The bacterial microbiota in inflammatory lung diseases

Gary B. Huffnagle [a,b,*], Robert P. Dickson [a]

[a] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA
[b] Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

ARTICLE INFO

ABSTRACT

Numerous lines of evidence, ranging from recent studies back to those in the 1920s, have demonstrated that the lungs are NOT bacteria-free during health. We have recently proposed that the entire respiratory tract should be considered a single ecosystem extending from the nasal and oral cavities to the alveoli, which includes gradients and niches that modulate microbiome dispersion, retention, survival and proliferation. Bacterial exposure and colonization of the lungs during health is most likely constant and transient, respectively. Host microanatomy, cell biology and innate defenses are altered during chronic lung disease, which in turn, alters the dynamics of bacterial turnover in the lungs and can lead to longer term bacterial colonization, as well as blooms of well-recognized respiratory bacterial pathogens. A few new respiratory colonizers have been identified by culture-independent methods, such as *Pseudomonas fluorescens*; however, the role of these bacteria in respiratory disease remains to be determined.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The past five years has seen a revolution in our understanding of the relationship between the microbial world and the lung environment during health and disease. The paradigm was that, during health, the lungs were sterile [1]. The evidence for this claim was based on over a century of defining the presence or absence of bacteria in a site by our ability to culture them from tissue samples. The emergence of DNA-based culture-independent detection techniques has overcome the hurdle of identifying the precise culture conditions for identification of bacteria that may exist in a tissue site, an approach that can exert highly selective growth pressures for those bacteria [2–5]. Using techniques of molecular microbial identification, no studies published have supported the claim that the lungs are sterile; bacterial DNA is always detectable in respiratory samples [4,6–12].

The upper compartments of the aerodigestive tract (mouth and nasal cavity) have been well documented to contain abundant bacteria. A number of earlier studies, some tracing back in the 1920s, demonstrated that microaspiration is common in healthy individuals [13–15], raising the idea that the lungs are continually exposed to bacteria from the upper airways. Most all of this work was performed with radiotracers applied to the nares of sleeping or sedated individuals. In one study, radioactive indium was used to study pharyngeal aspiration during sleep in 20 healthy subjects and 10 patients with depressed consciousness [14]. Almost half of the normal subjects and 70% of those with depressed consciousness aspirated during deep sleep. In those normal subjects who did not aspirate, they were noted to sleep poorly. In another study, a radioactive Tc tracer was deposited into the nasopharynx of 10 healthy sleeping subjects through a small catheter and standard lung scans were conducted immediately following final awakening. Microaspiration occurred commonly during sleep, was unrelated to sleep quality, and was variable within subjects that were studied on more than one occasion. Even more relevant to this review, they concluded that the "quantity aspirated is of an order of magnitude likely to contain bacterial organisms in physiologically significant quantities" [13]. Furthermore, as discussed by Quinn and Meyer in the 1920s, healthy human subjects, as well as other mammals, aspirate small amounts of liquids from the upper airways into the lower airways [15]. We have recently concluded studies that compared nasal, oral, lung and gastric microbiota within the same individual and have provided culture-independent microbiological support for the concept that microaspiration of upper airway microbiota is common in healthy individuals [16]. Thus, the lungs harbor bacteria even during health (the "lung microbiota"). As discussed below, the dynamics of immigration, elimination and growth of these microorganisms is markedly different from other sites of the aerodigestive tract.

2. Anatomy and microenvironments of the aerodigestive tract during health and disease

We have recently proposed the concept that the entire respiratory tract should be considered a single ecosystem extending from the nasal and oral cavities to the alveoli, which includes gradients and

niches that modulate microbiome dispersion, retention, survival and proliferation [3,17]. The surface area of the lungs is approximately 30 times that of the skin, and a recent estimate suggest that it contains a larger surface than that of the intestinal tract [18,19]. The airways and alveoli are continually exposed to the environment, with the linear distance from the nares to the alveoli being only about half a meter. Numerous studies, from many laboratories, on the lung microbiome during health and disease conceptually fit well into an ecological model based on the equilibrium model of island biogeography proposed by MacArthur and Wilson in 1963 [20]. Thus, we have proposed the "Adapted Island Model of Lung Biogeography" [3]. Using the concepts of this model, the composition of the lung microbiome can be predicted as arising from the outcome of three competing forces: immigration, elimination and the relative growth rates of its members. Using the language of this model, for a given site along the lower respiratory tract, the richness of bacterial species in a healthy lung will be the outcome of the immigration and extinction rates of the bacteria that originate from the upper airways, largely the oral cavity. During health, the lungs do not provide a hospitable environment for bacterial growth. Thus, any changes in the lung microbiota that occurs during disease must result from a change in one of these three forces.

Microbial immigration can occur via inhalation of air (which contains 10^4–10^6 bacterial cells per cubic meter even before reaching the microbe-dense upper airways [21]), microaspiration (which is ubiquitous among healthy subjects [13,14]), and direct dispersion along mucosal surfaces from the upper airways. We have recently demonstrated that there is high shared membership of bacterial species between the lung microbiome and that of the mouth [16], which is consistent with other studies [7,10]. The microbiota of healthy human lungs contrasts with that reported for air, suggesting that microaspiration contributes more to microbial immigration than does inhalation of airborne bacteria [13–15,22–25].

Microbes are cleared from the respiratory tract via mucociliary clearance, cough (frequent even among healthy subjects [26]) and innate and adaptive immunity. The distal alveoli are bathed in pulmonary surfactant, which also has bacteriostatic activity against some bacterial strains, further creating selective pressure on reproducing communities [27]. We have recently shown that the prominent members of the lung microbiome during health are cell-associated, either as extracellular adherent or intracellular organisms [28]. Less well-studied are the potential local microbial growth conditions within the respiratory tract. While the core body temperature is 37°, the air-exposed surfaces of the trachea and bronchi are significantly cooler, potentially expanding the permissive temperatures available to immigrating bacteria. Within a single lung, regional variation can be found in oxygen tension, pH, relative blood perfusion, relative alveolar ventilation, temperature, epithelial cell structure, deposition of inhaled particles and in the concentration and behavior of inflammatory cells [3,29–31], all of which may have demonstrable effects on microbial growth rates and provide growth niches. Thus, there is still much to be learned about the biotic and abiotic factors that shape the lung microbiome during health and disease, but the composition of the bacterial communities found in the lungs during health is under pressures from the constant influx, constant elimination and restricting growth conditions of the pulmonary environment. Changes in these forces during disease will change the lung microbiome, thereby contributing the pathologic processes of the lung diseases itself, which has been described as a self-reinforcing cycle of lung disease [3,17,32].

Despite the description of the lung microbiome as if it is a single type of community in healthy individuals, there is subject-to-subject variation in their microbiomes, thereby creating a range of "healthy" microbiomes [4,7–9,11,12,33]. However, shifts outside of this healthy spectrum are very evident in lung diseases, as exemplified in our recent study of the lung microbiome in lung transplant recipients [34]. When analyzed at the phylum level for relative abundance, the most common phyla consistently observed have been *Bacteroides*, *Firmicutes* and, to a lesser degree,

Proteobacteria. These are similar to those seen in concurrently collected samples from the oral cavity, but differ in relative abundance. Within these phyla, the most prominent genera observed in healthy subjects include *Prevotella*, *Veillonella*, and *Streptococcus*. While active cigarette smoking alters the microbial constitution of the upper airways [35], it has little effect on the lower airways [10]. To date, there have been no longitudinal analyses of serial respiratory specimens from healthy controls, so the relative stability or dynamic nature of the "normal" lung microbiome is unknown. Most of the longitudinal analysis of lung microbiota specimens to date has been performed on sputum specimens from patients with Cystic fibrosis (CF) [36–41]. In these studies, the microbial communities in the sputum of individual patients were relatively stable over time, even despite the development of clinical exacerbations and the administration of antibiotics [39,41]. Thus, while the spectrum of community compositions during health has initially been identified, it remains to be determined how dynamic the composition of the bacterial communities are in a single healthy lung over time.

Host microanatomy, cell biology and innate defenses are altered during chronic lung disease, which in turn, alters the dynamics of bacterial turnover in the lungs. Degenerative diseases such as emphysema and pulmonary fibrosis dramatically reduce the lumenal surface area of the lungs, even by as much as 90% [42,43]. Almost 75% of patients with advanced lung disease experience esophageal reflux and dysfunction, which increases the rate of bacterial microbial immigration by introducing an additional source of bacteria (stomach) [44,45]. Mucociliary clearance is impaired in chronic airway diseases such as CF, bronchiectasis and chronic bronchitis, thereby decreasing microbial elimination. In addition, baseline mucus production is increased, thereby providing both nutrient-rich growth environments as well as pockets of decreased oxygen concentration and increased temperature [46,47]. The importance of mucociliary clearance in controlling the airway microbiome was recently demonstrated in *Muc5b*−/− mice [48]. Muc5b was required for mucociliary clearance and its loss resulted in defects in control of bacterial colonization and in clearance of apoptotic macrophages. *Muc5b*−/− mice developed chronic airway infections by bacteria such as *Staphylococcus aureus*, *Streptococcus spp.*, and others. The single nucleotide polymorphism in *Muc5b* (rs35705950) is a risk factor for the development of interstitial pulmonary fibrosis and a recent study found that this polymorphism was independently associated with bacterial burden [49]. Inflammatory cell numbers in the alveolus and airway are increased in numbers and display higher levels of activation in individuals with chronic lung disease compared to that observed in healthy lungs, even in the absence of additional exacerbating stimuli [50,51]. Many therapies for chronic lung disease, such as supplemental oxygen, corticosteroids, and antibiotics have known or predicted effects on bacterial growth conditions, as well as affecting immigration and elimination [2,17].

Finally, during an exacerbation event in chronic respiratory diseases, all of these factors change even further. Exacerbations are periods of acute worsening of respiratory symptoms that arise abruptly, over hours to days, and generally prompt an escalation in medication therapy. During an exacerbation, hyperventilation accelerates the influx of airborne microbes and microaspiration and markedly lowers airway temperature while increased cough accelerates microbial efflux [52,53]. The number and activation state of inflammatory cells increases. Inflammatory mediators, catecholamines, increased temperature, glucose and free ATP have all been demonstrated to promote growth and virulence of selected respiratory bacterial isolates [47,54–58]. Bronchoconstriction alters regional oxygen concentration and pH while acute mucus production and vascular permeability increase local nutrient supply. Production of mucus in the airways introduces further gradients of local anoxia and hyperthermia, which selectively favor the growth of specific lung pathogens [46,47,59,60]. Thus, the host factors that control bacterial immigration, elimination and growth are altered during chronic lung disease and exacerbation stimuli can further drive this process.