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REVIEW

The 'microflora hypothesis' of allergic diseases

M. C. Noverr* and G. B. Huffnagle*†

*Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine and †Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Summary

Increasingly, epidemiologic and clinical data support the hypothesis that perturbations in the gastrointestinal (GI) microbiota because of antibiotic use and dietary differences in 'industrialized' countries have disrupted the normal microbiota-mediated mechanisms of immunological tolerance in the mucosa, leading to an increase in the incidence of allergic airway disease. The data supporting this 'microflora hypothesis' includes correlations between allergic airway disease and (1) antibiotic use early in life, (2) altered fecal microbiota and (3) dietary changes over the past two decades. Our laboratory has recently demonstrated that mice can develop allergic airway responses to allergens if their endogenous microbiota is altered at the time of first allergen exposure. These experimental and clinical observations are consistent with other studies demonstrating that the endogenous microbiota plays a significant role in shaping the development of the immune system. Data are beginning to accumulate that a 'balanced' microbiota plays a positive role in maintaining mucosal immunologic tolerance long after post-natal development. Other studies have demonstrated that even small volumes delivered to the nasopharynx largely end up in the GI tract, suggesting that airway tolerance and oral tolerance may operate simultaneously. The mechanism of microbiota modulation of host immunity is not known; however, host and microbial oxylipins are one potential set of immunomodulatory molecules that may control mucosal tolerance. The cumulative data are beginning to support the notion that probiotic and prebiotic strategies be considered for patients coming off of antibiotic therapy.

Introduction

In the United States, Canada, United Kingdom, Ireland, New Zealand and Australia, the incidence of allergic airway disease among 13–14-year old children is currently the highest in the world and ranges from 22% to 32% [1]. The high incidence in these countries is consistent with a world-wide trend in which the incidence of allergic airway disease in industrialized countries has increased over the past 40 years while remaining stable in developing countries [2]. This recent increase in the asthma rate, coupled with the dichotomy in the incidence of asthma between industrialized and developing countries, suggests that environmental changes are a major factor in the development of asthma [3–5]. Numerous studies have laid the foundation for the hypothesis that a lack of early microbial stimulation (infection or exposure) results in aberrant immune responses to innocuous antigens later in life, i.e. the 'hygiene hypothesis' [6–8]. In addition, other environmental factors such as differences in diet between industrialized vs. developing countries have been noted. This review focuses on an alternative interpretation of the data supporting the 'hygiene hypothesis.' The 'microflora hypothesis' proposes that perturbations in the gastrointestinal (GI) microbiota because of antibiotic use and dietary differences in 'industrialized' countries have disrupted the normal microbiota-mediated mechanisms of immunological tolerance in the mucosa, which has led to an increase in the incidence of allergic airway disease.

There is a significant amount of epidemiologic and clinical data supporting this altered microflora hypothesis. These include correlations between allergic airway disease and (1) antibiotic use early in life, (2) altered fecal microbiota and (3) dietary changes over the past two decades. Our laboratory has recently demonstrated that mice can develop allergic airway responses to allergens if their endogenous microbiota is altered at the time of first allergen exposure [9, 10]. In contrast, mice with normal microbiota do not develop allergic responses upon airway exposure to the allergens. These experimental and clinical observations are consistent with other studies demonstrating that the endogenous microbiota plays a significant role in shaping the development of the immune system [11–18]. There is currently a significant level of interest in the role of the endogenous microbiota as initiators of inflammatory diseases such as inflammatory bowel disease and arthritis [19, 20] and in how the immune system adapts to the presence of the microbiota in the GI tract during development [21–23]. However, we believe that it is now becoming clear that a 'balanced' microbiota plays a positive role in maintaining mucosal immunologic tolerance long after post-natal development, similar to ideas proposed by Rook and Brunet [24]. Thus, we predict that elements that

Correspondence: Gary B. Huffnagle, Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, University of Michigan Medical School, Ann Arbor, MI 48109-0642, USA.
E-mail: ghuff@umich.edu

disrupt the dynamics of the microbiota, such as antibiotics and diet, will disrupt mucosal tolerance.

Regulation of mucosal tolerance

Repeated antigen exposure in the airways does not result in enhanced reactivity; rather, it leads to decreasing responsiveness and the development of immunologic tolerance to the antigen [25–28]. Regulatory T cells (Treg) are the mediators of immunological tolerance and these cells possess anti-inflammatory capabilities (Fig. 1). Treg comprise a diverse group of cells that, even in small numbers, can suppress antigen-specific responses (largely via IL-10 and/or transforming growth factor-β). The type and maturation state of the antigen-presenting cell appears to play the most significant role in the development of Treg. Dendritic cells (DC) are the antigen-presenting cell primarily responsible for the antigen-specific activation of naïve T cells. While mature DC (activated by inflammatory signals) induce differentiation of inflammatory T cells (T helper [Th]1 and Th2), immature DC (absence of inflammatory signals) appear to induce the differentiation of Treg (Fig. 1) [29–31]. Resident DC in the airways are normally exposed to airborne antigens under non-inflammatory conditions that are believed to induce Treg responses to the antigens, thereby limiting T cell-mediated inflammatory responses. Interestingly, compared with immunogenic proteins, aeroallergens are micro-particulates that contain a number of moieties that are immunogenic and can stimulate inflammatory cytokine production by lung leucocytes when isolated in pure form [32]. However, aeroallergens induce minimal, if any, response in the lungs upon repeated exposure demonstrating that they are subject to tolerigenic mechanisms.

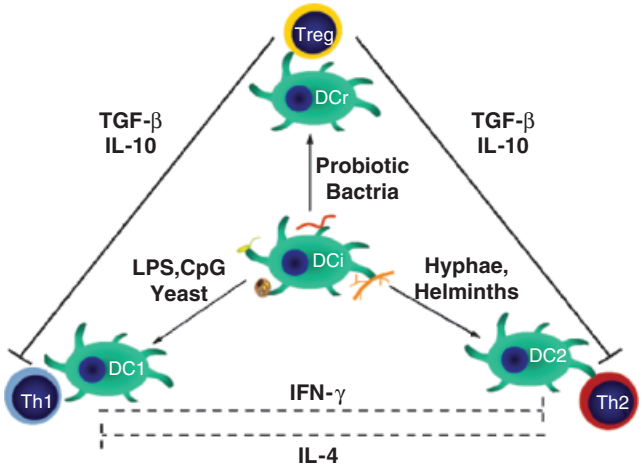

Fig. 1. T cell subset interactions and the role of dendritic cell (DC) maturation in the development of T cell subsets. Immature dendritic cells (DCi) can mature into regulatory (DCr), type 1 (DC1) and type 2 (DC2) phenotypes depending on the interaction between pathogen recognition receptor (PRR) and Toll-like receptor (TLR) binding to different microbes and microbial products. In addition, DC differentiation is also influenced by cytokines and oxylipins (prostaglandins and leukotrienes) in the local environment. These different DCs promote differentiation of naïve T cells into regulatory (Treg), type 1 (Th1) and type 2 (Th2) T cells, respectively. Treg can dampen both Th1 and Th2 cells by production of transforming growth factor-β and IL-10. Th1 and Th2 can counter-regulate each other via cytokine production.

Oral delivery of antigens also leads to the development of antigen-specific immunologic tolerance, a phenomena known as oral tolerance [33–35]. Oral tolerance to an allergen can block responses outside of the GI tract including the allergic response to that allergen in the lungs [36–38]. Similar to airway tolerance, oral tolerance is also mediated by Treg cells [27, 28, 34, 35]. The cellular mechanisms underlying oral tolerance are still an arena of intense investigation. Studies have demonstrated that (1) the normal microbiota is required for the generation of oral tolerance as it cannot be generated in germfree mice and (2) conventionalization of germ-free mice with normal microbiota can restore the ability to generate oral tolerance in these mice, indicating that tolerance continues to be regulated by the microbiota long after the post-natal period [11, 39].

Induction of oral tolerance has been suggested as a therapeutic strategy for treating asthma [27, 28, 34], but does oral tolerance normally play a role in down-regulating immune responses to inhaled allergens? The mucociliary architecture of the nasopharyngeal cavity and upper airways naturally sweeps all inhaled micro-particulates that stick to the mucus lining into the GI tract. Shortly after intranasal inoculation, fluids, particles and microbes introduced into the nasal cavity are largely found in the GI tract [40–42]. In mice, intranasal inoculation of a volume as small as 2.5 μL still largely ends up in the GI tract [41]. Thus, inhaled micro-particulates (which comprise the vast majority of aeroallergens) are also swallowed. Therefore, we propose that oral tolerance and airway tolerance are tightly linked and the GI tract acts as a 'sensor' for the development of tolerance to inhaled antigens. We propose that this 'sensor' system can be modified by genetics (affecting innate immune cells) but to an even greater extent by microbiota perturbations exerted by antibiotics and diet (Fig. 2).

Establishment and dynamics of the gastrointestinal microbiota

The GI tract of infants is sterile at birth but colonization begins upon delivery. GI colonization involves a succession of microbial populations waxing and waning as the diet changes and the host develops [43, 44]. Major factors affecting the nature of the early microbial populations are vaginal delivery vs. caesarian delivery, antibiotic use in the mother and bottle-feeding vs. breastfeeding. By adulthood, the microbial community generally stabilizes and is composed of both permanent members and transient colonizers, which are briefly introduced from an exogenous source. Both prokaryotic and eukaryotic microbes are present although bacterial species predominate. The majority of the bacterial species are strict anaerobes (97%), while only 3% are aerobic (facultative anaerobes). While an estimated 30–40 species predominate within the adult GI tract, the microbiota is composed of 400–1000 species, including approximately 60% unculturable species. The composition of the microbiota differs not only along the length of the GI tract, but also cross-sectionally, with different populations inhabiting the GI mucosa and lumen. The most common anaerobic genera in terms of concentration within the GI tract are *Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Clostridium* and *Lactobacillus*. Among the facultative anaerobes are the

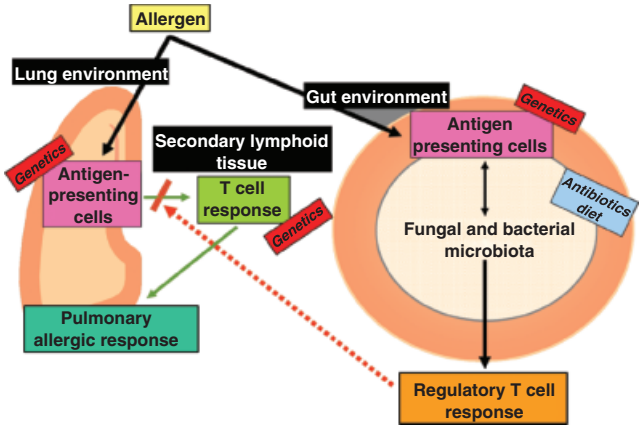

Fig. 2. Model for the regulation of pulmonary allergic responses by the microbiota and gastrointestinal (GI) immune responses. Inhaled aeroallergens are picked up by antigen-presenting cells in the lungs such as resident dendritic cells. The allergen can stimulate dendritic cell (DC) maturation (see Fig. 1) that can promote the development of allergen-specific T cells in the secondary lymphoid organs. These allergen-specific T cells are then recruited into the lungs where, upon encounter with allergen laden antigen-presenting cells, they drive the initial phases of the pathophysiology of the allergic response. Regulatory T cell (Treg) networks are necessary to prevent the development of this over-exuberant allergic T cell response in the airways. Inhaled aeroallergens are also swallowed because the anatomy of the sinuses and upper airways is designed to trap environmental microparticulates in the mucus layer and then "sweep" them into the throat where they are swallowed. Ingested allergens are then processed by antigen-presenting cells (DC) in the GI tract. In the anti-inflammatory environment of a healthy GI tract, these DC become regulatory DC (DCr) and promote the development of a Treg response to the allergen. The microbiota plays a key role in signaling for this anti-inflammatory environment and disruption of the normal balance, including increased yeast growth, prevent the development of the Treg response to the allergen. One potential set of signal molecules produced by the host and microbes in the GI tract are oxylipins. Antibiotics and diet will alter the microbiota balance while host genetics could modulate the response by altering the innate recognition response.

Gram-negative enteric bacteria (*Escherichia coli* and *Salmonella* spp.), the Gram-positive cocci (*Enterococcus*, *Staphylococcus* and *Streptococcus*) and fungal species (predominantly *Candida albicans*). Control of the microbiota populations occurs at the levels of microbe–microbe interactions (competitive exclusion), metabolic competition, host factors and host defenses such as IgA and defensin production [21–23]. As described below, antibiotics and diet can dramatically affect the stability of the microbiota populations.

The association between altered microbiota and allergic disease

Numerous studies indicate that the GI microbiota is different in atopic vs. non-atopic individuals and in industrialized vs. developing countries [45–51]. Sweden has a high incidence of allergic disease while Estonia has a low incidence. In a series of studies, it was shown that allergic children from either country have similar microbiota composition but the composition differs from non-atopic children. Atopy was associated with increased levels of aerobic microbes and decreased levels of anaerobic microbes, particularly lactobacilli, in fecal samples [45]. In a prospective study, it was noted that infants that developed allergies harbored decreased levels of *Bifidobacteria* and *Enterococcus* species but had increased levels of *Clostridium* species [46]. This is consistent with reports of decreased levels of bifidobacteria and Gram-positive organisms among the aerobic populations in infants with atopic eczema [49]. Individuals living an anthroposophic lifestyle abstain from antibiotic use and ingest fermented foods containing probiotic organisms [52]. Studies on this population of individuals also noted a decreased incidence of atopy compared with the surrounding community and fecal samples contained higher levels of lactic acid bacteria. As discussed below, antibiotic use and dietary differences, such as an increased proportion of refined foods in the diet and differences in fat intake, likely play a role in GI microbiota differences between industrialized and developing countries.

Role of antibiotics in microbiota dynamics and allergic responses

The major effects of antibiotic treatment on the microbiota are the direct effect of killing a large proportion of the microbiota and the indirect effect of decreasing colonization resistance within the GI tract. Colonization resistance is a multi-faceted mechanism whereby obligate anaerobic microbiota inhibit the overgrowth of potentially harmful exogenous or endogenous microbes. The end result of a reduction in colonization resistance can either be clinically asymptomatic (leading only to an imbalance in the microbiota), localized symptomatic (e.g. diarrhea) or systemic symptomatic (disseminated infection) [53]. In humans, yeast (*C. albicans*) infections of mucosal sites are one of the most common side effects of antibiotic therapy [53–57]. The ability of the bacterial microbiota to control or prevent *C. albicans* colonization is because of both competitive exclusion of favored niches and by production of growth-altering metabolites such as short chain fatty acids [58–62]. Thus, control of *C. albicans* by the normal microbiota (especially the probiotic species) is very important. Interestingly, changes in the microbiota populations can persist months after cessation antibiotic therapy and can result in long-term decreases in beneficial anaerobic organisms (*Bifidobacterium, Lactobacillus, Bacteroides*) and increases in potentially harmful microbes (Gram-negative aerobic enteric bacteria, the anaerobe *Clostridium difficile* and the yeast *C. albicans*) [55, 63–70].

Two lines of evidence support the concept that antibiotic use can be a major underlying factor in the development of allergic responses. The first are epidemiologic studies. A number of studies have identified a correlation between early antibiotic use in children and the subsequent development of allergy/asthma [71–74]. Other reports have identified a link between multiple ear infections early in childhood (which are treated with multiple courses of antibiotics) and the subsequent development of asthma [75]. Studies have also compared children of families with an anthroposophic lifestyle to children in neighbouring areas. Rates of allergy among anthroposophic children are also significantly lower and there is a correlation between the number of characteristic features of an anthroposophic lifestyle and decreasing risk of developing allergies [52]. Several features of the anthroposophic lifestyle are likely involved in promoting decreased rates of atopy (including a diet high in probiotics-fermented foods and restrictive use of vaccines, anti-pyretics and antibiotics) [76]. However, a study investigating anthroposophic children revealed that the use of antibiotic early in life was significantly associated with development of asthma

[74]. This indicates that antibiotic use within a cohort of children with similar lifestyles predisposes towards atopy. The second line of evidence is derived from national trends of antibiotic use vs. incidence of allergic disease in industrialized (high atopy, high antibiotic use) vs. developing countries (low atopy, low antibiotic use) [3–5]. While it is recognized that other interpretations exist for the correlative data described above, they are consistent with the hypothesis that antibiotic use may predispose an individual to developing allergic airway disease. Within families leading similar lifestyles and eating similar diets, there can exist atopic and non-atopic family members. It is therefore important to remember that genetics play an important role in determining whether atopic disease develops. However, what may be occurring is that environmental factors such as antibiotic use and diet (and subsequent effects on the GI microbiota) might work to uncover these genetic susceptibilities.

Role of the diet in microbiota dynamics and allergic responses

A significant amount of research effort was invested during the early 20th century in characterizing the effect of diet on the microbiota. This interest was in large part inspired by Eli Metchnikoff, who wrote extensively on the benefits of probiotic microbes in health. Some early experiments on the rodent microbiota demonstrated that it changed rapidly upon altering the diet [77, 78]. Perhaps even more relevant to the current health issues were later studies demonstrating that rodents fed an enriched bread diet exhibited a significantly delayed recovery of the microbiota ratios following antibiotic treatment compared to rodents fed a standard diet [78]. Prebiotics are food components that promote the growth of beneficial bacteria. Examples are prebiotic carbohydrates such as inulin and oligofructose, which stimulate growth of Bifidobacteria in the GI tract. In contrast, the simple sugar fructose stimulates growth of coliform and aerobic organisms in the GI tract [79]. These are a few of the experiments demonstrating that the composition of the host's diet plays a significant role in modulating species composition of the microbiota, as different species grow better on different substrates, which in turn can alter microbe–microbe interactions.

The role of diet in increasing or decreasing the incidence of allergic airway disease has been noted in a number of studies [80–84]. While antibiotic use in the Mediterranean countries of Spain, France, Italy and Greece is not necessarily different than that in the UK, Ireland or Australia, the asthma rates noted in the 1998 International Study of Asthma and Allergies in Childhood (ISAAC) report indicated that the incidence of asthma in these 'Mediterranean Diet' countries is significantly lower (Fig. 3) [1]. Significant attention has been paid to the role of dietary metabolites in direct immune system interactions during allergic responses [80, 81], but the diet also has a significant affect on the composition of the microbiota.

The role of fatty acids in allergic airway disease is not understood. There is a rough association between national polyunsaturated vegetable oil consumption and corresponding national incidence of atopy and asthma [82]. Another study of 10 European countries investigated the association

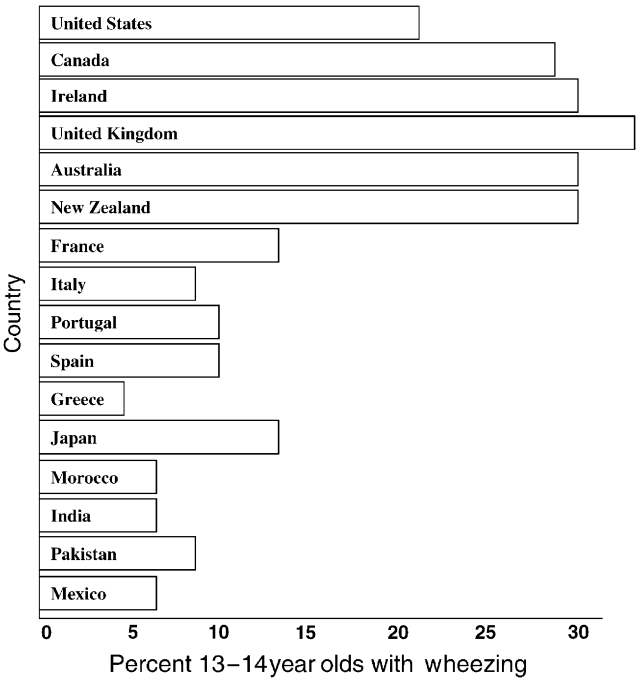

Fig. 3. Incidence of asthma in 13–14-year olds in selected countries around the world. The graph is based upon data from the 1998 International Study of Asthma and Allergies in Childhood (ISAAC) [1]. This graph highlights the lower incidence among Western European countries that follow a Mediterranean Diet.

between dietary trans-fatty acids and the prevalence of childhood asthma and allergies. There was a positive association between dietary trans fatty acids (expressed as percent of energy intake) and the prevalence of asthma, allergic rhinoconjunctivitis and atopic eczema [84]. Another example is a study of dietary fat intake vs. asthma in 478 men, 68 years of age, who were randomly selected from all the men born in Malmo, Sweden in 1914. The study concluded that men with asthma had a significantly higher intake of fat than men without asthma [85]. Generally speaking, these studies and others, discuss the possible role of dietary fats as substrates and modulators of leukotriene and prostaglandin production that would, in turn, augment allergic airway responses.

While dietary fatty acids may directly modify host responses, dietary fatty acid intake also plays a significant role in shaping the population dynamics of the microbiota. For example, a number of strictly anaerobic bacteria have strict requirements for long-chain fatty acids [86]. Thus, changes in dietary fats can alter one or more species of GI microbes, which in turn, can alter the numbers of other species of microbes by altering competitive exclusion dynamics. However, the argument continues to be circular in that the GI microbiota also plays a significant role in the metabolism of lipids and sterols, including biohydrogenation of sterols and fatty acids [87–89]. In the end, there is a tight relationship between dietary fat intake and modulation of GI microbiota dynamics. This raises the question of whether an alteration of GI microbiota populations by dietary fats is an underlying component of the dietary fat-asthma association.

An association has also been noted between higher dietary antioxidant intake and lower incidence of allergic airway

disease [80]. One class of antioxidants contains compounds such as vitamins C and E. However, another class of compounds includes polyphenols, which are found in high concentration in the skin of raw fruits and vegetables. A study in Italy demonstrated a correlation between high vegetable consumption and lower incidence of asthma [83]. Other studies have demonstrated an association between low fruit and vitamin C consumption and impaired lung function [80]. When antioxidant supplementation was examined as a preventative therapy pre-natally, differential results were observed. In atopic women, vitamin E supplementation was negatively associated with atopic disease in infants, while vitamin C was positively associated with atopy [90]. However, a separate study found that only vitamin C consumed as part of the diet (as opposed to a supplement) ended up in breast milk. In this study, results demonstrated that increased levels of vitamin C in breast milk was associated with a reduced risk of atopy in the infant. Therefore, an antioxidant-rich diet may be provide greater health benefits than antioxidant supplementation.

What is the mechanism underlying a protective effect of dietary antioxidants? One hypothesis is that membrane lipid peroxidation is elevated in the lungs of asthmatics, leading to cellular damage, the development of pathologic changes and hypersensitivity. Dietary antioxidants in the adult diet might protect against cellular oxidant damage [80]. However, many of the plant antioxidants (including polyphenols) belong to a class of compounds collectively called phytoalexins, which are plant host defense molecules produced in response to microbial attack [91]. Many plant polyphenols are potent inhibitors of the growth of a number of bacterial and fungal species and can also alter microbial metabolism. Thus, similar to dietary fatty acids, this raises the possibility that dietary antioxidants also alter the GI microbiota populations as an underlying component of the dietary antioxidant-asthma association.

One final diet–asthma association we wish to touch on is the association between bottle-feeding during infancy and increased risk of developing asthma. It was noted almost a century ago and confirmed in numerous other studies that there are significant differences in the GI microbiota between breastfed and bottle-fed infants [43, 92]. The chief difference between these two feeding regimens is that the microbiota of breastfed infants is composed mainly of lactic acid bacteria, while the microbiota of bottle-fed infants is more diverse, composed of a mixture of anaerobic bacteria as well as aerobic species [43]. Thus, the role of breastfeeding in protecting against atopic disease may also be related to the beneficial effects on the microbiota.

Experimental evidence that altered microbiota can promote the development of allergic airway disease

Our laboratory has recently developed a mouse model of antibiotic-induced GI microbiota disruption that is accompanied by stable increases in GI enteric bacteria and *C. albicans* levels [9, 10]. Using this model, we have addressed whether microbiota disruption can promote the development of an allergic airway response to mold spore (*Aspergillus fumigatus*) or ovalbumin (OVA) challenge. These studies utilized immunocompetent mice and did not involve previous systemic antigen priming as is typically used for breaking airway tolerance to these allergens. There was also no evidence of microbial growth in the lungs or inflammation in the GI tract in this model. The parameters measured included pulmonary eosinophilia, total serum immunoglobulin (Ig)E, lung leucocyte IL-5, IL-13 and IFN-γ, and goblet cell metaplasia. All of these parameters were significantly elevated in the microbiota disrupted mice (Fig. 4). Mice with unaltered microbiota did not develop an allergic response following intranasal challenge with either mold spores or OVA. The response did not develop in IL-13-deficient mice or mice that had been depleted of CD4 T cells. In addition, vigorous allergic airway responses could be generated in both C57BL/6 and Balb/c mice following microbiota disruption and antigen challenge but not in antigen-challenged 'normal microbiota' C57BL/6 and Balb/c mice. The presence of *C. albicans* in the GI tract was required to break airway tolerance. Thus, these studies demonstrate experimentally that antibiotic treatment, including fungal microbiota growth, can break airway tolerance to an aeroallergen such as mold spores or an experimental non-fungal allergen such as OVA.

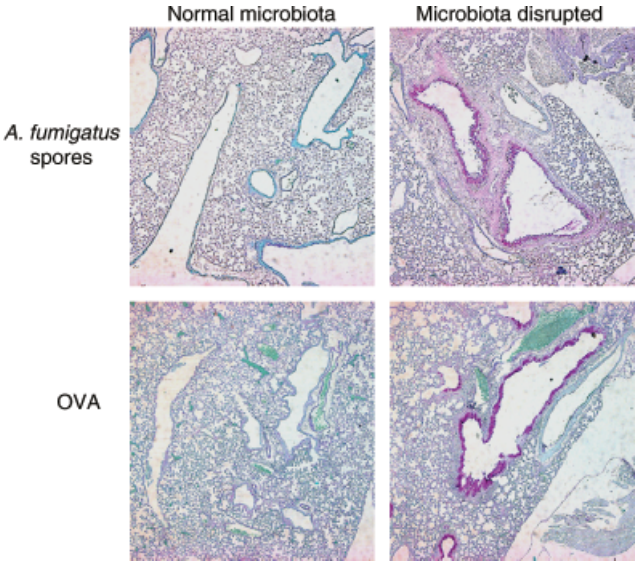

Fig. 4. Effect of gastrointestinal (GI) microbiota disruption on goblet cell metaplasia. One group of mice was treated with antibiotic in the drinking water for 5 days and then colonized in the GI tract with low levels of the yeast *Candida albicans*. The second group of mice was left untreated. For both groups of mice, the mice were exposed multiple times intranasally with either mold spores (*Aspergillus fumigatus*) or ovalbumin , beginning 2 days after cessation of the antibiotics in the first group. Approximately two weeks after the initial exposure, the lungs were harvested, fixed, sectioned and stained with Periodic-Acid Schiff stain to identify mucus-producing goblet cells (pink). Methyl green was used as a counterstain. These photographs illustrate the significant effect of microbiota disruption on promoting an allergic airway response to different types of allergens. Other parameters such as serum immunoglibulin E, pulmonary eosinophils, and IL-5/IL-13 production are also elevated by microbiota disruption during initial allergen challenge [9, 10].

Although these studies suggest a connection between the gut microbiota and pulmonary tolerance, the possibility that pulmonary tolerance may be directly affected by subtle changes in the upper respiratory tract microbiota as a result of antibiotic treatment cannot be ruled out. The bronchial mucosa and the GI mucosa use common mechanisms that allow these areas to discriminate among different antigens

and microbes in order to determine whether a tolerogenic or inflammatory response should follow [93, 94]. A recent study demonstrated that prior pulmonary viral influenza infection actually enhanced later allergen specific asthma in mice [95]. Pulmonary DCs isolated from lung after viral clearance were able to confer this allergic disease to recipient mice, indicating that similar mechanisms for maintenance and disruption of mucosal tolerance exist in the respiratory and GI tracts. The relative importance of the respiratory mucosa and the GI mucosa in controlling tolerance to inhaled/swallowed antigens remains to be determined.

What are the possible mechanisms underlying the break in tolerance during microbiota disruption?

This question clearly lies at the heart of the 'microflora hypothesis' of allergic airway disease. The most likely mechanism involves a break in the ability to generate Treg cells. Recall that antigens acquired by DCs in the absence of inflammation or in an anti-inflammatory environment such as a microbiota-balanced healthy GI tract preferentially stimulate the generation of Treg cells that can be recruited to the airways. Thus, it is our hypothesis at this point that microbiota disruption involves a disruption of this anti-inflammatory environment of the GI tract, where inhaled/swallowed micro-particulate antigens (aeroallergens) are acquired by DCs. The allergen-primed DCs then undergo maturation because of the stimulatory nature of the aeroallergen and a Treg response is not generated. In the lungs, the aeroallergen stimulates a mix of an inflammatory and a Th2 response, which is normally down-regulated by the Treg response, but is now left unchecked and develops into an allergic response in the airways.

Two possible mechanisms that may play a role in disrupting the anti-inflammatory environment of the GI tract are decreased short-chain fatty acid production by probiotic bacteria and increased oxylipin production by yeast. Short-chain fatty acids, such as butyric acid, are by-products of anaerobic fermentation by the normal probiotic members of the microbiota and these fatty acids possess anti-inflammatory activity [96–100]. Short-chain fatty acids can also inhibit *C. albicans* hyphal transformation, an important step in persistence of *C. albicans* on mucosal surfaces [58, 59]. Oxylipins are oxidized fatty acid metabolites that are produced by *C. albicans* and other fungi [101–103]. These oxylipins include leukotriene-like and prostaglandin-like molecules that can be synthesized *de novo* or via conversion of exogenous arachidonic acid by the yeast. Host-derived prostaglandins and leukotrienes are potent immunomodulatory molecules that can modulate innate and adaptive immune responses. Microbe-derived prostaglandin-like molecules are active on mammalian cells and can alter dendritic cell migration and biology [101]. The hypothesis of our current studies builds upon these observations and is investigating whether production of oxylipins is required for the immunomodulatory activity of *C. albicans*. Other potential mechanisms exist. However, it is intriguing that the fatty acid metabolite leukotriene C_4 (LTC_4) is required for dendritic cell movement from tissues to the lymph nodes, LTC_4 is secreted from cells via the action of a multi-drug resistance pump, and knockout mice deficient in one of the multi-drug resistance pumps spontaneously develop colitis, a disease believed to be caused by a deficient Treg response [104–106]. Thus, fatty acid metabolites from the host and the microbiota, which would be influenced by dietary fatty acid content, may play a critical role in maintaining the anti-inflammatory environment of the GI tract necessary for mucosal tolerance.

Probiotic therapy as treatment for atopic diseases

Probiotic supplementation has been practiced for over a century and has resulted in a litany of anecdotal evidence that suggests a connection to improved health. Probiotics are defined as live microbial supplements that exert a beneficial effect on health and are non-pathogenic. There is also some evidence that oral delivery of heat-killed saprophytic soil mycobacteria can down-regulate symptoms of allergic inflammation in animal models [107]. Current investigations of probiotic therapy provide statistical evidence that live microbial supplementation can produce positive results in both therapeutic and preventative ways. In regards to atopic diseases, the majority of human trials have focused on neonatal or infant subjects. In a randomized placebo-controlled study, *Lactobacillus casei GG* was effective in prevention of atopic eczema in children at-risk (one or more first-degree relatives with allergic disease) [108, 109]. Atopic eczema is often the earliest manifestation of subsequent allergic diseases in infants and children; therefore, a follow-up of this study was performed 2 years later. Interestingly, children who received prior probiotic supplementation exhibited protection from other atopic diseases that extended beyond infancy [108, 109]. A separate study demonstrated that the protection provided by early probiotic therapy also extends into adulthood [110]. In addition to post-natal therapy, pre-natal probiotic supplementation in pregnant women with a history of atopic disease reduced rates of atopy in their infants [111]. Other studies examined the ability of probiotics to treat atopic disease in infants. In these studies, probiotic supplementation decreased severity of atopic eczema, atopic dermatitis and food allergy compared with controls [112–117]. While these studies point to a role for modulation of the microbiota in children and infants as a preventative or therapeutic strategy against allergic disease, very little effort has been made to examine the role of probiotics in management allergy in adults. Among studies examining adults, probiotic supplementation resulted in a decrease in numbers of $CD34^+$ stem cell levels (an indicator for systemic allergic inflammation) [118]. In asthma patients, supplementation with yogurt containing probiotic *Lactobacillus* resulted in decreased pro-inflammatory cytokine levels and eosinophilia; however, clinical parameters of asthma were unchanged [119]. These results underscore the need for further clinical trials of probiotic and prebiotic therapy in adults with allergic disease.

Future perspectives

Allergic airway disease is a continuum of pathophysiologic changes in the host. It ranges from mild inflammation to severe inflammation and will ultimately result in structural changes in the airways and in the biology of the airway

smooth muscles and epithelium. It is clear from numerous studies that respiratory infection, airborne irritants, the nature of the allergen, exposure rates and genetics all influence the development and manifestation of asthma. However, it is our hypothesis that disruption of the normal microbiota breaks airway tolerance to aeroallergens (non-pathogenic micro-particulates that possess antigenic/inflammatory properties). Once tolerance is disrupted, other factors play a role in either promoting or preventing the development of airway hypersensitivity (genetics, infection). One of the difficulties in family association studies is that non-genetic effects are often difficult to discern from genetic effects. Diet, co-housing, physical interaction, non-life threatening communicable diseases and health practices are significantly more common between members of a family than between members of different families. These factors also influence an individual's microbiota. Thus, generating direct proof for the 'microflora hypothesis' of allergic disease will rely largely upon experimental animal models and well-controlled human intervention studies such as are now being proposed and carried out with probiotic therapies in children. The accumulating evidence also suggests that the medical establishment should more seriously consider the role of diet in chronic disease, think seriously about prescribing long-term antibiotics for non-life threatening conditions and also consider probiotic and prebiotic strategies for patients coming off of antibiotic therapy.

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