PERSPECTIVES

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Emerging pathogenic links between microbiota and the gut-lung axis

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Abstract | The microbiota is vital for immune system development and homeostasis. Changes to its composition and function, termed dysbiosis, in the respiratory tract and the gut have recently been linked to alterations in immune responses and to disease development in the lung. Here we review the microbial species normally found in the healthy gastrointestinal and respiratory tracts, their dysbiosis in disease and interactions with the gut-lung axis. Although this gut-lung axis is only beginning to be understood, emerging evidence indicates the potential for manipulation of the gut microbiota in the treatment of lung diseases.

Chronic lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD) are common and often occur together with chronic gastrointestinal tract (GIT) diseases, such as inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS)[1,2]. Up to 50% of adults with IBD and 33% of patients with IBS have pulmonary involvement such as inflammation or impaired lung function, although many patients have no history of acute or chronic respiratory disease[3,4]. Furthermore, COPD patients are 2-3 time more likely to be diagnosed with IBD[4]. Asthmatics have functional and structural alterations of the intestinal mucosa and COPD patients typically have increased intestinal permeability[2,5]. Although the mature GIT and respiratory tract have different environments and functions, they have the same embryonic origin and consequently have structural similarities. Thus, it is not unsurprising that the two sites might interact in health and disease (FIG. 1), but the underlying mechanisms are not well understood.

An emerging area of intense current interest is the influence of the microbiota (defined here as a microbial community occupying a defined area of activity[6]) on local and systemic host immunity. This is exemplified by germ-free mice, which lack a properly developed immune system and show mucosal alterations, both of which can be restored through

colonisation with gut microbiota[7,8]. The microbiome changes over time from birth, to adulthood and into old age, and in response to environmental factors, such as diet, and drug and environmental exposures[9].

In this ever-expanding field, researchers are now investigating how the local microbiota influence immunity at distal sites, in particular how the gut microbiota influence other organs such as the brain, liver or lung. This has led to the coining of terms such as the 'gut-brain axis' and 'gut-lung axis'. For example, antibiotic-induced alterations of the gut microbiota in early life increases the risk of developing allergic airway disease[10-13], which adds to our understanding of the links between exposure to microorganisms and allergy and autoimmunity (**Box 1**). The mechanisms by which the gut microbiota affect the immune responses in the lung, and *vice versa*, are being uncovered, but many questions remain. Here, we summarise the emerging role of the microbiota in the gut-lung axis, highlighting gaps in our knowledge and the potential for therapeutic intervention.

[H1] Microbiomes of the healthy gut and lung

The GIT remains by far the best-studied host-microbial ecosystem, partly due to its abundance of microorganisms and partly because the microbiota can be profiled through easily obtainable faeces. Both the abundance and diversity of the commensal microbiota generally increase along the GIT, and there are site-specific variations in the mucosa and the lumen[14,15]. These differences are governed by the prevailing environment, including pH, bile acid concentrations, digesta retention time, mucin properties and host defence factors[16]. Despite these variations, the GIT microbiome is dominated by four bacterial phyla, *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria;* with lesser and sporadic representation of others including *Fusobacteria*, *Verrucomicrobia* and *Spirochaetes*. This

'core' gut microbiome comprises up to 14 bacterial genera and 150 bacterial 'species', many of which have not yet been cultured[17-19].

We are beginning to understand the lung microbiota through programmes such as the lung HIV microbiome project, a multi-centre network examining both HIV-infected and uninfected persons with varying histories of lung and/or respiratory disease[20]. The lung has a large surface area with high environmental exposure, and is equipped with effective antimicrobial defences. Healthy lungs were long considered sterile, however, the advent of culture-independent approaches for microbiome profiling has resulted in the detection of microbial DNA within the lungs of healthy subjects[20,21]. These bacteria likely reached the lung from the oral cavity through micro-aspiration, as the taxonomic profiles of the two sites resembled each other[20,21]. Compared to surrounding sites, the lung has a reduced abundance of *Prevotella*-affiliated taxa and an enrichment of *Proteobacteria*, specifically *Enterobacteriaceae*, *Ralstonia*, and *Haemophilus*[20], which may result from host immunity and environment such as redox state and oxygen availability. The lung microbiota might not be resident in healthy individuals, but rather transiently recolonise by micro-aspiration and breathing. The lungs have a comparatively low bacterial biomass and remarkably similar microbial composition to adjacent sites, yet the lungs are continuously exposed to entering microorganisms and their environmental conditions differ vastly from other body sites. These observations support the hypothesis that entry and selective elimination of a transient microbiota is the major determinant of microbiome composition in the lung, as opposed to resident and expanding microorganisms. This does not negate the importance of host-microbiome interactions in the lungs, as evidenced by correlations between microbiome composition and pulmonary inflammation and disease[22]. Rather, it highlights the delicate balance of microbial exposure and elimination; the possibility of dysbiosis at oral sites preceding and/or causing dysbiosis in the lung and contributing to disease pathogenesis[20],

and; the importance in distinguishing whether bacterial DNA detected by culture-independent techniques is truly representative of viable bacteria in the lungs[23]. Technical challenges, such as low microbial biomass and bronchoscope contamination, constant seeding from oral and GIT sites and mucociliary and immune clearance have hindered the identification of a viable and resident, or a transiently recolonising microbiome in the lungs, as well as further research into host-microbiota interactions. Novel methods of sampling tissue with minimal contamination[24], longitudinal studies to identify temporal changes in microbiota, and the increasing use of metagenomic analysis to facilitate the cultivation of fastidious bacterial species[25] will provide a clearer picture of the role of the respiratory microbiota and allow for better design of interventional studies to develop a more complete understanding of host-microbiota interactions in the lung.

[H1] Interactions between the gut and lung

[H3] Interactions of microorganisms between the sites

The epithelial surfaces of the GIT and respiratory tract are exposed to a wide variety of microorganisms; ingested microorganisms can access both sites and microbiota from the GIT can enter the lung through aspiration. Both the gut and respiratory mucosa provide a physical barrier against microbial penetration, and colonisation with normal microbiota creates resistance to pathogens, for example through bacteriocins[16]. Furthermore, a rapidly expanding collection of gut commensal bacteria, including segmented filamentous bacteria (SFB), *Bifidobacteria* spp. and members of the colonic *Bacteroides* genus, induce the production of antimicrobial peptides, secretory IgA and pro-inflammatory cytokines. Non-pathogenic *Salmonella* strains downregulate inflammatory responses in GIT epithelial cells by inhibiting the ubiquitination of I-kappa-B-alpha[26], whereas *Clostridia* spp. also promote anti-inflammatory regulatory T-cell (Treg) responses in the colon[27]. In the respiratory tract,

Streptococcus pneumoniae and *Haemophilus influenzae* synergistically activate host p38 mitogen-activated protein kinase in a Toll-like receptor (TLR)-independent manner to amplify pro-inflammatory responses[28]. Conversely, non-pathogenic *S. pneumoniae* and other bacteria and their components can suppress allergic airway disease by inducing Tregs[29-32]. In lung transplant recipients, airway microbiota alters immunity in the lung. *Firmicutes* or *Proteobacteria*-dominated dysbiosis were associated with expression of inflammatory genes in pulmonary leukocytes, whereas *Bacteroidetes*-dominated dysbiosis was linked to a gene expression profile characteristic for tissue remodelling[33]. In both cell culture[33] and animal models[34], the inflammatory response induced by pathogenic species is greater than that induced by commensal microorganisms, indicating that the diverse lung microbiota protect against pathology by 'diluting' the more pro-inflammatory stimuli of pathogens. Although transfer of microorganisms from faecal suspensions has been used to determine the role of the gut microbiota, such techniques have not yet been used to transfer respiratory microbiota between animals, limiting our understanding of their roles.

Several studies show the effects of GIT colonisation with orally administered bacteria on lung function. Oral gavage of faecal suspensions in mice treated with broad-spectrum antibiotics improved survival rate and reduced lung damage induced by *S. pneumoniae* infection[35]. Even though the nature of this 'gut-lung axis' has been challenged due to potential confounding effects of faecal administration by oral gavage and antibiotic use[36], the concept warrants systematic and controlled evaluation. In infants, gut microbiota composition and caesarean section have been linked to atopic manifestations, and colonisation by *Clostridium difficile* at age 1 month was associated with wheeze and eczema throughout early life, and with asthma at 6-7 years[37]. Positive associations between the presence of 'beneficial' bacteria such as *Bifidobacterium longum* in the gut and a lower incidence of asthma have also been identified[38], although larger and longer studies are needed.

Considerable evidence suggests that host epithelia and other structural and immune cells assimilate information directly from microorganisms and from the concomitant local cytokine response to adjust inflammatory responses, and that this shapes immune responses at distal sites such as the lung[39,40] (FIG. 2). There is less evidence of direct transfer of microorganisms between sites, although the translocation of GIT bacteria to the lung has been observed in sepsis and acute respiratory distress syndrome where barrier integrity is compromised[41]. Additionally, some environmental factors such as dietary fibre can produce similar changes in the GIT and lung microbiota[40]. Whether this results from diet-driven changes in microbial metabolites, changes in innate immune responses, or a combination of both remains to be determined.

[H3] Microbial species-specific effects on host immunity

The crucial role of the microbiota in lung homeostasis and immunity is demonstrated by the poor outcomes of germ-free mice exposed to acute infections[42] and their susceptibility to allergic airway disease[43]. Current research is assessing the impacts of selected members of the commensal gut microbiota on systemic immunity including in the lung, as well as the use of probiotics and prebiotics to prevent and treat acute and chronic pulmonary disease (FIG. 3). For example, SFB in the gut, when present naturally or introduced by probiotic dosing or co-housing of mice, stimulated pulmonary Th17 responses and protection from *S. pneumoniae* infection and mortality[44]. Intriguingly, a respiratory microbiome enriched with oral-related taxa, such as *Prevotella, Rothia* and *Veillonella*, was associated with Th17-mediated immunity in the lungs of healthy humans[22], whether these links are correlative or causative remain unclear. Exposure of mice to dog-associated house dust altered the caecal microbiome, and in particular increased the abundance of *Lactobacillus johnsonii* and other *Firmicutes*-related lineages such as *Peptococcaceae* and *Lachnospiraceae*[45]. Both dog dust-

exposed mice and mice inoculated with *L. johnsonii* had reduced Th2 cytokine responses in the airways, which protected against exposure to respiratory syncytial virus and allergens such as ovalbumin. Other examples of microbial influences on host immunity include the ability of various *Bacteroides* spp. to expand Treg populations or bias the Th1/Th2 phenotype in either direction in a strain-specific manner, or the suppression of host inflammatory responses by the common bacterial metabolites short-chain fatty acids (SCFAs), which act through free fatty acid (FFA) receptors and/or epigenetic regulation of immune cells[46].

In related human studies, seropositivity to the gut-specific pathogen *Helicobacter pylori*, and in particular *cagA*$^{+}$ strains, has long been linked with reduced incidence of asthma and allergy[47,48,49]. Conversely, two recent meta-analyses suggest that *H. pylori* infection is positively associated with increased incidence of COPD and other chronic bronchial diseases[50]. Although these differences might be partly attributable to genetic, environmental and lifestyle factors, these findings raise the possibility that systemic immune responses triggered by *H. pylori* might have different roles in the aetiology of different lung disorders. Strain variations, in addition to the *cagA* expression, might also affect Treg responses[51].

Clearly, the incredible diversity and abundance of gut microbiota results in many immunomodulatory signals, which have considerable combined effects on host health. Although much has been uncovered about the activity of specific bacterial species, current research has only just begun to assess the structure-function relationships of the gut and lung microbiota with host immunity.

[H3] Components and metabolites of gut microbiota that influence the lung

Early studies showed that germ-free mice have reduced responsiveness to LPS-induced pathology and that this oral tolerance to microbial components was due to IL-10 mediated hypo-responsiveness; however, subsequent LPS exposure was no longer tolerated and the

immune response became similar to that seen in conventional mice[52,53]. Furthermore, a robust response to LPS by colonic macrophages could be restored by commensal microbiota[54].

Bacterial components can also have anti-inflammatory effects, attenuating GIT pathology. Polysaccharide A (PSA) from *B. fragilis* induces IL-10 expression by T-cells and protects against intestinal inflammatory disease caused either chemically or by *Helicobacter hepaticus* infection[55]. Sphingolipids, naturally occurring cell membrane components of many gut anaerobic genera including *Bacteroides*, reduce the number of invariant natural killer T cells in the colon, cells which have been implicated in the development of colitis[56]. The best-studied metabolites, SCFAs, are by-products of microbial fermentation of dietary fibre, have anti-inflammatory properties, are a source of energy for colonocytes, and regulate fatty acid and lipid synthesis in the host[57].

Much less is known about the influence of microbial components and metabolites on other sites, including the lung. Reductions in *Faecalibacterium, Lachnospira, Veillonella,* and *Rothia* in the gut, and the urine levels of some microbial bile acid metabolites correlate with the development of atopic wheeze in children, although whether they are a cause or a consequence of wheeze is not known[13]. Oral administration of SCFAs has been shown experimentally to alleviate allergic airway disease[40,58]. Microbial components and metabolites have been implicated in other disorders, such as tryptophan in brain health, PSA in the central nervous system and trimethylamine *N*-oxide in atherosclerosis, further highlighting their importance in extra-intestinal environments[56]. In studies of other diseases *Bacteroidetes* spp. were associated with early-onset autoimmune diseases, which may be a consequence of potent activation of immunity by the LPS of these bacteria[59].

[H1] The gut microbiota and lung diseases

[H3] Microbiota and asthma

An increased risk of asthma has been connected with the disruption of the gut microbiota in early life (Box 1), and several studies have sought to characterise the precise microbial constituents associated with the development of the disease in infants.

The overall community composition of the gut microbiome is not altered in infants at risk of asthma development, but subtle, transient changes in select taxa can be detected in the first few months of life[13,60]. Increased asthma risk has been associated with increased abundance of *B. fragilis* and total anaerobes in early life[61], as well as reduced microbial diversity[60], *Escherichia coli*[62], *Faecalibacterium*, *Lachnospira*, *Rothia* and *Veillonella* species[13], although these findings were not consistent across all studies. Additionally, although models of allergic airway disease support the existence of a critical developmental window early in life[43,63], only one study has provided direct evidence that restoring the altered gut microbiome through probiotic treatment can reduce asthma susceptibility[13].

Similarly, in adults, the overall composition of the faecal microbiome in allergic asthma does not differ from healthy controls[64,65]. There are taxa-specific differences, such as enrichment of *Bifidobacterium adolescentis*, which negatively correlated with the time since asthma diagnosis[64]. Interestingly, heat-inactivated *Bifidobacterium* spp. isolated from allergic infants induced greater pro-inflammatory responses than those from healthy individuals[66].

There are several proposed mechanisms through which the microbiota can attenuate the risk of asthma development. Infants at risk of developing asthma had reduced levels of LPS in their faeces[13], whereas PSA from *B. fragilis* protected against the development of allergic airways disease in mice by inducing IL-10 responses in T-cells[67]. *H. pylori* alleviated murine allergic airway disease in several ways, namely by direct activation of Tregs by neutrophil-activating protein[68], or indirectly through urease B subunit, which promotes tolerogenic reprogramming of dendritic cells[69]. Additionally, γ-glutamyl transpeptidase and vacuolating cytotoxin from *H. pylori* altered dendritic cell function, but did not require Tregs to alleviate

symptoms[70]. Commensal bacteria can also influence asthma development through the production and secretion of metabolites, specifically SCFAs. Asthma risk in infants was associated with reduced acetate concentration in faeces[13] and inversely correlated with serum acetate concentrations in their mothers when they were pregnant[58]. A high-fibre diet, which increased levels of SCFAs in serum and faeces, protected mice against the development of asthma symptoms, a phenomenon which could be replicated by direct administration of acetate or propionate prior to disease onset to promote tolerogenic immune responses in dendritic cells and Tregs[40,58]. The benefits of a high-fibre diet were associated with a reduced ratio of *Firmicutes:Bacteroidetes* and an enrichment of *Bacteroidaceae* in both the faeces and lung, which highlights the necessity of investigating microbial communities at several body sites for a complete understanding of the influence of microorganisms on host health. These studies did not directly explore the relationship between microbiome composition at the two sites, or the relative importance of the gut or lung microbiota in protection against disease. Such studies would be valuable in determining which body site to target with therapeutic interventions. An important but understudied area is the role of interactions between microorganisms in the development of asthma. For example, the loss of intestinal bacteria and outgrowth of commensal fungi triggered prostaglandin E_2-induced changes in alveolar macrophages and increased allergic airway inflammation[71]. Furthermore, gut helminth infection protected mice against allergic airway disease, which was associated with an increased abundance of *Lachnospiraceae* and other *Clostridiales* members, the production of SCFAs and subsequent robust Treg responses in the lungs[72]. Although the Treg-promoting capability of *Clostridia* spp. have previously been demonstrated in the colon[27,73], it is increasingly being explored for the treatment of diseases at other body sites, including asthma[74,75].

[H3] Microbiota and COPD

Respiratory microbiome research in COPD has assessed changes in the disease state, and with smoke exposure, a major risk factor for the development of this disease. Interestingly, although the lung microbiome is similar in healthy smokers and non-smokers, the oral microbiome differs substantially between the two groups[20]. As enrichment of lung microbiota with taxa from the oral cavity is associated with increased inflammation in smokers[76], it is plausible that changes in the oral microbiota and a failure to effectively clear aspirated microorganisms contribute to disease development, and may help explain why only a subset of smokers develop COPD. In any case, there are stark differences in the lung microbiome of COPD patients compared to 'healthy' smokers[77,78], which led to the proposal that the respiratory microbiome may be useful in the early diagnosis of COPD. In contrast, no study to date has investigated changes of the gut microbiome in COPD patients. Nevertheless, in 'healthy' smokers, the faecal microbiome is characterised by an increase in abundance of *Bacteroides-Prevotella* spp.,[79] and a reduced *Firmicutes*:*Bacteroidetes* ratio[80] compared to non-smokers. These changes in microbiota composition have been associated with intestinal inflammation and IBD[81,82]. Smokers also have a reduced abundance of *Bifidobacterium*[80,83], and hence may lose the anti-inflammatory effects often associated with this genus.

The causes of smoking-associated changes in microbiome composition are likely a combination of environmental, host and microbial changes such as intestinal and immune disruption, impaired clearance of pathogens[84,85], acidification of gastric contents[86] and ingestion of bacteria that occur in cigarettes[87]. Furthermore, cigarette smoke can directly affect the virulence of both bacteria[88] and fungi[89], as well as altering the growth and exopolysaccharide structure of known gut bacteria such as *Bifidobacterium animalis*[90], which may contribute to dysbiosis. Even following smoking cessation, many of these changes that

cause dysbiosis persist for prolonged periods, and thus any therapeutic intervention to restore the microbiota may potentially require repeated administration to avoid relapse.

In the absence of longitudinal or interventional studies, it is difficult to ascertain whether changes in the gut or respiratory microbiome are a cause, or a consequence of COPD. Most likely, both are true and operate simultaneously or at different stages of disease. Exposure to environmental stimuli and onset of disease cause dysbiosis, which in turn likely contributes to disease progression. In any case, defined probiotic use may benefit COPD patients, particularly if used as an early, preventative intervention. Oral *Lactobacillus casei* administration improved the previously defective function of peripheral natural killer cells in adult male smokers[91], whereas *Bifidobacterium breve* and *Lactobacillus rhamnosus* reduced lung pathology in a mouse model of COPD[92], and reduced inflammatory responses in macrophages exposed to cigarette smoke extract *in vitro*[93]. Similarly, a diet which increased SCFA production protected against elastase-induced inflammation and emphysema[94]. Although a causal relationship between SCFAs and protection in this study was not confirmed, both cigarette smoke[95] and environmental particulate matter[96] reduced SCFA concentrations in rodents, and cigarette smoke condensate reduced their production *in vitro*[90]. Furthermore, increased intestinal translocation of bacteria and their products occurred after exposure to particulate matter or development of COPD[2,96,97]. Bacterial toxins such as enterotoxin[98] or LPS[99] can contribute to the pathogenesis of COPD and microbiota-associated intestinal inflammation may become systemic and also contribute. The potential of SCFAs to improve intestinal barrier function may account for their benefits in animal models of COPD[100,101], although this is yet to be explored in clinical studies.

[H3] Microbiota and respiratory infections

The gut microbiota is broadly protective against respiratory infection, as its depletion or absence in mice led to impaired immune responses and worsened outcomes following bacterial or viral respiratory infection[35,42,102-104]. Administration of SFB improved resistance to *S. aureus* pneumonia[44] and *Bifidobacterium* spp. protected against both bacterial[105] and viral pulmonary infection in mice[104,106]. *Lactobacillus* and *Bifidobacterium*-based probiotics also improved the incidence and outcomes of respiratory infections in humans[107-110].

Several aspects of experimental design influence the results of infection studies, including the route of administration of bacterial ligands[103,111], the facility from which research animals are sourced[44], the type of antibiotic used for microbiota depletion[63,103] and the infecting pathogen. For example, herpes simplex virus type 2 or *Legionella pneumophilia* do not appear to be influenced by antibiotic-mediated microbiota depletion[103].

Nevertheless, several important mechanisms by which the gut microbiota promotes clearance of infection have been identified. Innate immune responses to bacteria in the lungs are greatly enhanced by exposure to NOD-like receptor and TLR agonists in the GIT, including peptidoglycan, LPS, lipoteichoic acid and CpG DNA[42,102,111]. Similarly, stimulation of TLRs by gut bacteria cell wall components and flagellin is necessary for effective adaptive immune responses to influenza[103,112], whereas the anti-inflammatory effects of oral SCFA administration are linked to reduced pulmonary pathology following both bacterial[105,113] and viral[114] infection in mice. However, microbiota can also drive gut pathology in pulmonary infection. Influenza virus infection in mice increased the number of lung-derived $CCR9^{+}CD4^{+}$ T-cells, which preferentially migrate to the GIT under the guidance of CCL25 expressed on intestinal epithelial cells[115]. This resulted in the outgrowth of *E. coli* and the induction of aberrant Th17 responses and intestinal damage.

[H3] Conclusions and perspectives

Many studies have identified the presence of a lung microbiome in health and disease. However, we believe that the healthy lung microbiota may be transient and best described as a progression of taxa influenced by adjacent body sites and the external environment, rather than an actively reproducing core resident community. This is not down-playing the importance of a transient microbiome in the healthy lung which could still have important roles in inflammatory responses whether viable or not. By contrast, the microbiota is more likely to be persistent and resident in the airways and lungs in respiratory disease, although whether it is a cause or consequence remain to be elucidated. Furthermore, the lung microbiota could affect or be affected by microbiota or immune responses at distal sites. The crosstalk between microorganisms and the host is complex and our current understanding of these interactions is only in its infancy. It is unlikely that any one of these interactions is solely responsible for the functions of the microbiota, and alterations of any part of these relationships may be enough to affect health and disease. It is unclear whether changes in the microbiota at one site affect many distal sites equally, or if these systemic effects might be specific to certain tissues. To date, no such broad study investigating these systemic widespread effects has been performed.

Gut-lung microbiota studies thus far have two major limitations: the first is discerning causative over correlative effects, the second is timing. Most studies have been associative. Furthermore, culture-independent identification of microbiota has not yet replaced the need to isolate and culture suspected opportunistic pathogens or probiotics in order to study their effects, and many members of the microbiota cannot be easily cultured. Thus, it is typically unclear whether changes observed in the microbiota are the cause or effect of disease. As for timing, most experimental data have described the role of the gut microbiota on the development of lung disease, and not in established lung disease. Longitudinal studies in humans and animals that associate changes in the microbiota with the severity of established

chronic lung disease are required. Research into manipulations of the microbiota during lung disease is necessary to improve our understanding and inform the development of novel therapies (Box 2).

Increasingly, microbiome research is moving towards defining the 'functional' microbiome. As taxonomic variation between sites and individuals is so large, and the microbiome consists of thousands of species, it is highly likely that there is redundancy between species in terms of their interactions with other microorganisms and in the metabolites they produce. Thus, next-generation '-omics' approaches are required to define how the microbiomes of the gut and lung interact with each other and influence health and disease.

In summary, lung microbiota in the healthy state may be transient and constantly re-seeded from the environment and cleared by the immune system, but may still influence health and disease. In respiratory diseases the lung microbiota likely becomes persistent and may be both a cause and consequence of the disease forming a pathogenic feedback loop. It is clear that bacterial components and metabolites in the gut and lung have the capacity to modulate systemic and local immunity, with specific taxa able to influence the pathogenesis of respiratory diseases such as asthma, COPD and respiratory infections. Such relationships have been identified in other respiratory diseases, such as cystic fibrosis[116], which, as a genetic disease, is a special case. Respiratory challenges with environmental factors such as pollution, cigarette smoke, antibiotics, and diet influence disease risk and likely drive pathogenesis through their ability to modulate microbiota composition, although the mechanisms of these effect remains unknown. Further longitudinal studies and improved interventional experiments will help to elucidate the role of the microbiota and gut-lung crosstalk in respiratory disease, and will potentially lead to the identification of new and effective avenues for treatment.

Box 1: The hygiene hypothesis and microbiota

In 1989, after observing an inverse correlation between the occurrence of hay fever and number of siblings, David Strachan coined the term 'hygiene hypothesis'[117]. He proposed that reductions in the incidence of infections during childhood altered the development of the immune system, leading to increased risk of allergic disease. Subsequent studies showed that growing up on a farm[118], attendance of child care[119] and exposure to dog-associated house dust[45] all protected against the development of asthma. This hypothesis was later modified to state that microbial exposure from commensal bacteria that had co-evolved with humans (as opposed to faster evolving viruses) were necessary to properly mature the immune system[120]. Both hypotheses have since been used to explain the rise of various autoimmune and allergic diseases that correlate well with the decrease in infectious diseases in affluent countries. This is now supported by substantial epidemiological evidence for asthma, hay fever[117], atopic dermatitis[121], type 1 diabetes mellitus[122] and multiple sclerosis[123].

Expansion of the gut microbiota begins immediately after birth and is heavily influenced by environmental factors, with species of the phylum *Actinobacteria* often dominant during infancy[124,125]. In this early window of life, changes in the microbiota may be linked with the development of chronic lung disorders arising in later life. The decline in exposure to infectious agents and changes in the microbiota has many causes, including improved hygiene and sanitation practices, provision of clean water, pasteurisation, and vaccination. Antibiotics directly cause dysbiosis and in infants this may increase susceptibility to chronic inflammatory diseases in later life[126]. Furthermore, the modern diet that is high in processed foods also affects the gut microbiota and may have a major but currently undefined role in these processes.

In addition, in areas where helminth infection is rare, the incidence of allergic disease is high. Those with chronic helminth colonisation show antigen-specific immune

hyporesponsiveness, with increased levels of IL-10 and suppressive Tregs. In addition, helminths can influence B-cell differentiation, IgE responses, natural killer T-cell activity and macrophage function, to downregulate immune responses and thereby protect the host against allergic disease[127].

Box 2: Future directions

Therapeutic efforts that involve the microbiota and are focused on gastrointestinal disorders are further advanced than endeavours targeting the gut-lung axis, or indeed targeting the lung in general. Whereas initial research has focused on associative studies between pathophysiology and microbiota composition, the next step is a shift to causal links, which will then indicate interventional strategies for microbiota-modifying or immunomodulatory therapeutics. A recent survey of the microbiota intellectual property landscape[128] showed that patent filings (dominated by food and nutraceutical companies and smaller biotechnology start-ups) were directed towards treating infectious diseases (for example, *Clostridium difficile* infection), digestive and metabolic disorders (IBD, type 1 diabetes), and to a lesser extent inflammatory and/or immune disorders. Products in development encompass faecal transplants and 'cocktails' of live microorganisms. In addition, there is interest in microbial metabolites and related designer small molecules to beneficially modulate host immune responses. For example there are several patents filed on small molecule agonists of FFAR2 (free fatty acid receptor 2), the host receptor for SCFAs[129-131]. FFAR2 is a G-protein coupled receptor, a class considered to be inherently 'drugable', which is expressed on neutrophils, eosinophils, and other immune cells and has been linked to exacerbated or unresolved inflammation in animal models of colitis, arthritis and asthma[132]. This provides a link between the SCFAs from fermentable dietary fibre and beneficial effects in inflammatory diseases such as asthma[58]. Receptor-targeted approaches such as this may be complementary

therapies to more traditional corticosteroids, and cytokine-directed treatments for pulmonary disorders.

Figure 1 | **Principles of gut-lung crosstalk in health and disease.** A healthy intestinal microbiota maintains a homeostatic local immune responses through the exposure of structural ligands (for example, LPS, peptidoglycan) and secreted metabolites (for example, SCFAs). Invading microbiota and absorbed metabolites influence circulating lymphocytes and contribute to the regulation of systemic responses. When the gut microbiota is disturbed, for example during infection or antibiotic exposure, the normal microbiota-derived signals are altered, leading to a transformed immune response. In early life, when the immune system is still developing, this disturbance can dramatically alter the way in which the immune system perceives its surroundings in later life, leading to chronic inflammatory disorders in the gut and lung. In adulthood, dysbiosis of the gut microbiota, for example through exposure to cigarette smoke, can cause systemic inflammation and an outgrowth of opportunistic pathogens, which can lead to chronic inflammation at distal sites. Although the specific taxa, ligands, metabolites and/or host responses may differ in specific disease situations, these broad principles outline the role of the microbiota in gut-lung crosstalk.

Figure 2 | **Structural and functional similarities and differences between the gut and lung.** The gut and airway epithelia have substantial differences in functional purpose and exist in different environments, yet they retain some anatomical similarities. Both are derived from the endoderm and consist of columnar epithelial cells with projections of microvilli (gut) or cilia (airway) that function as a physical barrier and as sentinels for the immune system in conjunction with associated lymphoid tissue. Both secrete mucus through goblet cells as well as secretory IgA (although less in the lung). The alveoli found in terminal airways in the lung differ substantially, consisting of squamous epithelial cells that secrete surfactant (type 2 alveolar cells) or function in gas exchange (type 1 alveolar cells). The similarities end here: the intestinal lumen is an oxygen-poor environment and functions to digest food and absorb nutrients. Movement of matter is unidirectional (mouth to anus), with the exception of reflux or vomiting. Furthermore, the pH, enzyme presence and structure vary along the GIT. In contrast, the airways and alveoli are oxygen-rich, and movement is bidirectional (inhalation and exhalation). The gut is of a relatively uniform 37°C, whereas airway temperature differs depending on the proximity to the pharynx. Thus, it is unsurprising that the microbial life in each environment is distinct. Changes in diet and exposure to therapeutics and environmental particulates can directly affect the composition of the microbiota. Both the gut and lung are able to influence each other's immune responses. Dendritic cells in the intestine and airways, and macrophages in the lungs, sample antigens in the lumen. Lymphocytes in the associated lymphoid tissues circulate through the lymphatic system to affect systemic immunity. Bacteria from the gut can travel to the lung through aspiration of vomit or oesophageal reflux. In times of dysbiosis, disturbed epithelial integrity may enable bacteria and their components and metabolites to enter the circulation causing systemic inflammation.

Figure 3 | **Immune system programming by microbiota.** Secreted and structural components of microbiota can influence the host immune response both locally and at distal sites. Microbial metabolites, such as short chain fatty acids (SCFAs), bind free fatty acid receptors or promote epigenetic changes in host leukocytes, which induce anti-inflammatory responses and reduce inflammation. Virulence factors from pathogenic bacteria, such as *Helicobacter pylori* or *Bacteroides fragilis*, can downregulate host immune responses, whereas structural components from commensal bacteria influence inflammatory responses through the activation of pattern recognition receptors. LPS, lipopolysaccharide; LTA, lipoteichoic acid; PSA, polysaccharide A; UreB, urease subunit beta; VacA, vacuolating cytotoxin A; GGT, gamma-glutamyl transpeptidase; NAP, neutrophil-activating protein.

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Competing interests statement

The authors declare no competing interests.

Glossary Terms

Microbiota: a microbial community occupying a defined area of activity

Key Points

- The gastrointestinal tract (GIT) and respiratory tract, while separate organs, are part of a shared mucosal immune system termed the gut-lung axis.
- The microbiota of the GIT and the respiratory tract are involved in the gut-lung axis, influencing immune responses both locally and at distant sites
- Current research has identified specific bacterial taxa, their components and metabolites which can influence host immunity.
- With greater knowledge of the gut-lung axis and microbial influences of immunity, great advances have been made in understanding the role of microbiota in respiratory diseases such as asthma, chronic obstructive pulmonary disease and respiratory infection.
- This newfound understanding has created a number of possible therapeutic strategies for the treatment or prevention of acute and chronic respiratory diseases. However, several technical challenges and unanswered questions remain.

Author biographies

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Kurtis F. Budden received his B. Biomedical Science (Honours) from The University of Newcastle, Australia. He is in the process of completing his PhD in Immunology and Microbiology under the supervision of Prof. Phil Hansbro at Hunter Medical Research Institute in conjunction with The University of Newcastle. He is currently investigating the

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Dr Gellatly completed her PhD at the University of British Columbia where she studied the functional genomics of the opportunistic respiratory pathogen Pseudomonas aeruginosa. She then completed a Post-doctoral Fellowship at the University of Newcastle, Australia, In Professor Hansbro's lab where she investigated the changes in the gut and lung microbiome in lung diseases, especially COPD. She is interested in all aspects of the host microbe relationship.

David LA Wood

David Wood is a bioinformatician who completed his Bachelor of Science (Hons I) at the Australian National University in 2003 and his PhD in mammalian transcriptomics and genome informatics at The University of Queensland. He is currently a post-doctoral research fellow at the Australian Centre for Ecogenomics investigating clinically-related host-associated microbial ecology.

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Matt Cooper completed his PhD in 1995 then spent 13 years in the UK, first at the University of Cambridge, then in start-ups and biotechnology companies. He returned to Australia in 2009 to work on therapies that block inflammation via the innate immune system, discovery and development of antimicrobials, rapid diagnostics and novel modulators of the human microbiome. He has over 20 patents and more than 200 scientific papers.

Mark Morrison

Professor Morrison is recognized for his translation of genomic and metagenomic datasets into biological frameworks, including novel organismal, diagnostic, and enzyme-based technologies. From 2006 he was a CSIRO science leader in metagenomics, as a stream leader for Gut Health in the Preventative Health National Flagship Research Program. He was one of CSIRO's five "Capability Platform leaders" (in Transformational Biology) 2007-13, before being appointed Chair in microbial biology and metagenomics, University of Queensland Diamantina Institute in 2013. He currently serves as Australia's science representative to the International Human Microbiome Consortium (IHMC) and hold Affiliate Professorships with The Ohio State University.

Philip Hugenholtz

Professor Hugenholtz is a microbiologist who has made contributions in the field of culture-independent analysis of microorganisms. He discovered and characterised numerous previously unrecognised major bacterial and archaeal lineages each with greater evolutionary divergence than animals and plants combined. He has participated in the development and application of metagenomics, the genome-based characterisation of microbiomes, which has revolutionised our understanding of microbial ecology and evolution. He has made several discoveries in environmental and clinical microbiology sometimes overturning decades of misdirected culture-based studies.

Philip M Hansbro

Professor Hansbro is a Chair in Immunology/Microbiology, NHMRC Principal Research Fellow, Associate Director, Research Centre for Lung Health, and Director and Chair of Research of the Thoracic Society of Australia and New Zealand. He leads internationally recognised research programs in COPD, asthma and bacterial and viral respiratory and reproductive infections, and microbiomes. He develops and interrogates novel mouse models and undertakes clinical studies of these important diseases to further our understanding of pathogenesis and develop novel therapies. He publishes extensively in influential journals and is regularly invited to present internationally, and has a substantial funding record.

Subject categories

Biological sciences / Microbiology / Microbial communities / Microbiome

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Health sciences / Anatomy / Gastrointestinal system / Microbiota

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Health sciences / Diseases / Respiratory tract diseases

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Biological sciences / Microbiology / Bacteria / Bacterial host response

[URI /631/326/41/2533]

Biological sciences / Immunology / Immunological disorders / Inflammatory diseases / Asthma

[URI /631/250/249/2510/31]

ToC blurb

The microbiota is central for host homeostasis and this affects not only the gut but also other organs, including the lung. In this Perspective, Hansbro and colleagues explore the role of the microbiota in the gut-lung axis and lung disease.