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Airway microbiota and acute respiratory infection in children

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Summary

Acute respiratory infection (ARI), such as bronchiolitis and pneumonia, is the leading cause of hospitalization for U.S. infants. While the incidence and severity of ARI can vary widely among children, the reasons for these differences are not fully explained by traditional risk factors (e.g., prematurity, viral pathogens). The recent advent of molecular diagnostic techniques has revealed the presence of highly functional communities of microbes inhabiting the human body (i.e., microbiota) that appear to influence development of local and systemic immune response. We propose a "risk and resilience" model in which airway microbiota are associated with an increased (risk microbiota) or decreased (resilience microbiota) incidence and severity of ARI in children. We also propose that modulating airway microbiota (e.g., from risk to resilience microbiota) during early childhood will optimize airway immunity, and thereby decrease ARI incidence and severity in children.

Keywords

acute respiratory infection; bronchiolitis; pneumonia; virus; microbiota; microbiome; *Moraxella*; *Haemophilus*; *Lactobacillaceae*; immune response; probiotics

Background

Acute respiratory infection (ARI), such as bronchiolitis and pneumonia, is a major public health problem for children in the U.S. and worldwide [1]. Indeed, ARI is the leading cause of infant hospitalizations in the U.S., accounting for 25% of all infant hospitalizations [2]. Respiratory viruses (e.g., respiratory syncytial virus [RSV], rhinovirus, influenza virus) are the most commonly detected causes of ARI [3,4]. ARI incidence varies among children; similarly, ARI severity also ranges from minor nuisance to fatal infection [5]. However, the reasons for these differences are not fully explained by traditional risk factors, such as prematurity and viral etiology [5].

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While common microbes are identifiable using conventional laboratory techniques (e.g., cultures), these pathogens represent only a small fraction of the microbes living within any one individual [6]. The recent advent of genome sequencing techniques has revealed the presence of highly functional communities of organisms inhabiting human – the *microbiota* (Table) – that represent up to 90% of all cells in the human body and appear to have a major influence on the development of local and systemic immune response [7]. However, it is largely unknown whether a specific composition of airway microbiota, in conjunction with viral pathogens, is linked to the incidence and severity of ARI.

The airway microbiota and ARI hypothesis

In the current article, we propose a "risk and resilience" model in which airway microbiota are associated with an increased (risk microbiota) or decreased (resilience microbiota) incidence and severity of ARI in children. In brief, we hypothesize that children with a higher abundance of *Moraxella* and *Haemophilus* species in the airway during infancy are associated with a higher incidence of subsequent ARI in early childhood, while those with a higher abundance of *Lactobacillaceae* (e.g., *Corynebacterium, Alloiococcus*) are associated with a lower incidence of ARI. We also hypothesize that children with a higher abundance of *Moraxella* and *Haemophilus* during viral ARI are at higher risk of a severe ARI while that those with a higher abundance of *Lactobacillaceae* are at lower risk of a severe ARI.

Intestinal microbiome and host immune response

For many years, microbiome research has focused on the intestinal tract, where bacteria are abundant and in regular contact with the food and drink that we ingest. Emerging evidence shows that the intestinal microbiota contribute to immune development and homeostasis. While microbiota shape the host immune system, the immune system controls the microbiota, leading to a symbiotic and mutualistic relationship between these commensal microbes and host immune system [8]. The clinical relevance of these inter-relations is growing clearer, with recent studies showing that disruption of balance in the microbiota (dysbiosis), microbiota-derived short-chain fatty acids and regulatory T cell responses is linked with inflammatory disease in the gastrointestinal tract (e.g., inflammatory bowel disease) [9]. Studies also suggested the link between dysbiosis of the intestinal microbiota, Th2-dominant immune response, and a more severe allergic response in distant mucosal locations – such the airways [10].

Airway microbiome and host immune response

Although the intestinal microbiota is being linked with lung health [11], much less is known about the role of actual airway microbiota [6]. One reason may be the widespread assumption that the lungs are sterile, yet recent studies show up to 2,000 bacterial genomes per cm^2 in the lung [12]. These findings should not surprise us since the airway tract (from nose to lung) is in contact with the external environment with every breath taken.

Although the scientific literature is sparse, several epidemiologic studies have reported associations between airway microbiota and ARI. For example, by applying the quantitative PCR technique to the nasal specimens from the RhinoGen cohort (n=380), Kloepfer et al.

found that *S. pneumoniae* or *M. catarrhalis* together with rhinovirus infection contributes to increased ARI incidence and severity in school-age children [13]. These cross-sectional data are particularly intriguing because, using a culture-dependent technique in the COPSAC cohort (n=411), Vissing et al. found that 1-month-old infants with bacterial colonization of the hypopharynx by *M. catarrhalis* or *H. influenzae* had an increased risk of subsequent occurrence of bronchiolitis or pneumonia by age 3 years [14]. Similarly, in the Childhood Asthma Study (CAS) cohort (n=234), Teo et al., found that early *Moraxella* colonization was associated with earlier first ARI during infancy and that both of *Moraxella* and *Haemophilus* colonization are associated with higher risk of lower respiratory infection [15]. In agreement with these epidemiological studies, an *in vivo* study using the upper airway mucosal lining fluid of neonates reported that *M. catarrhalis* and *H. influenzae* colonization of the airways is associated with an upregulated a mixed Th1/Th2/Th17-type inflammatory response of the airway mucosa [16]. Likewise, stimulation of dendritic cells with *Moraxella* and *Haemophilus* species induced 3- to 5-fold more IL-23, IL-10, IL-12p70 when compared with stimulation by the commensal airway bacteria [17]. In contrast to this small but supportive literature, Biesbroek et al. performed a post-hoc analysis of a small trial (n=60) and reported that healthy infants with *Moraxella*-abundant upper airway microbiota had a lower incidence of subsequent ARI [18]. The potential reasons for this discrepancy are unclear and likely multifactorial – e.g., a difference in the study population, setting, definition of outcomes, and analytic techniques (e.g., culture, quantitative PCR, and genome sequence). Despite this one negative study, these data collectively suggest to us that the presence of *Moraxella* and *Haemophilus* species is more common in children who develop ARIs with increased frequency or severity, and that *Moraxella*- and *Haemophilus*-abundant microbiota may be a risk factor for ARI (risk microbiota).

In contrast to risk microbiota, there may also be microbiota that protect against ARI: resilience microbiota. Studies have reported that high abundance of commensal *Lactobacillaceae* (e.g., *Corynebacterium, Dolosigranulum*) are associated not only with breastfeeding but with a lower frequency of acute otitis media and parental-reported ARI [19,20]. Likewise, the CAS cohort also demonstrated that *Lactobacillaceae* were less abundant in infants with ARI compared to healthy infants [15].

Although there are discrepancies in the admittedly sparse literature on airway microbiome and lung health, the available studies collectively suggest to us that airway microbiota are likely to affect lung health in early childhood, a crucial period of immune and structure development in the airways. Nevertheless, we recognize the ongoing confusion about the interrelation between ARI pathogens, airway microbiota, and host immune system. Further investigation on the role of airway microbiota in the incidence and severity of ARI by using advance sequence technologies (e.g., 16s rRNA and metagenomic sequencing) is warranted.

Evaluation of the hypothesis

To conduct studies capable of rigorously addressing the complexity of the proposed model underling the airway microbiota-ARI link, interdisciplinary collaborative research efforts – e.g., epidemiology, pediatric infectious diseases, virology, microbiome, and systems biology – are instrumental. For example, in a cooperative agreement with the National Institute of

Allergy and Infectious Diseases (U01 AI-87881; Camargo), our research group, the Emergency Medicine Network (EMNet; www.emnet-usa.org), is currently conducting a 17-center prospective cohort study with approximately 1000 racially and ethnically diverse U.S. infants hospitalized with bronchiolitis (~52% African-American or Hispanic). We collect nasopharyngeal aspirates and nasal swabs during the index hospitalization (median age, 3 months) and nasal swabs during healthy periods at approximately 9, 16 and 42 months of age. We also collect nasal swabs during clinically-significant ARI episodes through age 36 months. 16S rRNA gene compositional analysis of the longitudinally captured specimen from multiple time-points in early childhood enables to test hypotheses about diversity measures (α- and β-diversity measures) and specific genera. Additionally, metagenomic sequence of the specimen provides not only a "deep" genetic perspective on certain aspects of individual bacterial species – at a more granular level than 16S rRNA sequencing – but also their functional potential. Furthermore, EMNet is also conducting a nested case-control study to examine the role of the airway microbiome of children in the development of near-fatal bronchiolitis (i.e., bronchiolitis requiring mechanical ventilation) among the children enrolled into the 30th Multicenter Airway Research Collaboration Study (MARC-30; U01 AI-67693; Camargo). By sequencing nasopharyngeal aspirates collected during the children's hospitalization (16S rRNA gene and metagenomic sequence), we aim to identify airway microbiota associated with a higher or lower risk of near-fatal bronchiolitis.

These efforts would help us disentangle the complex web of ARI pathogens, airway microbiome, intermediates, confounders and effect modifiers in ARI pathogenesis. The use of a metagenomic sequence approach would enable to identify not only specific bacteria species protective against ARIs but also their functionality in the airway, which, in turn, will develop targeted preventive therapies (e.g., selected strains of probiotics) for children with ARIs.

Conclusions and future directions

The airway microbiota-ARI hypothesis, which began as an epidemiologic observation, now integrates evidence from mechanistic studies that provide biologically plausible explanations. Our model is unlikely to fully explain the variability in incidence and severity of ARI among children. However it is likely to serve as an essential starting point for further epidemiologic and interventional investigations of the interplay between the ARI pathogens, airway microbiota, host immune systems, and development of ARI in children. If future research supports our hypothesis, we believe that modulating airway microbiota (e.g., from risk to resilience microbiota) during early childhood will optimize airway immunity, and thereby decrease ARI incidence and severity.

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Table

Microbiome Glossary

16S rRNA gene sequence	Sequencing DNA within the hyper-variable regions of the 16S ribosomal RNA (16s rRNA) gene that enables identification of bacteria and archaea
Abundance	Extent to which 1 or more genera/species is abundant within the microbiota
α-diversity	Within-sample measures of similarity or dissimilarity (e.g., genus richness, Shannon diversity index and Simpson index)
β-diversity	Between-sample measures of similarity or dissimilarity (e.g., Sørensen index, Bray-Curtis index, unweighted and weighted Unifrac)
Dysbiosis	An imbalance in the microbes in a particular niche secondary to various changes (e.g., infection, use of antibiotics)
Genome	The complete set of genomic information for an organism including genes and non-coding sequences.
Microbiome	The collection of commensal, symbiotic, and pathogenic microbes (e.g., bacteria, archaea, fungi, viruses) and their genomes in the human body
Microbiota	All microbes that are found in a particular niche or region
Phylogenetic	Relating to the evolution of a species or group of organisms
Richness	The number of taxa present in a sample at a phylogenetic level
Metagenomic sequencing	Sequencing the total DNA of the ecosystem, with the advantage of providing information on the presence of bacteria, archaea, DNA viruses, eukarya, and their functionality
Taxa	A taxonomic category (e.g., phylum, genus, species)