

Role of Intestinal Flora in the Development of Allergy

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Abstract and Introduction

Abstract

Purpose of Review: The frequency of allergic diseases is increasing worldwide. Experimental and clinical studies have linked a reduced number of early infections to this trend. The gastrointestinal system, which comprises the largest lymphoid tissue and microbial reservoir of the body, has received more attention during the last few years as a potential determiner in the development of atopic disease.

Recent Findings: Alterations in intestinal microbiota have been detected both in infants suffering from allergic disease and in those later developing the disorder. Delay in the compositional development of *Bifidobacterium* and *Lactobacillus* in gut microflora was a general finding in allergic children. In a subsequent study, perinatal administration of lactobacilli halved the later development of atopic eczema during the first 2 years of life. Specific strains of the healthy gut microbiota have been shown to induce the production of IL-10 and transforming growth factor- β , which possess an important regulative role in the development of allergic type immune response. Probiotics also strengthen gut defense barrier mechanisms and reduce antigen load in the gut. Pattern recognition receptors in intestinal epithelial and antigen-presenting cells have been demonstrated to mediate a continuing dialogue between host and gut microbiota.

Summary: Despite several promising findings, the exact role of gut normal microbiota in the development of allergy remains to be elucidated. For successful interventions, more data concerning a communication between host and specific microbial species are needed.

Introduction

Allergy, manifested in atopic eczema, allergic rhinoconjunctivitis and **asthma**, represents an epidemic of rising importance in both the developed and developing world.^[1] Atopic eczema is typically the first manifestation of the disorder followed by the subsequent respiratory allergic diseases.^[2] The immunological basis of allergy originates from the over expression of type 2 T helper cells, secreting IL-4, IL-5 and IL-13, which results in allergic inflammation in affected organs.^[3] The hygiene hypothesis of allergy has related a reduced early microbial exposure to the rising frequency of allergic diseases.^[4] In this overview we present recent epidemiological and experimental data which have both renewed the bipolar Th1-Th2 concept in the development of allergy and extended the hygiene hypothesis more tightly to the gut microbiota.^[5*, 6*] The host-microbe crosstalk in the gut as a delicate immunomodulation of the immune system and a potential target for therapeutical interventions is also discussed.

Allergic Type of Immune Response

T helper cells play a central role in the adaptive immune responses. Murine and human T helper cells can be divided into two major subtypes, Th1 and Th2.^[3, 7*] Th1 cells are characterized by the production of INF- γ and are vital for cell-mediated immune responses, whereas Th2 cells promote humoral immunity, for example IgE production and eosinophilia, by secreting IL-4, IL-5, and IL-13. Th1 and Th2 responses are counter-regulative, that is cytokines produced by Th1 cells inhibit Th2 function and vice versa.^[3, 7*]

Both cell types are required for the comprehensive immune system and the inappropriate skewing of immune response to either Th1 or Th2 lineage can result in either autoimmune or allergic disease, respectively.^[5*, 7*] Th2-skewed immune response has been shown to be crucial for the maintenance of successful pregnancy and it also prevails at birth and during the first months of life.^[8-10] Postnatal exposure to microbial antigens elicits preferentially Th1 responses, which have been suggested to counterbalance Th2-polarized cytokine production in neonates.^[3, 11*] In the case of insufficient early microbial exposure, the production of Th2-type cytokines is further propagated leading to allergic disease.^[3, 10, 11*]

Expansion of the Th1-Th2 Dichotomy

Several findings suggest that an allergic type of immune responsiveness cannot merely be accounted for by the Th1-Th2 imbalance. First, the frequency of allergy is not increased in patients who have defects in the IL-12-dependent INF- γ pathway, that is in Th1 immune response.^[12] Second, there is a simultaneous rise in the incidence of Th1-mediated autoimmune diseases and Th2-mediated allergic diseases at the population level,^[13] and these disorders are also significantly associated with each other within individuals.^[14, 15] Third, allergen-specific Th1 cells did not counterbalance Th2-mediated allergic **asthma** in a mouse model but caused severe airway inflammation.^[16] In the same model, airway hyperreactivity and inflammation was, however, reversed by transforming growth factor- β (TGF- β) producing Th3 cells.^[17] These antiinflammatory T cells have also been found in humans.^[18, 19] Fourth, helminth infections and allergic diseases do not overlap, although both conditions are accompanied by a strong Th2 response. Indeed, intestinal parasite infections may have a protective effect against allergic disease.^[11*, 20, 21*] This protection may even overcome the opposite effect of IgE-mediated allergic hypersensitivity.

Scrivener and colleagues^[21*]⁴ demonstrated a significant correlation between **asthmatic** symptoms and *Dermatophagoides pteronyssinus* skin sensitization in the urban Ethiopian population. The same association was not found in the rural Ethiopian population in the presence of a high parasite load, despite a comparable sensitization to dust mite. An antiinflammatory cytokine, IL-10, has been proposed to mediate the protective effect.^[11*, 20-22*] This assumption is further supported by a recent study in mice in which IL-10 production induced by an enteric helminth infection protected against an allergic response to a dietary antigen.^[23**] A subset of T cells producing abundant amounts of IL-10 has been termed T regulatory cells 1 (Tr1).^[21*, 22*] Very recently, killed *Mycobacterium vaccae*-induced allergen-specific regulatory T cells producing IL-10 and TGF- β were shown to suppress experimental allergic airway inflammation.^[24**] This study and recent reviews have emphasized the vital suppressive/regulative role of these T cells, Th3 and Tr1 cells, in the development of both allergy and immunological tolerance to intestinal contents.^[5*, 25*] It has further been shown that both commensal and pathogenic microbes may activate not only Th1 and Th2 cells but also regulatory T cells, thus expanding the Th1/Th2 paradigm in the immunological basis of the hygiene hypothesis.^[22*, 24**]

Gut Microecology as a Potential Indicator of the Altered Microbial Environment

In addition to the above-mentioned studies concerning intestinal parasite infections, exposure to other food borne and orofaecal pathogens, including hepatitis A virus and *Toxoplasma gondii*, has been demonstrated to be associated with a reduced prevalence of allergic diseases.^[26, 27] This suggests that inadequate microbial stimulation of the gut-associated lymphatic tissue enhances the risk of atopic disease.

A lower prevalence of allergic sensitization has been reported in children from anthroposophic (Steiner) schools than in age-matched children from neighboring schools.^[28] Lifestyle factors associated with anthroposophy, including restrictive use of antibiotics and ample use of fermented foods containing an abundance of live lactobacilli, have been suggested to affect intestinal microflora, thereby decreasing the risk of allergy.^[28] Several epidemiological surveys have related early antibiotic use, a cause for disturbed gut microbiota, to increase in the frequency of later atopic disease.^[29-31] Interestingly, kanamycin treatment in mice during infancy, but not in older age, resulted in Th2-polarized immune deviation due to suppressed Th1 function.^[32] In a subsequent study by the same group, the Th2-skewed immune response induced by early antibiotic use was prevented by oral supplementation of intestinal probiotic bacteria.^[33**] These studies suggest that there is an early critical period when host-microbe interactions in the gut are more potent than later in life in order to induce maturational signals for the naïve immune system.

Gut Microbiota in Allergic Children

Intestinal microbiota in Estonian and Swedish healthy infants were found to have major differences. In brief, there were higher counts of lactobacilli and eubacteria in the former and increased numbers of clostridia in the latter group of children.^[34] Interestingly, the gut microbiota of the Estonian infants resembled in many aspects those of west European infants a few decades earlier.^[34] The intestinal microflora of allergic and non-allergic infants in Estonia and Sweden differed also significantly from each other. The allergic children were less often colonized with lactobacilli and bifidobacteria, whereas the non-allergic children had higher counts of coliforms and *Staphylococcus aureus*.^[35] The more detailed study of the composition of faecal bifidobacteria microbiota found that allergic infants harboured an adult like *Bifidobacterium* flora whereas healthy infants had typical infant *Bifidobacterium* flora.^[36]

Different *Bifidobacterium* species have also been shown to induce varying cytokine production, suggesting that rather than certain *Bifidobacterium* species than the whole genus may have immunoprotective effects against allergy.^[37*]

Intestinal Flora in Children Subsequently Developing Allergy

A prospective clinical study demonstrated that differences in neonatal gut microflora preceded the development of skin prick test reactivity to dietary and environmental antigens. Neonates who later developed skin prick test reactivity had higher counts of clostridia and lower counts of bifidobacteria in their faecal samples as analyzed by fluorescence in-situ hybridization.^[38] Differences in the bacterial culture of the stools were also observed during the first year of life between infants in whom atopic disease was and was not developing.^[39*] These studies suggest that healthy gut microbiota may have a crucial role for the maturation of the immune system to nonallergic mode. An original randomized placebo-controlled trial of 159 high-risk children demonstrated that perinatal administration of intestinal probiotic bacteria (*Lactobacillus* GG) halved the later development of atopic eczema during the first 2 years of life, thus corroborating the hypothesis.^[40]

Regulation of the Immune System by Intestinal Microbiota

During the first months and years of life the neonatal gastrointestinal tract is colonized with an adult-type pattern of indigenous gut microflora finally comprising approximately 10^{14} microorganisms, that is 10 times more than the number of eukaryotic cells in the adult body.^[41]

Microbial colonization commences immediately after birth, and all infants are initially colonized by *Escherichia coli* and streptococci.

The anaerobic genera *Bacteroides*, *Bifidobacterium* and *Clostridium* are established by the end of the first week of life. Formula-fed infants harbour the complex mixture of all these strains in faecal microbiota, whereas bifidobacteria and lactobacilli, prevail in breast-fed infants.^[42] After weaning, an adult-type pattern of intestinal flora gradually becomes established.

The successful maturation of the gut mucosal immune system as the most important part of the adaptive immune system requires instant and constant microbial stimuli from the gut microbiota.^[43] In experimental studies the lack or inadequacy of such stimuli has resulted in a decreased intestinal area, altered mucosal enzymes, defects in the intestinal barrier function, reduced inflammatory responses, a defective mucosal IgA system, and a deficient oral tolerance.^[41, 43, 44] Oral administration of ovalbumin to germ-free mice induced Th2-type cytokine- and antigen-specific IgE production. Reconstitution of the intestinal microflora with bifidobacteria during the neonatal period, but not in older age, resulted in oral tolerance, suggesting the importance of timing of the stimulus.^[44] Altered compositional development of the gut microflora in healthy infants has been shown to be associated with delayed maturation of humoral immune defense mechanisms, particularly of circulating IgA and IgM-secreting cells.^[45]

Probiotic Bacteria in Allergic Diseases

In addition to the above-mentioned preventive effect of *Lactobacillus* GG on atopic eczema,^[40] specific probiotic strains have successfully been used in the treatment of infants suffering from atopic eczema and cow's milk allergy.^[46, 47]

Antiinflammatory and antiallergic effects of probiotics, cultures of beneficial live microorganisms characteristic of the healthy infant gut microbiota, have recently been thoroughly reviewed.^[6*, 48*] The rationale of the probiotic approach in infants has been to simulate the microbiota of breast-fed infants and thus possibly yield long-term health-promoting effects.

Probiotics have been shown to reverse the increased intestinal permeability and enhance gut-specific IgA responses, thus promoting the gut defense barrier mechanisms which frequently are defective in children with atopic eczema and food allergy.^[6*, 48*] Certain probiotics have also been demonstrated to contribute to the processing of dietary antigens and to reduce their allergenicity *in vitro* and *in vivo*. Experimental and clinical studies indicate that probiotics dampen changes related to allergic inflammation by inducing Th1-, Th3- and Tr1-type cytokines.^[6*, 48*-50] Consumption of lactobacilli by pregnant and lactating mothers has also been shown to increase the amount of Th3-type cytokine, TGF- β , in breast milk.^[51*] Specific strains of the healthy gut microflora thus appear indispensable for the successful maturation of the immune system.

The Host-Microbe Crosstalk in the Gut

A single layer of epithelial cells covering the large gut mucosal surface, approximately the area of a tennis court in adults, is continuously exposed to huge amounts of dietary, environmental and microbial antigens. The gut-associated lymphatic tissue underlying the epithelia must constantly distinguish innocuous antigens present in food and gut commensals from invasive pathogenic microbes.^[7*, 25*, 52*] To meet the task, the gut mucosal immune system relies on the delicate balance between responsiveness and nonresponsiveness. Oral tolerance, that is peripheral nonresponsiveness to orally administered soluble protein antigens, has recently been completely reviewed.^[53]

Active cooperation of the innate and adaptive immune system is a prerequisite for normal mucosal immune responses against microbial antigens. Microbes entering the gastrointestinal tract confront several nonspecific and antigen-specific mucosal defense mechanisms including peristalsis, secretion of bile, acid, mucus, antibacterial peptides and IgA.

Microbes capable of penetrating this gut barrier are recognized by toll-like receptors (TLRs), a group of evolutionarily conserved pattern-recognition receptors, present, for example, in intestinal epithelial cells and antigen-presenting cells.^[25*, 52*, 54*] The TLRs are a part of the innate immune system and represent mammalian counterparts of the toll receptors originally found in *Drosophila*. Connection of these receptors by microbes leads ultimately to the production of proinflammatory cytokines by the activation of the transcription factor nuclear factor κ B (NF- κ B).^[25*, 52*, 54*] Antigen-presenting cells, such as macrophages and dendritic cells, and T cells orchestrate specific mucosal immune responses which seem to preferentially induce suppressive Th3 and Tr1 responses in the gut.^[7*, 25*] The definite maturation of a naïve T cell into the Th1-, Th2-, Th3- or Tr1-type effectors cell, however, depends on a complex interplay between antigen, antigen presenting cell, environmental cytokines and the expression of certain regulatory transcription factors and cell surface receptors.^[7*, 55, 56] Interestingly, intestinal inflammation caused by Th1 cytokines has been shown to up regulate the expression of TLRs in the gut, thus from a functional perspective connecting TLRs closely with the adaptive arm of the immune system.^[57**, 58**]

More than 10 members of the TLR family have been discovered, each of them possessing specificity towards microbial surface structural determinants.^[52*, 54*] For example, TLR4 is activated by lip polysaccharide present in Gram-negative bacteria, while TLR2 is specific for the lipoteichoic acid component of Gram-positive cell walls. TLR9 recognizes unmethylated cytosine phosphate-guanosine (CpG) dinucleotides, which are 20 times more common in the DNA of bacteria than in higher vertebrates.^[52*, 54*] Both the commensal flora and pathogens have these essential microbial structural elements for recognition by the TLRs.^[25*, 52*]

There are, however, a few potential reasons why commensal bacteria in the luminal flora normally do not cause constant inflammation in the gut by the activation of the immune system via TLRs.

First, commensals have to be able to penetrate the superficial gut defense barrier mechanisms to reach either epithelial cells where TLRs are preferentially situated basolaterally or antigen-presenting cells in the lamina propria. Second, the expression of TLRs is sparse both in normal intestinal epithelial cells and in macrophages in the lamina propria.^[57**, 59] Third, intestinal macrophages lack both CD14, a pattern recognition receptor involved in the response to lipopolysaccharide, and CD89, the receptor for IgA, resulting in reduction in lipopolysaccharide and IgA-mediated activities.^[60*] Fourth, two nonpathogenic strains of *Salmonella*, *S. typhimurium* Pho P^c and *S. pullorum*, have been demonstrated to induce a signal that inhibited the NF- κ B pathway and prevented inflammatory response.^[61] It has been suggested that the commensal flora, including probiotics, may possess evolved secreted effector proteins with similar antiinflammatory effects.^[52*] Alternatively, potential differences in the molecular patterns of commensals and pathogens may result in the activation of antiinflammatory and proinflammatory cytokines, respectively.^[25*]

Despite tight mucosal control, some nonpathogenic microbes do gain access to the gut-associated lymphatic tissue, thus enabling direct contact with the immune system.^[25*] Such a dialogue may peak during early postnatal life because of the immature gut barrier function.^[43] Moreover, some TLRs are expressed on enterocytes, especially during foetal life but also in childhood, despite a sparse expression in normal adult gut.^[58**, 59, 62, 63**] *Lactobacillus* GG, a probiotic strain capable of inducing the production of Th1, Th3 and Tr1 cytokines, particularly activates the NF- κ B pathway in human macrophages.^[64]

It is tempting to speculate that the early preventive effect of the strain on atopic eczema^[40] might at least partly be mediated by the activation of the NF- κ B pathway via TLRs. In parallel, lack of any effect of *Lactobacillus* GG on birch-pollen allergy in young adults^[65] might be partly due to the properties of the healthy adult gut discussed in the preceding paragraph. The effects of normal gut microbiota on the immune system may also increase during intestinal inflammation when the integrity of the gut defense barrier is breached and the expression of TLRs is unregulated.^[57**, 58**]

To date, the role of pattern-recognition receptors in the development of allergy has mostly remained unexplored. A promoter polymorphism of the CD14 gene has been linked with a more severe allergic phenotype.^[66, 67] It has been shown that a close interaction between CD14 and TLR4 participates in lipopolysaccharide signaling leading to activation of the NF- κ B pathway.^[68] Breast milk contains very high amounts of the soluble form of CD14, which has been demonstrated to mediate the activation of the innate immune responses by lipopolysaccharide in intestinal epithelial cells.^[69] The authors postulated that soluble CD14 in breast milk may modulate local innate and adaptive immune responses in the neonatal intestine. Interestingly, a recent study demonstrated that reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the later development of allergic sensitization and atopic eczema.^[63**]

Conclusion

Recent data indicate a dependence of healthy host-microbe interaction for the maturation of the naïve immune system. These data further suggest that adequate microbial exposure may prevent the subsequent development of atopic disease. A prerequisite for such a preventive effect is clearly that the microbial exposure be early and universal, since the majority of infants even in the developed countries are spared from allergic disease. The exposure should also elicit an immune response. The discovery of the pattern-recognition receptors has presented us with a new tool to explore the host-microbe crosstalk in the gut-associated lymphatic tissue. Despite accumulating evidence, the exact role of gut microbiota in the development of allergy is far from clear. More studies are needed to explore the interplay between the host and specific bacterial species in the gut, particularly in allergic diseases. Due to preliminary encouraging results, more clinical trials with known and new potential probiotic strains are called for in the fight against the profusion of allergic diseases.

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Abbreviation Notes

NF- κ B, nuclear factor κ B; TGF- β , transforming growth factor- β ; TLR, toll-like receptor; Tr1, T regulatory cells 1

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