

The mechanism or mechanisms driving atopic asthma initiation: The infant respiratory microbiome moves to center stage

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Developments over the last 5 to 10 years, principally from studies on comprehensively phenotyped prospective birth cohorts, have highlighted the important role of viral respiratory tract infections during infancy and early childhood, particularly those occurring against a background of pre-existing sensitization to perennial aeroallergens, in driving the development of early-onset atopic asthma. Although debate surrounding the mechanism or mechanisms governing this causal pathway remains intense, demonstration of the capacity of pretreatment with anti-IgE antibody to blunt seasonal virus-associated asthma exacerbations in children provides strong support for the underlying concept. However, emerging data appear set to further complicate this picture. Notably, a combination of culture-based studies and complementary population-wide bacterial metagenomic data suggests that parallel host-bacteria interactions during infancy might play an additional role in modulating this causal pathway, as well as contributing independently to pathogenesis. These and related issues surrounding development of immune competence during the crucial early postnatal period, when these pathways are maximally active, are discussed below. (*J Allergy Clin Immunol* 2015;136:15-22.)

Key words: *Asthma, atopy, infancy, viral infections, nasopharyngeal microbiome*

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It is increasingly recognized that the cause and pathogenesis of allergic disease involve multiple factors beyond the classical IgE-associated inflammatory mechanisms traditionally thought of as its defining hallmarks. Moreover, it is also now acknowledged that key events that determine risk for development of chronic

Abbreviations used

AMDC:	Airway mucosal dendritic cell
ARI:	Acute respiratory illness
CAS:	Childhood Asthma Study
DC:	Dendritic cell
fLRI:	Febrile lower respiratory tract illness
HrV:	Human rhinovirus
LRI:	Lower respiratory tract illness
mDC:	Myeloid dendritic cell
MPG:	Microbial profile group
pDC:	Plasmacytoid dendritic cell
PNA:	Postnasal aspirate
RSV:	Respiratory syncytial virus
Treg:	Regulatory T

allergic disease frequently occur many years in advance of manifestation of persistent symptoms. Recognition of these facts represents an important watershed in the continuing saga of allergy drug development because they markedly alter perceptions of the range of potential therapeutic targets and the available relevant treatment (particularly preventive treatment) windows. This review focuses on atopic asthma as an archetypal example of allergic disease and examines in particular the role of early childhood factors in the underlying pathogenic process.

ALLERGIC SENSITIZATION TO AEROALLERGENS: WHEN, WHERE, AND HOW?

The initial priming of T_H2 memory that underlies expression of allergic symptoms resulting from ongoing exposure to aeroallergens in adults can be tracked back to early childhood in most cases. Cross-sectional studies in the 1990s first demonstrated age-dependent increases in serum titers of aeroallergen-specific IgE in children, which in many cases appeared to begin increasing during the first few years of life.^{1,2} A variety of evidence (reviewed by Holt³), particularly demonstration of the presence of apparently aeroallergen-specific T_H2 cells in cord blood,⁴⁻⁶ suggested that initial priming of relevant T_H2 memory can in many cases occur *in utero*. However, follow-up studies on the responding T cells identified them as immunologically naive *recent thymic emigrants* with functionally immature antigen receptors that enabled them to bind (and as a consequence become transiently activated by) specific aeroallergens.⁷ The majority of responsive recent thymic emigrant cells undergo rapid apoptosis after activation and do not apparently give rise to long-lived memory cells, although a small subset can be

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Terms in boldface and italics are defined in the glossary on page 16.

“rescued” by **common γ -chain** cytokines if present at sufficiently high levels within the local microenvironment.⁷ Further studies suggested that stable T_H2 memory generation does not usually commence until around the second half of postnatal year 1.⁸

More recent studies on serum IgE antibodies in infants and young children have reignited debate on this question based on claims that aeroallergen-specific IgE of fetal as opposed to maternal origin can be detected at low levels in cord blood by using highly sensitive assay methodology.⁹ However, the evidence supporting the fetal origin of this antibody is indirect, and its validity has been challenged by other evidence.¹⁰ Moreover, prospective tracking of postnatal IgE titers in serum of individual members of a large birth cohort using samples collected repeatedly over the first 5 years of life strongly suggests that IgE antibody production against aeroallergens rarely begins before age 6 months.^{8,11} Additionally, these studies demonstrate that a prominent feature of these early IgE responses is that they occur, at least initially, in almost all subjects, including those who remain nonatopic throughout subsequent childhood.¹¹ In these latter subjects

IgE titers typically wax and wane cyclically within the concentration range of 0.01 to 0.35 kU/L (ie, less than the accepted sensitization threshold). Based on evidence from the experimental literature,^{12,13} this cycling process reflects underlying competition between allergen-specific T_H2 and regulatory T (Treg) cell populations within individual aeroallergen-specific memory responses. In nonatopic subjects this cross-regulatory process is eventually dominated by Treg cells, and as a result, T_H2 cell proliferation is terminated and aeroallergen-specific “tolerance” is established, accompanied by waning of downstream specific IgE production, which in many cases is permanent.

The cellular mechanism or mechanisms that govern this competition for tolerance/sensitization to aeroallergens has been partially elucidated. The key cell population in this context is the network of airway mucosal dendritic cells (AMDCs) responsible for local immune surveillance, which were first described in the Holt laboratory^{14,15} and subsequently further characterized by many other groups (reviewed by Holt et al¹⁶ and Lambrecht and Hammad¹⁷). These cells are responsible for transmission of

GLOSSARY

CCR2: A gene that encodes 2 isoforms of a receptor for monocyte chemoattractant protein 1 (CCL2), a chemokine that specifically mediates monocyte chemotaxis. Monocyte chemoattractant protein 1 is involved in monocyte infiltration in patients with inflammatory diseases, such as rheumatoid arthritis, as well as in the inflammatory response against tumors and inflammatory responses in the lung.

COMMON γ -CHAIN (γ_c OR CD132): Also known as IL-2 receptor subunit γ (IL-2RG), the common γ -chain is a cytokine receptor subunit common to the receptor complexes for the following interleukin receptors: IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors. The γ_c glycoprotein is a member of the type I cytokine receptor family expressed on most lymphocyte populations, and its gene is found on the X-chromosome of mammals.

FCR: A protein found on the surfaces of certain cells, including B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils, and mast cells, that contributes to the protective functions of the immune system. Fc receptors bind to antibodies that are attached to infected cells or invading pathogens. Their activity stimulates phagocytic or cytotoxic cells to destroy microbes or infected cells.

FC ϵ RI: The high-affinity receptor for the Fc region of IgE, an antibody isotype involved in allergy disorders and parasitic immunity. Fc ϵ RI is a tetrameric receptor complex consisting of 1 α (Fc ϵ RI α , antibody-binding site), 1 β (Fc ϵ RI β , which amplifies the downstream signal), and 2 disulfide bridge-connected γ chains (Fc ϵ RI γ , the site where the downstream signal initiates). It is constitutively expressed on mast cells and basophils and is inducible in eosinophils. A different form is expressed on myeloid cells, comprising 1 α and 2 γ chains.

HUMAN TYPE I INTERFERONS: A large subgroup of interferon proteins that help regulate the activity of the immune system. The mammalian types are IFN- α , IFN- β , IFN- κ , IFN- δ , IFN- ϵ , IFN- τ , IFN- ω , and IFN- ζ . IFN- α and IFN- β are secreted by many cell types, including lymphocytes (natural killer [NK] cells, B cells, and T cells), macrophages, fibroblasts, endothelial cells, osteoblasts, dendritic cells, and others. They stimulate both macrophages and NK cells to elicit an antiviral response and are also active against tumors.

IFN- γ : Also known as type II interferon, IFN- γ is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial, and protozoal infections. It is an important activator of macrophages and inducer of MHC class II molecule expression. IFN- γ is produced predominantly by natural killer and natural killer T cells as part of the innate immune response and by CD4 T_H1 and CD8 cytotoxic T lymphocyte

effector T cells once antigen-specific immunity develops. The importance of IFN- γ in the immune system stems in part from its ability to inhibit viral replication directly and most importantly from its immunostimulatory and immunomodulatory effects.

IL-12: A cytokine produced by dendritic cells and macrophages in response to Toll-like receptor stimulation. IL-12 is involved in the differentiation of naive T cells into T_H1 cells. It is known as a T cell-stimulating factor, which can stimulate the growth and function of T cells. It stimulates the production of IFN- γ and TNF- α from T cells and natural killer cells, and reduces IL-4-mediated suppression of IFN- γ .

MHC CLASS II MOLECULES: A family of molecules found mainly on antigen-presenting cells, such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. Antigens presented by class II peptides are derived from extracellular proteins, which are acquired by means of endocytosis or phagocytosis, and loading of fragments of processed protein antigens onto MHC class II molecules occurs intracellularly after biochemical processing of the antigens.

MYELOID DENDRITIC CELL (MDC): An antigen-presenting cell most similar to monocytes, which act as messengers between the innate and adaptive immune systems by processing antigen and presenting it on the cell surface to the T cells of the immune system. mDCs are major stimulators of T cells and secrete IL-12.

PBMC: Any blood cell having a round nucleus (as opposed to a lobed nucleus). Comprised of lymphocytes, monocytes, macrophages, and dendritic cells, these blood cells are a critical component in the immune system to fight infection.

PLASMACYTOID DENDRITIC CELL (PDC): Cells with certain characteristics similar to mDCs but that look like plasma cells. pDCs produce high amounts of IFN- α and play a key role in defense against microbial infections.

RECENT THYMIC EMIGRANTS: T cells that first exit the thymus in a phenotypically and functionally immature state. In particular, they express antigen receptors that lack the fine specificity of those on mature T cells, and as a result, they bind a much broader range of peptides.

16S RNA GENE DEEP SEQUENCING: A common sequencing method used to identify and compare bacteria present within a given sample that has become a well-established method for studying phylogeny and taxonomy of samples from complex microbiomes or environments that are difficult or impossible to study.

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aeroallergen-specific signals from the airway mucosal surface to the central immune system and for “programming” the ensuing balance between the T_H2 and Treg cell components of resultant immunologic memory. This balance ranges between the extremes of Treg cell dominance (absolute tolerance/no evidence of IgE production) and strong T_H2 memory dominance (ie, multiple sensitizations/high IgE/high asthma risk).^{18,19} However, a very common outcome is an apparently “mixed” memory response in which sufficiently high levels of T_H2 memory persist to drive ongoing IgE production but not to mediate the repeated cycles of aeroallergen-induced acute-phase (IgE-*FcR*-mediated) plus late-phase (T_H2 effector-mediated) responses responsible collectively for the severe airways inflammation necessary to drive asthma pathogenesis. The existence of such balance but seemingly “benign” T_H2 memory responses is evident from data from some of the large birth cohort studies exemplified in Fig 1. These data come from the 14-year respiratory follow-up of our RAINE cohort,²⁰ in which approximately 40% of the 1380 subjects assessed were sensitized to the perennial indoor allergen house dust mite but only a minority of these exhibit wheezing symptoms. As shown in Fig 1, the symptomatic subjects are most frequently seen in the top quartile of sensitized “ T_H2^{hi} ” subjects on the basis of IgE titers, but even in this subgroup, only approximately 1 in 3 are affected, and the majority are clearly “physiologically tolerant” to continuing aeroallergen exposure but obviously not “immunologically tolerant.”

Based on experimental model data,^{12,13,21} this state of physiologic tolerance is likely to reflect the underlying balance between T_H2 memory and Treg memory populations in sensitized/asymptomatic subjects, in which Treg cell-associated mechanisms operate with sufficient efficiency to silence T_H2 -dependent airway inflammatory responses before they cross the clinically relevant intensity threshold, although not with sufficient efficiency to eradicate the T_H2 memory component. Of particular note, the same population of AMDCs that initially sets the T_H2 /Treg cell balance in aeroallergen-specific T_H memory (ie, the tolerance vs memory “decision”) are also responsible for triggering T_H2 cell and/or associated Treg cell reactivation in response to aeroallergen inhalation in subjects in whom a component of T_H2 memory persists. It is of interest that recent studies²¹ suggest that a genetically determined deficiency in the antigen-sampling activity of AMDCs might play a significant role in the increased susceptibility to both primary sensitization and subsequent symptom expression after sensitization that is the hallmark of the T_H2^{hi} (ie, IgE^{hi}/ T_H2 cytokine^{hi}) immunophenotype, which is characteristic of an important subgroup of asthmatic patients.

Additionally, it is of interest that unlike corresponding dendritic cell (DC) networks in other peripheral tissues, which are established rapidly postnatally, experimental animal studies indicate that the AMDC population develops relatively slowly in number and function between birth and biological weaning age,^{22,23} and a similar pattern appears in human subjects.²⁴ Moreover, it is noteworthy that the age-dependent increase in the density of this network during infancy appears to be accelerated by exposure to inhaled microbial stimuli, including live virus.^{23,24} In this context the primary role of AMDCs in local immune surveillance of the airway mucosa is detection and ensuing initiation of adaptive immunity against microbial pathogens. It is known that susceptibility to severe respiratory tract infection is maximal during infancy,²⁵ and the “immaturity” of this network during this period is a likely important risk determinant in this regard.

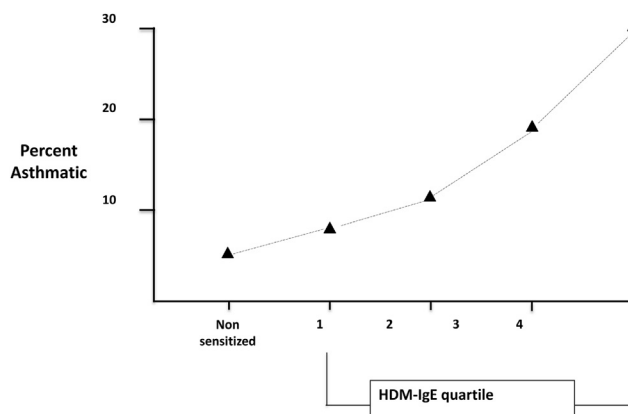


FIG 1. Immunologic versus physiologic tolerance to house dust mite (HDM) allergen in a birth cohort: 452 sensitized subjects placed in quartiles on the basis of HDM-specific serum IgE titers.

As discussed below, early severe respiratory tract infections are also important risk factors in early atopic asthma pathogenesis, and the asthma-promoting effects of these infections might be mediated through this same DC-associated pathway.

An additional factor that is relevant in this context is the kinetics of overall immune development. It is recognized that between birth and weaning, multiple innate and adaptive immune mechanisms that are central to inflammatory pathways triggered by infectious and atopic stimuli are transiting at highly variable rates from the functionally attenuated fetal-like state toward adult-equivalent levels of immune competence (reviewed by Walker et al²⁶). Of particular relevance to this discussion are observations linking sluggish postnatal maturation kinetics with increased susceptibility to subsequent development of atopic and/or asthma-related phenotypes. Immune functions identified in this context include the capacity to produce *IL-12*^{27,28} and *IFN-γ*,²⁹⁻³¹ *MHC class II* expression on myeloid cells,³² and numbers/functions of circulating Treg cells³³ and DCs.³⁴⁻³⁶ Likewise, susceptibility to severe respiratory tract infection in infancy has also been linked to decreased T_H1 -associated cytokine production,³⁷⁻³⁹ decreased circulating DC numbers,³⁶ and an imbalance between production of regulatory/effector cytokines within adaptive immune responses.⁴⁰ Young children with the high-risk phenotype also display suboptimal responsiveness to common childhood infectious disease vaccines.^{41,42}

ROLE OF VIRAL RESPIRATORY TRACT INFECTION IN ASTHMA CAUSE AND PATHOGENESIS

Interest in the role of respiratory tract infections in asthma was first stimulated by observations relating to established disease, notably that viral infections frequently trigger asthma attacks in children and adults with a pre-existing diagnosis of asthma.⁴³ Moreover, it is clear that viruses can function as independent risk factors in this regard, as can aeroallergens, but it has become increasingly evident over time, as data flow from multiple asthma birth cohorts internationally,^{11,18,20,44-49} that the underlying inflammatory pathways can interact. The key observations in this regard relate to the RAINE⁵⁰ and Childhood Asthma Study (CAS)⁵¹ Australian birth cohorts and the Childhood Origins of Asthma Study (COAST) cohort from the United States⁵²; in each case early allergic sensitization and early and severe viral

lower respiratory tract infections during infancy independently increase the risk for a subsequent diagnosis of persistent asthma, but the risk is greatest in children who experience both types of respiratory insults. Crucially, for this apparent synergy to occur, aeroallergen sensitization needs to precede the relevant infectious episodes^{11,51,52}; that is, it is not sufficient to simply express the at-risk T_H2^{hi} phenotype (which could in theory attenuate overall capacity to mount effective T_H1 -dependent antimicrobial immunity), but rather the subjects are required to be actively producing IgE against aeroallergens, which implies that this interaction is a direct and active process. Moreover, this scenario also might apply in relation to established asthma in older age groups. In particular, severe asthma exacerbations in school-age children resulting in hospitalization are invariably triggered by viral infections, and more than 80% of affected children are atopic (reviewed by Sly et al⁵³), whereas at the lower end of the asthma severity spectrum, the frequency and intensity of infection-associated lower respiratory tract illnesses (LRIs) and attendant loss of asthma control during the fall virus season are higher among atopic asthmatic children relative to their nonatopic asthmatic counterparts.⁵⁴

Two related pathways have been described that might account for these observations. First, as initially reported in mouse models,^{55–57} influenza and parainfluenza infections lead to upregulation of expression of the myeloid version of the high-affinity IgE receptor (*FcεRI*) on lung DCs; the precise mechanism or mechanisms involved remain to be elucidated but can include signals from a subset of incoming neutrophils recruited to the infection site.⁵⁶ It was suggested that cross-linking of this receptor with IgE directed against viral antigens might trigger local T_H2 cytokine production by virus-specific T_H2 memory cells,⁵⁷ but the existence of the latter remains unproved. Alternatively, it has been shown that upregulation of this receptor in the parainfluenza model in animals presensitized to house dust mite allergen was associated with development of hyper-responsiveness to local allergen challenge, resulting in enhanced local production of T_H2 cytokines and reduced lung function.⁵⁵

The operation of a comparable pathway in human subjects is suggested by airway biopsy data demonstrating the presence of *FcεRI*⁺ DCs in the bronchial mucosa of asthmatic patients⁵⁸ and in particular that this expression increases during periods of active disease.⁵⁹ Recent studies on acute severe asthma exacerbations in children resulting in hospitalization have shed some light on the underlying pathways involved.⁶⁰ *PBMCs* sampled from these children at admission and after convalescence were used for genome-wide expression profiling, which defined a large panel of genes that were differentially expressed in association with the exacerbation event. The most prominent signals detected were gene signatures on bone marrow–derived myeloid cells (particularly *myeloid dendritic cells [mDCs]*, *plasmacytoid dendritic cells [pDCs]*, and monocytes) downstream of *human type 1 interferons* and the T_H2 cytokines IL-4 and IL-13. The circulating monocyte population exhibited classical IL-4/IL-13–dependent “alternative activation” gene signatures, and enhanced expression of *FcεRIα* was detected on the surface of both mDCs and pDCs by using flow cytometry.⁶⁰ The most prominent gene upregulated in myeloid cells was that encoding the chemokine receptor *CCR2*, which is associated with lung homing.⁶¹ In addition, these cells were actively expressing the gene encoding *FcεRIγ*,⁶⁰ which is rate limiting in the process of uploading the functional high-affinity IgE receptor (a combination of the

FcεRIα and *FcεRIγ* chains: *FcεRIαγγ*) on the surfaces of myeloid cells.⁶² In this regard it is recognized that atopic subjects constitutively hyperexpress *FcεRIα* on the surfaces of myeloid cells,⁶³ but the capacity to express fully functional receptor is limited by the availability of the intracellular *FcεRIγ* chain.⁶²

As noted above, the most prominent gene signatures expressed by bone marrow–derived myeloid cells were downstream of IL-4/IL-13 and type 1 interferons.⁶⁰ This implies that during exacerbations, these cytokines are translocated into the bone marrow from the inflamed airways, where they contribute to the programming of immature myeloid precursors. Based on the demonstration of trafficking of cytokine-secreting activated T_H2 cells to the bone marrow immediately after bronchial allergen challenge of atopic subjects,⁶⁴ it is likely that these exacerbation-associated T_H2 and type 1 interferon signals are likewise borne by migrating cells. Moreover, parallel *in vitro* studies on cultured blood monocytes derived from atopic children demonstrated that *FcεRIα* and *CCR2* were strongly upregulated by the T_H2 cytokines, whereas *FcεRIγ* expression was induced by type 1 interferons.⁶⁰

In the scenario described here, exacerbation-associated signals into the bone marrow result in marked upregulation of the *FcεRIγ* chain gene and further amplification of *FcεRIα* expression, thus equipping these cells optimally for expression of functional *FcεRI* on arrival into inflamed airways tissues,⁶⁰ where they replenish the resident airway mucosal mDC population, which turns over very rapidly during acute viral infection.^{65,66} DCs thus armed with fully functional *FcεRI* are recognized to express 10- to 100-fold increased capacity to present allergens to T cells by virtue of their capacity to use receptor-bound IgE to more efficiently sample incoming aeroallergen to which the infected subject is co-exposed^{62,67}; a plausible result in this case is local triggering of a T_H2 cytokine “storm” through activation of mucosal trafficking aeroallergen-specific T_H2 memory cells, which are abundant in sensitized children. A limitation of our studies to date on virus-associated exacerbations is their reliance on data from profiling of circulating cell populations in the process of their migration to the lung and airways. More direct evidence in support of this general pathway comes from studies involving expression profiling of sputum-derived cells collected from children during viral triggered exacerbations, which have also revealed prominent T_H2 (including *FcεRI*)–associated gene signatures.⁶⁸

In addition to this *FcεRI*-dependent pathway mediated mainly by mDCs, recent evidence points to the operation of a second related pathway involving pDCs. In this case surface expression of *FcεRI* and its subsequent cross-linking after IgE binding leads to rapid downregulation of type 1 interferon production.⁶⁹ The pDC population is one of the most important sources of the type 1 interferons that play a key role in viral clearance, and hence one consequence of upregulation of *FcεRI* on these cells might be prolongation of infection. Additionally, as well as being inflammatory in its own right, the wave of enhanced T_H2 cytokine production stimulated by upregulation of *FcεRI* expression on airway mucosal mDCs might attenuate the capacity of these cells to generate sterilizing T_H1 -associated immunity directed against the triggering virus, which is analogous to the antagonistic effects described for T_H2 cytokines in regard to herpes and dengue virus infection.^{70,71} It is also noteworthy that additional *in vitro* evidence suggests that type 1 interferons might play a dualistic role in regulating *FcεRI* expression on mDCs because in addition to inducing *FcεRIγ* expression, at high concentrations, this cytokine antagonizes IL-4/IL-13–induced *FcεRIα* chain expression⁶⁰

and as such might provide a homeostatic “negative feedback” signal that mitigates overshoot of the host response into the immunopathologic zone.

Indirect but compelling proof of concept supporting the operation of an FcεRI-dependent pathway in the immunopathology underlying acute severe asthma exacerbations has been provided from recent findings from the US Inner City Asthma Consortium. In particular, they have demonstrated that continuous treatment of high-risk children with anti-IgE antibody essentially abolishes the annual virus seasonal peak of asthma exacerbations seen in untreated children during the fall,⁷² and a number of studies are in progress internationally to validate these findings.

THE EMERGING ISSUE OF THE RESPIRATORY MICROBIOME

Questions relating to the cataloging of respiratory pathogens that contribute to the underlying immunopathogenesis of atopic asthma and their ranking based on relative contribution to disease development has until recently focused almost exclusively on viral pathogens. The 2 main viruses identified in this context are respiratory syncytial virus (RSV) and human rhinovirus (HrV), which are the 2 most common respiratory viral pathogens afflicting infants and preschoolers, and although much attention has recently switched to HrV-C,⁷³ there remains spirited ongoing debate surrounding the comparative magnitude of the contribution of RSV.^{74,75} However, emerging studies from multiple investigators have recently widened this debate to include consideration of the potential role of bacterial pathogens.

The initial finding that has served to reignite interest in this question was the demonstration that the lower airway surface in even healthy subjects is virtually littered with bacterial genomes at baseline; that is, there might be a “resident” microbiota present throughout the conducting airways in addition to that present in the nasopharynx, from which the lower airway populations are likely derived.⁷⁶ Moreover, cross-sectional studies suggest that there might be both qualitative and quantitative differences in the lower airway bacterial populations between asthmatic and nonasthmatic adults and children, including the mix of potentially pathogenic and benign strains present.^{76–78} In particular, the overall bacterial burden in the lower airways appears higher in asthmatic patients.^{79,80} Additionally, preliminary studies focusing on a small number of culturable organisms suggest that early postnatal colonization of the nasopharynx with known pathogens measured at a single time point during infancy might be a risk factor for subsequent asthma development and accompanying enhancement of T_H2-associated immunity.⁸¹ Moreover, detection of pathogenic bacteria during rhinovirus infection in older asthmatic and nonasthmatic children has been associated with increased severity of ensuing respiratory tract illness, including asthma exacerbations.⁸²

These observations are consistent with a possibility that has been debated over many years, notably that viral respiratory tract infections weaken local defenses against opportunistic bacterial pathogens that are ubiquitous in the environment, enhancing the risk for breaching of mucosal barriers by these secondary pathogens and the resultant amplification of ensuing immunoinflammatory responses and associated tissue damage (reviewed by Holt and Sly⁸³). Relevant to this possibility, it has recently been demonstrated that infants and schoolchildren with atopic asthma symptoms display aberrant patterns of humoral immunity against common respiratory bacterial pathogens. This includes reduced

production of specific IgG₁ antibody levels that are central to bacterial clearance^{84,85} and also reduced levels of specific IgE directed against particulate antigens on bacterial pathogens, which are produced in relatively high titers by nonatopic and asymptomatic atopic subjects.⁸⁶

Collectively, these findings emphasize the necessity of obtaining an accurate picture at the population level of the full spectrum of bacterial strains that constitute the respiratory tract microbiome and their population dynamics across the age range during which asthma development is most commonly initiated. The first asthma birth cohort to yield such data is the Perth CAS cohort,⁸⁷ which has been used for the first detailed characterization of the respiratory microbiome throughout the crucial period of early infancy, during which respiratory tract infections exert their strongest asthma-promoting effects.^{50–52} This study focused on the nasopharynx, which serves as a reservoir for microbes associated with acute respiratory illnesses (ARIs). A key feature of this study was the capturing of approximately 100% of respiratory tract infection events in the cohort of 234 infants at high risk of allergy/asthma, comprising physician assessment in conjunction with collection and cryostorage of postnasal aspirate (PNA) samples for future (viral/bacterial) analyses; for reference purposes, repeated PNA sampling was also performed at several time points during the year when the infants were infection free. The study population was rigorously monitored prospectively over the ensuing 5 years, with a final follow-up at their 10th birthdays, accumulating a comprehensive record of their developing immunophenotypes and accompanying clinical phenotypes relevant to allergy and asthma.

Characterization of nasopharyngeal microbiome composition throughout year 1 in the cohort using **16S recombinant RNA gene deep sequencing**, together with initial analysis of how this relates to short- and long-term clinical phenotypes, has been completed,⁸⁷ focusing on a subsample of 1021 PNAs from year 1 in the cohort comprising 534 collected during ARI episodes and 487 collected when patients were symptom free (“healthy”). The resultant 193×10^6 16S rRNA sequences were initially classified into operational taxonomic units, and the dominant phyla identified were Proteobacteria (48%), Firmicutes (38%), and Actinobacteria (13%).

Salient findings from additional analyses⁸⁷ were as follows. First, the microbiome during the first year comprises 6 major genera, each of which is dominated by a single major operational taxonomic unit consistent with the species *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, or *Alloicoccus otitidis*. These in turn cluster with their own semiunique mix of minor genera to define individual microbial profile groups (MPGs).

Second, *Staphylococcus*, *Alloicoccus*, and *Corynebacterium* MPGs predominated in the “healthy” PNAs. This pattern altered radically during ARI, which were dominated by *Haemophilus*, *Streptococcus*, and/or *Moraxella* MPGs accompanied by reduced frequency of the “healthy” MPGs.

Third, environmental factors exerted significant effects on the nasopharyngeal microbiome, most notably selection for the disease-associated genera (in “healthy” samples) by antibiotic treatment and exposure to daycare.

Fourth, across the cohort, both RSV and HrV were independently associated with ARI symptom expression, and after controlling for virus, *Haemophilus*, *Streptococcus*, and/or *Moraxella* MPGs remained highly significantly associated with ARI ($P < 1 \times 10^{-15}$). Moreover, these MPGs and RSV (but not HrV) were also

independently associated with both the spread of ARI to the lower respiratory tract and the ensuing expression of febrile symptoms.

Fifth, in contrast, at the population level, wheeze during LRI events was not significantly associated with any single pathogen or combination of pathogens, but consistent with earlier analyses from both the COAST and CAS cohorts discussed above, HrV (most notably the C subtype) was associated with wheeze during LRIs only in the “high-risk” group of children who had sensitization during infancy.

Sixth, of note, febrile lower respiratory tract illness (fLRI) in year 1 was associated with increased risk for progression to chronic wheeze across the whole cohort (36% prevalence at age 5 years in children experiencing ≥ 1 events from a baseline of 20% in fLRI-free subjects), whereas corresponding HrV-C–associated wheeze during LRIs conferred increased risk only in the early sensitization group (30% prevalence). Moreover, these LRI effects appear independent and additive because the prevalence in children experiencing both types of severe LRI was 58% at 5 years.

Seventh, time to first severe LRI event also appears important, with earlier fLRIs occurring among children with chronic wheeze. Relevant to this, early colonization with *Streptococcus* species (which was again more common in the early sensitization group) was strongly associated with both earlier first LRI and with the risk for subsequent development of chronic wheeze.

INTERSECTING CAUSAL PATHWAYS IN EARLY ASTHMA PATHOGENESIS

The emerging data on the potential role of opportunistic bacterial pathogens in asthma development builds on earlier

findings from many groups who have demonstrated the major contributions of viral infections and atopic sensitization in this process. As discussed above and summarized on the right side of Fig 2, airways inflammation triggered by viral lower respiratory tract infection or sensitization/repeated exposure to aeroallergens during infancy and the preschool years can independently promote development of a wheezing phenotype, but in most cases the result is intermittent (often transient) wheeze. However, in children experiencing both pathways, particularly when sensitization occurs early, the risk for progression to persistent wheeze attracting a diagnosis of asthma by early school age is much higher. This interaction between underlying inflammatory pathways appears mediated, at least in part, through FcεRI-dependent mechanisms operative in both the airway mucosa and bone marrow, resulting in *inter alia* enhanced T_H2 cytokine-mediated inflammation at the infection site and resultant interference with viral clearance mechanisms. However, as shown in Fig 2 (left), it is becoming apparent that this picture underestimates the underlying complexity of these causal pathways.

Notably, in the “high-risk” CAS cohort during year 1, distinct bacteriomes occupy the nasopharynx at (healthy) baseline versus during acute viral infections, and the bacterial pathogens that emerge at that time appear to play a range of direct and indirect roles in what is normally considered virus-driven respiratory illness, including promotion of infection spread to the lower airways. Moreover, these bacterial pathogens additionally exert independent effects, including intensification of associated airways inflammation (marked by fever symptoms), thus increasing the risk for subsequent persistent wheeze. These findings relating to the role of the nasopharyngeal microbiome in infants likely represent

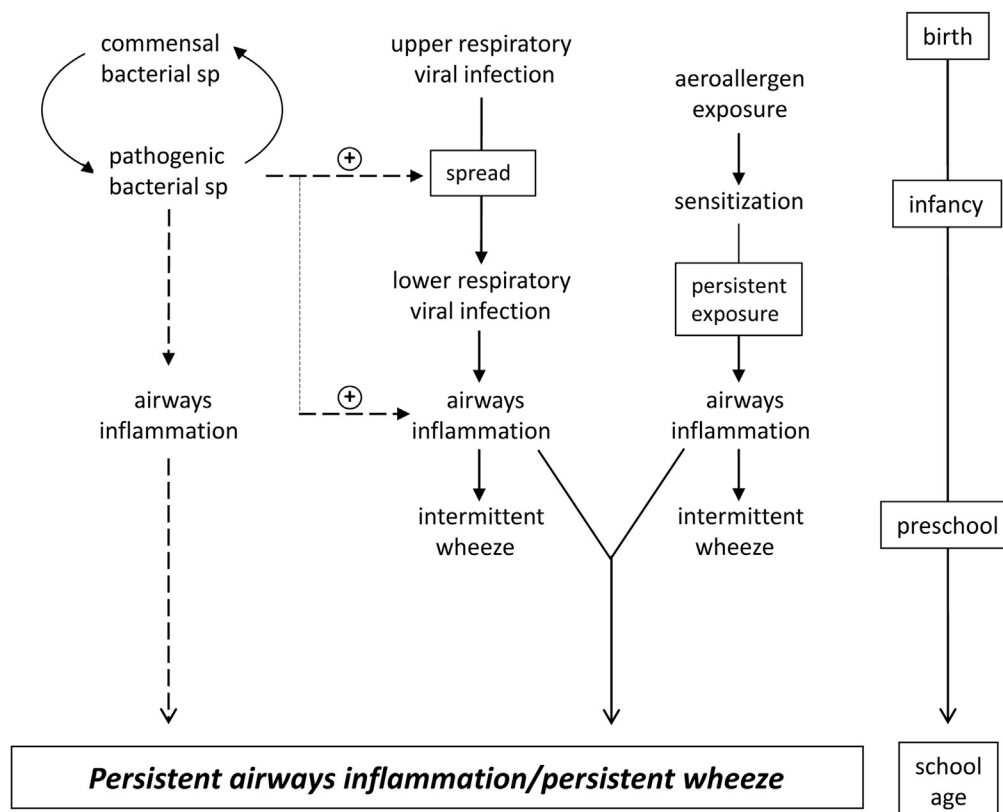


FIG 2. Causal pathways leading to development of persistent wheeze during the preschool years: emerging data on the contribution of the nasopharyngeal microbiome.

the tip of the iceberg and will be expanded and refined as further data emerge from similar studies that are in progress in other asthma cohorts. In this regard analyses are also proceeding on 16S sequencing data from a further approximately 2000 PNAs from the CAS cohort spanning years 2 to 5, during which the complexity of the nasopharyngeal microbiome will undoubtedly increase.

In conclusion, the emerging data relating to bacterial communities in the upper airways in infants and susceptibility to severe LRIs provide tantalizing clues relating to the potential role of these organisms in the early stages of asthma development, but at this stage, they raise more questions than they answer, including the following. Does the replacement of commensal bacterial species in the nasopharyngeal microbiome of infants with known pathogens precede the onset of local viral infection or instead result from the latter? Why is the effect on risk for asthma development of early colonization of the nasopharynx with pathogens, such as *Streptococcus* species, inversely related to age at colonization? Why is this more common in children who subsequently have aeroallergen sensitization? Is there a critical threshold of aeroallergen-specific IgE production that must be crossed before subjects experience "atopy-enhanced" airways inflammation triggered initially by viral or bacterial pathogens? A deeper understanding of these and related issues will hopefully develop in the near future as this exciting area of asthma research expands.

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