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The role of gut microbiota in the pathogenesis and management of allergic diseases

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Abstract. **Allergy is defined as a hypersensitivity reaction due to specific antibody-mediated or cell-mediated immunologic mechanisms. Epidemiological studies are showing a dramatic increase of allergies in industrialized countries in the last few decades, while remaining stable in developing countries.**

In 1989 Strachan, hypothesized that the increase in allergic disorders was the result of a lack of infections in early infancy, and in 1998 Wold suggested that, rather than a decrease in viral or bacterial infections, an altered normal intestinal colonization pattern in infancy, could be responsible for the increase in allergies.

Germ-free mice were shown to mount an exaggerated allergic airway reaction compared with that seen in colonized mice, indicating the important role of microbe-host interactions in the development of allergic diseases.

Infants with food allergies are found to exhibit an imbalance between "beneficial"and potentially harmful bacteria, i.e., decreased *Lactobacilli, Bifidobacteria* and *Enterococcus* species and increased coliforms, Staphylococcus aureus and Clostridium species, suggesting that microbial inhabitants of the human body, may play either a pathogenic or protective role in allergies.

Based on this data, many clinical trial addressing the use of probiotics in the context of allergic disorders, have been conducted in children. However, currently, no conclusive item may be drawn.

Key words:
Gut microbiota, Food allergy, Probiotics.

Introduction

The concept of "allergy" was originally introduced in 1906 by the Viennese pediatrician Clemens von Pirquet, after he noted that some patients were hypersensitive to normally innocuous entities such as dust, pollen, or certain foods. Pirquet called this phenomenon "allergy" from the ancient Greek words αλλος *allos* meaning "other" and εργον *ergon* meaning "work"[1]. A major breakthrough in understanding the mechanisms of allergy was the discovery of the antibody class named immunoglobulin E (IgE), first isolated by Ishizaka and co-workers in the 1960s[2]. One more cornerstone in the history of allergy was the classification designed in 1963 by Philip Gell and Robin Coombs, who described four types of hypersensitivity reactions, known as Type I to Type IV hypersensitivity[3]. With this new classification, the word "allergy" was restricted to type I hypersensitivities (also called immediate hypersensitivity), which are characterized as rapidly developing reactions.

Actually, allergy is defined as a hypersensitivity reaction due to specific antibody-mediated or cell-mediated immunologic mechanisms. The term hypersensitivity describes objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal (i.e. non-allergic) persons. Sensitization to allergens derived from food, pollen, house dust mite, etc. is thought to be a prerequisite for initiating the allergic march.

Worldwide, sensitization to foreign proteins in the environment is reported in up to 40% of the population. Of note, food allergy prevalence reaches up to 6% among children and 3% among adults. In USA allergic diseases represent the 6th most common chronic disorder, thus making them a major healthcare problem[4]. Overall epidemiological studies are showing a great increase of allergies in industrialized countries in the last few decades, while remaining stable in developing countries[4].

In 1989 Strachan[5] hypothesized that the increase in atopic diseases was the result of a lack of infections in early infancy. This hypothesis was based upon the observation that infants with higher number of siblings were at decreased risk for developing atopy. Although sib-ship size, and other indirect markers of microbial exposure such as rural and farm-living were consistently shown to be associated with a decreased risk of

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developing allergies, studies of the association between viral and bacterial infections and allergy were less consistent. In 1998 Wold[6] suggested that, rather than a decrease in viral or bacterial infections, an altered normal intestinal colonization pattern in infancy, which fails to induce immunological tolerance, could be responsible for the increase in allergies.

The opening idea of a potential role of the gut microbiota in the pathogenesis of allergic disease was based on the observations that: (i) oral tolerance is difficult to achieve in germ-free animals; (ii) combined administration of lipopolysaccharide and food antigens increases the tolerizing effect of feeding; (iii) bacterial toxins may break oral tolerance[7].

Since these initial observations, several studies linking the composition of gut microbiota to the pathogenesis of allergy have been conducted.

Gut microbiota and immune response

Our gut harbors the majority of mammalian-associated microbes. The fetal intestine is sterile and bathed in swallowed amniotic fluid. Following delivery, the colonization of the intestine by a variety of microorganisms begins[8]. Gastrointestinal colonization involves a succession of bacterial populations waxing and waning as the diet changes and the host develops. This assemblage of bacteria inhabiting the gut is usually referred to as the commensal intestinal microbiota. Each human adult harbors approximately 10^{14} bacteria in the gut, which is about 10 times the number of cells making up the human body. There are at least 400-500 different bacterial species and these species can again be divided into different strains, highlighting the enormous complexity of this ecosystem[9]. Factors influencing the intestinal microbiota composition can be divided into host factors (such as pH, transit time, bile acids, pancreatic enzymes and mucus composition), non-host factors (such as nutrients, medication and environmental factors), and bacterial factors (such as adhesion capacity, enzymes and metabolic capacities)[10]. The bacteria in the gut interact with their human host, and although some bacteria are potentially pathogenic and can become a source of infection and sepsis, this host-bacterial interaction is mainly symbiotic and health-conferring[11].

Changes in gut microbial composition have been reported in patients with allergic diseases[12]. Alterations of the intestinal microbiota might precede the development of allergic manifestations, supporting the hypothesis that microbial dysbiosis is not only a consequence but also a cause of allergy. Potential reasons for microbial dysbiosis in allergic subjects lie in complex individual-specific interactions between genetic predispositions and environmental factors, such as birth delivery mode, diet, hygiene, and medications. Birth delivery mode, for instance, markedly influences initial microbial colonization of newborns. Natural birth, which results in immediate exposure of the child to the mother's vaginal and fecal microbiota, is associated with a reduced incidence of allergies compared with that seen in children born by means of cesarean section[12].

The revised hygiene hypothesis[13] states that reduced microbial exposure in early childhood results in an increased TH2/TH1 response ratio and in defective regulatory immune mechanisms that contribute to a higher incidence of immune-mediated diseases, such as allergies, in developed countries.

The immune system of neonatal mice and humans is thought to have a TH2 bias. Allergic diseases are traditionally associated with pronounced or dysregulated TH2 responses. Indeed, stimulation of allergen-specific T cells by allergen-derived peptides, presented by dendritic cells, results in differentiation of CD4+ T cells into TH2 cytokine-producing cells. TH2 cells produce interleukin (IL)-4, IL-13 and IL-5, which coordinately regulate the allergic response. In detail, IL-4 directs the differentiation of T cells towards a TH2 cytokine profile and acts as a growth factor for the expression of these cells. In addition IL-4 together with IL-13 regulate the synthesis of immunoglobulin IgE by B cells. Finally, IL-5 regulates the differentiation and egress of eosinophils from the bone marrow into the blood.

Studies of human infants indicate that this TH2 response gradually diminishes during the first 2 years of life in non-allergic individuals. In the search for a plausible immunological foundation for the hygiene hypothesis, it has been suggested that the developing immune system needs a TH1 stimulus from the environment, that is exposure to pathogens in early life, to decrease the TH2/TH1 response ratio, thus avoiding the development of allergy[14]. Many bacterial infections, including mycobacteria, can provide just such a stimulus. Indeed, *Bacille Calmette Guerin* infection ameliorates experimental allergic asthma in mouse models, and the administration of killed *Mycobacterium vaccae* can ameliorate the

severity of atopic dermatitis in children[15-16]. Exposure to pathogens, such as *Helicobacter pylori* or parasites has also been associated with reduced incidence of allergic diseases[17,18].

Studies in germ-free mice[19] have identified important interactions between the intestinal microbiota and the development of Treg cells, both locally in the gut and systemically. Germ free mice express lower numbers of Treg cells in both mesenteric and peripheral lymph nodes compared with conventional mice, and these Treg cells produce less IL-10. Thus, oral tolerance could not be established in germ free mice because of the impaired suppressive function of their Treg cells and the reduced production of TGF-β and IL-10.

Animal model studies

The "microflora hypothesis" has been proposed as an alternative explanation for the development of allergic diseases. Consistent with a decreased TH1/TH2 ratio, germ-free mice were shown to mount an exaggerated allergic airway reaction compared with that seen in colonized mice, indicating the important role of microbe-host interactions in the development of allergic diseases. Ovalbumin challenge in sensitized germ-free mice resulted in increased infiltration of lymphocytes and eosinophils into the airways and increased local levels of ovalbumin specific IgE and typical TH2 cytokines compared with those seen in colonized mice. Whereas increased allergic reaction correlated with reduced secretion of bronchial IgA, reduced numbers of plasmacytoid dendritic cells and alveolar macrophages, and increased numbers of basophils, numbers of Treg cells and levels of regulatory cytokines were unchanged[20]. In addition to exaggerated allergic airway reaction, germ-free mice seem to be more susceptible to IgE-mediated cow's milk allergy. Indeed, germ-free mice were more responsive to oral sensitization and were characterized by greater reduction in body temperature (clinical cow's milk allergy symptom) and higher blood levels of mast cell protease 1 and β-lactoglobulin-specific IgG1 after oral challenge with β-lactoglobulin compared with colonized mice. Interestingly, whereas sensitization had no significant effect on dominant intestinal bacterial groups, severe allergic disease was associated with low counts of cecal *staphylococci* (culture analysis), suggesting that subdominant species might play protective roles[21].

Treatment of normal 3-week-old mice with the broad-spectrum antibiotic, kanamycin, resulted in the elimination of all Gram-negative bacteria in the stool, and a subsequent shift to a TH2 response, suggesting that intestinal bacteria are involved in controlling the TH2 response[22]. This data has been further supported in a study by Nowerr et al[23]. The authors, by using a mouse model of antibiotic-induced gastrointestinal microbiota disruption, to address whether microbiota disruption can promote the development of an allergic airway response to mold spore (*Aspergillus fumigatus*) or ovalbumin challenge, found that mice with unaltered microbiota did not develop an allergic response following intranasal challenge with either mold spores or ovalbumin. Moreover, vigorous allergic airway responses can 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[24].

Finally, a very recent study[25] demonstrated that mice with food allergy exhibited a specific gut microbiota signature (*Lachnospiraceae, Lactobacillaceae, Rikenellaceae, Porphyromonadaceae*), capable of transmitting disease susceptibility.

In summary, the use of gnotobiotic mice and experimental allergy models has been very useful in testing the causative role of microbiota in shaping regulatory immune responses in the gut and the development of allergic responses.

Human studies

Back to the early 1980s, Russian scientists[26] linked food allergy to abnormal intestinal microbiota. The authors examined 60 infants with dermatological syndrome, caused by food allergy and reported a deficiency of *Bifidobacteria* and *Lactobacilli* combined with an increase of *Enterobacteriaceae*. In 1999, the first case-control study conducted on 2-year-old allergic and non-allergic infants from Estonia and Sweden found that allergic infants were less often colonized by *Lactobacilli* compared with non-allergic infants in both countries. In contrast, the allergic children harbored higher counts of facultative aerobic microorganisms, especially *coliforms* in the Estonian and *Staphylococcus aureus* in the Swedish children[27]. In a follow-up study, the same research group prospectively evaluated the intestinal microbiota composition in relation to

the development of allergy. The prevalence of colonization by *Bifidobacteria* was consistently lower throughout the first year of life in infants who developed allergy compared with those who did not[28].

Since then, several culture-dependent technique based studies have reported differences in the composition of the microbiota of infants who develop allergic diseases. Overall, infants with food allergies are found to exhibit an imbalance between "beneficial" and potentially harmful bacteria, that is, decreased *Lactobacilli, Bifidobacteria and Enterococcus* species and increased *coliforms*, *Staphylococcus aureus and Clostridium* species[7]. However, the culture-dependent techniques used in these studies makes them vulnerable to bias.

The development of molecular techniques to investigate ecological microbial communities has provided the microbiologists with a wide variety of new techniques to study the human intestinal microbiota. In a case-control study using Fluorescence In Situ Hybridization to characterize the gut microbiota, no differences in concentrations of specific genera were found between healthy infants and atopic infants. However, higher *Bacteroides* counts and lower counts of B*ifidobacteria* were associated with more severe dermatitis[29]. In a cohort study[30] prospectively following up 76 high-risk infants during their first year of life, bacterial fatty acid profiles in fecal samples collected at age 3 weeks significantly differed between infants in whom atopy was or was not developing. Two case-control studies[31,32] nested within the prospective National Asthma Campaign Manchester Asthma and Allergy Study showed no differences in the dominant fecal microbiota and the *Bifidobacteria* and *Lactobacilli* composition between sensitized wheezy and non-sensitized non-wheezy 4-year old children. The first large-scale study, aimed to address the association between the gut microbiota and atopic diseases, was carried out in 2007 by KOALA Birth Cohort Study in the Netherlands. Colonization with *Escherichia coli* was associated with an increased risk of developing eczema in a concentration-dependent manner. Furthermore colonization with *Clostridium difficile* was associated with an increased risk of developing eczema, recurrent wheeze and becoming sensitized[33]. Very recently Ismail et al[34], by analyzing 98 infants at high risk of allergic disease, who were followed prospectively to age 12 months, found that a more diverse intestinal microbiota in the first week of life is associated with a reduced risk of subsequent eczema in infants at increased risk of allergic disease.

Another debated issue in the field of microbiota implications in allergy, is whether vertical transmission of bacteria from mother to infant is important in the development of allergy. Rudi et al[35], recently showed that the mother's bacterial composition correlated significantly to the child's IgE sensitization state at the age of 2 years. High *Escherichia coli* and low *Bacteroides fragilis* levels in the mother were negatively correlated, while low *Escherichia coli* and high *Bacteroides fragilis* were positively correlated to IgE levels. These interesting findings support the hypothesis that allergy could partly be communicable, being transferred from mother to infant through the gut microbiota.

In summary, human studies strongly suggest that microbial inhabitants of the human body, may play either a pathogenic or protective role in allergies and may also influence the severity of allergic diseases.

Probiotics in the prevention and management of allergic diseases

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts[36]. They are proposed to exert either direct protective effects on the host or to modulate the intestinal microbiota in a protective way.

Many different bacterial strains or mixtures of them, have been used until now in clinical trials to assess the protective effects in the context of allergic sensitization and allergic diseases. Starting from the first publication in 1997, over 30 randomized, double-blind, placebo-controlled clinical trials, including above 3000 individuals, have been conducted to study the effects of various probiotics on prevention and treatment of allergic diseases[37-40].

Administration of *Lactobacillus casei* GG to the mothers before and after (via breastfeeding) delivery prevents atopic eczema, as well as other atopic diseases that develop later, in children at risk[41,42]. Protection from allergic diseases provided by oral administration of non-pathogenic *Escherichia coli* in early life was shown to extend to adult life[43].

Finally, a number of studies have been performed using probiotics to treat preexisting atopic disease in infants. These studies found that

the administration of *Lactobacilli* was able to decrease the severity of various allergic diseases, including atopic eczema, atopic dermatitis, and food allergy in these children[37-40]. However, a meta-analysis of data from clinical studies concluded that probiotics cannot be generally recommended for the treatment of eczema or the prevention of allergies[44]. As far as concern the development, incidence or severity of asthma in children at high risk, to date there is no evidence supporting probiotic therapies[45]. On the other hand, studies using animal models have found that oral administration of certain *Lactobacilli* and *Bifidobacterium* species were able to modulate allergic responses in the respiratory tract apparently via induction of regulatory T cells, suggesting that it may be possible to treat asthma using an optimal combination of probiotics[46].

A recent position paper from the World Allergy Organization[47] stated that "probiotics do not have an established role in the prevention or treatment of pediatric allergy. No single probiotic supplement or class of supplements has been demonstrated to efficiently influence the course of any allergic manifestation or long-term disease or to be sufficient to do so".

Most clinical trials using probiotics in the context of allergic disorders, have been conducted in children. The administration of probiotics to adult subjects for allergies have not proven to be successful. It is conceivable that only a developing immune system and a not-yet established microbiota composition may benefit from probiotic administration. Nevertheless a very recent double-blinded, parallel, randomized placebo-controlled trial, carried out in adults suffering from seasonal allergic rhinitis, concluded that the administration of the probiotic Nestlé culture collection (NCC)2818 *Bifidobacterium lactis* strain mitigated immune parameters (TH-2 cytokines secreted by stimulated blood lymphocytes and percentages of activated CD63 expressing basophils) and improved allergic symptoms during seasonal exposure[48].

Therefore, additional large and well-designed clinical intervention trials are needed before any recommendation concerning the use of probiotics for the prevention or therapy of allergic diseases.

Conclusions

The steep increase in allergy prevalence during the last decades has been attributed to changes in environmental factors. Gut microbiota is now regarded as the major player in the development of allergic disease. Therefore, any external factor that can alter the gut microbiota balance, such as diet or antibiotic treatment, should be viewed as a potential risk factor for development of these inflammatory diseases. In addition, the relationship between gut microbiota, immunity, and disease is very complex, since the same commensal bacteria can induce either a protective or pathogenic response, a response depending on the susceptibility of the individual. Characterization of microbiota beyond phylogenetic diversity analysis is mandatory to identify alterations of microbial functions that contribute to early immune disturbances in allergic patients.

After a decade of clinical research in the field, the use of probiotics for the prevention or treatment of allergic diseases, at present, no general recommendations for their use in clinical practice can be given. Thus, the clinical relevance of candidate probiotics, suitable in the management or prevention of allergic disease, must be determined in high-quality clinical studies.

Conflict of interest

The Authors declare that they have no conflict of interests.

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