. Chronic Aspiration of Gastric Fluid Induces the Development of Obliterative Bronchiolitis in Rat Lung Transplants. Am J Transplant (2008) 8(6):1614–21. doi: 10.1111/j.1600-6143.2008.02298.x.

Hartwig MG, Appel JZ, Li B, Hsieh CC, Yoon YH, Lin SS, et al. Chronic Aspiration of Gastric Fluid Accelerates Pulmonary Allograft Dysfunction in a Rat Model of Lung Transplantation. J Thorac Cardiovasc Surg (2006) 131(1):209–17. doi: 10.1016/j.jtcvs.2005.06.054.

Fischella PM, Davis CS, Lowery E, Pittman M, Gagermeier J, Love RB, et al. Pulmonary Immune Changes Early After Laparoscopic Antireflux Surgery in Lung Transplant Patients With Gastroesophageal Reflux Disease. J Surg Res (2012) 177(2):e65–73. doi: 10.1016/j.jss.2012.03.066.

10.1016/j.ccm.2010.10-001.

Walker N, Badri L, Wettlaufer S, Flint A, Sajjan U, Krebsbach PH, et al. Resident Tissue-Specific Mesenchymal Progenitor Cells Contribute to
Fibrogenesis in Human Lung Allografts. Am J Pathol (2011) 178(6):2461–9. doi: 10.1016/j.ajpath.2011.01.058

159. Boers JE, Ambergen AW, Thunnissen FB. Number and Proliferation of Clara Cells in Normal Human Airway Epithelium. Am J Respir Crit Care Med (1999) 159(5 Pt 1):1585–91. doi: 10.1164/ajrccm.159.5.9806044

160. Hiemstra PS, Bourdin A. Club Cells, CC10 and Self-Control at the Epithelial Surface. Eur Respir J (2014) 44(4):831–2. doi: 10.1183/09031936.00089214

161. Barnes PJ. Club Cells, Their Secretory Protein, and COPD. Chest (2015) 147 (6):1447–8. doi: 10.1378/chest.14-3171

162. Wong AP, Keating A, Waddell TK. Airway Regeneration: The Role of the Clara Cell Secretory Protein and the Cells That Express it. Cytotherapy (2009) 11(6):676–87. doi: 10.1016/j.jcyt.2009.03.007

163. Liu Z, Liao F, Scozzi D, Furuya Y, Pugh KN, Hachem R, et al. An Obligatory Role for Club Cells in Preventing Obliterative Bronchiolitis in Lung Transplants. JCI Insight (2019) e124732. doi: 10.1172/jci.insight.124732

164. Faust HE, Golden JA, Rajalingam R, Wang AS, Green G, Hays SR, et al. Short Lung Transplant Donor Telomere Length Is Associated With Decreased CLAD-Free Survival. Thorax (2017) 72(11):1052–4. doi: 10.1136/thoraxjnl-2016-209897

165. Naikawadi RP, Green G, Jones KD, Achtar-Zadeh N, Mieleszko JE, Arnould I, et al. Airway Epithelial Telomere Dysfunction Drives Remodeling Similar to Chronic Lung Allograft Dysfunction. Am J Respir Cell Mol Biol (2020) 63 (4):490–501. doi: 10.1165/rcmb.2019-0374OC

166. Husain AN, Siddiqui MT, Holmes EW, Chandrasekhar AJ, McCabe M, Radvany R, et al. Analysis of Risk Factors for the Development of Bronchiolitis Obliterans Syndrome. Am J Respir Crit Care Med (1999) 159 (3):829–33. doi: 10.1164/ajrccm.159.3.9806099

167. Jungraithmayr W, Ji L, Yang L, Werner W, and Korom S, Hersberger M. Increased T-Bet to GATA-3 Ratio During Acute Allograft Rejection in the Rat Lung. Transplant Proc (2009) 41(10):4316–20. doi: 10.1016/j.transproceed.2009.08.057

168. Jaramillo A, Smith CR, Maruyama T, Zhang L, Patterson GA, Mohanakumar T. Anti-HLA Class I Antibody Binding to Airway Epithelial Cells Induces Production of Fibrogenic Growth Factors and Apoptotic Cell Death: A Possible Mechanism for Bronchiolitis Obliterans Syndrome. Hum Immunol (2003) 64(5):521–9. doi: 10.1016/S0198-8859(03)00038-7

169. Estenne M, Hertz MI. Bronchiolitis Obliterans After Human Lung Transplantation. Am J Respir Crit Care Med (2002) 166(4):440–4. doi: 10.1164/rccm.200201-003PP

170. Lama VN, Belperio JA, Christie JD, El-Chemaly S, Fishbein MC, Gelman AE, et al. Models of Lung Transplant Research: A Consensus Statement From the National Heart, Lung, and Blood Institute Workshop. JCI Insight (2017) 2(9): e93121. doi: 10.1172/jci.insight.93121

171. Neurohr C, Kneidinger N, Ghiani A, Monforte V, Knoop C, Jaksh P, et al. A Randomized Controlled Trial of Liposomal Cyclosporine A for Inhalation in the Prevention of Bronchiolitis Obliterans Syndrome Following Lung Transplantation. Am J Transplant (2022) 22(1):222–9. doi: 10.1111/ajt.16858

172. Covington M, He X, Scuron M, Li J, Collins R, Juvekar A, et al. Preclinical Characterization of Itacitinib (INCB039110), A Novel Selective Inhibitor of JAK1, for the Treatment of Inflammatory Diseases. Eur J Pharmacol (2020) 885:173505. doi: 10.1016/j.ejphar.2020.173505

173. Lau CL, Beller J.P, Boys J.A, Zhao Y, Phillips J, Cosner M, et al. Adenosine A2A Receptor Agonist (Regadenoson) in Human Lung Transplantation. J Heart Lung Transplant (2020) 39(6):563–70. doi: 10.1016/j.healun.2020.02.003

174. Miyamoto E, Takahagi A, Ohsumi A, Martinu T, Hwang D, Boonstra KM, et al. Ex Vivo Delivery of Regulatory T Cells for Control of Alloimmune Priming in the Donor Lung. Eur Respir J (2022) 59(4):p.2100798.. doi: 10.1101/2021.02.07.430098

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| Term | Definition |
|------|------------|
| ACR  | acute cellular rejection |
| ADCC | antibody-dependent cell-mediated cytotoxicity |
| AMR  | antibody mediated rejection |
| APC  | antigen presenting cell |
| ARAD | Azithromycin-responsive allograft dysfunction |
| BAL  | bronchial lavage |
| BOS  | bronchiolitis obliterans syndrome |
| Breg | regulatory B cell |
| CARV | community-acquired respiratory virus |
| CCL11 | C-C motif chemokine ligand 11 |
| CD   | cluster of differentiation |
| CLAD | chronic lung allograft dysfunction |
| CMV  | human cytomegalovirus |
| CXCL10 | chemokine C-X-C motif ligand 10 |
| DAMP | damage-associated molecular pattern |
| DSA  | donor-specific antibody |
| EBV  | Epstein-Barr virus |
| EMT  | epithelial mesenchymal transition |
| FEV1 | one-second forced expiratory |
| GERD | gastroesophageal reflux disease |
| HLA  | human leukocyte antigen |
| IFN-γ | interferon-γ |
| IL   | interleukin |
| ILC  | innate lymphoid cell |
| IRI  | ischemic reperfusion injury |
| AK-1 | Janus kinase 1 |
| LAD  | lymphocytic airway disease |
| MHC  | major histocompatibility complex |
| NK   | natural killer |
| NKT  | natural killer T cell |
| NRAD | neutrophile reversible allograft dysfunction |
| PaO2/FiO2 | arterial oxygen partial pressure to fraction of inspired oxygen ratio |
| PCD  | primary graft dysfunction |
| PM10 | Particulate matter under 10 micros in size |
| RAS  | restricted allograft syndrome |
| RORγT | retinoic acid-related orphan receptor-γ |
| TGF-β | transforming growth factor-β |
| TNF-α | tumor necrosis factor-α |
| Treg | regulatory T cell |