The Microbiome and Development of Allergic disease

Susan V. Lynch[1] and **Homer A. Boushey**[2]

[1]Division of Gastroenterology, Department of Medicine, University of California San Francisco, CA 94143

[2]Division of Pulmonary/Critical Care and Allergy/Immunology, Department of Medicine, University of California San Francisco, CA 94143

Abstract

Purposes of review—First, to review how the global rise in prevalence of asthma prompted studies of the relationships between microbial exposure in early infancy, the rate and pattern of development of immune function, and the development of allergic sensitization and of wheezing in childhood. And, second, to review how those studies laid the groundwork for a possible strategy for primary prevention of asthma through manipulation of the microbiome of the gastrointestinal and/or respiratory tracts.

Recent findings—Atopy and asthma are complex diseases thought to result from a "gene-by-environment" interaction; the rapidity of their rise in prevalence points to a change in environment as most likely causal. Epidemiologic studies noting associations between events in infancy and later development of atopic diseases have suggested that their rise in prevalence is related to a deficiency in microbial exposure in early life. The findings from birth cohort studies of humans and from interventional studies of mice converge in suggesting that a deficiency in microbial colonization of the respiratory or gastrointestinal tract by certain commensal microbes results in skewed development of systemic and/or local immune function that increases susceptibility to allergic sensitization and to viral lower respiratory infection. Recent studies are now honing in on identifying the microbes, or collection of microbes, whose collective functions are necessary for induction of immune tolerance, and thus of reduced susceptibility.

Summary—Atopy and asthma appear to have their roots in an insufficiency of early life exposure to the diverse environmental microbiota necessary to ensure colonization of the gastrointestinal and/or respiratory tracts with the commensal microbes necessary for induction of balanced, toleragenic immune function. Identification of the commensal bacteria necessary, now ever closer at hand, will lay the groundwork for the development of strategies for primary prevention of atopic disease, especially of childhood asthma.

Keywords

atopy; asthma; microbiome; immune function

INTRODUCTION

That interest in the causes of asthma is now expressed by high-level governmental agencies [1,2] reflects widespread recognition of the need for an effective strategy for primary prevention of the disease, a recognition driven by the dramatic increase in disease prevalence over the past 60 years [3]. Asthma is now one of the most common chronic diseases in the world [4], with especially high prevalence in "westernized" countries, where it is the most common cause of hospitalization and of chronic disabling disease in school-age children [3,5]. While effective therapies for controlling asthma are available, none is curative, and the disease often persists from early childhood through adult life [6]. Because most asthma in children is associated with atopy - clinical or laboratory evidence of allergic sensitization [7] - interest has naturally turned to the origins of atopy and to the causes of asthma in atopic children [8].

That these interests might be fulfilled is tantalizingly suggested by convergence of studies by researchers with widely different skills and perspectives. Epidemiologic studies of human populations and laboratory studies of animal models of allergic sensitization and inflammation point to interactions between the external environment, the human microbiome (the multi-species microbial communities that exist in the human host), immune function, and exposure to certain respiratory viruses in the first years of life as underlying predisposition to allergic sensitization and to the development of asthma. Review of evidence of the central role played by the gastrointestinal and/or bronchial microbiome in these interactions is the focus of this brief review.

EPIDEMIOLOGY

The prevalence of different allergic diseases – "hay fever," asthma, and food allergy – has increased globally, but the rate and pattern of increase has not been uniform among the diseases nor across different regions of the world [9]. In general, the highest prevalence rates have been in Western Europe, North America, Japan, Australia and New Zealand, but prevalence rates are rising even in developing countries with largely agrarian economies [10]. The relationship between gross national product and allergic disease prevalence is not linear, however, for it weakens above a threshold level [11]. In fact, the relationship between socio-economic status and asthma is inverse in the United States of America, where poor, inner-city populations suffer both higher prevalence and greater severity of allergic asthma [12,13]. Thus, while atopy and asthma have a heritable component, what is inherited seems to be susceptibility to disease development, and the diseases are regarded as a consequences of a "gene by environment" interaction [14], the causes of the increase in prevalence are thought to lie in the environmental component of the equation.

HYGIENE HYPOTHESIS AND ITS EVOLUTION

Strachan interpreted his seminal observation of an inverse relationship between hay fever and family size and birth-order as indicating that the rising prevalence of hay fever reflected a decrease in the frequency of childhood infections [15]. Known colloquially as the "Hygiene Hypothesis," the concept has since evolved to state that the rise in allergic diseases

is an unintended consequence of reduction in microbial *exposure* or *colonization*, rather than microbial *infection* in early life. This evolution was driven by cross-sectional and longitudinal studies showing no relationship between the frequency or severity of early childhood infectious illnesses and the later development of allergy or asthma [16], and by a wealth of studies showing protective effects from conditions plausibly associated with enhanced beneficial microbial exposure, and inductive effects in environments depleted of such microbial contact. An incomplete list of protective conditions in early-life include residence on a farm with domestic livestock [17,18], growing up in a household with dogs [19,20] exposure to endotoxin [21,22], vaginal birth [23], breast feeding [24] and consumption of unpasteurized "farm milk," [25]. Inductive conditions include pre-natal antibiotic treatment or of the child in infancy [26], delivery by Caesarian section [23], and early formula feeding [27] (though it is unclear whether this remains true of newer pro- or pre-biotic enriched formulas).

Also driving this evolution in the hygiene hypothesis were studies prompted by the collapse of the Iron Curtain and the unexpected discovery that atopic diseases were significantly less prevalent in genetically similar populations in former Eastern versus Western Europe [28], where, as Rook observed, exposure to microbes has decreased substantially in the post-industrial revolution era [29]. This idea underlay culture-based studies of stool samples from infants born in countries in the two regions which showed that infants in Estonia, where atopic diseases were uncommon, and Sweden, where they were much more prevalent, were microbiologically distinct. The major differences were high counts of *Lactobacillus* and *Enterococcus* in Estonian babies and of *Bacteroides* and *Clostridium* in Swedish babies [30].

These cross-country comparisons were complemented by cross-condition comparisons of the bacteria cultured from stool of children with and without atopy and/or asthma. An example is again provided from comparison of fecal bacteria in 2 year olds from Estonia and Sweden [31]. Allergic children in both countries were less often colonized with *Lactobacillus*. In contrast, allergic children harbored higher counts of aerobic microorganisms particularly coliforms and *Staphylococcus*. A follow-up prospective birth cohort study of newborns in the same two countries showed that demonstrable atopy at age 2 was associated with differences in stool microbiota as early as the first month of life, long before any clinical indications were apparent [32]. These findings were confirmed by larger birth cohort studies again showing differences in the bacterial composition of stool samples collected in the first weeks of life and atopic manifestations at age 1 or 2 years, calling attention to the association of increased abundance of *Clostridium*, and *Escherichia* species and of decreased *Bifidobacteria* with eczema, wheeze, and allergic sensitization [33].

With respect to asthma, if not to hay fever, the findings from cross-sectional and birth cohort studies of associations between events in early life and later development of disease challenge one interpretation of the original statement of the hygiene hypothesis: that the rise in prevalence could reflects a decrease in the severity of childhood infections (rather than in their frequency, as Strachan actually stated) [15]. Two landmark cohort studies of children at risk for development of atopic disease have shown a robust association between development of severe illness from RSV or HRV infection in the first year of life and the

later development of asthma, especially in children found to be atopic at age one [34,35] (see also Beigelman in this issue for detailed review).

Individual summary, or even reference to the many other studies shaping the refinement and restatement of the hygiene hypothesis through the first years of the 21st century is not possible in this brief review, but their collective contributions can be summarized by stating (1) that the roots of the rise in prevalence in allergic sensitization and asthma stem from modern, post-industrial changes in environmental exposures in westernized countries, (2) that these changes affected early life gut bacterial microbiome composition, and (3) that these changes in gut microbiota alter functional microbiome-host interactions that re-shape historical patterns of immune responses to allergen exposure and respiratory viral infection.

GUT MICROBIOTA AND IMMUNE FUNCTION

The importance of gut microbiota in the development of the host immune system was first shown by studies of germ-free (GF) mice, which have poorly developed gut and mesenteric lymphoid tissue, decreased production of IgA, skewed T-cell populations in gut tissue, and impaired resistance to infection [36] [37]. These abnormalities in immune function can be reversed by introduction of gut bacteria, but only if done early in life [38].

That manipulation of gut microbiota of mice can induce or reverse the patterns of immune dysfunction associated with allergy has been demonstrated by studies showing that introduction of specific species of *Clostridium* (Clades IV or XIVA) and *Bacteroides* enhances production of the T-regulatory cells essential for immune tolerance [39,40]. An excess, rather than a deficiency, of other microbes may also be associated with asthma-like changes. Mice deficient in induced regulatory T cells (iTregs) spontaneously develop T2-type pathologies; they exhibit macrophage and neutrophil infiltration of the bronchial mucosa, goblet cell and smooth muscle hyperplasia, mucin hyper-secretion, and impaired lung function [41]. The gut micorbiome of iTreg-deficient mice is altered, with enrichment of members of the TM7 phylum and of *Alistipes* species [41]. Thus, the presence of specific bacteria appear to be critical to the proliferation of T-helper subsets in mice, and a deficiency in these species or an excess of other species in the developing gastrointestinal microbiome may favor development of the impaired or imbalanced immune function that underlies allergic disease.

EARLY LIFE ENVIRONMENT, GUT MICROBIOME, AND CHILDHOOD ALLERGIC ASTHMA

The linkage between maternal exposure to domestic animals throughout pregnancy and protection against allergic asthma [42] may be mediated through fetal exposure to microbes. Although the uterine environment was once thought to be sterile, recent studies have indeed described a placental microbiome [43]. Murine studies have also shown that maternal intranasal introduction of a bacterium isolated from cowsheds, *Acinetobacter lwoffii F78*, protected against induction of airway allergic sensitization and inflammation by ovalbumin challenge in their offspring [44]. A second study showed that introduction of four intestinal bacteria associated with lowered rates of allergic asthma in a birth cohort study

(*Lachnospira, Veillonella, Faecalibacterium, and Rothia)* into maternal mice also induced resistance to allergic sensitization and inflammation [45].

Whatever microbial exposures might occur *in utero*, the birthing process represents an abrupt introduction of the neonate to a diverse microbial world. Vaginally delivered infants are colonized by a microbiome similar to that of their mother's vaginal tract, enriched with *Snethia* and *Lactobacillus* species [46]. Caesarian-born infants (who face significantly higher risk of allergic disease [47]) typically begin life with a microbiome similar to that of their mother's skin, enriched with *Staphylococcus* and *Streptococcus* species [46]. Because the species that initially colonize an ecosystem frequently determine ecosystem conditions, thus dictating the types of organisms that co-colonize or succeed them the niche, this difference in early inoculum could lead to microbiomes deficient in the commensal species necessary for appropriate immune development.

Exposure to household pets, especially to dogs, in early life is associated with lower rates of allergic sensitization and asthma in childhood [19,20]. Reasoning that this protection might be mediated by pet-associated microbes, Fujimura et al undertook a 16S rRNA-based examination of dust collected from dog, cat, and no-pet households. They found richer, more diverse bacterial communities in the house dust from residences with dogs compared to residences with no pets. Many of the more abundant bacterial taxa have previously been detected in the human gut microbiome [48]. In the bacteria-depleted house-dust, they additionally found a wider range of fungal species, which themselves have been associated with allergic disease development [49–51].

A more recent study suggests some surprising additions to the list of animals whose presence in a household appears to be protective against the development of asthma. This study again applied 16s rRNA-based methods to examine the bacterial communities present in house dust collected from the homes of poor, inner-city households that had enrolled a newborn in a longitudinal birth cohort study [Urban Environment and Childhood Asthma (URECA] study) of associations with the development of the rates of allergy and asthma in an environment whether the rates of allergy and asthma - and the levels of cockroach and mouse allergen – are high [52]. The house dust was collected when the infants were 3–6 months of age. The dust from residences in which the child later developed atopy alone or atopy and recurrent wheeze exhibited reduced bacterial diversity and richness. Surprisingly, these same house dust samples often had low levels mouse, cockroach and cat allergen. In fact, the children exposed to the highest levels of both microbes and allergena had the lowest rate of development of atopy and recurrent wheeze, whereas those exposed to the lowest levels of both had the highest rates. This raises the possibility that it is early life exposure to the combination of environmental microbes and allergens that promotes immune tolerance. Another interpretation, however, is that the levels of allergen were simply markers of the presence of the creatures (cockroaches, dogs, cats) responsible for introducing protective bacterial species into the environment. The house dust samples from the "protective" environments were in fact significantly enriched for a large number of gastrointestinal-associated bacteria, including obligate endosymbionts of cockroaches. Taken together, these findings suggest that rodents or cockroaches in inner city environments may serve a function similar that served by dogs in other environments, enhancing the diversity of bacteria

accessible to the developing infant gut microbiome and thus shaping the pattern of development of immune function away from disposition to allergic sensitization.

Fujimura and colleagues further enhanced the concept that household microbiota represent a determinant of risk of development of allergy and asthma in their study of the effects of supplementing mice with house dust collected from a dog-keeping household. These animals showed significantly reduced responses to sensitization and airway challenge with cockroach allergen [53], as reflected by lowered airway expression of Th2 cytokines (IL-4 and IL-13), mucin secretion (assessed by Gob5 gene expression), and improved airway histology compared to control, unsupplemented animals or those supplemented with house dust from a pet-free household. The gut microbiome of the protected mice exhibited significant enrichment for ~100 bacterial taxa, including one represented by *Lactobacillus johnsonii*. Fujimura et al. then showed that supplementation of mice with *L. johnsonii* protected them against both cockroach and ovalbumin airway sensitization and challenge. Moreover, airway protection extended beyond common allergens, since *L. johnsonii* supplementation also afforded protection against infection with respiratory syncytial virus. In addition to the reductions in airway Th2 responses, protected animals exhibited significantly reduced numbers of activated dendritic cells in their mesenteric lymph nodes, indicating that changes in the gut microbiome influence the capacity of antigen presenting cells to activate local T-cell populations, a plausible mechanism of induction of immune tolerance.

Candidates for the mechanism by which gut microbiota might alter immune function must include short-chain fatty acids produced through microbial fermentation of complex carbohydrates [54]. Their importance in the context of allergic disease was recently illustrated by Trompette et al. [55] who fed mice either a low- standard, or high-fiber diet prior to reeated nasal exposure to house dust mite (HDM) extract. Mice fed a low-fiber diet exhibited significantly increases in lung tissue IL-4, IL-5, IL-13 and IL-17A, airway mucus production and goblet cell hyperplasia, circulating IgE and total HDM-specific IgG1. In contrast, mice fed a high-fiber diet exhibited significantly lower cytokine concentrations and a normal mucin phenotype. The gut microbiome of these animals differed; animals on the low-fiber diet showed increased abundance of *Firmicutes*, especially those belonging to the *Erysipelotrichaceae* family, while animals on a high fiber diet were enriched for *Bacteroidaceae* and *Bifidobacteriaceae*. Since SCFAs were found to be significantly increased in the high-fiber diet animals, the investigators supplemented other animals with one of these, propionate, and showed increased Foxp3$^+$CD25$^+$ CD4$^+$ T-reg cell numbers and enhanced hematopoiesis of DC precursors in propionate-supplemented animals. Further support for a role of the gut microbiome in allergic disease development comes from a recent study by Arrieta et al., who demonstrated in ~300 neonatal and infant fecal samples from a large birth cohort study that neonatal bacterial community dysbiosis is associated with atopy and recurrent wheeze development in childhood, indicating that perturbations to very early-life gut microbiome composition and, presumably, concomitant microbial dysfunction, underlies childhood atopy [45]. They further found that the fecal samples collected at 3-months of age in the infants who went on to develop atopy and wheeze contained lower level of acetate, a short-chain fatty acid. As described above, they also found that resistance to allergic sensitization and inflammation was induced in the offspring

of maternal mice fed the four intestinal bacteria most strongly associated with lowered rates of allergic asthma [45].

EARLY LIFE ENVIRONMENT, BRONCHIAL MICROBIOME, AND ASTHMA DEVELOPMENT

This brief review has focused on the possible role of the gut microbiome in shaping the pattern and rate of development of immune function toward the development of allergic sensitization and asthma. That bacteria colonizing the airways in the neonatancy and infancy might also shape the response to allergen exposure and to viral respiratory infection is also possible [56], but is beyond the scope of this review, especially since it is elegantly addressed by A. Beigelman in this edition.

CONCLUSION

Emerging data offers a "common ground" hypothesis for the rising prevalence of atopy and asthma, in which the gut and respiratory microbiomes, whose composition and function are influenced by environmental influences ranging from diet to antimicrobial administration to early local environmental microbial exposures, play a significant role in disease development. Studies of animal models of allergy support the existence of a gut-airway axis and offer insights into the mechanisms by which the activities of the gut microbiome influences immune responses at remote mucosal sites. Hence, strategies aimed at shaping microbial community composition and function, particularly in the neonatal phase of life, may offer a novel strategy for prevention of childhood allergic asthma [8,11].

Acknowledgments

Abbreviations

HRV	Human rhinovirus
IL	Interleukin (e.g., IL-4, IL-13)
iTregs	Induced Regulatory T cells (develop from conventional mature CD4^{+} T cells outside of the thymus)
RSV	Respiratory syncytial virus
SCFA	short-chain fatty acid
Tregs	regulatory T cells
URECA	Urban Environment and Childhood Asthma

16S rRNA   16S ribosomal RNA, a component of a subunit of prokaryotic ribosomes, used for reconstructing phylogenies

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KEY POINTS

- The rapidity of increase in the prevalence of atopic disease, especially of asthma, suggests a change in environment in westernized societies is somehow related.

- Epidemiologic studies suggest that early life exposure to sources of rich, diverse microbial populations is protective.

- Interventional studies of mice show that the composition of gut microbiota shapes the rate and pattern of development of immune function, and that introduction of particular bacteria or of particular collections of bacteria can reduce susceptibility to allergic sensitization and inflammation and to viral respiratory infection.

- Identification of the functions of these "protective" microbes or microbial collections – possibly their production of short-chain fatty acids - may lay the groundwork for strategies for primary prevention of asthma and other atopic diseases.