Gut Microbiota and Allergic Disease

New Insights

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Abstract

The rapid rise in childhood allergies (atopy) in Westernized nations has implicated associated environmental exposures and lifestyles as primary drivers of disease development. Culture-based microbiological studies indicate that atopy has demonstrable ties to altered gut microbial colonization in very early life. Infants who exhibit more severe multisensitization to food- or aero-allergens have a significantly higher risk of subsequently developing asthma in childhood. Hence an emerging hypothesis posits that environment- or lifestyle-driven aberrancies in the early-life gut microbiome composition and by extension, microbial function, represent a key mediator of childhood allergic asthma. Animal studies support this hypothesis. Environmental microbial exposures epidemiologically associated with allergy protection in humans confer protection against airway allergy in mice. In addition, gut microbiome–derived short-chain fatty acids produced from a high-fiber diet have been shown to protect against allergy via modulation of both local and remote mucosal immunity as well as hematopoietic antigen-presenting cell populations. Here we review key data supporting the concept of a gut–airway axis and its critical role in childhood atopy.

Keywords: allergy; microbiome; gastrointestinal tract; built environment; short chain fatty acids

(Received in original form July 20, 2015; accepted in final form October 13, 2015)

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Ann Am Thorac Soc Vol 13, Supplement 1, pp S51–S54, Mar 2016
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DOI: 10.1513/AnnalsATS.201507-451MG
Internet address: www.atsjournals.org

Historical Relationships between Childhood Allergic Sensitization and Infant Gut Microbiology

Atopic sensitization (allergy) is considered the strongest risk factor for childhood asthma development in Westernized nations (1), and therefore it is unsurprising that its rise in prevalence over the past several decades is associated with a parallel increase in asthma (2). Atopy is consistently associated with elevated total (and allergen-specific) serum IgE. Until relatively recently, arbitrary cutoffs, for example, equal to or exceeding 0.35 kU L^{-1}, were used to define atopic status, with those falling below this breakpoint considered nonatopic, whereas those above it were deemed atopic. More recently a less ambiguous and more statistically rigorous approach, latent class analysis, which clusters subjects on the basis of their pattern of allergic sensitization, has been used to objectively define patient subgroups. Using this approach and samples from a large birth cohort, Havstad and colleagues identified four distinct classes of subjects at age 2: (*1*) Low to no sensitization (n = 457), (*2*) highly sensitized (n = 16), (*3*) milk and egg sensitized (n = 91), or (*4*) peanut and inhalant sensitized (n = 30) (3). Moreover, the authors demonstrated that children in group 2, who exhibit multiple early-life sensitizations to a range of food and aero-allergens, for example, egg, peanut, dog, cat, and cockroach, are at higher risk of subsequently developing physician-diagnosed asthma at age 4 (odds ratio, 5.3; 95% confidence interval, 1.6–17.4), compared with those who are either nonsensitized or lowly sensitized to one or two of these allergens (3). This indicates that the use of statistical approaches to stratify subjects on the basis of their profile of allergic sensitization offers improved capacity to predict clinical outcomes. Moreover, it also indicates that children who are predominantly multisensitized in very early life incur a significantly increased risk of asthma development in later childhood.

Childhood sensitization has previously been linked to changes in the relative abundance of specific gut microbial species in early life. Kalliomäki and colleagues demonstrated that 3-week-old neonates who possessed significantly higher fecal burden of *Clostridium difficile* and a higher ratio *of C. difficile* to *Bifidobacterium*, had a significantly higher rate of atopy development (as defined by skin prick testing to a range of food and aero-allergens and diagnosis of atopic dermatitis) (4). In a separate study, infants at 1 month of age in

whom IgE-associated eczema was developing exhibited a significantly higher relative abundance of fecal *Escherichia coli* (5). In addition to demonstrable differences in the relative abundance of specific fecal microbial species, epidemiological studies have identified several risk factors associated with the development of allergic sensitization. These include early-life antimicrobial exposure (6), caesarean birth (7), formula feeding (8, 9), lack of maternal exposure to pets or livestock during pregnancy (10), and maternal consumption of antimicrobials during pregnancy (11). It should be noted that the relationship linking formula-feeding with allergic disease was based on studies of formula that was not supplemented with prebiotics. More recent studies have demonstrated that prebiotic-supplemented formula increases *Bifidobacterium longum* abundance (characteristically depleted in allergic children [12]) and overall diversity of this protective genus, to levels beyond that observed in breast-fed infants (13). Risk factors associated with allergic sensitization, which have been identified and are conspicuously concentrated in the pre- and early postnatal phases of life, plausibly influence neonatal (and maternal) microbiome composition, that is, the mixed-species communities of microbes that exist in and on, and interact with, the human host. This has been demonstrated for antimicrobial administration, which acutely depletes gut microbiome diversity (14) and for particular infant formulas, which deplete critical commensal *Bifidobacterium* populations in the infant gut (15–17). Collectively, these studies have suggested that the foundation for childhood allergic sensitization may lie, at least in part, in early-life gut microbiome perturbations during the critical period of microbiological and immunological development.

Early-Life Gut Microbiome Development

DNA-based microbiome profiling studies have described the presence of a placenta-associated (18) and cord blood–associated (19) microbiome, indicating *in utero* exposures to, at the very least, microbial DNA, a potent ligand for innate Toll-like receptor signaling. This may explain the relationship between maternal exposures in pregnancy and the risk of allergic disease development in childhood. Birthing represents an important microbiological exposure as has been demonstrated by Dominguez-Bello and colleagues, who showed that vaginally delivered infants are colonized on their skin and in their gastrointestinal tract by microbes such as *Sneathia* and *Lactobacillus* species, which represent members of the mother's vaginal tract microbiome (20). Caesarean-born infants exhibit a compositionally distinct bacterial colonization pattern, dominated by skin-associated species such as *Staphylococcus* and *Streptococcus* species, and skin and gut assemblages most similar to the skin microbiome of their mothers (20). Incidentally, caesarean section delivery represents a risk factor for allergic disease development, with section-delivered infants exhibiting a higher risk ratio for eczema and sensitization compared with vaginally born subjects (7).

In healthy infants, the gut microbiome becomes increasingly diverse over the first 3 years of life, at which point the phylogenetic distribution of bacterial species present largely resembles that of adults (21). The accumulation of bacterial species and their encoded functions obeys the central tenets of primary succession, a well-established series of ecological events that occur during the initial colonization of a previously pristine environment. The species that initially colonize an ecosystem frequently define ecosystem conditions, which in turn can influence subsequent temporal patterns of co-colonization and ecosystem productivity. Hence, a developing hypothesis in the field of allergy research is that colonization of the neonatal gut microbiome by invasive microbial species not typically found in the earliest stages of life in this niche may lead to increased competition with, and prevent colonization by, commensal microbial species found in the healthy infant gut microbiome, thus influencing the subsequent chronosequence of microbial colonization. This raises the possibility that the patterns of early aberrant microbial colonization in the gut, as described by Penders and colleagues (5) and Kalliomäki and colleagues (4), are hallmarks of a chronologically dysbiotic microbiome, enriched in potentially pathogenic species and lacking commensal species necessary for appropriate immune and physiological development as well as maintenance of immune homeostasis.

The Built Environment as a Source of Microbial Inocula for the Developing Gut Microbiome

As mentioned previously, the infant gut microbiome accumulates bacterial diversity over the first several years of life, although the source of this bacterial diversity remains unknown. In Westernized nations individuals spend protracted periods (almost 90%) in the built environment, with approximately 70% of time spent in their personal residences (22), leading to the hypothesis that the built environment may represent a key source of microbes for the developing infant gut microbiome. Indeed, specific built environments have been shown to protect against allergy; infants raised in households in which dogs or cats are present have a significantly lower risk of allergic disease development in childhood (10). Fujimura and colleagues examined the microbiological content of households with a dichotomous risk for allergic disease based on pet ownership and showed that pet-owning households were significantly increased in the number of types of bacteria present and possessed far fewer types of fungi (23). These findings are consistent with other, lower-resolution studies, which have also demonstrated that environments with no pets present are depleted of bacteria (24–26). A more recent study of the bacterial communities present in inner city household dust during the first year of life of children whose recurrent wheeze and atopic status at age 3 is known, produced similar findings. Residences in which the children developed atopy or atopy and recurrent wheeze exhibited reduced bacterial diversity and richness (number of types of bacteria detected) (27). Interestingly, in this study the most allergy-protective environments had significantly increased exposure both to bacteria and to specific allergens (mouse, cockroach, and cat) in early life. This raises the possibility that it is the combined early-life exposure to environmental microbes and allergens that promotes immune tolerance. However, the finding that specific allergens associated with animal or insect presence were associated with allergy protection raises an alternative possibility—that these specific animals or insects introduce protective bacterial species into the environment. This concept is supported by both the Fujimura

and Lynch studies (23, 27), in which the house dust of allergy-protective environments was significantly enriched for a large number of gastrointestinal-associated bacteria, including obligate endosymbionts of cockroaches in the latter (inner city) study (27). These data support the notion that pets, or even rodents in inner city environments, enhance the diversity of bacterial species available for inoculation of the developing infant microbiome, appropriate development of which is associated with protection against allergic sensitization.

Clearly one mechanism by which altered environmental exposures may influence allergic disease development is via changes in gut microbiome composition and function. Fujimura and colleagues (28) demonstrated, using a murine model of airway allergic sensitization, that exposure of animals to dog-associated house dust resulted in a significant reduction in airway expression of helper T-cell type 2 cytokines (IL-4 and IL-13) and in mucin secretion (as defined by histology and *gob-5* gene expression) compared with control, unsupplemented animals or those supplemented with house dust from a residence with no pets present. Protected animals exhibited significant gut microbiome compositional changes and were enriched for approximately 100 distinct bacterial groups, including one represented by *Lactobacillus johnsonii*. Supplementation of mice with *L. johnsonii* protected them against both cockroach and ovalbumin airway sensitization and against a viral respiratory pathogen, respiratory syncytial virus. In addition to significant reductions in airway helper T-cell type 2 responses, protected animals exhibited significantly reduced numbers of activated dendritic cells in the mesenteric lymph nodes, indicating that changes in the gut microbiome associated with *L. johnsonii* supplementation influence the capacity of antigen-presenting cells to activate local T-cell populations, a plausible mechanism of induction of immune tolerance.

Indeed, other murine studies have demonstrated the capacity for specific gastrointestinal species to promote particular adaptive immune responses. For example, early-life introduction of a cocktail of 46 chloroform-resistant, spore-forming *Clostridium* clade IV and XIV species (29) or, in an independent study, a diversity of bacteria (30), significantly decreased circulating IgE concentrations (elevated serum IgE is a hallmark of atopy) in adulthood. Interestingly, this protection against allergic sensitization was not observed if the microbial species introduced occurred in adult animals, supporting the concept that early-life microbial colonization represents a formative influence on allergic disease susceptibility. Moreover, the *Clostridium* cocktail induced antiinflammatory $CD4^+Foxp3^+$ T-regulatory (Treg) cells and decreased airway IL-4 concentrations after ovalbumin challenge, indicating that appropriate gastrointestinal microbial colonization in early life leads to enhanced capacity to down-regulate pro-allergic responses.

Gut Microbiome Metabolism as a Mechanism of Protection against Allergic Sensitization

More recently, insights into the mechanisms underlying the capacity of the gut microbiome to prevent allergic response have implicated microbial-derived metabolites, especially short-chain fatty acids (SCFAs) such as butyrate (31), acetate, and propionate (32) as key drivers of T-cell subset proliferation and activity. In addition to acting as an essential energy source for gastrointestinal colonocytes, SCFAs are antiinflammatory and increase significantly upon induction of colonic $CD103^+FoxP3^+$ cells and IL-10 production (32, 33). The G protein–coupled receptor 43 (GPR43; also known as free fatty acid receptor 2 [FFAR2]) binds SCFAs and mediates their effect on colonic Tregs (32). GPR43 is essential for SCFA signaling; $Gpr^{-/-}$ mice exhibit significantly lower IL-10 expression (32) and increased allergic airway inflammation (34). Gastrointestinal microbes generate SCFAs through fermentation of complex dietary carbohydrates. Trompette and colleagues (35) demonstrated that mice fed a low-fiber diet before nasal exposure to house dust mite extract exhibited significantly increased IL-4, IL-5, IL-13, and IL-17A in lung tissue, increased mucus production and goblet cell hyperplasia in their airways, and higher levels of circulating IgE. In contrast, mice fed a high-fiber diet exhibited significantly lower cytokine concentrations and a normal mucin phenotype. Fiber intake influenced the gut microbiome composition; low-fiber animals became enriched for *Erysipelotrichaceae*, whereas a high-fiber diet promoted the relative abundance of specific *Bacteroidaceae* and *Bifidobacteriaceae* species. In subsequent studies, supplementation of animals with the SCFA propionate led to increased $Foxp3^+CD25^+CD4^+$ Treg-cell numbers and enhanced hematopoiesis of dendritic cell precursors (35). These seminal studies indicate that gut microbial SCFA production not only down-regulates proinflammatory responses at the site of allergen insult, but that circulation of these metabolites also influences bone marrow–derived antigen-presenting cell precursors, indicating a mechanism by which gut microbial–derived metabolites may reprogram the immunological tone of the mammalian ecosystem.

Thus the emerging data support the existence of a gut–airway axis. It also indicates that gut microbiome composition and metabolic activity, which are influenced by environmental microbial exposures and dietary intake, not only impact local gastrointestinal immune activation status, but also dictate immunological responses and define cellular populations at both remote mucosal sites and in the hematopoietic compartment. Hence the gut microbiome may represent a critical target for the prevention or management of allergic asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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