

## Role of microbiota on lung homeostasis and diseases

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The lungs, as a place of gas exchange, are continuously exposed to environmental stimuli, such as allergens, microbes, and pollutants. The development of the culture-independent technique for microbiological analysis, such as 16S rRNA sequencing, has uncovered that the lungs are not sterile and, in fact, colonized by diverse communities of microbiota. The function of intestinal microbiota in modulating mucosal homeostasis and defense has been widely studied; however, the potential function of lung microbiota in regulating immunity and homeostasis has just begun. Increasing evidence indicates the relevance of microbiota to lung homeostasis and disease. In this review, we describe the distribution and composition of microbiota in the respiratory system and discuss the potential function of lung microbiota in both health and acute/chronic lung disease. In addition, we also discuss the recent understanding of the gut-lung axis, because several studies have revealed that the immunological interaction among the gut, the lung, and the microbiota was involved in this issue.

### lung, microbiota, homeostasis, lung disease

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## INTRODUCTION

The microbiota represents the complex collections of microorganisms including bacteria, viruses, parasites, and fungi colonizing the body surfaces exposed to the outside world. The diversity and composition of microbiota are determined by many factors, including host genetics, environmental factors, and host immunity (Rooks and Garrett, 2016). In health, the microbiota is beneficial for metabolism, development of the immune system and protection against pathogens, and in turn, the immune system will affect the composition of the microbiota (Alegre et al., 2014; Honda and Littman, 2016).

In recent years, the intestinal microbiota has become the subject of extensive research, and our knowledge about the components of intestinal microbiota and their potential function is rapidly growing. The human intestinal tract harbors over 100 trillion microbial cells that contribute to physiology, metabolism, nutrition and immune functions (Glenwright et al., 2017). Dysbiosis of intestinal microbiota has been linked to gastrointestinal diseases, such as inflammatory bowel disease (IBD) and obesity (Round and Mazmanian, 2009). Intriguingly, intestinal microbiota is also found to influence other organs, such as brain, liver, and lung, which led to the coining of the concept of the gut-lung axis (Bird, 2012; He et al., 2017; Young et al., 2016).

The functions of intestinal microbiota have been widely studied and recognized. However, our understanding of

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the functions of microbiota at other body sites is still in its infancy. Clearly, the skin, the upper respiratory tract, and the genitourinary tract also harbor diverse communities of microbiotas as well as site-specific immune networks that are involved in maintaining barrier function and local immune homeostasis (Belkaid and Tamoutounour, 2016; Brubaker and Wolfe, 2017; Taylor et al., 2016). The textbook told us that “the normal lungs are free from bacteria”. However, the culture-independent techniques of microbial identification indicate the presence of a lung resident microbiota in mammals (Bassis et al., 2015; Morris et al., 2013). In humans, lung microbiota have been identified in healthy donors and in patients with chronic pulmonary disease, and the composition of lung microbiota is similar as the microbiota in the upper respiratory tract, but the number is lower, likely resulting from transient entry rather than independent communities with indistinguishable structure (Charlson et al., 2011). The studies for potential functions of lung microbiota in regulating specific immunity and homeostasis have just begun. Many questions need to be answered, such as what are the barrier and immune functions of lung microbiota? What is the influence of changed lung microbiota on lung health and disease? What is the possibility that using therapeutic microbiota to cure chronic pulmonary diseases? In this review, we will describe the recent understanding of lung microbiota and its relevance for pulmonary health and disease.

### PHYSIOLOGICAL FUNCTION AND HOMEOSTASIS MAINTENANCE OF RESPIRATORY SYSTEM

The respiratory system is comprised by a series of organs that are responsible for taking in oxygen and expelling carbon dioxide. For the sake of convenience, the respiratory system is divided artificially into two functional parts: the upper respiratory tract and the lower respiratory tract. The upper respiratory tract consists of nostrils, nasal cavities, pharynx, epiglottis, and larynx. The lower respiratory tract includes trachea, bronchi, bronchioles, and lungs. The primary function of the lungs is to transfer oxygen from the air to the blood and to release carbon dioxide from the blood to the air. Surprisingly, Lefrancais et al. newly found that the lungs are a primary site of terminal platelet production and account for approximately 50% of total platelet production in mice, which means that the lungs are an organ with considerable hematopoietic potential (Lefrancais et al., 2017). The breath function of the lung determines its structure that opens to the exterior environments, which makes the lungs face the persistent challenge from foreign substances (Bai et al., 2016; Siu et al., 2014). However, it is surprising that the inflammatory response seldom occurs in the lungs even though considering the large surface areas of the lungs and the huge volumes of air inspired on a daily basis (Tian et al., 2016; Wissinger et

al., 2009). How could the lungs ignore or tolerate harmless stimuli to prevent potentially fatal immunopathology?

An immune response initiates only when a pathogen is sufficiently dangerous that it exceeds a specific threshold of immune response. The threshold of immune response is determined by many factors including environmental factors, genetics, diet, stress, age, and even preceding inflammatory events. Thus, the threshold is varied between different individuals and even in different parts of the same individual (Shekhar et al., 2017; Snelgrove et al., 2011; Wissinger et al., 2009). The condition of the lungs demands the threshold of immune response higher to avoid overmuch and excessive inflammatory response. To achieve this purpose, the lungs execute multiple site-specific immune regulatory strategies to restrain inflammation. For example, compared with macrophages from other parts of body, alveolar macrophages, which are dominated in airway (>95%), express lower levels of MHC Class II and costimulatory molecules and display a suppressive phenotype by secreting interleukin-10 (IL-10) and transforming growth factor beta (TGF- $\beta$ ) (Thepen et al., 1994); airway epithelial cells secrete high levels of TGF- $\beta$ , IL-10 and granulocyte-macrophage colony-stimulating factor (GM-CSF) to limit DC responsiveness and alveolar macrophage activation (Li and Flavell, 2008); pattern recognition receptors, especially Toll-like receptors, play a critical role in activating the innate immune response and subsequent adaptive immune response. Studies found that although TLR4 molecules are constitutively expressed in human alveolar and bronchial epithelial cells, they mainly express intracellularly rather than on the cell surface (Guillot et al., 2004).

Recently, with the development of the culture-independent technique for microbiological analysis, many studies uncovered that, except the upper respiratory tract, the lower respiratory tract including the lungs is also colonized by diverse communities of microbiota (Dickson et al., 2016; Man et al., 2017). The studies of intestinal microbiota have shown that microbiota could provide essential health benefits to the host by regulating immune homeostasis (Chung and Kasper, 2010). Given the beneficial function of intestinal microbiota to the intestinal tract, we cannot help but wonder if the microbiota in the lungs has the similar function as intestinal microbiota to promote the development of the lung immune system and maintain lung immune homeostasis?

### THE ORIGIN AND COMPOSITION OF MICROBIOTA IN THE LUNGS

Historically, traditional culture-based studies and classic teaching indicated that the normal lungs are free from bacteria, and this notion has persisted in contemporary medicine (Dickson et al., 2016). However, in the past 20 years, several culture-independent techniques based on the direct amplification and analyses of 16S-rRNA have been developed to study

environmental microorganisms and the microbiota in human (Su et al., 2012). The application of culture-independent techniques has uncovered that the lungs are not sterile and, in fact, colonized by diverse communities of microbiotas (Segal and Blaser, 2014). The microbiotas in human body primarily include six phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Cyanobacteria* (Cui et al., 2014; O'Dwyer et al., 2016). The upper respiratory tract is colonized by a huge number of anaerobes and tenfold more aerobic bacteria (Bassis et al., 2015; Charlson et al., 2011). The most common bacterial phyla in the lungs are *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* after analyzing the bacteria in bronchoalveolar lavage (BAL) from a healthy adult with culture-independent techniques. The dominant genera in the lungs include *Prevotella*, *Veillonella*, *Pseudomonas*, *Fusobacteria*, and *Streptococcus* (Beck et al., 2012).

Where is the lung microbiota from, and what is the relationship of lung microbiota with microbiota in other part of the body? Using culture-independent methods based on RNA/DNA sequencing or microarrays, the investigation of lung microbiota composition does not require culture of individual microbes. However, sampling the lungs for microbiota sequencing is a technical challenge considering lung microbiota with a low biomass, and sampling lung microbiota by bronchoscopy would introduce a theoretical risk of contamination from both mouth and nose (O'Dwyer et al., 2016). Charlson et al. used two bronchoscopes to collect samples up to the glottis, followed by serial bronchoalveolar lavage and lower airway protected brush, which could limit the risk of contamination. By analyzing the composition of the microbiota in oral wash, bronchoalveolar lavage fluid (BALF), nasal swab, and gastric aspirate samples, they found that the respiratory tract harbors a homogenous microbiota that decreases in biomass from the upper respiratory tract to the lower respiratory tract, and the lung microbiota more closely resemble the oral and nose microbiota (Charlson et al., 2011), implying that the lung microbiota might originate from the upper respiratory tract by breath. In addition, the composition and diversity of the lung microbiota are mainly influenced by three factors: (i) the type and number of microbes immigrating into the lungs, (ii) the elimination rate of microbes from the lungs, and (iii) the reproduction rates of the microbe itself in the lungs (Bassis et al., 2015; Charlson et al., 2011).

## THE MICROBIOTA IN LUNG HOMEOSTASIS MAINTENANCE

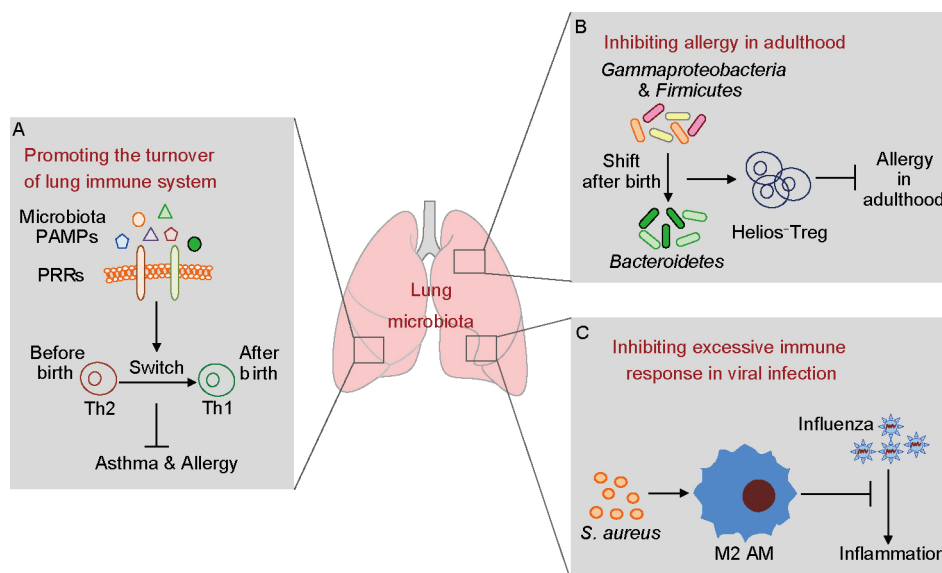
### Promote the turnover of lung immune system

The studies on intestinal microbiota have proved that the microbiota benefits the host by improving the mucosal

structure and function, shaping both the innate and adaptive immune systems, and providing the protection against harmful pathogen infection (Rooks and Garrett, 2016; Zhang and Liang, 2016). The gut-associated lymphoid tissues including Peyer's patches, isolated lymphoid follicles, and mesenteric lymph nodes are underdeveloped in Germ-free mice (Nakanishi et al., 2015; Round and Mazmanian, 2009). However, there are no reports to show that the lung microbiota had similar effects on the development of pulmonary mucosa-associated lymphoid tissue (Gallacher and Kotecha, 2016). For maintaining the homeostasis of intestinal system, pattern recognition receptors (PRRs) sense microbial compounds and induce the differentiation of regulatory T cells (Treg) and Th17 cells (Atarashi et al., 2013; Lochner et al., 2011; Shaw et al., 2012; Song et al., 2016). Similarly, PRRs in the lungs could also sense microbial compounds from lung microbiota and shift naive T cells to Th1 cells but not Th2 cells. Before birth, the unsound pattern of immune system is dominated by Th2 cells. After birth, the polarization of naive T cells in the lungs will switch from Th2 phenotype to Th1 phenotype, which will protect against neonatal asthma and allergic disease (Lloyd and Hessel, 2010) (Figure 1A). Germ-free and specific-pathogen-free mice mount immune response development toward Th2 type and display susceptibility to house dust mite-induced allergic asthma (Remot et al., 2017). Mucosal administration of innocuous whole bacteria or component such as lipopeptide, peptidoglycan, LPS or DNA can induce Th1 immune response and protect mice against asthma and allergy (Saeedi et al., 2015).

### Inhibit excessive immune response in acute infection

As discussed above, for maintaining the homeostasis of intestinal system and inhibit excessive inflammatory response, intestinal microbiota promote and maintain the differentiation of Treg cells. In the lungs, the bacterial load increased during the first two weeks after birth, and the bacterial phyla shifts from *Gammaproteobacteria* and *Firmicutes* towards *Bacteroidetes*. The changes of the microbiota were associated with the development of Helios-negative Treg cells in the lungs in a PD-L1-dependent manner. Absence of microbiota or blockade of PD-L1 caused exaggerated inflammatory response to allergens through to adulthood (Gollwitzer et al., 2014) (Figure 1B). In our previous studies, we found that the microbiota in upper respiratory tract also provided protection against lethal inflammation in the lungs caused by influenza infection in a TLR2- and alveolar macrophage-dependent manner. Priming SPF mice with TLR2-ligand<sup>+</sup> *Staphylococcus aureus*, which commonly colonizes the upper respiratory tract in human, promoted the differentiation of M2 macrophages with immunosuppressive function, which then significantly reduced influenza-mediated inflammatory response in the lungs (Wang et al., 2013) (Figure 1C).



**Figure 1** (Color online) The lung microbiota play roles in lung homeostasis maintenance. A, Similar as intestinal microbiota, lung microbiota might also be recognized by pattern recognition receptors (PRRs) and then promote the polarization of naïve T cells in the lungs from Th2 to Th1 after birth to protect against neonatal asthma and allergy. This issue needs to be determined. B, In neonate's lungs, the bacterial load increases, and the bacterial phyla shifts from *Gammaproteobacteria* and *Firmicutes* towards *Bacteroidetes*. The changes of the microbiota are associated with the development of Helios-negative Treg cells in the lungs that subsequently inhibit the exaggerated inflammatory response to allergens through to adulthood. C, *Staphylococcus aureus* (*S. aureus*), a common microbiota in upper respiratory tract and lung, promote the differentiation of M2 alveolar macrophages then provide protection against lethal inflammation in the lungs caused by influenza infection.

## THE MICROBIOTA IN LUNG DISEASE

The relevance of microbiota to intestinal health and disease has been widely demonstrated. Dysbiosis of intestinal microbiota is involved in the pathogenesis of chronic bowel diseases including chronic inflammatory bowel diseases (IBD), ulcerative colitis (UC), and Crohn's disease (CD) (Macfarlane et al., 2009; Matsuoka and Kanai, 2015). Recently, the lung microbiota was also suggested to contribute to lung disease, and the changes of the lung microbiota will affect the risk of disease, the response to drugs, and the clinical outcomes (Lynch, 2016). There are many factors, such as anatomical injuries, pathological effects, physiological changes, and immune system defects, which could disrupt the lung microbiota and result in chronic lung diseases (Marsland and Gollwitzer, 2014). Chronic lung diseases mainly include asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and idiopathic pulmonary fibrosis (IPF).

### Asthma

Asthma is a chronic and multifactorial disease and thought to be caused by a combination of genetic and environmental factors including air pollution and allergens (Ghosh et al., 2015). Asthma is more popular in developed countries, which means that the living environment has great impact on the etiology of asthma by altering the diversity and composition of the microbiota in the lungs. Ege et al. have reported that children who grow up in traditional farms are exposed to a

wider range of environmental microbes and show a low risk of asthma compared with the children in reference group (Ege et al., 2011). Many studies on asthma patients have identified that the composition of lung microbiota in patients is different from healthy controls (Hilty et al., 2010; Marri et al., 2013). There are more frequent *Proteobacteria* and less frequent *Bacteroidetes* in asthma patients, which might be an accurate predictor of this disease. Thus, the composition of the lung microbiota and the interaction between lung microbiota and host are important for the etiology and development of asthma.

### Chronic obstructive pulmonary disease (COPD)

COPD is a type of obstructive lung disease characterized by long-term poor airflow (Tan et al., 2016; Tan et al., 2014). Many studies on the relationship between lung microbiota and COPD have found that the lung microbiotas in patients with mild and moderate COPD are similar as the lung microbiota in healthy controls (Sze et al., 2014), which is different from asthma patients who have detectably changed lung microbiota even with mild disease (Marri et al., 2013). The change of lung microbiota only can be found in patients with advanced COPD (Dickson et al., 2016). In patients with advanced COPD, there are more frequent *Proteobacteria* or *Firmicutes* and less frequent *Bacteroidetes* (García-Núñez et al., 2014; Wu et al., 2014), which is similar to the shift of the microbiota in asthma patients. However, asthma and COPD are different diseases in the lungs, implying that, besides the change of the lung microbiota, other factors also contribute

to the disease and play more important roles than the microbiota.

### Cystic fibrosis

CF is an inherited disorder that mainly affects the lungs. The syndrome of CF in the lungs displays a progressive development of bronchiectasis and obstructive lung disease (Stenbit and Flume, 2011). The relationship between lung microbiota and the pathogenesis of CF is still controversial. Specific respiratory pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, can be detected increased in sputum from almost all young CF patients during both clinical stability and exacerbations (Ramsey, 1996), so the exacerbations of CF has long been considered as a result of bacterial infection. However, some studies found that antibiotic therapy has no significant influence on the process of the disease (Hurley et al., 2012; Smith et al., 2003). Therefore, CF exacerbation is not associated with increased bacterial density or decreased diversity, and the relationship between lung microbiota and CF pathogenesis might be more complex than we thought before.

### Idiopathic pulmonary fibrosis (IPF)

IPF is a chronic fatal remodeling lung disease characterized by a progressive decline in lung function. There are many evidences supporting that the etiology and progression of IPF are related to the change of the lung microbiota and bacterial infection (Folcik et al., 2014; Molyneaux et al., 2014). An increased bacterial burden could be detected in the BALF of IPF patients compared with controls, and there were a relatively increased abundance of *Streptococcus*, *Pneumococcus*, or *Staphylococcus* taxonomic groups found in human and mouse model (Collard et al., 2007; Han et al., 2014). Moreover, host defense and innate immunity also showed to play a role in IPF disease progression. Defective TLR3 signaling leads to aberrant inflammation and promotes IPF disease progression (O'Dwyer et al., 2013).

Thus, it is clear that the lung microbiota is involved in the etiology or/and progression of chronic lung diseases. However, there are still several questions that remain to be answered. For example, what are the reasons that cause the change of the lung microbiota in chronic lung disease? Is the change of the lung microbiota a cause or consequence of chronic lung disease? Meanwhile, with the in-depth study of the relationship between lung microbiota and respiratory disease, manipulating lung microbiota might become a rational and promising way for therapies. Excitingly, exposure to bacteria and their products by airway route has already been demonstrated to be useful to control allergic airway inflammation in adult mice. Nembrini et al. reported that pulmonary exposure to *Escherichia coli* results in a suppression of allergic airway inflammation through the recruitment of  $\gamma\delta$  T

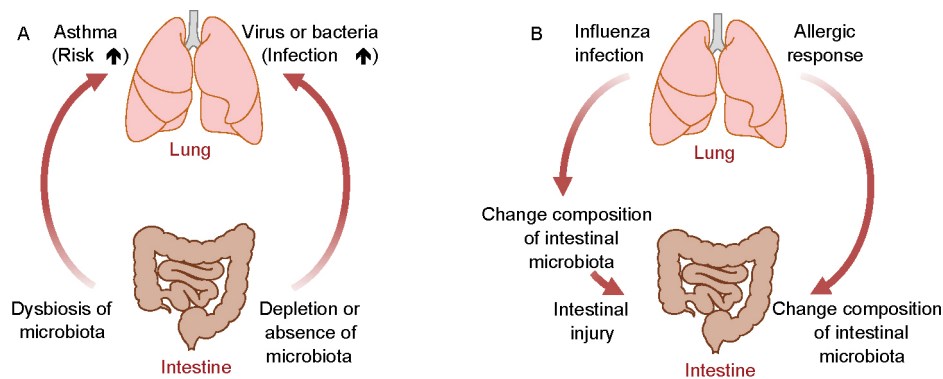
cells in a TLR4-dependent manner (Nembrini et al., 2011). Hagner et al. found that intranasal treatment of farm-derived *Staphylococcus sciuri* W620 could protect mice against both ovalbumin (OVA)- and house dust mite extract (HDM)-induced airway hyperresponsiveness by inhibiting IL-12 expression in mature DCs in a TLR2- and NOD2-dependent manner (Hagner et al., 2013). These two examples show us a possible way to take advantage of lung microbiota to prevent or change disease progression in human, and there are more bacteria that could be selected as the target with increasing knowledge of lung microbiota.

## THE CONCEPT OF GUT-LUNG AXIS

Microbiota plays a critical role in maintaining the homeostasis of the colonized organs or tissues. However, more and more studies found that the local microbiota changes could influence the immunity at the distal tissues, especially the interaction between the intestinal tract and respiratory tract (Budden et al., 2017; Schleiermacher and Hoffmann, 2007; Trompette et al., 2014). Chronic lung disorders, such as asthma, COPD, and CF, all display a component of intestinal disease manifestation and respiratory viral infections are usually accompanied by intestinal symptoms (Keely et al., 2012), which implies the existence of the immunological link between the guts and the lungs, referred to as gut-lung axis.

### Intestinal microbiota influences lung immunity

Increasing evidence shows that the complex interactions between the intestinal microbiota and host immune system are important not only for the intestinal local but also for other organs or tissues. Dysbiosis of the intestinal microbiota is linkage to the pathogenesis and progression of chronic lung diseases, such as asthma. Disruption of the intestinal microbiota in early life could increase the risk of the development of asthma, and restoring the changed intestinal microbiota via probiotic treatment could attenuate the risk (Arrieta et al., 2015; Kozakova et al., 2016; Liu and Marc Rhoads, 2016). In addition, the intestinal microbiota is also broadly protective against respiratory infection (Tamburini and Clemente, 2017). Depletion or absence of intestinal microbiota would lead to impaired immune responses following viral or bacterial respiratory infection. Ichinohe et al. found that intestinal microbiota played a critical role in the generation of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and antibody responses after respiratory influenza virus infection (Ichinohe et al., 2011). Chen et al. found that depleting intestinal microbiota by antibiotic treatment would increase the bacterial counts in blood and the lungs and the mortality of mice following respiratory *Escherichia coli* infection (Chen et al., 2011) (Figure 2A).



**Figure 2** (Color online) The bridge function of microbiota in the gut-lung axis. A, Dysbiosis of the intestinal microbiota is linkage to the pathogenesis and progression of asthma, and depletion or absence of intestinal microbiota leads to impaired immune responses following viral or bacterial respiratory infection. B, Respiratory influenza infection changes the composition of intestinal microbiota and causes intestinal immune injury, and the allergic response in the lungs affects the composition of the intestinal microbiota.

### Lung inflammation influences intestinal microbiota and causes disease

Evolving literature on microbiota suggested that the gut-lung axis is bi-directional, resembling a loop that can be stimulated from two sites. Although little is known about the influence of lung microbiota on intestinal microbiota and intestinal immunity, some studies demonstrated that lung inflammation could affect intestinal microbiota and cause disease. In our previous study, we found that respiratory influenza infection caused immune injuries in both respiratory and intestinal mucosal tissues. Further studies showed that there was no influenza virus in the intestinal tract after viral infection by an intranasal route, which ruled out the possibility that influenza virus infected and caused immune injury at intestinal local directly. Finally, we found that CCL25/CCR9 axis mediated the recruitment of lung-derived CCR9<sup>+</sup>CD4<sup>+</sup> T cells into the intestinal tract, which then changed the composition of intestinal microbiota and caused intestinal immune injury (Wang et al., 2014). Another study also found that a locally induced pulmonary allergic response could also affect the composition of the intestinal microbiota, and vice versa, the changed intestinal microbiota support inflammation in the lungs (Vital et al., 2015) (Figure 2B).

### The common mucosal immune system

More than 30 years ago, McDermott and Bienenstock found that donor-derived mesenteric lymph node B cells distributed in most mucosal tissues of recipient mice after adoptive transfer, while peripheral lymph node B cells only returned to their original site, which led to the coining of the concept of “The common mucosal immune system” (McDermott and Bienenstock, 1979). It suggested that the mucosal immune system is a system-wide organ and immune cells interplay among different mucosal tissues (Gill et al., 2010; Wang and Tian, 2015). Although the concept was presented over 30 years ago, some questions remain to be answered, such as how do the different mucosal sites communicate with each other?

What kinds of immune cells and molecules are involved in this process? We think that the gut-lung axis is a part of the common mucosal immune system, and the studies and findings of the gut-lung axis will help us to know more about the common mucosal immune system.

### CONCLUSION

With the advent and development of culture-independent techniques, the microbiota was found in the lungs, which is contrary to the old notion that the lungs are a sterile organ. At the same time, many new questions arise. The studies about intestinal microbiota have shown that it plays a critical role in the development of local and even systemic immune system, but there is no evidence to support that the lung microbiota has a similar function. The dysbiosis of lung microbiota is linked to the lung chronic disease, but it is not clear whether dysbiosis is a cause or a consequence of immune dysregulation and disease initiation or progression. In the future, we may constitute a healthy lung microbiota community to further our understanding of the complexity of lung microbiota as well as their genetic and metabolic potential and even manipulate the lung microbiota as a potential therapeutic way to treat chronic lung disease.

**Compliance and ethics** The author(s) declare that they have no conflict of interest.

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