Host–Pathogen Interactions and Chronic Lung Allograft Dysfunction

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Abstract

Lung transplantation is now considered to be a therapeutic option for patients with advanced-stage lung diseases. Unfortunately, due to post-transplant complications, both infectious and noninfectious, it is only a treatment and not a cure. Infections (e.g., bacterial, viral, and fungal) in the immunosuppressed lung transplant recipient are a common cause of mortality post transplant. Infections have more recently been explored as factors contributing to the risk of chronic lung allograft dysfunction (CLAD). Each major class of infection—(1) bacterial (*Staphylococcus aureus* and *Pseudomonas aeruginosa*); (2) viral (cytomegalovirus and community-acquired respiratory viruses); and (3) fungal (*Aspergillus*)—has been associated with the development of

CLAD. Mechanistically, the microbe seems to be interacting with the allograft cells, stimulating the induction of chemokines, which recruit recipient leukocytes to the graft. The recipient leukocyte interactions with the microbe further up-regulate chemokines, amplifying the influx of allograft-infiltrating mononuclear cells. These events can promote recipient leukocytes to interact with the allograft, triggering an alloresponse and graft dysfunction. Overall, interactions between the microbe–allograft–host immune system alters chemokine production, which, in part, plays a role in the pathobiology of CLAD and mortality due to CLAD.

Keywords: bacteria; virus; fungus; lung transplant; chronic lung allograft dysfunction

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Lung transplantation is now considered to be a therapeutic option for patients with advanced-stage lung diseases. Unfortunately, due to post-transplant complications, both infectious and noninfectious, it is only a treatment and not a cure. The various “insults” to the lung allograft can be broadly described as alloimmune dependent (e.g., acute cellular rejection and antibody-mediated rejection) and alloimmune independent (ischemia-reperfusion injury/primary graft dysfunction, gastroesophageal reflux disease, air pollution, and infectious insults such as bacterial, viral, and fungal microbes) (1). The lung transplant inherently alters lymphatics, vascular flows, and the cough reflex, which, when combined with methylprednisolone as well as other immunosuppressive therapies, increases the recipient’s risk of microbial infections. The microbes can alter the

allograft inflammatory environment and can have different outcomes depending on the allograft’s intrinsic inflammatory milieu. The interactions between the microbe–allograft–host immune system play a critical role in the pathogenesis of chronic lung allograft dysfunction (CLAD) and is thus the focus of this review.

Bacteria Interaction with an Allograft Milieu Enriches for Chemokines Increases the Risk for CLAD

Bacterial infections, including asymptomatic colonization, are common post lung transplantation (2–6). The most frequent gram-positive bacterial infection in the post–lung transplant period is *Staphylococcus aureus* (2–6). In a

single-center lung transplant cohort that included 596 patients, *S. aureus* infection was found in 18% of the lung transplant recipients (7). *Staphylococcus*-infected recipients had increased rates of acute and chronic lung allograft rejection as well as mortality throughout the first 3 postoperative years. This study raised questions regarding the molecular mechanisms through which *Staphylococcus* infection leads to increased morbidity and mortality post lung transplant. Interestingly, in nontransplant rodent models, *S. aureus* infection has been demonstrated to induce acute lung injury and mortality, in part by increasing CXC chemokines (e.g., the two N-terminal cysteines of CXC chemokines are separated by one amino acid, represented in this nomenclature with an “X”). These glutamic acid–leucine–arginine (ELR)⁺ CXC chemokines caused the recruitment

of injurious neutrophils that led to lung parenchymal damage. Using rodent lung transplant models of primary graft dysfunction as well as acute and chronic rejection, these ELR⁺ CXC chemokines were found to have a bimodal function (8, 9). Early on, they were important via their interaction with neutrophils expressing the receptor CXCR2, which contributed to the influx of allograft neutrophils, which took part in both ischemia reperfusion injury and early acute rejection (8, 9). Later on, these ELR⁺ CXC chemokines were interacting with endothelial cells expressing CXCR2, which promoted angiogenesis (8, 9). This angiogenesis was found to be critical for supporting fibroblast proliferation and transdifferentiation to myofibroblasts and eventually caused allograft airway fibro-obliteration (8, 9). Collectively, these rodent studies demonstrated that specific chemokines are involved in allograft dysfunction.

The above observations led to human lung transplant studies that evaluated *S. aureus* isolation and its interaction with ELR⁺ CXC chemokines during the pathogenesis of human CLAD (3). We used a three-state Cox semi-Markovian model to account for the complexities of human observational studies (3). The Markovian model measures the intensity of moving from one state to another and allows for the comparison of the covariate effects on each transition state or outcome (e.g., healthy to CLAD, healthy to death, or CLAD to death), without imposing censoring on competing outcomes. Furthermore, the Cox semi-Markovian modification adds the ability to take into account the time in a state. For instance, the longer a patient is in healthy lung transplant state, the less likely the lung transplant recipient is susceptible to the development of CLAD. In addition, this Cox semi-Markovian modification accounts for the entry time into a state, or, explained another way, has a built in time-dependent analysis when transitioning from one state to another (e.g., healthy to CLAD). Importantly, sensitivity analyses using the more stringent and laborious competing risk, mixed effects, and joint modeling generated similar results. In this human study, 209 lung transplant recipients were evaluated, 62 of whom

had *S. aureus* isolated, of whom 32 developed CLAD, and 15 of those with CLAD then died. From the original 62 patients with *S. aureus* isolation, 5 died without developing CLAD, transitioning directly from the lung transplant state to death (3). We evaluated available bronchoalveolar lavage fluid (BALF) for the ELR⁺ CXC chemokines CXCL1 and CXCL5 concentration on the basis of their prominent angiogenic abilities as well as their proficiency to chemoattract neutrophils, all of which should be important in the pathobiology of bacterial infections and CLAD. Acute rejection, BALF CXCL5 concentrations, and the interaction between BALF CXCL5 and *S. aureus* all impacted the intensity of the transition from lung transplant to CLAD. Conversely, the direct transition from lung transplant to death was not driven by *S. aureus* isolation. Furthermore, BALF CXCL5 concentrations increased the intensity of going from CLAD to death, whereas *S. aureus* isolation did not. Collectively, *S. aureus* isolation from the lung allograft had state-specific effects after lung transplant, and only when interacting with elevated BALF CXCL5 concentrations did it augment the risk of CLAD. This suggests that *S. aureus* isolation, in conjunction with a lung allograft inflammatory milieu concentrated with ELR⁺ CXC chemokines (and presumably neutrophils), initiates allograft injury that eventually leads to CLAD.

The most common gram-negative bacteria isolated post lung transplant is *Pseudomonas* (2, 4–6). Surprisingly, using culture-independent techniques, a negative association was found between the abundance of *Pseudomonas* species and the diagnosis of bronchiolitis obliterans syndrome (BOS) (5). However, a more in-depth analysis using culture-independent techniques on BAL specimens from almost 60 lung transplant recipients demonstrated that there are two distinct *Pseudomonas* species that can flourish in the lung allograft microbiota (2). The two distinct species included *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. Only *P. aeruginosa* was associated with poor clinical outcomes, such as symptoms consistent with acute infection, BAL

neutrophilia, increased bacterial burden, and decreased bacterial diversity (2). These studies suggest that *P. aeruginosa* has pathogenic potential post lung transplant.

Using standard clinical microbacterial culture methods, studies have shown some disagreement for the role of *P. aeruginosa* and the development of CLAD. For instance, in a single-center study involving 59 lung transplant recipients with cystic fibrosis, persistent graft colonization with *Pseudomonas* was associated with an increased risk of CLAD (10). Another retrospective study involving 92 lung transplant recipients, 26 with and 66 without cystic fibrosis, showed post-transplant airway colonization trended toward being an independent risk factor for CLAD in multivariate analysis (11). Conversely, another study involving 155 lung transplant recipients demonstrated that only “*de novo*” *Pseudomonas* (e.g., cultures positive for *Pseudomonas* only after transplantation) was associated with the development of BOS, whereas those with “persistent” culture positivity (e.g., *Pseudomonas* isolations before and after transplantation) was not (12). Collectively, this suggests that *P. aeruginosa* pathogenicity may be related to the interaction between *P. aeruginosa* with the lung allograft as well as the host immune responses.

On the basis of the above studies, *P. aeruginosa*, as well as its interaction with the ELR⁺ CXC chemokines, was evaluated using a Cox semi-Markovian analysis (4). This study involved 260 lung transplant recipients; 93 had *P. aeruginosa* isolated, of whom 50 developed CLAD, and 31 of those with CLAD then progressed to death. From the original 93 patients with *P. aeruginosa* isolated, 12 transitioned directly from the lung transplant state to death. The transition from lung transplant to CLAD was enhanced by acute rejection, BALF CXCL5 concentrations, and the interaction between *P. aeruginosa* and BALF CXCL1 concentrations. The *P. aeruginosa* effect on this transition was due to symptomatic pulmonary infection rather than colonization. *P. aeruginosa* infection also increased the intensity of movement from lung transplant to death. Transition from CLAD to death was impacted by the time spent in then

healthy post-transplant state, BALF CXCL5 concentrations, and the interactions between *P. aeruginosa* colonization and BALF concentrations of CXCL5. This study suggests that *P. aeruginosa* pneumonia is a strong risk factor for driving a patient from the healthy lung transplant state to a CLAD state. However, even *P. aeruginosa* colonization that occurs in conjunction with an allograft enriched with ELR⁺ CXC chemokines does increase the intensity of transition from CLAD to death. Overall, this study suggests that patients with elevated allograft concentrations of ELR⁺ CXC chemokines are particularly vulnerable to movement from a healthy lung transplant state to CLAD and from the CLAD to death state if the patient is further challenged with an infectious insult.

Viral Infections of the Lung Allograft Lead to Dysregulated Chemokine Expression and the Development of CLAD and Mortality due to CLAD

Pulmonary cytomegalovirus (CMV) infection (CMVI) and disease (CMVD) are associated with reduced long-term survival post lung transplantation; however, the specific biologic mechanisms remain unclear (13–15). Previous studies have demonstrated that increased expression of CC chemokines occurs during lung allograft dysfunction in humans as well as in rodent models of alloreactivity and lung allograft rejection (16–19). Therefore, the role of pulmonary CMV up-regulating the expression of multiple CC chemokines and their associations with lung allograft dysfunction was explored (14). Using a nested case-control study at a single center, the concentrations of CC chemokines were measured in BALF from lung transplant recipients with CMVI and CMVD and in healthy lung transplant control subjects. There was a trend toward increased BALF concentrations of CCL3 during pulmonary CMVI. BALF concentrations of CCL2 and CCL5 were significantly elevated during pulmonary CMVI and CMVD. Interestingly, elevated concentrations of CCL3 in BALF were protective regarding survival. Furthermore, elevated concentrations of CCL2 in BALF predicted the development of CLAD, whereas elevated levels of CCL5 in BALF predicted an increase in mortality post

lung transplant. These studies suggest that specific CC chemokines are acting differently during pulmonary CMV. CCL2 is important in recruiting mononuclear phagocytes that are phenotypically distinct by virtue of their expression of CCR2 and are profibrotic, leading to lung allograft fibroplasia (17). CCL5 expression from the lung allograft leads to the infiltration of lymphocytes and other mononuclear cells that are important in perpetuating an alloreactive response (16). CCL3 may be protective during pulmonary CMV by its ability to recruit polyfunctional CD4⁺ T cells and CD8⁺ T cells that express IL-2, tumor necrosis factor- α , and IFN- γ (16, 20–23), which allows for the rapid killing of CMV before collateral lung allograft damage occurs. Overall, altered concentrations of specific CC chemokines during pulmonary CMV were associated with future clinical outcomes and suggest a possible utility for BALF CC chemokines as biomarkers for guiding risk assessment during pulmonary CMV. Furthermore, alteration of these chemokines may be a therapeutic approach during CMV to improve clinical outcomes.

Although CMV was the first virus to demand respect for its influence on allograft dysfunction and mortality, more recent studies have shown that community-acquired respiratory viruses (CARV) can accelerate the development of lung allograft dysfunction, but the immunologic mechanisms are poorly understood (13, 15, 18, 19, 24). The chemokine receptor CXCR3 and its chemokine ligands, CXCL9, CXCL10, and CXCL11, have all been shown to play roles in the immune response to viruses as well as in the pathogenesis of CLAD (25, 26). The impact of CARV infection on CXCR3/ligand biological axis was explored in a single-center, case-control study (1:2 match cohort) (e.g., two healthy lung transplant recipient control subjects for every one CARV case) matched for duration post lung transplant (27). The BALF concentration of each CXCR3 chemokine was found to be increased during CARV infection. Among CARV-infected patients, a high BALF concentration of either CXCL10 or CXCL11 was predictive of a greater decline in FEV₁ 6 months later. Mechanistically, CXCL11 was found to be predominately expressed on endothelial cells and was important in recruiting mononuclear

cells to the allograft vasculature (1). CXCL9 and CXCL10 were more compartmentalized to the lung epithelial cells, thus pulling the mononuclear cell from the allograft vasculature to the airways and alveoli, where BOS and restrictive allograft syndromes occur (1). Moreover, the BALF CXCR3/ligand biological axis provides prognostic information, and this may have important implications for the development of novel treatment strategies to modify outcomes after CARV infection.

Fungal Colonization of the Lung Allograft Is Associated with the Development of CLAD and Mortality due to CLAD

Multiple bacterial and viral infections have been linked with the development of CLAD, as described above. However, the lung allograft airways, alveolar ducts, and alveoli are frequently colonized by *Aspergillus* species post lung transplant (28, 29). This led to the hypothesis that *Aspergillus* colonization of the lung allograft may promote the development of CLAD and may decrease survival post lung transplantation. Thus, a retrospective study involving all lung transplant recipients in a single center was performed (29). *Aspergillus* colonization was defined as a positive culture from BALF or two sputum cultures positive for the same *Aspergillus* species, in the absence of invasive pulmonary *Aspergillus*. *Aspergillus* colonization was associated with CLAD and CLAD-related mortality in Cox regression analyses. *Aspergillus* colonization preceded the development of stage 0-pCLAD by a median of 184 days and stage 1 or greater CLAD by a median of 261 days. Moreover, in a multivariate Cox regression model, *Aspergillus* colonization was a distinct risk factor for CLAD, independent of acute rejection. These data suggest a potential causative role for *Aspergillus* colonization in the development of CLAD post lung transplantation and raise the possibility that strategies aimed to prevent *Aspergillus* colonization may help delay or reduce the incidence of CLAD.

The above results were expanded on in a large two-center study involving 298 recipients at the University of California, Los Angeles and 482 recipients at Duke University Medical Center (28). *Aspergillus* species were grouped by conidia

diameter less than or equal to 3.5 μm (small conidia: *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus*, and *Aspergillus flavipes*) and greater than 3.5 μm (large conidia: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ustus*, and *Aspergillus clavatus*). Cox models were used to assess the relationship of small and large conidia colonization with clinical outcomes. Pre-CLAD colonization with small conidia *Aspergillus* species, but not large, was a risk factor for CLAD ($P = 0.002$; hazard ratio, 1.44; 95% confidence interval, 1.14–1.82), along with acute rejection, single lung transplant, and *P. aeruginosa*. Colonization with small conidia species also associated with risk of death ($P = 0.03$;

hazard ratio, 1.30; 95% confidence interval, 1.03–1.64). This demonstrates in two large independent cohorts that colonization with small conidia *Aspergillus* species increases the risk of CLAD and death. Prospective evaluation of strategies to prevent *Aspergillus* colonization of the allograft is warranted, with the goal of preventing CLAD and mortality after CLAD.

Conclusions

Overall, infection post lung transplantation influences the outcome of the lung transplant recipient regarding CLAD and mortality after CLAD. Mechanistically,

some microbes directly increase the expression of specific injurious chemokines (e.g., CC chemokines and IFN-inducible ELR^- CXC chemokines), whereas other microbes have a synergistic response when entering an allograft enriched with ELR^+ CXC chemokines that are known to eventually cause allograft dysfunction as well as lead to premature mortality. Therapies directed toward eradicating specific microbes in conjunction with altering the allografts' inflammatory milieu may lead to improved clinical outcomes post lung transplantation. ■

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

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