nature reviews microbiology

https://doi.org/10.1038/s41579-024-01048-8

Review article

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The gut–airway microbiome axis in health and respiratory diseases

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Abstract

Communication between the gut and remote organs, such as the brain or the cardiovascular system, has been well established and recent studies provide evidence for a potential bidirectional gut–airway axis. Observations from animal and human studies indicate that respiratory insults influence the activity of the gut microbiome and that microbial ligands and metabolic products generated by the gut microbiome shape respiratory immunity. Information exchange between these two large mucosal surface areas regulates microorganism–immune interactions, with significant implications for the clinical and treatment outcomes of a range of respiratory conditions, including asthma, chronic obstructive pulmonary disease and lung cancer. In this Review, we summarize the most recent data in this field, offering insights into mechanisms of gut–airway crosstalk across spatial and temporal gradients and their relevance for respiratory health.

Sections

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Introduction

Respiratory health has long been related to the presence and activities of both cellular (bacterial, fungal) and acellular (viral) microbial pathogens with the capacity to disrupt epithelial barriers and instigate inflammatory responses[1,2]. While these microorganism–host interactions remain a cornerstone of acute and chronic respiratory infection and disease, a growing body of evidence indicates that airway mucosal responses are not only governed by local respiratory microorganisms but are also influenced by activities of the gut microbiome. Recent evidence indicates that the gut–airway axis may be bidirectional and that respiratory infection and chronic airway inflammation can also influence the composition and, particularly, the metabolic activity of the gut microbiome, leading to longer-term consequences for respiratory health.

Airway and gut microbiomes can modulate immunity at remote mucosal sites through cell-associated and secreted compounds. These microbial products shape immune function through signalling pathways, including Toll-like receptors[3], nucleotide-binding oligomerization domain-like receptors[4] and G protein-coupled receptors (GPCRs)[5]. Microbial-derived metabolites also influence epigenetic modifications[6], leading to longer-lasting regulatory control on the transcriptional activity of host cells[3]. Such interactions at one mucosal site can impact remote mucosal immune function via migratory immune cells and circulating antigens[7]. Additionally, microorganisms and microbial-derived products may enter the circulation directly or via extracellular vesicles, which are small lipid-bound particles that can carry immunomodulatory cargo and can be detected at various sites across the human body[8]. Thus, recent efforts in the field have focused on understanding how microorganisms at the gut–airway axis, which sense and respond to inhaled and ingested environmental cues, may influence respiratory health via their dynamic interactions with local and systemic host immunity.

Large population-based human microbiome studies of pulmonary disease have demonstrated the existence of distinct microbial colonization patterns and metabolic features that co-associate with specific disease endotypes[9]. These studies have also identified exposures that influence respiratory disease risk, the majority of which are known to exert strong selective pressure on gastrointestinal and respiratory microbiome function and activity. Critically, recent observational human studies have also revealed relationships between early-life gut and airway microbial colonization and respiratory health outcomes in later childhood, significantly expanding our understanding of the spectrum of microbial strategies that tune host immunity across the gut–airway axis and over developmental time. Improved understanding of microorganism–host interactions in the gut and airway across human lifespan offers a novel framework for respiratory disease prevention and treatment. This Review summarizes the latest literature addressing gut–airway crosstalk and its implications for human health. We specifically discuss early-life development of the gut and airway microbiota and the known mechanisms by which gut microbiomes influence airway immune function with a focus on respiratory viral infections. Microorganism–host interactions at these two large mucosal surfaces are critically important to respiratory health, not only because they have improved our understanding of disease heterogeneity but also because they have revealed therapeutic targets for pulmonary disease in both airway and gut microbiomes.

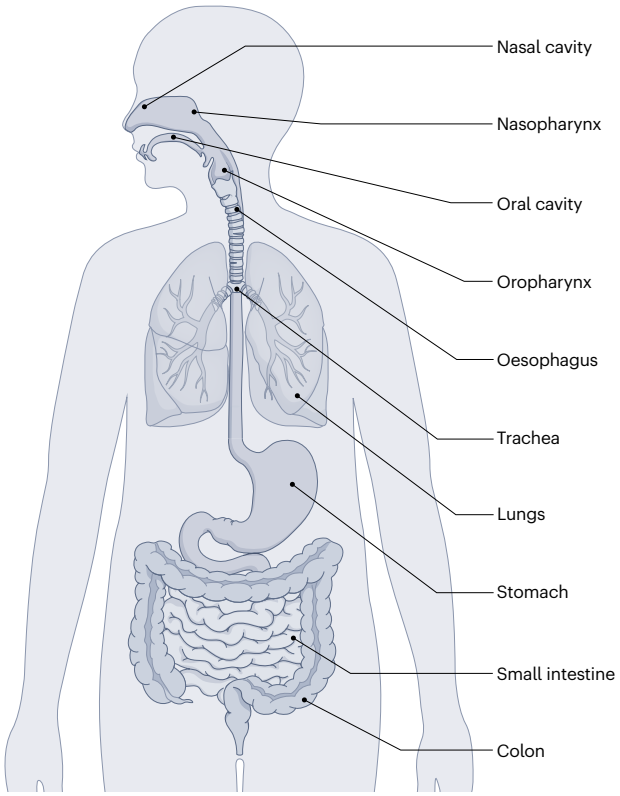
Development of the gut and airway microbiota

Independent early-life studies of the gut and airway provide evidence that both mucosal surfaces are rapidly colonized by microorganisms in the postnatal period[10–12]. While the distal gut harbours the largest number and most diverse microbiome in the human body, clear compartmentalization of microbial phylogeny is observed along the length of the gastrointestinal tract[13], reflective of the idiosyncratic function (for example, mastication, digestion and absorption) and related intrinsic ecosystem conditions (such as mucin secretion and immune responses) that exist within each gastrointestinal niche. Despite housing a significantly lower microbial burden and diversity, the respiratory tract also exhibits microbial niche specialization, with the nasopharynx harbouring the highest burden of microorganisms. Microbial load decreases upon transition into the lower airways in healthy individuals (Fig. 1). Both the human gut and airways exhibit distinct environmental conditions for microbial colonization, with markedly different oxygen levels, nutrient availability and pH, which contributes to the establishment of niche-specific microbiomes and immune system interactions[14].

In humans, development of the faecal microbiome occurs over the first several years of life with characteristic domination by members of the Firmicutes and Bacteroidetes phyla typically observed by 3 years of age[15,16]. However, functional evolution of gut microbiomes continues throughout childhood and into adult and senior years[17,18] and relates to health status. For example, microbial-derived amino acids in the circulation deviate across human populations and have been shown to predict survival rates in elderly individuals[18]. It is well established that a variety of environmental factors, including antimicrobial administration[19], diet[20], season[21], maternal microbiome[22], gestational age[23], mode of delivery[24], pet[25] or farming exposures[26], and microbial infection[27], shape microbiome composition and function both in the intestine and airway (Fig. 2). Such exposures, coupled with intrinsic niche-specific conditions, microorganism–microorganism[28] and microorganism–host[29] interactions, result in a relatively small number of reproducible microbiota compositions that are evident in specific airway and gut niches, are age-dependent and relate to respiratory disease outcomes[9,30].

Longitudinal studies of human airway microbiota development are sparse. However, a small number of studies provide tantalizing evidence that early-life microbiome development also occurs in the upper airways and that distinct respiratory microbiota trajectories in early childhood relate to maternal immune status, heritable microorganisms and epigenetic modifications to neonatal immunity[6,31]. The composition of the early-life upper airway microbiota can predict respiratory infections in infants and exacerbations of bronchiectasis, a chronic lung condition where the airways are widened, increasing the risk of infection[32]. More specifically, infant upper airway microbiota, dominated by *Moraxella* spp., *Haemophilus* spp. or *Streptococcus* spp., are associated with increased risk of febrile viral infection and subsequent allergy and recurrent wheeze development in later childhood[33]. In vitro and in vivo studies suggest that short-chain fatty acids (SCFAs) and SCFA-producing microorganisms may also contribute to airway diseases, such as cystic fibrosis, by degrading mucins, in turn providing nutrients for pathogens otherwise unable to efficiently obtain nutritional substrates in the lungs[34,35].

Human birth cohort studies indicate that very-early-life exposures, including prenatal and early postnatal experiences such as maternal prenatal nutrition, adversity and stress[36] as well as caesarean section delivery[24], shape both gut and airway microbiomes and pulmonary disease risk. Larger human studies indicate that a relatively small number of microbiota compositions are evident at specific body sites and relate to factors such as age[9], long-term dietary exposures[37]



	Microbial load	Dominant microorganisms
Nasal cavity	~10^3	*Corynebacterium, Moraxella, Staphylococcus, Streptococcus* species
Nasopharynx	~10^3	*Corynebacterium, Dolosigranulum, Haemophilus, Moraxella, Neisseria, Staphylococcus, Streptococcus* species
Oral cavity	~10^3	*Actinomyces, Corynebacterium, Lautropia, Rothia, Streptococcus* species
Oropharynx	~10^6	*Bacteroides, Fusobacterium, Leptotrichia, Neisseria, Streptococcus, Prevotella, Rothia, Veillonella* species
Oesophagus	NA	*Haemophilus, Neisseria, Prevotella, Streptococcus, Veillonella* species
Trachea	NA	*Acinetobacter, Corynebacterium, Haemophilus, Moraxella, Neisseria, Pseudomonas, Staphylococcus, Streptococcus* species
Lungs	~10^2	*Tropheryma, Prevotella, Streptococcus, Veillonella* species
Stomach	~10^{1-3}	*Actinomyces, Enterococcus, Fusobacterium, Haemophilus, Helicobacter, Lactobacillus, Leptotrichia, Prevotella, Rothia, Streptococcus, Veillonella* species
Small intestine	~10^{1-8}	*Actinomyces, Bacteroides, Bifidobacterium, Clostridium, Enterococcus, Escherichia, Gemella, Lactobacillus, Neisseria, Prevotella, Propionibacterium, Streptococcus* species
Colon	~10^{10-12}	*Akkermansia, Alistipes, Bacteroides, Bifidobacterium, Clostridium, Enterobacter, Escherichia, Helicobacter, Klebsiella, Lactobacillus, Prevotella, Ruminococcus* species

Fig. 1 | Compartmentalization of microbial phylogeny in the gut and airways. Both the respiratory and gastrointestinal tracts exhibit microbial niche specificity with distinct but relatively reproducible microbial burden and characteristic colonizing genera evident in each compartment. In healthy individuals, the lung microbiome composition is determined by the balance between microbial immigration and elimination. Species belonging to *Moraxella*, *Haemophilus*, *Staphylococcus*, *Corynebacterium* and *Streptococcus* are the most commonly detected dominant genera in the upper respiratory tract of humans. In the gastrointestinal tract, microbial burden gradually increases from proximal to distal sites, ranging from ~10^1 to 10^3 colony formation units (CFUs) ml^{-1} in the stomach to ~10^{10} to 10^{12} CFUs ml^{-1} in the distal colon[130]. Microbial diversity in the human stomach is limited. The low pH of the gastric lumen limits the types of microorganisms that can colonize, selecting for acid-resistant bacterial populations such as *Prevotella*, *Streptococcus*, *Veillonella* and *Rothia* species. From the small intestine towards the colon, oxygen levels gradually decrease, promoting colonization primarily by *Bacteroides*, *Clostridium*, *Enterococcus* and *Lactobacillus* species. Colonization in the colon is characterized by *Bacteroides*, *Prevotella*, *Alistipes*, *Akkermansia* and *Ruminococcus* species. The unit by which bacterial density is measured varies per niche; the density measures in the nasal cavity and nasopharynx are shown as an estimated number of bacteria per nasal swab[131], and the densities in the oropharynx and the lungs represent the estimated number of bacteria per millilitre of oral wash[132] or bronchoalveolar lavage[132,133], respectively. NA, no available data.

and, importantly, respiratory health status[9]. These data suggest that differences in neonatal airway microbial colonization and microbiota development contribute to such outcomes. To date, the mechanisms by which the airway microbiota contribute to the development of respiratory disease remain largely unknown.

Although most pulmonary diseases manifest in the lower airways, this compartment is more difficult to sample (Box 1). Nonetheless, evidence suggests that the microbiota of the human lower airway more closely resembles that of the oropharynx rather than that of inhaled air, the nasopharynx or the lower gastrointestinal tract[14]. In adults, the bacterial burden (that is, the quantity of bacteria, which is distinct from diversity) in the lower airways predicts mortality and disease progression of idiopathic pulmonary fibrosis and responsiveness to inhaled antibiotics in patients with bronchiectasis[38,39]. Sputum microbiota diversity has also been found to predict mortality in patients with chronic obstructive pulmonary disease (COPD)[32]. Reduced diversity of the lung and gut microbiota in mechanically ventilated patients is associated with lower survival rates[40,41], and increased lung bacterial burden is predictive of chronic rejection and death in patients with lung transplants[41,42]. Thus, the data, though sparse, indicate that airway microbiomes develop in early life and that local airway microbial colonization in both the upper and lower airways exhibits a sustained relationship with respiratory disease development and outcomes throughout the life course (Box 2). These studies highlight that microbiome states within both the gut and the airways are related to age, environmental factors and respiratory disease. The data also suggests that the application of finer resolution microbiome profiling tools, such as shotgun metagenomics, untargeted metabolomics and proteomics, in parallel with immune profiling will enable more precise assessments of disease risk and facilitate stratification of patients into distinct endotypes, enabling interventions specifically tailored to their microborganism–immune dysfunction.

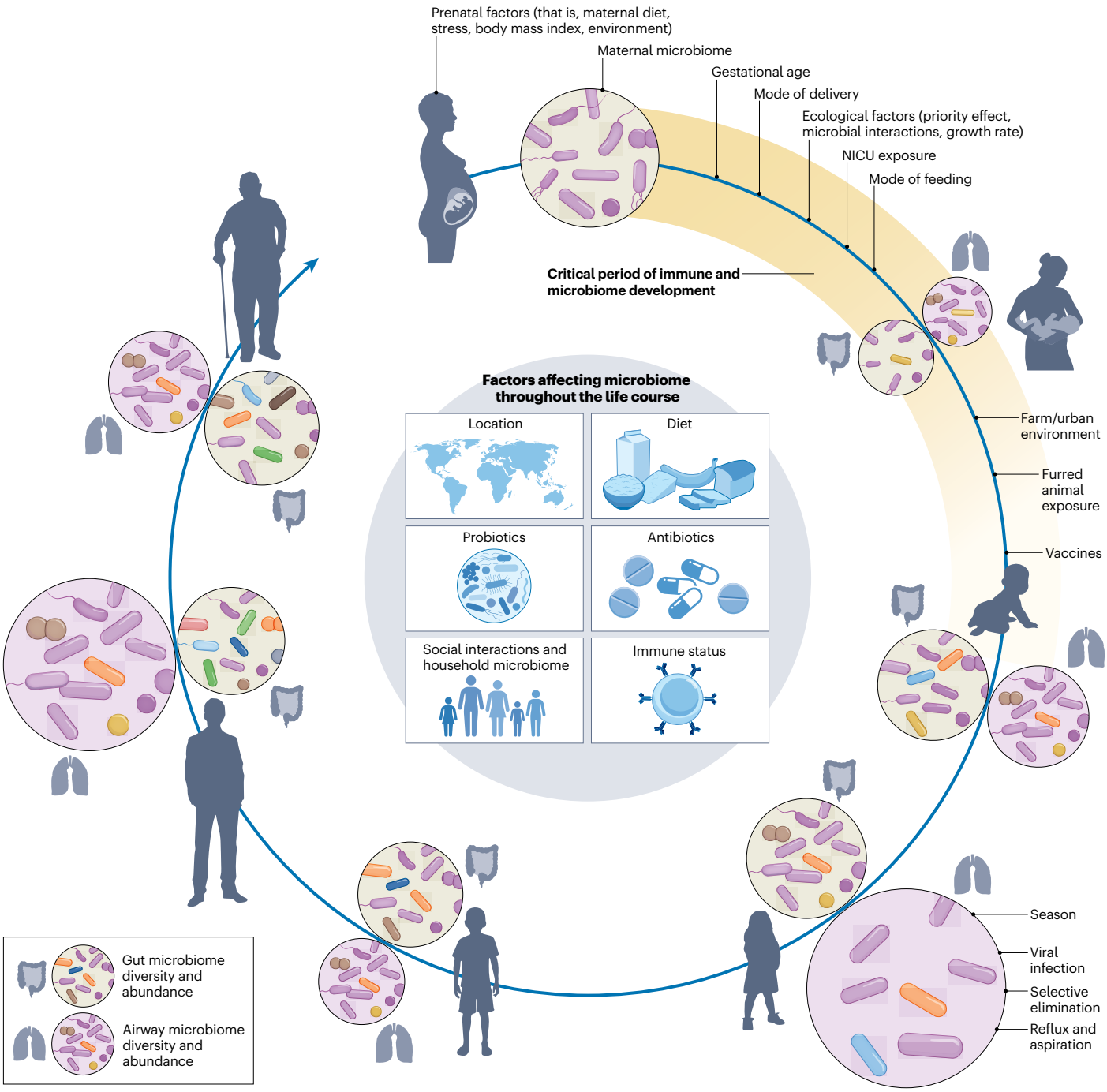


Fig. 2 | Factors affecting human gut and airway microbiome development through the lifespan. Environmental factors, including maternal stress, gestational age and mode of delivery, shape neonatal and early-life human microbiome composition and, by extension, immune development (yellow shading) during a critical 'window of opportunity' in early life. Ecological factors, including priority effect, which refers to the initial order or timing of microbial arrival to the niche in early life, microorganism–microorganism–host interactions and microbial growth rates also shape microbiome development. The gut microbiome (green shading) diversifies in early life and typically becomes phylogenetically stable by 3 years of age[16]. Microbial composition and abundance in the airways (purple shading) are dynamic throughout the lifespan and are affected by factors such as season, viral infection, reflux, aspiration and microbial competition. The functional evolution of the gut and airway microbiomes continues to be shaped by factors such as diet, location and antimicrobial environmental exposures throughout the life course, with consequences for health status. NICU, neonatal intensive care unit.

Mechanisms of gut–airway crosstalk

The microbiota plays an essential role in the education, development and function of the immune system, both locally and systemically. Several factors have been shown to exert their functions along the gut–lung axis, including systemic dissemination of microbial-derived immunomodulatory metabolites, extracellular vesicles and epigenetic modification of immune cells[43–45]. Probably, the best-studied example of microbial-derived immunoregulatory metabolites are SCFAs, which are amongst the most abundant products of gut microbiome metabolism and play an important role in immune modulation[46].

Diet has a substantial influence on shaping the gut microbiome[20]. Reciprocally, the pan-microbial gene pool, particularly that encoded by the distal gut microbiome, dictates the production of diet-derived microbial metabolites that modulate both local and remote immune function[47]. Notably, free-living mammals, such as wild mice, have heightened immune activation compared with laboratory mice, and laboratory mice colonized with pathogens exhibit immune profiles more closely resembling those of free-living animals[48].

Murine studies demonstrate that gut microbiome metabolic reprogramming mediated by oral introduction of *Lactobacillus johnsonii* to the gut provides protection against acute airway infection by respiratory syncytial virus (RSV)[49]. Airway protection mediated by *L. johnsonii* was characterized by reduced expression of type 2 cytokines IL-4, IL-5 and IL-13 by airway T helper cells, decreased activation of dendritic cells, and increased frequency of regulatory T (T_{reg}) cells. Notably, only animals supplemented with *L. johnsonii* exhibited the capacity to rapidly produce a broad range of anti-inflammatory lipids in response to airway RSV infection[49]. This indicates that oral administration of *L. johnsonii* led to changes in the capacity of the mouse gut microbiome to produce or stimulate the production of anti-inflammatory lipids, which downregulate the inflammatory response to viral infection and mitigate airway pathology. Moreover, this reprogramming of airway immune responses could be recapitulated in murine bone marrow-derived dendritic cells exposed to docosahexaenoic acid (a polyunsaturated fatty acid (PUFA) found to be highly enriched in the plasma of *L. johnsonii*-supplemented mice) prior to RSV infection[49]. In human populations, a large randomized clinical trial of prenatal supplementation with omega-3 long-chain PUFA and/or vitamin D resulted in supplement-associated microbiota changes in the infant airways at 1 month of age but not in the infant faecal or maternal vaginal microbiota, indicating that prenatally supplemented PUFAs exert their effect on microbial colonization primarily in the airways[50]. This latter observation points to immunometabolism, defined as the tuning of immune cell function by distinct programmes of cellular metabolism[51], as a strategy by which microorganisms plausibly shape systemic immunity and thus dictate the colonization landscape and microorganism–host interactions. Immunometabolism, which sits at the intersection of metabolic signalling and cell fate, offers a framework for how the gut microbiome, which irrevocably contributes to the local and systemic metabolic pool, may shape immune function both in the gut and in the distal airways.

Short-chain fatty acids

Probably the best-studied example of microbial-derived immunoregulatory metabolites are SCFAs, which are amongst the most abundant products of gut microbiome metabolism. Human population studies have provided evidence that both faecal and systemic SCFA concentrations directly relate to respiratory disease. For example, in 1-year-old children, elevated faecal concentrations of SCFAs, particularly propionate and butyrate, are associated with reduced risk for asthma development[52]. In adults, reductions in SCFA concentrations relate both to the presence and severity of COPD[53] and a reduced abundance of butyrate-producing bacteria has been associated with non-small-cell lung cancer[54] (Fig. 3).

Reduced abundance of fermentative microorganisms that produce airway-protective SCFAs is widely attributed to changing dietary habits and reduced consumption of complex plant polysaccharides, particularly in Western nations where the incidence of chronic inflammatory diseases is especially high[55]. Indeed, poor maternal diet during pregnancy is a risk factor for severe lower respiratory infections for the offspring in infancy[56]. Animal models have provided insights into the mechanisms by which a fibre-depleted diet promotes susceptibility to respiratory infection. Mice fed a low-fibre diet during pregnancy bore pups with enhanced lower respiratory infection severity due to delayed recruitment of plasmacytoid dendritic cells and perturbation of T_{reg} cell expansion in the lungs[57]. In this in vivo study, a low-fibre prenatal diet also altered the composition of the maternal milk microbiome, reduced secretion of the dendritic cell growth factor FMS-like tyrosine kinase 3 ligand (Flt3L) by neonatal intestinal epithelial cells, and impaired downstream plasmacytoid dendritic cell haematopoiesis. Supplementation with propionate-producing

Box 1

Technical challenges in studying the microbiome of the lower airway

While the upper airway microbiota is relatively accessible and easy to sample, specimen collection from the lower respiratory tract is challenging and typically based on bronchoalveolar lavage or expectorated sputum. Both lower airway sampling methods carry a high risk of cross-contamination by microorganisms in the oral cavity and upper respiratory tract[130]. However, several studies have shown that properly executed bronchoalveolar lavage sampling with protected specimen brushings is minimally impacted by microbial contamination from the upper respiratory tract[134,135].

Distal respiratory samples frequently harbour DNA signatures of anaerobic oral microorganisms, including *Prevotella* and *Veillonella* members, albeit at a much lower burden compared with that observed in the mouth or upper airways[136,137]. Because the oropharynx and the tracheobronchial tree are contiguous, concerns regarding oral contamination of lower airway samples are valid[138]. However, a key study evaluated the lung tissue microbiota of patients with mild to moderate chronic obstructive pulmonary disease using surgically collected lung samples. This study detected prototypical oral and nasal bacteria in the lung tissue, confirming aspiration as a primary source of lower airway microbial species[139]. Low concentrations of airway microbial nucleic acids also make microbiome analysis approaches (such as shotgun metagenomics or transcriptomics) challenging, hindering functional assessments of relationships between airway microbiomes and respiratory disease.

Box 2

Prenatal and early-life microbiome–immune system interactions shape respiratory health

The prenatal period lays the foundation for fetal immune development. In humans, fetal immune activation and memory development are evident by the second trimester, with a diversity of T cell effector-memory phenotypes observed in the fetal intestine[149]. Mechanisms underlying fetal immune development include transplacental transfer of immunoglobulins, cytokines, metabolites, extracellular vesicles and antigenic micro-chimaeric cells[150]. Studies of human fetal intestinal tissue indicate that encounters with viable microorganisms can occur in utero[151], which have been shown to promote transcriptional responses that include epithelial expression of antimicrobial genes[152] and memory T cell programming[149,153]. Longitudinal studies of lung function have demonstrated that children diagnosed with asthma by age 7 years exhibited significant airflow deficit as neonates, which ultimately progressed during early childhood. Thus, a large proportion (40%) of lung function deficit associated with asthma is present at birth[154], with additional decline occurring in early childhood. This has led to the hypothesis that development of pathogenic airway and/or gut microbiomes, primed in part by prenatal exposures, may be responsible for neonatal lung function deficits characteristic of this population.

Emerging evidence suggests that the trajectory leading to chronic childhood airway disease can begin during pregnancy, with an increased risk linked to exposures that disrupt the microbiome such as prenatal antimicrobial use, infection and inadequate nutrition[155]. Studies of mother–infant dyads provide evidence to suggest that maternal prenatal immune status relates to respiratory disease in the offspring[156]. Furthermore, maternal prenatal immune status was linked to specific methylation marks on cord blood mononuclear cells. The majority of these epigenetically modified loci are known to be antimicrobial response elements, and longitudinal sampling and profiling of the airway microbiota in these infants further revealed this deficit in neonatal antimicrobial immune function associated with altered development of the airway microbiome.

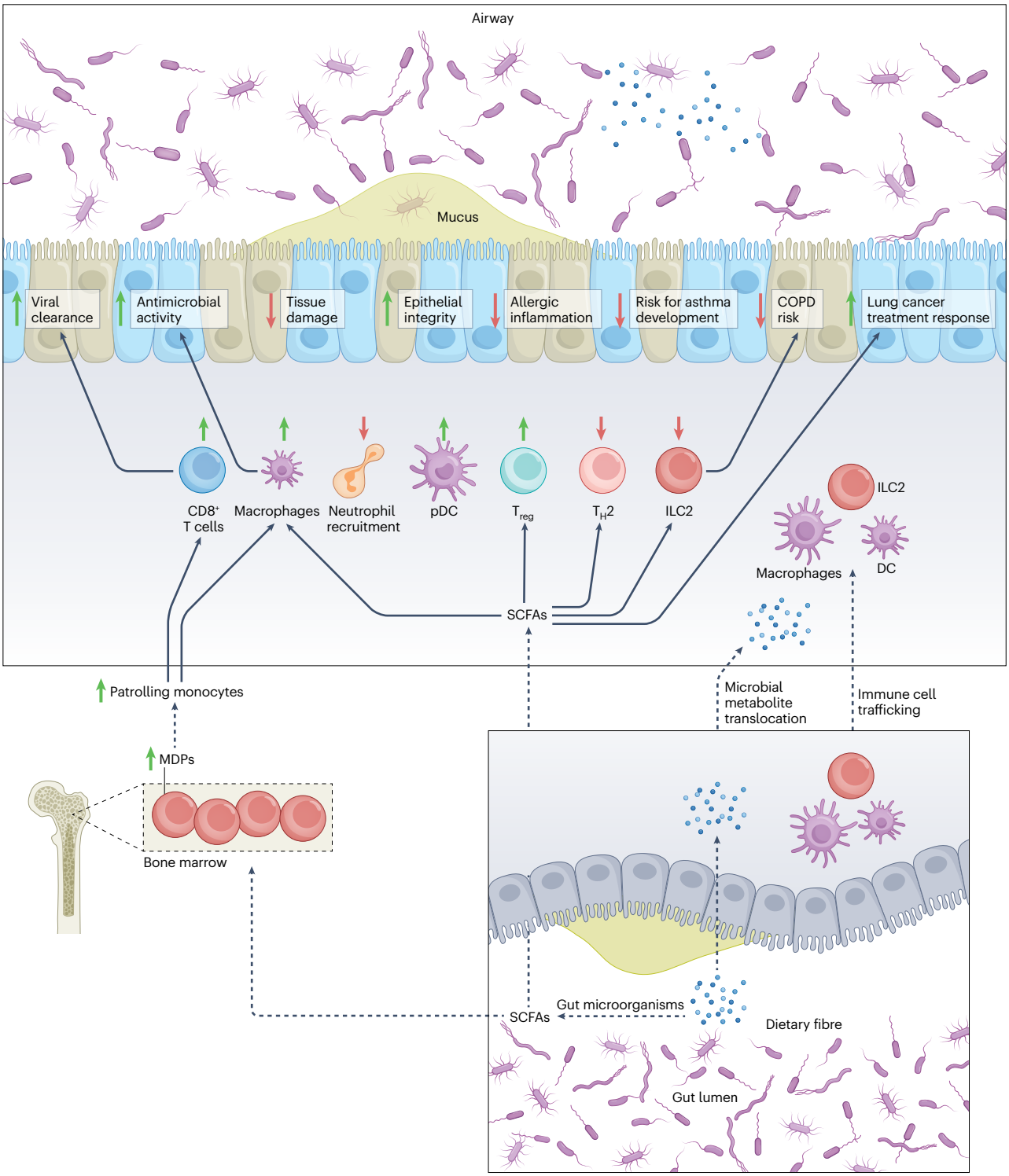
The interplay between microbial colonization in early life and trained immunity in the context of chronic respiratory disease has generated considerable interest. Maternal microbiomes are the primary source of neonatal microbial species[157]. Intergenerational microbial transmission may play a key role in chronic airway disease development in childhood, with most of the evidence coming from birth cohorts focused on allergy and asthma[31]. Neonates at high or low risk of asthma development exhibit distinct meconium microbiota and delayed gut microbiota diversification[158]. A recent study on mother–infant dyads demonstrated that the composition of the prenatal vaginal microbiota is related to maternal diet and stress levels and that the variance in heritable vaginal bacteria, that is, those shared between paired vaginal and infant stool samples, relates to both maternal and infant atopy status[31]. These data provide the first evidence indicating that inherited microbial functions transmitted from mother to child may shape early-life immune function and risk of airway disease in childhood, a framework that may well be applicable to additional childhood airway diseases. Moreover, the relationship between the gut microbiome and respiratory health is maintained throughout childhood and adult years, suggesting that prenatal and early postnatal microbial exposures and colonization events may shape longer-term respiratory health. Multiple studies demonstrated that the adult airway and gut microbiome relates to a range of respiratory conditions in adulthood, including chronic obstructive pulmonary disease[159], cystic fibrosis[108], SARS-CoV-2 severity[160], tuberculosis[161] and lung cancer[162].

bacteria isolated from the milk of high-fibre diet-fed mothers or with propionate alone restored Flt3L expression and plasmacytoid dendritic cell haematopoiesis in the gut, thus protecting against severe lower respiratory infections[57]. Additionally, propionate produced by the gut microbiota has demonstrated protective effects against lung injury induced by zinc oxide nanoparticles in an animal model of acute lung injury[58].

Similar observations have been reported in independent studies in which mice were fed with fermentable dietary fibre, resulting in protection against airway allergen challenge through the production of SCFAs[59] or viral influenza infection[60]. In the latter study, airway protection in mice was associated with increased numbers of lymphocyte antigen 6 complex ($Ly6c^-$) patrolling monocytes, which increased the frequency of alternatively activated macrophages. These macrophages exhibited limited ability for expressing chemokine (C-X-C motif) ligand 1 (CXCL1), a key factor controlling the recruitment of innate immune cells in the airways. Dampened CXCL1 production resulted in reduced airway neutrophil recruitment and tissue damage. In parallel, SCFAs produced by microbial fermentation activities in the gut increased $CD8^+$ T cell effector function against the viral pathogen by altering their cellular metabolism[60] (Fig. 3).

In a separate mouse model of acute exacerbation of COPD, treatment with oral butyrate reduced the proportion of inflammatory type 2 innate lymphoid cells in the colon and lung tissues, thereby ameliorating disease[61]. SCFAs translocate from the gut into the bloodstream, where they can reach distal organs[62]. There are limited reports of elevated concentrations of SCFAs in the airways, suggesting that circulating concentrations do not typically accumulate in airway tissue or are rapidly metabolized by airway cells. However, SCFAs have been detected in sputum samples of patients with cystic fibrosis in millimolar concentrations, where they positively correlate with sputum neutrophil count and impaired nitric oxide production[35].

Circulating SCFAs can also reach the bone marrow, where they can promote haematopoiesis and resolution of airway inflammation, as well as restoration of homeostasis in the lung[63]. This indicates that the airway-protective effect of SCFAs may be due more broadly to their



capacity to exert a systemic effect on immunometabolic tone. In mice, SCFAs maintain and reinforce intestinal epithelial integrity, reduce inflammation, and prevent pathogen invasion at both gastrointestinal and airway mucosal surfaces[64]. Animal studies showed that SCFAs also serve as a significant source of energy for colonocytes via β-oxidation and the tricarboxylic acid cycle[46], thus dictating the metabolic state of local and migratory immune cell populations. SCFAs can signal through GPCRs to activate anti-inflammatory signalling cascades[55] and, in the case of butyrate, suppress dendritic cell activation, increase Forkhead box P3 (FoxP3)$^{+}$ T_{reg} cell polarization, and promote epigenetic

Fig. 3 | Crosstalk between the lung and the gut microbiotas. Epithelial cells serve as a primary barrier in both the gut and airways. Secondary defences are facilitated via mucosal immunity scattered throughout the intestinal epithelium and lamina propria[80]. These defence mechanisms prevent microbial translocation to extraintestinal sites[80]. Gut microorganisms, gut microbial metabolites and immune cells trained in the gut environment can travel to the lungs and impact airway immunity. Short-chain fatty acids (SCFAs) are the best-studied microbial-derived immunomodulatory metabolites. SCFAs are produced by microbial metabolism of complex polysaccharides and have been shown to promote airway-protective phenotypes via modulation of both innate (neutrophils, macrophages and dendritic cells (DCs)) and adaptive (virus-specific $CD8^+$ T cells, regulatory T (T_{reg}) cells and T helper 2 (T_H2) cells) immune cells and by programming bone marrow-derived cell populations. SCFA-mediated immune modulation includes enhanced antimicrobial activity, viral clearance, maintenance of epithelial integrity, and the mitigation of tissue damage and allergic inflammation within the airways. Studies on human populations reveal an association between elevated SCFA concentrations and a decreased risk of developing asthma or chronic obstructive pulmonary disease (COPD) as well as positive outcomes in lung cancer treatment. In the graphical representation, black solid arrows symbolize the impact of SCFAs on immune cells and airway physiology, while dashed arrows indicate the translocation of SCFAs, microbial metabolites and immune cells to distant organs, including the lungs and bone marrow. ILC2, type 2 innate lymphoid cells; MDPs, monocyte-dendritic cell progenitors; pDC, plasmacytoid dendritic cells.

modifications that promote FoxP3 expression[65]. SCFA-induced immune modulation may also be detrimental in the case of infection. An increase in serum SCFAs has been associated with increased *Mycobacterium tuberculosis* susceptibility in a human cohort of individuals with HIV infection. In vitro, in response to *M. tuberculosis* antigen stimulation, SCFA induced interferon-γ (IFNγ) and IL-17A production in peripheral blood mononuclear cells isolated from individuals with HIV infection and undergoing antiretroviral therapy. The presence of SCFAs in the lungs was correlated with increased oral anaerobes, such as *Prevotella* spp., and with T_{reg} cells triggered by *M. tuberculosis* antigens[66].

Linoleic acid metabolites

As the field progresses, a growing number of additional gut microbial-derived metabolites have been shown to exert an effect on airway immunity. The linoleic acid metabolite dihomo-γ-linoleate is found in elevated concentrations in the faeces of infants at low risk of developing allergy and asthma in later childhood. Conversely, infants at high risk (who possess a distinct gut microbiota composition from those at lower risk) exhibit elevated faecal concentrations of an alternate linoleic acid metabolite 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME)[30]. This indicates that microbial metabolic activities in the nascent gut microbiome dictate the fate of linoleic acid metabolism. Shotgun metagenomic studies have confirmed that 12,13-diHOME can be produced by epoxide hydrolase-encoding strains of *Enterococcus faecalis* and *Bifidobacterium bifidium* in the infant gut. Significantly increased copy numbers of bacterial epoxide hydrolases with the demonstrated capacity to produce 12,13-diHOME have been observed in infants who subsequently develop atopy or allergy in childhood[67]. In vivo, peritoneal delivery of 12,13-diHOME or oral supplementation with an *Escherichia coli* strain engineered to produce this lipid exacerbates allergic inflammation in the airways. Airway inflammation mediated by 12,13-diHOME is characterized by decreased frequency of T_{reg} cells expressing IL-10 in the airways and increasing circulating immunoglobulin E (IgE) levels in mice[67]. Linoleic acid, an omega-6 PUFA, has become highly prevalent in Western diets[68], suggesting that increased substrate availability in the context of a dysfunctional gut microbiome leads to the production of lipid mediators in the gastrointestinal tract that exacerbate airway inflammation. Linoleic acid can also be metabolized to produce arachidonic acid (a substrate for both cyclooxygenase and lipoxygenase enzymes that produce the eicosanoid prostaglandins), thromboxanes and leukotrienes, all of which are involved in the pathophysiology of allergic sensitization and asthma[69]. As a lipid class, linoleic acid metabolites are emerging as a potent group of gut microbial-derived metabolites that act along the gut–airway axis to regulate airway immunity. Though multiple isomers of linoleic acid exist, the predominant metabolic product produced by healthy human intestinal *Bifidobacteria*, *Clostridium* or *Propionibacterium* species is conjugated linoleic acid (CLA)[70]. Mice fed CLA exhibit significantly reduced airway hyperresponsiveness induced by allergens, along with reduced concentrations of IL-5 and a decreased number of eosinophils in bronchoalveolar lavage fluid[71]. In adult individuals who are allergic to birch, oral supplementation with CLA resulted in improved airway clinical symptoms and reduced plasma levels of granulocyte–macrophage colony-stimulating factor and eosinophil-derived neurotoxin concentrations[72]. Thus, emerging evidence indicates that strain-level gut microbial metabolism of dietary substrates, particularly those that are in excess in Westernized diets and whose metabolic products are known to influence airway physiological and immunological responses, play a role in the regulation of airway response to both antigenic and pathogenic encounters.

Epigenetic mechanisms

Microbial-derived metabolites, particularly SCFAs, also shape epigenetic marks, thus influencing transcriptional activity and the function of immune cell populations. For example, when macrophages are exposed to butyrate, they exhibit enhanced antimicrobial activity, particularly due to antimicrobial peptide production. This is associated with both a shift in macrophage metabolism and the inhibition of histone deacetylase 3 (HDAC3)[73], which regulates epigenetic modifications via chromatin remodelling[74]. In a large human study, children at the highest risk of asthma development possessed a module of cord blood mononuclear cell methylation marks, enriched for antimicrobial response elements. Consistent with these epigenetic modifications, cord blood mononuclear cells from these neonates were significantly diminished in their capacity to respond to lipopolysaccharide stimulation and exhibited a distinct airway microbiota developmental trajectory over the first 3 years of life. Airway microbiota in these children was characterized by early colonization with *Haemophilus* spp. followed by the later establishment of members of the *Moraxella* genus[15]. This pattern of postnatal bacterial colonization has been associated with the subsequent development of childhood asthma in several studies[15,33]. Independently, in older children diagnosed with asthma, *Haemophilus*-dominated or *Moraxella*-dominated airway microbiota also associate with a higher frequency of pulmonary exacerbation[9,75]. Thus, epigenetic modifications at birth, particularly those in antimicrobial response elements, may shape the microbial colonization landscape in early development, leading to long-term consequences for respiratory health. An independent study provided further support for this concept by demonstrating that upper airway microbial composition in infancy was associated with the development of allergic

rhinitis in childhood and that this association was mediated, at least in part, through altered DNA methylation patterns in upper airway mucosal cells[45].

Extracellular vesicles

Beyond the products of microbial metabolism, microbial cellular constituents can also travel along the gut–airway axis to influence airway biology. Extracellular vesicles, which are secreted from the majority of cell types (microbial and human) examined to date[44], facilitate cell–cell communication and can be detected in the circulation[76]. Extracellular vesicles carry cargo, including microbial pathogen-associated molecular patterns such as cell wall components, lipoproteins, carbohydrates or nucleic acids[77]. Recognized by pattern recognition receptors on immune cells, pathogen-associated molecular patterns promote immune cell activation and release of inflammatory mediators. Microbial extracellular vesicles, more commonly termed membrane vesicles or outer membrane vesicles, are detectable in body fluids and are related to airway diseases. For example, membrane vesicles produced by *Staphylococcus aureus* induce neutrophilic inflammation in the airways via T helper 1 and T helper 17 cell responses[78], while those produced by *Akkermansia muciniphila* strains in the gut microbiome promote epithelial barrier integrity through the upregulation of occludin expression[79]. α-Galactosyl ceramide, a glycolipid that can be produced by a strain of the the gut bacterium *Bacteroides fragilis*[80], has been shown to mitigate gut bacterial translocation to the mesenteric lymph nodes, restore tight junction protein expression, and attenuate gut and lung injury through upregulation of IL-4 and IL-10 in an animal model of gut ischaemia–reperfusion injury[81].

Gut and airway microbiomes in respiratory viral infections

Respiratory viral infections, including Influenza A virus (IAV), RSV and coronavirus disease (SARS-CoV-2), can induce gastroenteritis-like symptoms, leading to disrupted gut microbiome composition and metabolic productivity. Perturbed gut microbiomes are, in turn, associated with susceptibility to respiratory infection supporting a bidirectional communication system between these two mucosal sites that governs antiviral immunity[43,82].

Gut microbiome perturbation due to, for example, antibiotic usage, can distally influence airway immunity during respiratory viral infections. Several in vivo and human clinical studies have now shown that antibiotic-mediated gut microbiota disturbance increases the severity of viral respiratory infections such as influenza[43]. Antibiotic treatment impaired both innate and adaptive antiviral immune responses in mice, which substantially delayed viral clearance following respiratory infection[83]. Genome-wide transcriptional profiling of macrophages isolated from antibiotic-treated mice exhibited reduced expression of genes associated with antiviral immunity, including defective type I and type II interferon responses and impaired capacity to limit viral replication[84]. Additional studies have highlighted the role of the gut microbiota on the production of type I interferons by the lungs[85] that also control other respiratory viral infections, including SARS-CoV-2 (refs. 86,87).

Animal studies have shown that gut commensals and their metabolites regulate antiviral immunity at the respiratory mucosa via distinct mechanisms, including the generation of virus-specific $CD4^+$ and $CD8^+$ T cells and antibody responses following respiratory virus infection[83]. For example, the gut microbial-derived metabolite desaminotyrosine, produced through flavonoid metabolism, promotes protective airway innate immunity against influenza virus infection by enhancing the interferon-regulated responses of pulmonary phagocytes, which are key to viral clearance[88]. Independently, it has been demonstrated that gut microbiome expansion of *Bifidobacterium animalis* is associated with improved survival following respiratory IAV infection in mice[89]. Faecal microbiota transplantation (FMT) experiments have demonstrated that the gut microbiota from mice that survived IVA infection provided protection to recipient antimicrobial-treated or germ-free animals infected with the virus[89]. Moreover, supplementation of animals with *B. animalis* alone was sufficient to protect mice against IAV infection. Airway protection was associated with alterations to carbohydrate, lipid, energy, nucleotide, and amino acid metabolism and, more specifically, with the capacity of *B. animalis* to synthesize valine, isoleucine, lysine and coenzyme A. Oral supplementation with valine or intraperitoneal injection of coenzyme A also protected mice against respiratory IAV infection[89], providing further support that the severity of IAV infection relates to the metabolic capacity of the gut microbiota. Indeed, a diet rich in fibre reduced viral load and pulmonary inflammation in mice infected with RSV, an effect that is dependent, at least in part, on the production of acetate by the intestinal microbiota. Acetate diffuses into the circulation and activates the GPCR GPR43 expressed by stromal cells, which augments type I interferon antiviral responses[85]. Type I interferon signalling via the IFN1 receptor was found to be essential for acetate antiviral activity in pulmonary epithelial cell lines and for the protective effect of acetate in RSV-infected mice. Activation of GPR43 in pulmonary epithelial cells reduced virus-induced cytotoxicity and promoted antiviral effects via an IFNβ response[85,90].

Due to the global pandemic, SARS-CoV-2 has come to the forefront of acute respiratory infections and has prompted intense research efforts into its pathology both in the airways and the gastrointestinal tract[91]. SARS-CoV-2 was detected in rectal swabs in approximately 80% of patients, even after their nasopharyngeal samples were virus negative, suggesting that the distal gut represents an extrapulmonary site for viral persistence[92]. Studies in small intestinal organoids showed that SARS-CoV-2 replicates in enterocytes[93]. Associations between gut microbiome composition and concentrations of inflammatory markers in patients with SARS-CoV-2 infection indicate that, like for influenza severity, the modulation of host immunity by the gut microbiome influences SARS-CoV-2 clinical outcomes[94]. In human studies, SARS-CoV-2 infection has been shown to alter the abundance of key immunomodulatory gut microorganisms, including *Enterococcus faecium*, *Roseburia intestinalis* and *A. muciniphila*, with parallel changes to gut barrier integrity[95,96]. Genome-resolved metagenomic analysis of faecal samples identified 33 metagenome-assembled genomes that showed differential distribution across patient groups with mild, moderate, and severe or critical SARS-CoV-2 infection[97]. The gut microbiome of patients with SARS-CoV-2 infection presented an impaired capacity for SCFA and L-isoleucine biosynthesis that was sustained even after the resolution of infection. These microbial functions significantly correlated with the host immune response and the severity and outcome of respiratory viral infection[98]. Mouse models of SARS-CoV-2 have confirmed that airway infection alters the expression of immune-related and infection-related genes in gut epithelial cells[99]. In mice, airway infection with SARS-CoV-2 induced perturbation of the gut microbiome and was associated with decreased numbers of Paneth cells, increased markers of intestinal epithelial barrier permeability and with goblet cell hyperplasia. Perturbations to the gut microbiome caused by airway infection resulted in the proliferation of opportunistic

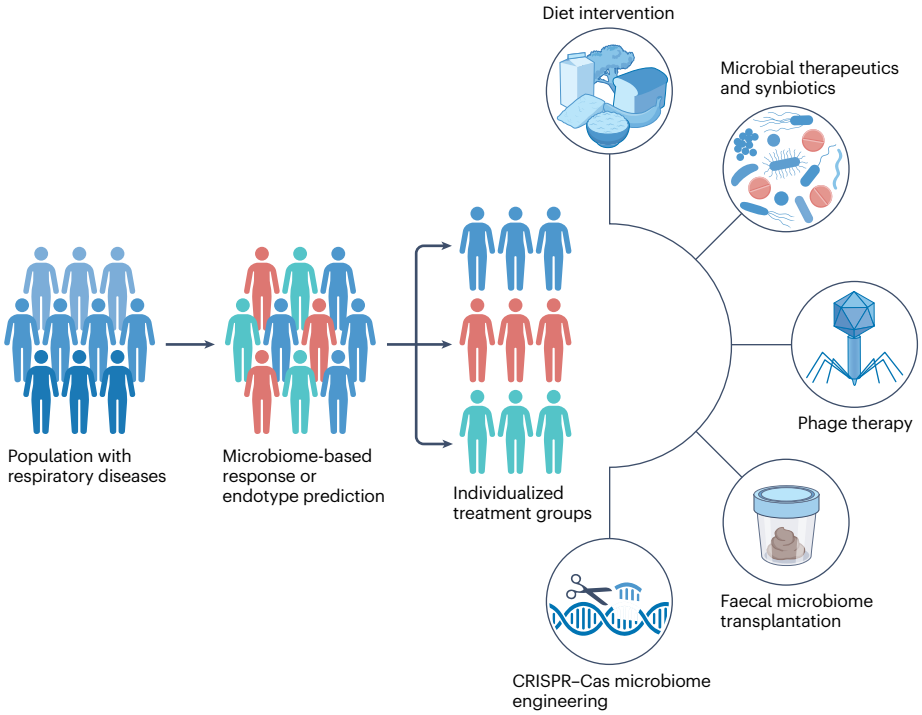


Fig. 4 | The future of microbiome medicine. Endotype discovery is a relatively new concept in respiratory diseases and refers to the identification of distinct subtypes of disease based on underlying biological mechanisms rather than just on the observable clinical symptoms. Disease endotypes can help tailor treatment approaches to specific patient groups with the goal of improving outcomes. As human microbiome studies become larger, the capacity to employ artificial intelligence and deep learning approaches to identify microbial functional features that predict or differentiate respiratory clinical outcomes is ongoing. Coupling such microbiome-based patient stratification methods with a range of microbiome manipulation technologies, like precision dietary interventions, live cellular microbial therapeutics, CRISPR–Cas-mediated microbiome engineering, phage therapy and faecal microbiota transplantation, represents a major focus of the field of gut–airway microbiome research.

bacterial pathogens, with evidence pointing at bacterial translocation into the systemic circulation and the occurrence of bacteraemia in severe cases[100].

Therapeutic interventions

Analysing the gut and respiratory microbiomes, particularly in the context of immune function and clinical data, offers an opportunity to identify distinct patient subsets and/or respiratory disease endotypes. Identifying disease endotypes is crucial for personalized medicine and for the development of targeted and more effective interventions tailored to the specific pathogenic mechanisms at play within a patient or group of patients. This knowledge becomes instrumental in the context of microbiome-based therapeutic interventions targeting the gut–lung axis. Emerging evidence for the gut–airway axis has led to therapeutic interventions aimed at leveraging microbiome manipulation to improve respiratory health (Fig. 4). To date, these approaches have largely focused on the manipulation of the gut microbiome and have included dietary as well as live microbial supplementation strategies. In mouse models, modulation of the gut microbiome through either live microbial supplementation or dietary interventions was shown to protect the airways against allergen challenge or viral respiratory infection[49,60,101]. These studies also provided evidence for intergenerational airway protection; prenatal treatment of mice with viable microorganisms, like *L. johnsonii* or *Helicobacter pylori*, promoted airway protection in the offspring, mediated in part, by the capacity to induce T_{reg} cell responses following respiratory microbial pathogen challenge[102,103]. In a 3-month-long, randomized, double-blind, placebo-controlled trial involving 55 adult patients with asthma, daily oral supplementation with *Bifidobacterium lactis* (as an adjuvant to inhaled corticosteroid treatment) was associated with significant reductions in fractional exhaled nitric oxide concentrations and significant improvement in asthma control test scores compared with the placebo arm of the trial[104]. While the intervention did not lead to significant reductions in circulating IgE, it increased serum concentrations of 5-dodecenoic acid[105] (a lipid with inhibitory activity against cyclooxygenase I and II) and tryptophan (a potent immunomodulatory amino acid whose metabolic products are implicated in allergic asthma)[106,107]. In children with cystic fibrosis, daily oral supplementation for 12 months with the commercially available probiotic species *Lactobacillus rhamnosus* GG was associated with a reduced rate of pulmonary exacerbations, improved pulmonary function, lower intestinal inflammation and fewer antibiotic administrations. However, these positive outcomes were observed only in those patients who, following *L. rhamnosus* GG supplementation, developed a gut microbiome dominated by *Bifidobacteria* (NCT01956916)[108,109].

FMT represents the opposite end of the microbial reconstitution spectrum. A small number of FMT trials aimed at treating respiratory diseases are ongoing. These include FMT to potentiate anti-PDL1 efficacy in lung cancer (NCT05502913 (ref. 110), NCT04924374 (ref. 111) and NCT04951583 (ref. 112)) (Box 3), to treat pulmonary sarcoidosis (a systemic inflammatory disease characterized by the enlargement of lymph nodes and formation of granulomas; NCT04924270 (ref. 113)) or to improve systemic inflammatory tone in patients with SARS-CoV-2 infection (NCT05873348 (ref. 114)). It remains to be determined how these interventions influence respiratory health and if these effects are sustainable. However, preclinical animal studies provide promising findings; FMT and a high-fibre diet were observed to attenuate the development of emphysema, a lung disease induced by cigarette smoking, by suppressing inflammation and apoptosis[115].

Additional efforts to manipulate the microbiome focus on the development of defined, multi-species live microbial therapeutic consortia. The aim of these is to reintroduce species and, more importantly, functional traits that have been eroded from perturbed microbiomes to

Box 3

Gut and lung microbiome association with ICI treatment response in lung cancer

Mounting evidence supports a role for both airway and gut microbiomes in lung cancer (extensively reviewed in ref. 140) and in treatment outcomes. Immune-checkpoint inhibitors (ICIs) targeting PD1 and PDL1 induce remission in a proportion of patients with lung cancer[141], and there is evidence to suggest that the microbiome affects expression of immune-checkpoint molecules[142]. Patients who are non-responsive to ICIs exhibit a distinct gut microbiome composition to that of patients responsive to treatment[143]. Furthermore, antibiotic treatment was shown to inhibit ICI efficacy, suggesting a role of the gut microbiome in potentiating the efficacy of ICI immunotherapy[144]. In vivo studies using animal models have demonstrated that faecal microbiome transplantation using faeces from ICI-responsive patients but not from patients who are non-responsive to ICIs ameliorates the antitumour effect of PD1 blockage in recipient animals[145]. The microbiome from patients responsive to treatment had a higher relative abundance of ileal *Akkermansia muciniphila*, which restored PD1 blockage efficacy in an IL-12-dependent manner and by increasing infiltration by $CCR9^{+}CXCR3^{+}CD4^{+}$ T lymphocytes into mouse tumour beds. However, the specific mechanisms by which *A. muciniphila* elicits this effect on antitumour immunity remain unclear[145]. In a separate PD1 blockage study, patients responsive to treatment harboured a higher faecal microbiome diversity prior to treatment and exhibited stable microbiota composition during the treatment. The lung microbiota has also been shown to induce inflammation associated with lung adenocarcinoma by activating lung-resident γδT cells[146]. Germ-free or antibiotic-treated mice are protected from lung cancer development whereas, in specific-pathogen-free mice, the presence of airway microorganisms stimulated inflammation through myeloid differentiation primary response 88 (MyD88)-dependent IL-1β and IL-23 production from myeloid cells. This prompts proliferation and activation of γδT cells that produce IL-17, leading to tumour cell proliferation[146]. Recently, patients with higher faecal microbiome diversity were found to experience prolonged progression-free survival — a length of time during and after a cancer treatment during which the disease does not worsen — compared with those who evidenced lower diversity[147]. Gut microbiota composition and functionality after the initiation of concurrent chemoradiotherapy have also been associated with progression-free survival in patients with non-small-cell lung cancer[148]. In the past few years, the microbiome has emerged as a critical component in immunotherapies and cancer management. The efficacy of immunotherapy and chemotherapy has been shown to be influenced by antibiotic administration during ICI treatment. Despite these many studies demonstrating significant associations between microbiome and lung cancer treatment outcomes, the molecular mechanisms linking the microbiota, host immunity and malignancies remain to be elucidated. Such insights could lead to the development of microbiome-based adjuvant therapies, increased efficacy and reduced health-care costs.

promote airway health. Again, this field is nascent, and a limited number of trials are ongoing. Nonetheless, these highly controlled human interventional trials in patient populations with respiratory disease offer the opportunity to better understand microbiome features and functional mechanisms that associate with treatment efficacy. Such efforts would also warrant the development of microbial prognostics to identify, prior to treatment, individuals for whom the intervention is efficacious, moving the field towards precision microbiome medicine.

Nutritional interventions for respiratory diseases, such as COPD and asthma, have generated mixed results[116,117]. Different trials have attempted to address the effect of specific nutritional supplements to improve respiratory health. For example, prenatal vitamin D or PUFA supplementation reduced the incidence of childhood croup (NCT00798226)[118,119]. Nutraceuticals are referred to as food or food-derived elements with substantial health benefits beyond nutrition. Those that are enriched with antioxidant and anti-inflammatory properties, and when consumed as part of a balanced diet, are associated with improved pulmonary function, reduced lung function decline and reduced risk of COPD[117]. However, the varied results of such interventions may be explained, at least in part, by inter-personal variation in microbiome functional traits, particularly the capacity to metabolize dietary supplements to produce respiratory protective metabolites. Mechanistic studies in humans and mice have demonstrated that the response to dietary or microbial interventions is, in large part, dictated by the pre-existing intestinal microbiome[13,47]. These observations have prompted the emergence of the precision microbiomics field, which leverages functional features of the gut microbiome to predict responses to specific dietary constituents, with the ultimate goal of generating diets and interventions tailored to individual microbiomes[120].

Bacterial CRISPR–Cas and other genome editing systems are distributed widely amongst microorganisms[121] and have revolutionized the capacity to precisely engineer genomes. CRISPR–Cas-edited haematopoietic stem and progenitor cells, engineered to disrupt the gene promoters that promote sickle cell disease (a genetic disorder characterized by abnormal haemoglobin in erythrocytes), have been recently successfully used in a first-in-human clinical trial to treat the condition (NCT04443907)[122,123]. Additional engineered cell types have also shown promise in human trials[124] and include chimaeric antigen receptor or T cell receptor engineered T cells for cancer malignancies, haematopoietic stem cells involved in leukaemia and autoimmune deficiency syndrome, and induced pluripotent stem cells to treat diabetes. The early success of precision cellular engineering interventions to treat human disease opens the possibility of expanding this technology to engineer traits in or out of human microbiomes. CRISPR–Cas technology has been used to successfully engineer microbial cells, including bacterial and fungal species that have traditionally been reticent to genome engineering[125]. Moreover, microorganisms — both bacterial

and viral species — are being leveraged as delivery systems for CRISPR–Cas cargo, including phage-based systems to target intracellular pathogens[126,127], a common trait amongst bacterial respiratory pathogens[128]. Animal models indicate that specific genes in microbial species can be engineered in situ in complex mammalian microbiomes. For example, phage-delivered CRISPR–Cas9-mediated depletion of a target fluorescent gene in *E. coli* strains in the gut microbiome successfully resulted in gene excision, even under conditions of competitive colonization with isogenic non-fluorescent *E. coli* strains[129]. Though nascent, the potential for precision engineering of gut and airway microbiomes, particularly in the context of chronic respiratory disease, is substantial. Additionally, there is immense opportunity to understand the contribution of specific microbial genes within complex microbiomes directly in their natural environments and how such genes impact host physiological features or disease outcomes. Equally important, the opportunity to tailor the technology to precision engineer specific microbial traits within patient microbiomes and adapt such interventions on a patient-specific basis is highly appealing.

Conclusions and outlook

Increasing evidence that both gut and airway microbiomes interactively regulate respiratory health has led to novel insights into the molecular and cellular mechanisms by which this crosstalk is facilitated. Identification of gut microbial-derived metabolites that shape immune tone both locally and systemically and the increasing recognition that gut microbiome features relate to airway disease treatment outcomes offer the possibility to develop novel microbial prognostics, diagnostics and interventions for a range of respiratory conditions. While the field holds great promise, there is much more work to be done to understand the implications of such developments across heterogeneous patient groups.

Published online: 22 May 2024

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Acknowledgements

This work was supported by S.V.L.'s research programme, which is funded by the National Institutes of Health/National Institute of Allergy and Infectious Disease awards AI128482, AI148104, UG3OD023282 and AI089473 and by Lyda Hill Philanthropies, Acton Family Giving, the Valhalla Foundation, Hastings/Quillin Fund — an advised fund of the Silicon Valley Community Foundation, the CH Foundation, Laura and Gary Lauder and Family, the Sea Grape Foundation, the Emerson Collective, Mike Schroepfer and Erin Hoffman Family Fund — an advised fund of the Silicon Valley Community Foundation and the Anne Wojcicki Foundation through the Audacious Project at the Innovative Genomics Institute, Berkeley California. M.Ö. is supported by postdoctoral T32 fellowship 2T32DK007762-46. The authors thank H. Steininger, M. Bacino and S. Vylkova for their internal review of this article.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

S.V.L. is a board member and consultant for the biotechnology company Siolta Therapeutics, Inc. and holds stock in the company. She also consults for Sanofi. M.Ö. is supported in part by NIH Training Grant T32-DK007762.

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Peer review information *Nature Reviews Microbiology* thanks Benjamin Wu and Michael Combs for their contribution to the peer review of this work.

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