New Tools for Old Problems: Gastroesophageal Reflux Disease and the Lung Allograft Microbiome

Chronic lung allograft dysfunction (CLAD) has two main phenotypes, obstructive and restrictive, and is the principal cause of late morbidity and mortality after lung transplantation (LTx) (1, 2). Major risk factors include acute cellular rejection, lymphocytic bronchiolitis, and antibody-mediated rejection, but infections with pathogenic bacteria and viruses likely play a significant role. Whether, and how, gastroesophageal reflux disease (GERD) fits into this causal relationship remains unproven, but a link between aspiration of gastroesophageal contents, especially bile acids, has been proposed. The advent of high-throughput metagenomic techniques has brought the promise of understanding these complex relationships by in-depth analysis of lower respiratory tract microbial composition and load (3). The lexicon used in this somewhat niche field is slowly creeping into common parlance, but some explanations are useful. *Microbiota* describes the specific collection of microorganisms (fungi, bacteria, viruses, and archaea) in a particular environment, whereas *microbiome* refers to the microbiota and their genes—an important distinction, as the latter requires nonculture techniques that may detect new and important potential pathogens (4). The microbiome project, a strategy to understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease, was first described in 2007 (5). Three recent reviews have highlighted the rapidly developing body of knowledge regarding the microbiome in the LTx recipient with the hope of deciphering and conquering the root causes of allograft damage, particularly CLAD (6–8). Key studies analyzing bronchoalveolar lavage fluid (BALF) have demonstrated that respiratory tract microbial communities in LTx recipients differ in structure and composition from healthy subjects (9). Furthermore, a gradient of phenotypic subsets of myeloid-derived suppressor cells (MDSCs) exists within the lung, with a higher proportion of immunosuppressive MDSCs in proximal airways, compared with proinflammatory MDSCs in distal airways (10). It is important to note that patients who developed CLAD or died had differences in lung bacterial communities compared with those of patients who survived and remained CLAD-free, in part because of bacterial burden (11). However, an analysis of airway brushings in CLAD found that, although infection was associated with decreased microbial α-diversity, neither infection nor α-diversity was associated with small airway gene expression (12).

In this issue of the *Journal*, Schneeberger and colleagues (pp. 1495–1507) present an exhaustive single-center retrospective analysis of biobanked BALF data collected every 3 months over a 1-year period post-LTx in two highly selected groups with and without GERD to determine microbial composition, density, markers of inflammation, and associations with acute lung allograft dysfunction (ALAD) and CLAD, using cluster analysis of communities (13). Similar data were provided from a small subset of patients from another institution after early surgical intervention with Nissen fundoplication. The authors found that GERD was more commonly associated with a high-density, oropharyngeal taxa–enriched BALF, but with lower inflammatory cytokine levels than pathogen-dominated BALF. Patients with GERD had greater diversity of microbial density over the first year as well as delayed recovery of microbial diversity. Somewhat surprisingly, GERD status was not associated with inflammation, ALAD, or CLAD, although these were associated with "community state type" (CST).

CST describes a group of community states with similar microbial phylotype composition and abundance. Unlike CSTs in the female genital tract, where distinct communities occur, allograft CSTs represent a continuum, as the authors display in a Bray-Curtis plot (*see* Schneeberger and colleagues' Figure 1; Reference 13). Nevertheless, cluster analysis demonstrated significant differences in diversity and density between CSTs. CST1 was the highest bacterial density state and was enriched with *Prevotella* and *Veillonella*, common oropharyngeal taxa. CST2 was characterized as a low-bacterial density state with the greatest evenness and enrichment with *Streptococcus* and *Tannerella*, whereas CST3 was often dominated by a single pathogenic taxon, such as *Staphylococcus* or *Pseudomonas*, and had the highest variability in density and diversity over time. Microbial diversity was strongly associated with proinflammatory cytokines, independently of GERD status, at all measured time points. The most striking correlation was between proinflammatory cytokine levels and CST3, even at the same bacterial density. Patients with CST3 were more likely to have ALAD or develop CLAD, but this was not associated with GERD.

Importantly, transitions between CSTs occurred frequently; this aspect has not been demonstrated conclusively before, but it accords with the development of ALAD, often due to acute infection. Overall, there were distinct parallels with Das and colleagues, who described four distinct compositional states or "pneumotypes" (14). The predominant "balanced" pneumotype was characterized by a diverse bacterial community with moderate viral loads and host gene expression profiles suggesting immune tolerance. The other three pneumotypes were either microbiota depleted, or dominated by potential pathogens and were linked to increased immune activity, lower respiratory function, and increased risk of infection and rejection.

There are inherent limitations of retrospective studies of biobanked samples of BALF, especially between institutions, and it is questionable as to how much the Nissen fundoplication data add to the core message. The sole focus on the bacterial component of the microbiome, in the absence of examining other components, represents a lost opportunity, as does the parsimonious clinical analysis that hinders thorough assessment of potential confounding variables, including the impact of therapies used for GERD, ALAD,

and CLAD, especially the impact of antibiotic therapies for pathogenic taxa and antirejection therapies that might promote dysbiosis. CLAD phenotypes were not provided to allow consideration of airway versus parenchymal pathology, nor were the implications of single versus bilateral LTx explored exhaustively. Whether protective bacterial genera exist to assist in homeostasis and prevent dysbiosis is not elaborated but is a potential area of great interest in septic lung disease states and, possibly, in GERD (15). Notably, the analysis was developed using an "extreme phenotype" that excluded two-thirds of LTx recipients, so caution is warranted in interpretation. Future studies in the intermediate GERD group, to test the relationships identified here, might prove informative.

Notwithstanding these criticisms, the results provide powerful new information regarding the impact of GERD on the bacterial component of the pulmonary microbiome and demonstrate how new-generation technologies can advance our understanding of the many factors affecting the health of the lung allograft. On the basis of the aforementioned findings, perhaps the time has come to reconsider the paradigm of GERD management and focus on the pathogenic components of the pulmonary microbiome for which compelling evidence is accumulating regarding their role in inflammation, injury, and CLAD. ∎

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Lung Allograft Dysfunction: Does Aspiration Hold the Key?

Despite advances in management, the long-term survival of lung transplant recipients remains poor compared with that of other solid

organ transplant recipients. Chronic lung allograft dysfunction (CLAD), defined as a substantial decline in FEV_1 that persists over at least 3 months after transplantation, is the leading cause of death in the late posttransplant period (1). Although CLAD is a heterogeneous disorder, multiple studies have linked CLAD to various clinical factors, including the lung microbiome. CLAD has been associated with gastroesophageal reflux disease (GERD) (2, 3), lung inflammation (4), increased lung bacterial biomass (5), and changes in lung microbiome composition (5–8). Although mouse studies have shown that microaspiration of oral commensals provokes a normal protective lung immune response (9), it is unknown if this beneficial