

The microbiota of the respiratory tract: gatekeeper to respiratory health

Wing Ho Man^{1,2*}, Wouter A.A. de Steenhuijsen Piters^{1,3*} and Debby Bogaert^{1,3}

Abstract | The respiratory tract is a complex organ system that is responsible for the exchange of oxygen and carbon dioxide. The human respiratory tract spans from the nostrils to the lung alveoli and is inhabited by niche-specific communities of bacteria. The microbiota of the respiratory tract probably acts as a gatekeeper that provides resistance to colonization by respiratory pathogens. The respiratory microbiota might also be involved in the maturation and maintenance of homeostasis of respiratory physiology and immunity. The ecological and environmental factors that direct the development of microbial communities in the respiratory tract and how these communities affect respiratory health are the focus of current research. Concurrently, the functions of the microbiome of the upper and lower respiratory tract in the physiology of the human host are being studied in detail. In this Review, we will discuss the epidemiological, biological and functional evidence that support the physiological role of the respiratory microbiota in the maintenance of human health.

Microbiota

The microorganisms (including bacteria, archaea and single-celled eukaryotes) and viruses that inhabit a particular niche.

Anterior nares

Openings in the nose that connect the external environment and the nasal cavity.

¹Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, Lundlaan 6, 3584 EA Utrecht, The Netherlands.

²Spaarnse Gasthuis Academy, Spaarnepoort 1, 2134 TM, Hoofddorp, The Netherlands.

³The University of Edinburgh/ MRC Centre for Inflammation Research, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK.

*These authors contributed equally to this work.

Correspondence to D.B. D.Bogaert@ed.ac.uk

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Microbial communities have co-evolved with humans and our ancestors for millions of years and they inhabit all surfaces of the human body, including the respiratory tract mucosa. Specific sites in the respiratory tract contain specialized bacterial communities that are thought to have a major role in the maintenance of human health. In the past decade, next-generation sequencing has led to major advances in our understanding of the possible functions of the resident microbiota. So far, research has largely focused on the gut microbiota and gut microbiota-derived metabolites, and their influence on host metabolism and immunity. However, recent studies on microbial ecosystems at other body sites, including the respiratory tract, reveal an even broader role for the microbiota in human health¹.

The respiratory tract is a complex organ system that is divided into the upper respiratory tract (URT) and the lower respiratory tract (LRT). The URT includes the anterior nares, nasal passages, paranasal sinuses, the nasopharynx and oropharynx, and the portion of the larynx above the vocal cords, whereas the LRT includes the portion of the larynx below the vocal cords, the trachea, smaller airways (that is, bronchi and bronchioli) and alveoli. The primary function of the respiratory tract in human physiology is the exchange of oxygen and carbon dioxide. For this purpose, the adult human airways have a surface area of approximately 70 m², which is 40 times larger than the surface area of the skin². This entire surface is inhabited by niche-specific bacterial communities, with the highest bacterial densities observed in the URT (FIG. 1). Over the years, evidence for the roles that

bacterial communities in the URT have in preventing respiratory pathogens from establishing an infection on the mucosal surface and spreading to the LRT has accumulated. For most respiratory bacterial pathogens, colonization of the URT is a necessary first step before causing an upper, lower or disseminated respiratory infection³. Inhibition of this first step of pathogenesis for respiratory infections by the resident microbiota, a process that is also called 'colonization resistance', might be of paramount importance to respiratory health. Furthermore, if a pathogen has colonized the mucosal surface, it might be beneficial to both the microbial community and the host that these pathogens are kept at bay, preventing their overgrowth, inflammation and subsequent local or systemic spread⁴. In addition to this symbiotic relationship, the respiratory microbiota probably has a role in the structural maturation of the respiratory tract⁵ and in shaping local immunity^{6,7}.

Current research questions address how the healthy respiratory microbiota is established and what ecological and environmental factors govern its development. Concurrently, the broad range of functions of the respiratory microbiome is starting to become clear. In this Review, we focus on the role that the respiratory microbiota has in the development and maintenance of human respiratory health.

Anatomical development and the microbiota

Anatomical development and physiology. The development of the structures of the human respiratory tract is

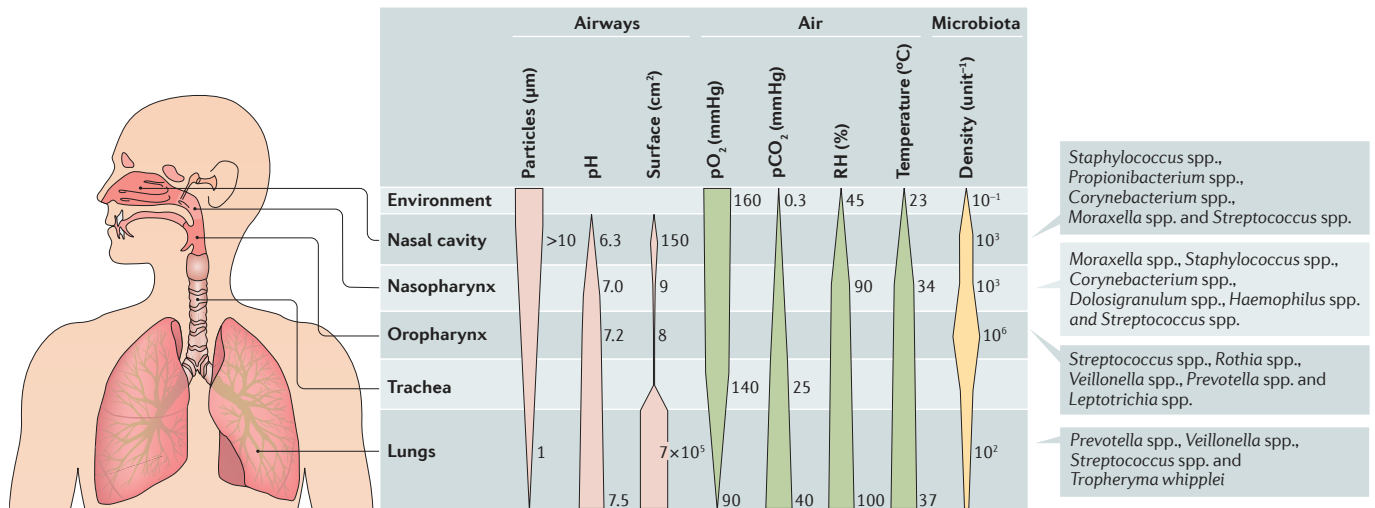


Figure 1 | Physiological and microbial gradients along the respiratory tract. Physiological and microbial gradients exist along the nasal cavity, nasopharynx, oropharynx, trachea and the lungs. The pH gradually increases along the respiratory tract^{177–180}, whereas most of the increases in relative humidity (RH) and temperature occur in the nasal cavity^{181–183}. Furthermore, the partial pressures of oxygen (pO₂) and carbon dioxide (pCO₂) have opposing gradients¹⁸⁰ that are determined by environmental air conditions and gas exchange at the surface of the lungs^{181,184,185}. Inhalation results in the deposition of particles from the environment into the respiratory tract; inhaled particles that are more than 10 µm in diameter are deposited in the upper respiratory tract (URT), whereas particles less than 1 µm in diameter can reach the lungs. These particles include bacteria-containing and virus-containing particles, which are typically larger than 0.4 µm in diameter¹⁸⁶. These physiological parameters determine the niche-specific selective growth conditions that ultimately shape the microbial communities along the respiratory tract. The unit by which bacterial density is measured varies per niche; the density in the environment is depicted as bacteria per cm³ (indoor) air¹⁸⁷, density measures in the nasal cavity and nasopharynx are shown as an estimated number of bacteria per nasal swab⁷⁴, and the densities in the oropharynx and the lungs represent the estimated number of bacteria per ml of oral wash^{57,74} or bronchoalveolar lavage (BAL)^{57,74,153}, respectively.

Colonization

The act of settlement and reproduction of organisms that are subject to selective pressure.

Symbiotic relationship

A close biological interaction between two different species.

Microbiome

All of the genetic content of a microbial community.

Nasal placodes

A thickening of the embryonic head ectoderm that occurs in the fifth embryonic week and marks the start of the formation of the nose and nasal cavity.

Oropharyngeal membrane

A transient bilaminar (ectoderm and endoderm) membrane that appears in the fourth embryonic week during the development of the primitive mouth and pharynx.

Lung buds

A pair of endodermal outgrowths of the foregut that develop into the larynx, trachea and lungs.

Nasopharynx-associated lymphoid tissue

(NALT). One of the anatomical locations of mucosa-associated lymphoid tissue (MALT), which, in humans, consists of the lymphoid tissue of Waldeyer’s pharyngeal ring, including the adenoids (the unpaired nasopharyngeal tonsil) and the paired palatine tonsils.

a complex multistage process that begins in the fourth week of gestation with the development of the nasal placodes, the oropharyngeal membrane and the lung buds^{8,9}. The anatomy of the URT at birth is substantially different from the configuration in adults owing to the higher position of the larynx, which results in a large nasopharynx relative to the oropharynx¹⁰. In addition, the lack of alveoli in the newborn lungs underlines the immaturity of the LRT at birth. Indeed, the formation of alveoli begins in a late fetal stage and their development continues throughout the first three years of life¹¹. By adulthood, many distinct subcompartments have developed in the respiratory tract, each of which has specific microbial, cellular and physiological features, such as oxygen and carbon dioxide tension, pH, humidity and temperature (FIG. 1).

Microbiota and the morphogenesis of the respiratory tract. Similar to the anatomical development of the respiratory tract, the initial acquisition of microorganisms marks the establishment of the respiratory microbiota in early life. The establishment of the respiratory microbiota is thought to have an effect on the morphogenesis of the respiratory tract. Indeed, germ-free rodents tend to have smaller lungs¹² and a decreased number of mature alveoli⁵. The latter finding was supported by experiments in which the nasal cavities of germ-free mouse pups were colonized with *Lactobacillus* spp., after which the number of mature alveoli normalized⁵. Intriguingly,

the nasopharyngeal-associated lymphoid tissue (NALT) also develops mostly after birth, which suggests that its development requires environmental cues — for example, from the local microbiota¹³.

Development of healthy microbiota. In contrast to the long-standing hypothesis that we are born sterile, it was recently suggested that babies acquire microorganisms *in utero*^{14,15}, although this suggestion is controversial¹⁶. Irrespectively, the transfer of maternal antibodies and microbial molecules *in utero* markedly influences post-natal immune development^{17,18}. This, in turn, primes the newborn for the substantial exposure to microorganisms that occurs after birth. During the first hours of life, a wide range of microorganisms can be detected in the URT of healthy term neonates^{19,20}. At first, these microorganisms are nonspecific and are of presumed maternal origin. During the first week of life, niche differentiation in the URT leads to a high abundance of *Staphylococcus* spp., followed by the enrichment of *Corynebacterium* spp. and *Dolosigranulum* spp., and the subsequent predominance of *Moraxella* spp.²⁰. Microbiota profiles that are characterized by *Corynebacterium* spp. and *Dolosigranulum* spp. early in life, and *Moraxella* spp. at 4–6 months of age, have been shown to correlate to a stable bacterial community composition and respiratory health^{21,22}.

Birth mode and feeding type are important drivers of the early maturation of the microbiota, with children who are born vaginally and/or are breastfed transitioning

towards a presumed health-promoting microbiota profile more often and more swiftly^{20,23}. These findings were corroborated by epidemiological findings that showed breastfeeding-mediated protection against infections²⁴, which is presumably a consequence of the transfer of maternal antibodies¹⁸ and beneficial microorganisms in breast milk, such as *Bifidobacterium* spp. and *Lactobacillus* spp.^{25,26}. Conversely, the development of the respiratory microbiota can be disturbed, for example, through the use of antibiotics, which are commonly used in young children to treat infections²⁷. Antibiotic perturbations were characterized by a decreased abundance of presumed beneficial commensal bacteria, such as *Dolosigranulum* spp. and *Corynebacterium* spp. in the URT of healthy children^{22,28,29}. This, in turn, might increase the risk of respiratory tract infections following antibiotic treatment³⁰. In addition, season, vaccination, presence of siblings, day-care attendance, exposure to smoke and prior infections can also affect the infant microbiota^{22,31–35}, which indicates that the microbiota during early life is dynamic and affected by numerous host and environmental factors (FIG. 2). Host genetics seems to have a minor effect on the URT microbiota in healthy individuals, only influencing nasal bacterial density and not the composition of the microbiota³⁶. By contrast, the composition of the sputum microbiota seems to be influenced equally by host genetics and environmental factors³⁷.

Although the gut microbiota matures into an adult-like community during the first 3 years of life³⁸, the time that is required to establish a stable respiratory microbiota remains to be determined. Although niche differentiation occurs as early as 1 week after birth²⁰, the respiratory microbiota evolves throughout the first few years of life^{21,33,39}. After the respiratory microbiota is established, antibiotic treatment remains an important perturbing factor of the microbial equilibrium

throughout life⁴⁰. Active smoking also affects the microbial communities in the URT^{37,41}; however, in the LRT, smoking has no clear influence on the composition of the microbiota⁴². Interestingly, it has been suggested that the niche-specific differences disappear again in the elderly⁴³.

Remarkably, not only exposure to beneficial bacteria seems to be important but also the timing of these exposures seems to play a crucial part in the maintenance of respiratory health, as especially aberrant respiratory colonization patterns in infancy seem to be a major determinant of respiratory disease later in life^{21,22,44}. This could be due to the effect of host–microbial interactions in immune education during early life⁶. It has been proposed that the dynamic nature of the developing microbiota early in life might provide a window of opportunity for the modulation of the microbiota towards a beneficial composition⁴⁵; however, the extent of this period of time is currently unknown.

The microbiota of the upper respiratory tract

Gatekeeper to respiratory health. The URT consists of distinct anatomical structures that have different epithelial cell types and is exposed to various environmental factors. These diverse micro-niches are colonized by specialized bacterial communities, viruses and fungi.

The anterior nares are closest to the external environment and are lined with a skin-like keratinized squamous epithelium, including serous and sebaceous glands, the latter of which produces sebum, which leads to the enrichment of lipophilic skin colonizers, including *Staphylococcus* spp., *Propionibacterium* spp. and *Corynebacterium* spp.^{46–48}. Bacteria that are frequently found in other respiratory niches, including *Moraxella* spp., *Dolosigranulum* spp. and *Streptococcus* spp., have also been observed in the anterior nares^{29,43,48,49}. The nasopharynx is located deeper in the nasal cavity and

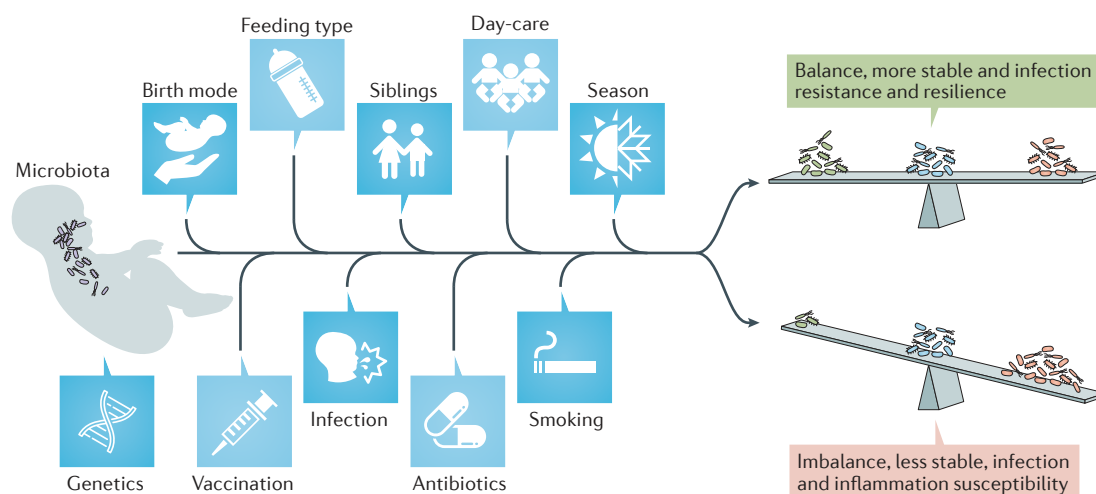


Figure 2 | Host and environmental factors that influence the respiratory microbiota. During early life, microbial communities in the respiratory tract are highly dynamic and are driven by multiple factors, including mode of birth, feeding type, crowding conditions and antibiotic treatment. Together, these host and environmental factors can change the composition of the microbiota towards a stable community at equilibrium that is resistant to pathogen overgrowth, or, conversely, an unstable community develops that is predisposed to infection and inflammation.

is covered by a stratified squamous epithelium that is punctuated by patches of respiratory epithelial cells. The composition of the bacterial communities in the nasopharynx is more diverse than in the anterior parts⁵⁰ and demonstrates considerable overlap with the anterior nares; it also contains *Moraxella* spp., *Staphylococcus* spp. and *Corynebacterium* spp. However, other bacteria more typically inhabit the nasopharyngeal niche, most notably *Dolosigranulum* spp., *Haemophilus* spp. and *Streptococcus* spp.^{20–22,33}. The oropharynx, which is lined with a non-keratinized stratified squamous epithelium, has more diverse bacterial communities than the nasopharynx⁴¹, which are characterized by streptococcal species, *Neisseria* spp., *Rothia* spp. and anaerobes, including *Veillonella* spp., *Prevotella* spp. and *Leptotrichia* spp.^{39,41,51,52}.

In addition to bacterial inhabitants, PCR-based studies suggest the extensive presence of viral pathogens in the URT. These studies have reported an overall detection rate of 67% for respiratory viruses in healthy asymptomatic children, including human rhinovirus (HRV), human bocavirus, polyomaviruses, human adenovirus and human coronavirus^{31,53}. However, recent advances in metagenomics have revealed that the entire respiratory virome contains many other viruses. For example, the recently discovered *Anelloviridae* family was identified as the most prevalent virus family in the virome of the URT^{54,55}. Moreover, the healthy URT has a mycobiota that includes *Aspergillus* spp., *Penicillium* spp., *Candida* spp. and *Alternaria* spp.^{56,57}. Although the size of the respiratory mycobiome is unknown, the gut and skin mycobiomes are approximated to comprise 0.1% and 3.9%, respectively, of the total microbiome in their corresponding niches^{47,58}.

Environmental pressures, as well as microorganism–microorganism and host–microorganism interactions, influence the composition of the bacterial ecosystem in the human host and, as a consequence, its function. For various macroscale ecosystems, such as forests and coral reefs, it is well established that greater biodiversity increases the efficiency by which ecological communities are capable of using essential resources⁵⁹. Similarly, the diversity of specific microscale ecosystems in the human host, such as the gut microbiota, has been associated with health outcomes. For example, increased diversity of intestinal bacteria has been linked to the absence of inflammatory bowel disease, obesity⁶⁰ and resistance against acute infections by enteropathogens⁶¹. Conversely, at other body sites, such as the vagina, low bacterial diversity is considered ‘healthy’ as it is associated with decreased incidences of bacterial vaginosis^{62,63} and premature birth⁶⁴, which highlights the niche-specific effect of biodiversity on human health. In the respiratory tract, evidence indicates that acute URT infections, such as acute otitis media (AOM)^{29,65}, and mucosal inflammation in chronic rhinosinusitis⁶⁶ are associated with a decrease in the diversity of local bacterial communities. However, other studies report a less clear association between diversity and respiratory health, which suggests that the composition of bacterial communities, in a niche-specific ecological context, also affects respiratory

health⁵². Moreover, certain members of the microbiota, known as ‘keystone species’, may have exceptionally large beneficial effects on ecosystem balance, function and health⁶⁷. Potential keystone species in the URT microbiota are *Dolosigranulum* spp. and *Corynebacterium* spp., as they have been strongly associated with respiratory health and the exclusion of potential pathogens, most notably *Streptococcus pneumoniae*, in several epidemiological and mechanistic studies^{21,29,68,69}.

A primary function of any microbial ecosystem is to elicit a state of symbiosis, providing ‘colonization resistance’ against pathogens^{4,70}. The principal mechanism that underlies colonization resistance is that members of a diverse local microbiome probably use all of the nutrients that are available, thereby preventing pathogens from finding the necessary resources for colonization. Although cross-sectional surveys have demonstrated associations between decreased diversity and pathogen colonization, no direct evidence exists that demonstrates that increased microbial diversity in the respiratory tract can protect against the acquisition of pathogens. However, specific members of the microbiota have been identified that can actively exclude pathogens from the nasopharyngeal niche. For example, *Staphylococcus epidermidis* was shown to exclude *Staphylococcus aureus* and destroy pre-existing biofilms through the secretion of serine proteases⁷¹. Furthermore, colonization resistance may be enhanced by interactions with the host immune system. For example, neutrophils seemed more able to kill *S. pneumoniae* following priming with *Haemophilus influenzae*⁷².

The URT is generally considered to be a major reservoir for potential pathogens, including *S. pneumoniae*, to expand and subsequently spread towards the lungs, which could potentially lead to a symptomatic infection³. Thus, establishing and maintaining a balanced microbiota in the URT that is resilient to pathogenic expansion and invasion could prove vital for respiratory health. The mechanisms that underlie a healthy respiratory microbiota, as well as specific microbiota–host interactions that support this, are considered below.

Healthy lungs and their microbiota

The LRT comprises the conducting airways (the trachea, bronchi and bronchioles) and the alveoli, in which gas exchange takes place. The conducting airways are lined with a similar respiratory epithelium to that found in the URT, with the epithelial cells gradually shifting towards a cuboidal shape along the respiratory tree. The alveoli in the lungs are lined with functionally distinct alveolar epithelial cells. In contrast to the URT and other human mucosal sites, the LRT has traditionally been considered as sterile; however, recent studies that used next-generation sequencing discovered a wide range of diverse microbial species in samples taken from the LRT. Potential contamination of low-density specimens remains a major concern when carrying out these types of study and caution is required when interpreting the results (BOX 1).

Source of the lung microbiota. In healthy individuals, bacteria enter the lungs by direct mucosal dispersion and micro-aspiration from the URT⁷³. Culture-independent

Biodiversity

The composite of species richness (the number of species present in an ecosystem) and evenness (the equitability of the abundance of these species).

Acute otitis media

(AOM). An acute-onset viral and/or bacterial infection of the middle ear.

Chronic rhinosinusitis

A common condition that is typified by prolonged inflammation of the paranasal sinuses.

Keystone species

A sole species that is typically not highly abundant but is disproportionately important in maintaining the organization and structure of an entire community.

Biofilms

Microorganisms embedded in a self-produced matrix of extracellular polymeric substances that are adherent to each other and/or a surface.

Bronchoalveolar lavages

(BALs). A technique in which fluid that contains bronchoalveolar cells is obtained by infusing and extracting saline during bronchoscopy.

Mucosal dispersion

The separation and scattering of organisms from the mucosa.

Micro-aspiration

Subclinical aspiration of small droplets.

Box 1 | Technical challenges in respiratory microbiome research

Respiratory microbiome research faces general and niche-specific challenges. The absence of uniform laboratory practices (sample storage, DNA isolation and choice of 16S rRNA variable region) and bioinformatics and data analysis pipelines limits the potential to carry out accurate comparative or meta-analyses. Although different research questions require different approaches, more energy should be invested into the development of standardized operating procedures that are comparable to, for example, the Earth Microbiome project protocol¹⁵².

A specific challenge in microbiota surveys of the respiratory tract is the low density of bacterial communities that are found there, particularly in healthy individuals; densities as low as 10^2 – 10^3 bacteria ml^{-1} have been reported in bronchoalveolar lavages (BALs) of healthy individuals^{74,153}. Such low quantities of DNA preclude whole-genome sequencing, which results in hindered taxonomic resolution and functional interpretation of microbiome data. Furthermore, sampling of the lower respiratory tract (LRT) is cumbersome and is typically based on BAL or the collection of expectorated sputum. Both sampling methods carry a high risk of cross-contamination of the LRT samples with resident bacterial communities in the upper respiratory tract (URT). Distinguishing between authentic LRT communities and URT contamination is further complicated because of the anatomical link between both niches. Once bacterial DNA has been extracted, the quantity can be so low that contamination from environmental DNA invalidates the results^{154,155}. The development of standard operating procedures, including the careful use of appropriate negative controls at different stages of the sampling and laboratory workflow, can help to identify and exclude sequences from contaminating sources^{20,156}.

microbiota studies have confirmed that the lung microbiota largely resembles the URT microbiota when studied in healthy individuals^{74–76}. The oropharynx seems to be the main source of the lung microbiota in adults⁷⁴, whereas in children the source is more likely to be both the nasopharynx and oropharynx⁷⁶. This might be due to the difference in the anatomy of the URT and the frequent increased production of nasal secretions in children, which both probably enhance the dispersal of microorganisms to the lungs. Another potential source of bacteria in the LRT is the direct inhalation of ambient air, albeit, to date, its direct influence on the lung microbiome is unknown. The contribution of the gastric microbiota to the microbial community in the LRT through gastric–oesophageal reflux has, until now, been suggested to be negligible⁷⁴.

Composition of the lung microbiota. As LRT sampling is particularly challenging in young infants (BOX 1), current data on the composition and development of the neonatal LRT microbiota is limited to samples from intubated prematurely born infants^{77–79}. These studies showed that the LRT microbiota of premature infants is dominated by pathogenic *Staphylococcus* spp.^{78,79}, *Ureaplasma* spp.⁷⁹ or *Acinetobacter* spp.⁷⁷, which highlights the lack of complexity in these developing bacterial communities.

In healthy children and adults, a unique microbial community in the lungs was found that contained many of the bacteria that are common to the URT. A study in young children reported that although the lung microbiota was distinct from the microbiota of the URT, it was dominated by species that are also present in the URT, including *Moraxella* spp., *Haemophilus* spp., *Staphylococcus* spp. and *Streptococcus* spp., but lacked other typical URT species, such as *Corynebacterium* spp. and *Dolosigranulum* spp.⁷⁶. The adult lung microbiota

seems to be dominated by genera in the phyla Firmicutes (including *Streptococcus* spp. and *Veillonella* spp.) and Bacteroidetes (including *Prevotella* spp.)^{42,75,80}. Interestingly, *Tropheryma whippelii* seems enriched only in the LRT, which suggests that this might be one of the few bacterial species that is not derived through dispersal from the URT^{42,75,80}.

Studies of the LRT virome have revealed a high prevalence of members of the *Anelloviridae* family, in addition to a high frequency of bacteriophages^{81–83}. Furthermore, the healthy lung mycobiome was found to be predominantly composed of members of the *Eremothecium*, *Systenostrema* and *Malassezia* genera, and the Davidiellaceae family, with common fungi in the URT detected only in low abundance^{57,84,85}.

Although there are subtle regional variations of physiological parameters in the lungs (for example, of oxygen tension, pH and temperature), which, in theory, could affect microbial selection and growth, spatial microbial diversity in the lungs of healthy individuals seems almost absent^{75,80,86}. This supports the hypothesis that, in health, the lung microbiota is a community of transiently present microorganisms that are derived from the URT, rather than a thriving, resident community as is commonly found in chronic respiratory diseases^{80,87,88}. Correspondingly, a recently proposed ecological model, the adapted island model, postulates that the composition of a healthy lung microbiota is determined by the balance of microbial immigration and elimination^{80,88}. Regardless, to date, the exact function that the lung microbiome has in establishing and maintaining respiratory health is unclear, although it probably contributes substantially to mucosal immune homeostasis (BOX 2).

Interbacterial relationships

Next-generation sequencing studies have revealed valuable information on both positive and negative microbial associations. By comparing sequencing data with mechanistic work, ecological interaction networks between microbial community members, or between the microbiota and the host or environment, can be partially reconstructed.

Associations between members of the microbiota can signify direct mutualism or commensalism (positive interactions), or antagonism (negative interactions). Positive interactions have been described primarily for members of the oropharyngeal microbiota; as such, *Veillonella* spp. were shown to induce streptococcal biofilm growth in a species-specific manner, presumably owing to shared quorum sensing systems⁸⁹. These communication systems also seem to affect positive interactions between commensal and pathogenic members of the *Streptococcus* clade⁹⁰, and between the nasopharyngeal community members *Moraxella catarrhalis* and *H. influenzae*⁹¹. Other mutualistic or commensal interactions in the nasopharyngeal microbiota exist, as illustrated by interactions between *Corynebacterium* spp. and *Staphylococcus* spp. The relationship between these species is complex and its directionality is probably species-specific or even strain-specific; *Corynebacterium accolens* and *S. aureus* mutually induce each other's

Mutualism

An interaction between two species in which each species benefits (win-win).

Commensalism

An interaction between two species in which one species benefits and the other is unaffected (win-neutral).

Antagonism

An interaction between two species in which one species is inhibited or adversely affected by another species, comprising: amensalism (lose-neutral), predation and parasitism (win-lose) or competition (lose-lose).

Quorum sensing

A communication system between bacterial cells that is capable of triggering microbial group behaviour (for example, the formations of biofilms) once a certain threshold of signalling molecules is reached.

Box 2 | Specific host–microbiota interactions that contribute to tolerance

The respiratory microbiota has been hypothesized to control mucosal immunity in early life and contribute to immune tolerance. For example, members of the Bacteroidetes phylum, such as *Prevotella* spp., decrease lung inflammation, neutrophil recruitment and the production of Toll-like receptor 2 (TLR2)-mediated pro-inflammatory cytokines compared with *Haemophilus influenzae* in a mouse model¹⁵⁷, which could be related to the number of acyl side chains on their respective lipopolysaccharide (LPS) molecules^{158–160}. Furthermore, *in vitro* activation of epithelial TLRs and nucleotide-binding oligomerization domain (NOD)-like receptors induced the release of antimicrobial peptides, such as β -defensin 2 (REF. 138), which could potentially influence the composition of the upper respiratory tract (URT) microbiota¹⁶¹. The production of these antimicrobial peptides is stimulated by T helper 17 (T_H17) cells¹⁶², which, in turn, were shown to be induced by specific microbial species¹⁶³.

Intriguingly, immune signalling in the URT was shown to elicit responses in distally located mucosal tissues; intranasal inoculation of *Staphylococcus aureus* led to the TLR2-induced recruitment of monocytes to the lungs, in which they differentiated into immunosuppressive alveolar macrophages and subsequently dampened influenza virus-induced inflammatory responses¹⁶⁴. Intranasal administration of *Lactobacillus plantarum* led to TLR2 and NOD2 receptor-mediated protection against lethal pneumovirus infection in the lungs of mice¹⁶⁵. In addition, gut microbiota-induced priming of innate immune cells at the intestinal mucosa was shown to affect respiratory health; for example, through NOD1 receptor-mediated activation of the neutrophils that are required for the clearance of *Streptococcus pneumoniae* in the URT^{72,166}. Furthermore, in germ-free mice, inoculation with microorganisms was shown to be essential for the recruitment of dendritic cells to the lungs¹⁶⁷, and the priming of CD8⁺ T cells¹⁶⁸. Crosstalk between dendritic cells and T cells induces the release of immunoglobulin A (IgA) at the mucosal interface, which prevents pathogens from interacting with the epithelium and selects for a heterogeneous composition of the gut microbiota, facilitating the expansion of regulatory T cells (T_{reg} cells)¹³³ (FIG. 3).

The most convincing evidence that the lung microbiota reciprocally affects local immune responses came from a study in healthy adults. In this study, specific lung bacteria (including *Prevotella* spp. and *Veillonella* spp.) were associated with an increased number of lymphocytes in bronchoalveolar lavage (BAL) fluid, T_H17 cell-mediated lung inflammation and a diminished TLR4 response by alveolar macrophages¹²⁶. Correspondingly, a positive correlation between the relative abundance of members of the phylum Proteobacteria and both alveolar and systemic inflammation was described for patients with acute respiratory distress syndrome (ARDS)¹⁶⁹.

growth through an unknown molecular mechanism⁵⁰, whereas there are mixed reports about the interactions between *Corynebacterium pseudodiphtheriticum* and *S. aureus*^{46,50,92}. Furthermore, antagonistic relationships have been identified, such as those between *S. aureus* and *S. pneumoniae*³², that may, in part, be explained by the production of pneumococcal hydrogen peroxide, which results in lethal bacteriophage induction in *S. aureus*^{93,94}. Human experimental colonization with the commensal *Neisseria lactamica* reduces existing *Neisseria meningitidis* colonization and even protects against new meningococcal acquisition, although the exact mechanisms that underlie this antagonistic relationship are unknown⁹⁵.

It could be postulated that, especially in early life, the human host may nourish and promote specific members of the microbiota, such as *S. aureus*, to benefit from the wide range of antimicrobial molecules that it produces; this could aid the human host in its defence against invading pathogens⁹⁶. The fact that *S. aureus* is present in almost all infants but only sporadically causes disease at this age could, in turn, be related to specific microbial interactions; for example, co-occurrence with *Corynebacterium striatum* was shown to increase the commensal behaviour of

S. aureus and decrease its virulence in an *in vivo* infection model⁹⁷. Furthermore, interactions between species in the *Staphylococcus* genus might help to prevent *S. aureus* from overgrowing as well; for example, its colonization is hindered by serine protease activity in *S. epidermidis*⁷¹ and by the production of lugdunin by *Staphylococcus lugdunensis*, which is a natural antibiotic that is also active against other potential pathogens⁹⁸.

Members of the microbiota might also modulate each other's growth in an indirect manner; for example, through outer membrane vesicle (OMV)-mediated immune evasion⁹⁹, or by using specific properties of the local environment, as shown for *C. accolens*, which converts host triacylglycerols into free fatty acids (FFAs) that, in turn, limit pneumococcal growth⁶⁸. A second example of these mechanisms is the frequent co-occurrence of *Corynebacterium* spp. and *Dolosigranulum* spp. in the nasopharynx^{20,22,69}, in which *Dolosigranulum* spp. might be responsible for the acidification of the local environment, which, in turn, may facilitate the expansion of *Corynebacterium* spp.; however, a direct interaction between these species cannot be ruled out. Given the low density and presumably transient microbiota in the LRT, it could be speculated that the diversity of this microbiota is shaped by interbacterial relationships to a lesser extent than bacterial communities in the URT, although little is known about the level of proximity and likelihood of interbacterial effects.

Bacterial associations that are detected in epidemiological surveys may also indicate the existence of joint host or environmental drivers and not the presence of direct or indirect microbial interactions per se. For example, the co-occurrence of *C. accolens* and *Propionibacterium* spp. on the lipid-rich mucosa of the anterior nares⁴⁸ could be explained by solely their joint lipophilic nature. Furthermore, epidemiological data suggest positive associations between *M. catarrhalis*, *H. influenzae* and *S. pneumoniae* that could be mediated by biological interactions or be based on their shared association with crowding conditions (for example, the presence of young siblings and day-care attendance) and/or their frequent asymptomatic co-presence with respiratory viruses^{53,100}.

Effect of the virome and mycobiome

Bidirectional viral–bacterial interactions. Perhaps the best-known historical example of viral–bacterial interactions in the respiratory tract comes from the Spanish flu pandemic in 1918–1919, when millions of individuals died from secondary bacterial pneumonia after an initial infection with influenza A virus¹⁰¹. In addition, in the absence of disease, epidemiological studies have suggested the presence of viral–bacterial interactions (reviewed in REF. 102). The biological mechanisms that underlie these bidirectional interactions have been extensively studied, although mostly for viruses and bacteria that are known to cause respiratory diseases¹⁰².

One of the main modes of action by which respiratory viruses are thought to predispose individuals to bacterial disease is through the disruption of the airway–epithelial barrier, which facilitates the adhesion

Regulatory T cells

(T_{reg} cells). A subpopulation of T cells that modulate the host immune system and are pivotal in the maintenance of tolerance.

Acute respiratory distress syndrome

(ARDS). A clinical phenotype that occurs in patients who are critically ill and is characterized by overt lung inflammation in response to various pathologies, including trauma, sepsis and pneumonia.

Toll-like receptor

An evolutionary conserved transmembrane protein that has a crucial role in innate immune responses against invading pathogens.

T helper 17 cells

T helper cells that are characterized as preferential producers of interleukin-17 (IL-17), mediate host defence mechanisms to various infections, and are involved in the pathogenesis of several autoimmune disorders.

Lipopolysaccharide

(LPS). The main constituent of the cell wall of Gram-negative bacteria and a potent Toll-like receptor 4 (TLR4) ligand.

of bacterial pathogens^{103–105}. Furthermore, it was demonstrated that influenza virus infection enhances colonization (especially by pneumococci) by liberating host-derived nutrients¹⁰⁶ and by decreasing mucociliary clearance¹⁰⁷. In addition, respiratory viruses can modulate innate and adaptive immune responses in the host, thereby promoting bacterial colonization and infection; for example, by impairing monocyte activity¹⁰⁸, the extended desensitization of alveolar macrophages for Toll-like receptor (TLR)-ligands¹⁰⁹, suppressing phagocytic capacity of alveolar macrophages¹¹⁰, and by inhibiting the production of antimicrobial peptides that is induced by T helper 17 cells¹¹¹.

Vice versa, respiratory bacteria can also promote viral infection through numerous pathways^{112–116}. For example, the upregulation of adhesion receptors, such as intercellular adhesion molecule 1 (ICAM1), was shown to increase the binding of HRV and respiratory syncytial virus (RSV) to epithelial cells and amplify pro-inflammatory responses^{114–116}. These findings were substantiated by a recent clinical study that showed that nasopharyngeal colonization by *S. pneumoniae* and *H. influenzae* in infants is associated with an amplified systemic RSV-induced host immune response, plausibly resulting in more severe RSV infection¹¹⁷.

Conversely, the presence of specific bacterial species in the respiratory microbiota may impede viral infections. These interactions can be either direct^{118,119} or indirect through the host immune system. For example, infection by influenza virus was shown to be less efficient following immune priming by lipopolysaccharide (LPS)-mediated TLR4 activation of innate immune cells^{120,121}.

In fact, some studies suggest that LPS signalling is necessary for appropriate immune crosstalk and immune ‘readiness’ for future encounters with viruses^{122,123}.

In general, the infection of bacteria by bacteriophages seems to be omnipresent. This phenomenon has even resulted in the evolution of a diverse range of antiviral defence mechanisms in commensal bacteria¹²⁴. Consequently, selective infection of specific bacterial strains may regulate the composition of the bacterial community and may facilitate the adaptation of the bacterial community to novel environments by preserving its diversity¹²⁵. A recent study also reported a broad overlap between species-specific bacteriophages and the bacterial community diversity in the lungs, which suggests that substantial interactions between the microbiota and bacteriophages exist in the healthy respiratory tract as well¹²⁶.

Fungal–bacterial interactions. Mechanistic insight into the interactions between fungi, bacteria and the host during health is scarce. However, it has been demonstrated *in vitro* and *in vivo* that the formation of biofilms by *S. aureus*, *Streptococcus* spp. and *P. aeruginosa* damages respiratory epithelia, which enables fungal biofilms to develop^{127–129}. Furthermore, *P. aeruginosa* stimulates the growth of *Aspergillus fumigatus* through sensing volatile metabolites at a distance¹³⁰. Conversely, *Candida albicans* was shown to increase the prevalence of *P. aeruginosa* in mice by impeding the production of reactive oxygen species (ROS) by alveolar macrophages¹³¹. To date, the exact role and breadth of the mechanisms by which fungi contribute to a healthy equilibrium in the respiratory tract have unfortunately remained unstudied.

Although studies suggest the importance of both the respiratory virome and mycobiome in respiratory health, there is a considerable knowledge gap in their exact contributions to health compared with the role of the bacterial microbiome. However, current evidence provides an important basis for further in-depth analyses of the interactions that exist between bacteria, viruses and fungi, as well as the effect of host and environmental factors on these interactions.

Host–bacterial interactions

As there are a vast number of commensals and potential pathogens that inhabit the mucosa of the respiratory tract, a delicate equilibrium has to be maintained between immune sensing and tolerance of non-pathogenic commensals, and the containment of resident pathogens and new invaders. This fine balance is of specific importance to the LRT, as gaseous exchange is absolutely essential for human life and the lungs are exceptionally susceptible to damage from inflammatory responses. Below, we will provide an overview of the immune components that have a role in immune homeostasis in the URT and lungs. A detailed discussion of host–bacterial interactions and their role in immune homeostasis, organogenesis and immune education is provided in BOXES 2,3, respectively. In addition to bacteria, viruses may also promote host immune homeostasis (discussed in BOX 4).

Box 3 | An early window of opportunity

There is increasing evidence that early environmental and microbiota-derived cues are of paramount importance for the development of lymphoid tissue in neonates and ultimately shape the host immune system in the long term. For example, nasopharyngeal-associated lymphoid tissue (NALT) organogenesis is only initiated in the first week of life and is stimulated by cholera toxin, which suggests that NALT organogenesis may require microbiota-derived signals¹³. Similarly, the exposure of neonatal mice to lipopolysaccharide (LPS) led to the formation of bronchus-associated lymphoid tissue (BALT), which was not observed when mice were only exposed later in life¹⁷⁰. Furthermore, it was demonstrated that neonatal, but not adult, bacterial colonization attracts activated regulatory T cells (T_{reg} cells) to the skin and is necessary for the induction of immune tolerance to skin commensals¹⁷¹. Similarly, the lung microbiota promotes the transient expression of programmed death ligand 1 (PDL1) in dendritic cells during the first two weeks of life, which is necessary for the T_{reg} cell-mediated attenuation of allergic airway responses⁷. Furthermore, hypermethylation of the CXC-motif chemokine ligand 16 (*Cxcl16*) gene in the lungs of germ-free mice increases the expression of CXCL16 and the accumulation of invariant natural killer T cells (NKT cells), which are known for their role in inflammation and asthma⁶. Transplantation of the microbiotas from normal mice at neonatal, but not adult age, prevented the accumulation of NKT cells, which abrogated disease in these mice (FIG. 3).

Altogether, these data suggest that the presence of a respiratory microbiota within a specific developmental period is crucial for shaping the adaptive immune response to commensals in adult life and coordinating the delicate balance between host, microbiota and the environment towards a long-term equilibrium. Although this early period of development can be regarded as a susceptible window for aberrant microbial colonization that could lead to the induction of immune disorders, importantly, this same phase of development may also provide a window of opportunity to intervene.

Box 4 | Viral–host interactions

Persistent viral infections naturally occur in humans and may regulate innate and adaptive immunity. In serum, there is an estimated daily turnover of more than 10^9 anellovirus particles, which is thought to induce continuous immune surveillance and influence inhabitation by other microorganisms¹⁷². Similarly, chronic infection with herpesviruses, which have co-evolved with mammals for millions of years¹⁷³ and can be detected in more than 90% of humans¹⁷², protects against bacterial infections by increasing the basal expression of interferon- γ (IFN γ) and facilitating the activation of macrophages¹⁷⁴. Likewise, acute infection with common respiratory viruses activates innate immune pathways that remain active after the virus has been cleared. For example, infection with Sendai virus in mice is associated with the interleukin-13 (IL-13)-dependent activation of natural killer T cells (NKT cells) and lung macrophages and subsequent airway hyper-reactivity¹⁷⁵. Similarly, early infection with respiratory syncytial virus (RSV) leads to impaired regulatory T cell (T_{reg} cell) function in mice, which increases the risk of allergic airway disease¹⁶⁹. These findings are further supported by data from a human infant cohort study, in which persisting immune dysregulation was still detected one month after acute RSV infection¹⁷⁶.

The respiratory tract is exposed to large quantities of airborne particles from the environment. The first line of defence is the mucus layer of the nasopharynx and conducting airways. The mucus traps these particles, including microbial pathogens, which are then cleared through ciliary action towards the oral cavity. In addition, the glycoproteins in the mucus accommodate resident microorganisms and prevent infection¹³², as evidenced by the decrease in antibacterial cytokines and the presence of phagocytosis-impaired macrophages in the lungs of mucin-deficient mice¹³³.

The mucus layer contains immunoglobulin A (IgA) produced by activated B cells¹³⁴ and can preclude pathogens from inhabiting the mucosal surface and interacting with epithelial surface receptors. IgA is also hypothesized to be involved in the regulation and selection of commensal microorganisms and establishing mutualistic host–microbial interactions^{135,136}. Similarly, alveolar surfactant has an important role in lung innate immunity, as a deficiency in surfactant protein A has been associated with decreased bacterial phagocytosis and killing by alveolar macrophages¹³⁷.

The next line of defence is the epithelial cell layer, which is essential for the spatial segregation of the microbiota and the underlying lamina propria. The respiratory tract epithelium produces various antimicrobial substances that contribute to barrier function, including human β -defensin 2 (REF. 138). Pharyngeal and lung epithelial cells, as well as macrophages and dendritic cells, have various receptors to sense the microbiota, including innate pattern recognition receptors (PRRs), such as TLRs and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors)¹³⁸, which are central to balancing the activation of downstream inflammatory signalling and the maintenance of immune tolerance. The epithelium in the URT is supported by mucosa-associated lymphoid tissue (MALT), which is populated with microfold cells that transport microorganisms from the epithelium to the lamina propria, where they can activate dendritic cells¹³⁹. In the lungs, dendritic cells are located within and directly below the alveolar epithelium, where they continuously sample the alveolar space¹⁴⁰. They subsequently present

processed antigens to different subsets of T cells in the lung-draining lymph node, which initiates adaptive immune responses.

Anti-inflammatory alveolar macrophages are vital in lung immune homeostasis and for regulating the crosstalk between epithelial cells, dendritic cells and T cells (reviewed in REFS 141,142). These cells dampen TLR-induced inflammatory signals in epithelial cells¹⁴³, suppress inflammation by inhibiting dendritic cell-mediated activation of T cells^{144,145} and induce regulatory cells¹⁴⁶ (FIG. 3).

In conclusion, host–microbiota interactions influence different aspects of immune system development and contribute to immune maturation, immune tolerance and resistance to bacterial infection.

Conclusions and perspectives

The development of massive parallel sequencing¹⁴⁷ has provided us with extensive insights into the microbial ecology of human body habitats, including the respiratory tract. Studies have shown that different ecological niches in the respiratory tract are occupied by diverse microbial communities that could act as gatekeepers to respiratory health. Further studies will be required to understand the pressures that shape these communities, their precise functions and contributions to human health. Efforts should focus on reductionist approaches to understand the underlying mechanisms involved in environment–microorganism, microorganism–microorganism and microorganism–host interactions in their authentic ecological context. The use of *in vitro* models that enable the manipulation of specific bacterial, host or environmental factors could substantially advance our understanding of the respiratory microbiota. In addition, *in vivo* optical imaging techniques will help to visualize host–microbiota or intra-microbiota interactions in their spatial context in health and disease¹⁴⁸. Data derived from these approaches could be used in mathematical models to reconstruct bacterial interactions and study host and environmental forces that govern microbial behaviour¹⁴⁹.

In addition to the in-depth studies of highly complex and context-dependent interspecies and host–microbiota interaction networks, holistic approaches remain important. Although studies on the composition of the respiratory tract microbiota did not show substantial differences between different developed countries, the question as to whether comparable host and environmental factors regulate the respiratory microbiota of individuals living in low/middle-income countries remains an important open question. The high burden of infectious and inflammation-related diseases in developing areas of the world might at least, in part, be related to compositional changes in the respiratory microbiota and vice versa¹⁵⁰. Most progress can be expected from large cohort studies, in which the microbiota of healthy individuals and individuals who have an increased risk of infectious respiratory diseases is longitudinally characterized. In parallel, multi-omic (for example, transcriptomic and metabolomic) and clinical data should be integrated to study the crosstalk between host and microorganism,

Inhabitation

The presence or occupancy of organisms.

Mucus

A viscous secretion that is produced by goblet cells and is composed of a diverse range of mucin proteins.

Mucin

A class of gel-forming glycoproteins that give mucus its viscosity.

Alveolar surfactant

A mixture of proteins and lipids that reduce surface tension and prevent alveolar collapse, and, additionally, have antimicrobial and anti-inflammatory properties.

Lamina propria

A layer of loose connective tissue that is located directly underneath the epithelium.

β -Defensin 2

An antimicrobial peptide that is produced by epithelial cells in the respiratory tract following microbial stimulation.

Nucleotide-binding oligomerization domain-like receptors

(NOD-like receptors). Intracellular, innate pattern recognition receptors that recognize molecular fragments in peptidoglycan (a constituent of the bacterial cell wall).

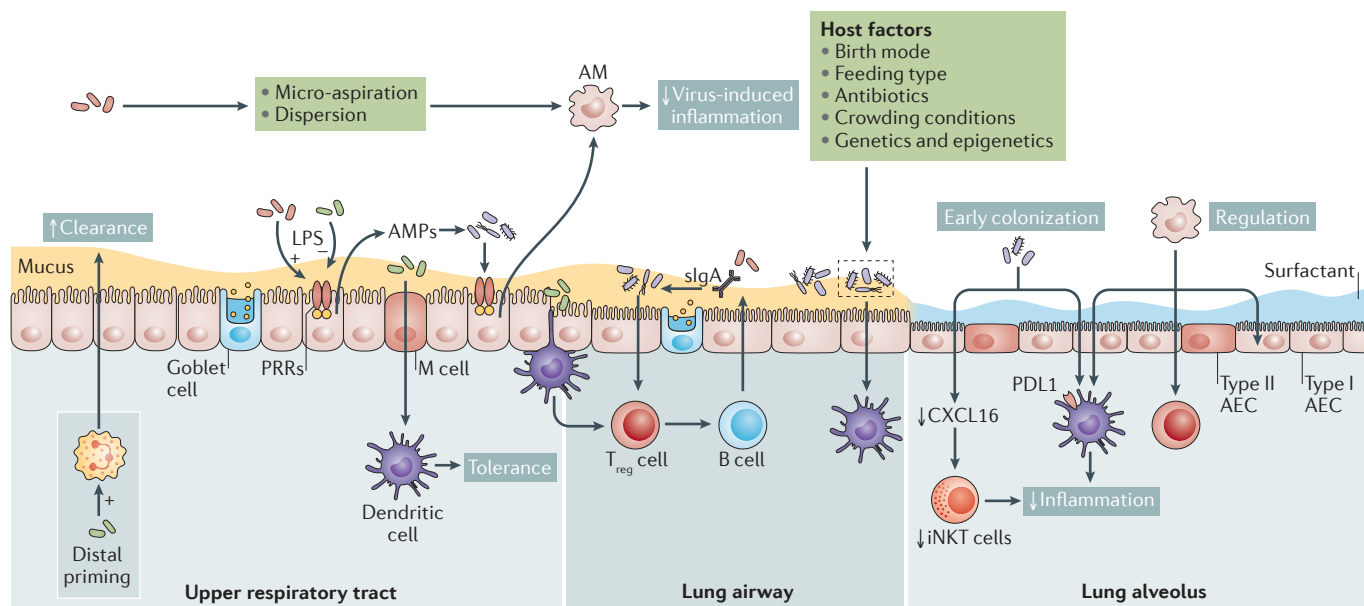


Figure 3 | Host–microbiota interactions in the respiratory tract. Host–microbiota interactions in the respiratory tract occur mostly at the mucosal surface. Resident microorganisms prime immune cells either locally or systemically; these include epithelial cells, neutrophils and dendritic cells, which all contribute to the clearance of pathogens. Moreover, microbial signalling is necessary for the recruitment and activation of regulatory cells, such as anti-inflammatory alveolar macrophages (AMs) and regulatory T cells (T_{reg} cells). Locally, the host will respond to microbial colonization through the release of antimicrobial peptides (AMPs) and secretory immunoglobulin A (sIgA). Sensing of the microbiota involves microfold (M) cells that activate tolerogenic dendritic cells. In addition, alveolar dendritic cells can directly sample luminal microorganisms. Together, these pathways lead to the regulation of inflammation and the induction of tolerance, which, in turn, shape resident bacterial communities. It is also plausible that early bacterial colonization is key to long-term immune regulation, which is illustrated by the microbiota-induced decrease in hypermethylation of the CXC-motif chemokine ligand 16 (*Cxcl16*) gene, which prevents the accumulation of inducible natural killer T cells (iNKT cells), and by the programmed death ligand 1 (PDL1)-mediated induction of tolerogenic dendritic cells (BOX 3). This tolerant milieu, in turn, contributes to the normal development and maintenance of resident bacterial communities, which are also influenced by host and environmental factors (FIG. 1). AEC, alveolar epithelial cell; LPS: lipopolysaccharide; PRR: pattern recognition receptor; URT, upper respiratory tract.

microbiota function, and the effect of environmental factors on the composition of the microbiota. Consequently, advances in bioinformatics will be required to appropriately combine and analyse multiple high-dimensional datasets. Methods to analyse complex combinatorial data sets are sparse, but the field is rapidly progressing by applying machine-learning algorithms and time-resolved data modelling. A multidisciplinary approach to extract patterns and associations from these studies could culminate in individualized risk assessment and preventive

personalized medicine, as illustrated by a study in which dietary interventions that were made based on the gut microbiota led to the improved control of post-meal glucose levels⁵¹. Microbiota-based interventions are likely to be most beneficial in young children, as a window of opportunity within which the local microbiota primes specific features of the immune system seems to exist. Interventions during this impressionable period could redirect an aberrant developmental route, potentially influencing long-term respiratory health.

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Competing interests statement

The authors declare no competing interests.