

Evidence of Probiotics in Prevention of Allergy and Asthma

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Abstract: Previous research into the causes of allergic diseases was mostly focussed on potential risk factors in the environment, with little success, however. Over the past 10 years, focus has therefore more been directed against protective factors that could enhance the development of tolerance to allergens which were previously encountered early in life, but are now lost in modern affluent societies. In particular, the role of childhood infections has been discussed, but so far these studies have not been conclusive.

Recent epidemiological studies and experimental research suggest that the microbial environment and exposure to microbial products in infancy modifies immune responses and enhances the development of tolerance to ubiquitous allergens. The intestinal microflora may play a particular role in this respect, as it is the major external driving force in the maturation of the immune system after birth and animal experiments have shown it to be a prerequisite for normal development of oral tolerance. The composition of the microflora differs between healthy and allergic infants and in countries with a high and low prevalence of allergies. These differences are apparent within the first week of life, or even in the maternal vaginal flora during pregnancy and thus precede clinical symptoms. The use of live microorganisms that might be beneficial to health has a long tradition and the safety is well documented. Prospective intervention studies, in which the gut flora was modified from birth have yielded encouraging results and may suggest a new mode of primary prevention of allergy in the future.

Keywords: Asthma, allergy, prevention, probiotics, microflora, lactobacilli.

INTRODUCTION

It is almost generally accepted that there is a pronounced increase in the incidence and prevalence of allergic diseases in many countries, at least during the last 40-50 years and probably well before that. It is also generally accepted that the development of asthma and other allergic diseases is strongly influenced by genetic components. As the human genotype would not have changed appreciably over a short time period, the search for the causes of the allergy epidemic has focussed on environmental factors. Initially, the search was more or less limited to identifying risk factors, but recent research has shifted attention from triggers of sensitisation and factors provoking allergic disease to factors that may programme the initial susceptibility to allergic diseases. This shift in focus from "Why do people develop allergy?" to "Why are we not all allergic?" is largely a consequence of increased understanding of how immunity develops after birth. Allergic diseases are now by many seen as a result of a prolonged period after birth with an immature immune system and as a consequence of decreased immune stimulation. Current search for risk factors emphasises the absence in modern society of factors, which might stimulate the immune system, allowing it to develop protective responses, but also to establish appropriate control of these responses.

It has been suggested that modern living is associated with too little microbial stimulation early in life and that

allergic disease and autoimmune disease could be regarded as a consequence of a "microbial deprivation syndrome" [1]. According to this hypothesis, microbes are essential for the development of a normal immune regulation. In this chapter, the possible role of the intestinal microbiota in the modulation of immune responses to allergens and subsequent development of asthma and allergy will be discussed.

INFECTION, MICROBIAL PRODUCTS AND ALLERGY

For many years, all kinds of infectious diseases were seen as potential triggers of allergy. However, the Canadian paediatrician John Gerrard observed in 1976 that the prevalence of allergy was low in the Metis community in Northern Canada, while the prevalence of infections was high [2]. He suggested that atopic disease is the price paid by some members of the white community for their relative freedom from diseases due to viruses, bacteria and helminths. In 1989, Strachan reported that the risk of allergic diseases was lower with increasing number of siblings [3]. This formed the basis of the 'hygiene hypothesis', according to which siblings are a surrogate measure of early life infections, which were suggested to protect against allergic diseases. Since then, over 20 studies have confirmed an inverse relationship between sibship size and allergy [4, 5]. These studies do not provide any evidence for a protective role of infections, however, as it is equally possible that the relationship is a consequence of altered maternal immunity induced by multiple pregnancies [6]. An altered maternal immunity in turn, has an impact on her offspring and may influence the development of allergy [7].

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While respiratory infections are established as major triggers of bronchial obstruction and wheezing in infancy, their role for subsequent asthma and allergy is controversial. An analysis of the possible relationship between severe or systemic infections early in life and development of asthma and allergic disease lies beyond the scope of this review, however.

Exposure to various microbial products early in life has been suggested to explain the low prevalence of allergy among children of farmers [8, 9] and the reported inverse relationship between levels of endotoxin house dust and prevalence of allergy [10-12]. In the study by Böttcher *et al.* [10], however, an inverse relationship between positive skin prick tests and endotoxin levels in house dust was only observed in Sweden, but not in Estonia, where the endotoxin levels were generally higher and the prevalence of allergy is low. This study indicates that endotoxin may not be directly related to protection against allergy, but rather be a surrogate marker for other factors.

THE INTESTINAL MICROBIOTA

The biological and medical communities increasingly realise that the microflora of the large gut may have important roles in both human health and disease. The perspective of the human colon in health and disease is by no means a new concept, as the Russian scientist Elia Metchnikoff already a century ago indicated the clinical importance of the host colonic microflora. He also suggested that certain live microorganisms might promote health. Despite this, there was for many years modest interest in this concept among researchers and it is only over the last 10 years that microbial ecology has again become a major research area. It is now generally accepted that the bacterial microflora of the human gut is an integral component of the host defence system. This has generated considerable interest in the functional food/nutraceutical industry and among clinicians.

The intestinal tract performs many different functions. In addition to absorption and digestion it is also the body's largest organ of host defence. Part of the intestinal mucosal barrier function is formed by a common mucosal immune system, which provides communication between the different mucosal surfaces of the body [13]. The total mucosal surface area of the adult human gastrointestinal tract is up to 300m², making it the largest body area interacting with the environment. It is colonised with over 10¹⁴ microorganisms, weighing over 1 kg and corresponding to more than 10 times the total number of cells in the body.

The gastrointestinal tract of the newborn baby is sterile and soon after birth it is colonised by numerous types of microorganisms. Colonisation is complete after approximately one week, but the numbers and species of bacteria fluctuate markedly during the first three months of life. There is a continuous interaction between the microbial flora and the host, comprising a dynamic eco system that, once established, is surprisingly stable under normal conditions. Environmental changes, e.g. a treatment period with antibiotics only temporarily change the composition of the microflora. The intestinal colonisation with a balanced microflora is of major importance for the maturation of the

immune system, as clearly shown in studies of germfree animals.

MICROBIOTA AND IMMUNE FUNCTION

The gut microbiota is thus the quantitatively most important source of microbial stimulation and may provide a primary signal for driving the postnatal maturation of the immune system and the induction of a balanced immunity [13]. As microorganisms have been present through the evolution of man, this has taken place in close interaction with the microbial environment. There is mounting evidence that commensal microbes acquired during the early postnatal period are required for the development of tolerance, not only to themselves, but also to other antigens [14]. For example, Th2- mediated immune responses are not susceptible to oral tolerance induction in germ free mice [15]. Oral tolerance was only induced after the introduction of components of the normal microflora.

It is recognised that interaction with microbes, especially the normal microbial flora of the gastrointestinal tract, is the principal environmental signal for postnatal maturation of T-cell function (in particular the Th1 component) [16]. Recognition of these signals is mediated by a series of TOLL-like receptors expressed on cells of the innate immune system, and other receptors such as CD14, and it is noteworthy that a polymorphism in the CD14 gene has recently been associated with a high level of IgE [17].

Rook and Stanford suggested two major syndromes that could be the result of inadequate microbial stimulation early in life [1]. One was inadequate priming of T helper cells, leading to an incorrect cytokine balance. The second suggestion was a failure to fine-tune the T cell repertoire in relation to epitopes that are cross-reactive between self and microorganisms. The authors coined the expressions "input deprivation syndrome" and "uneducated T-cell regulation syndrome". This hypothesis could be supported by comparative global studies showing that not only allergy, but also type-1 diabetes and celiac disease are associated with a "Western life style" [18]. Thus, modern society is associated with an increasing incidence of immunologically mediated diseases, which is considered to be mediated by both Th and Th2 type immune mechanisms. The simultaneous increase in the incidence of both Th1 mediated autoimmune diseases and Th2 dependent allergies can not be explained merely by an imbalance between the two arms of immunity. Rather, an immunological explanation for the increase would focus on the role of immune regulation of both Th1 and Th2 type immunity and the induction of tolerance early in life. This seems to be largely mediated in the microenvironment of the mucosae and adjacent lymphoid tissues [19, 20].

If indeed microbes were essential for normal development of immunity, what microbes would be expected to be essential for this process? It would seem unlikely that the postnatal maturation of a balanced immune system would be driven primarily by pathogens, i.e. by stimuli that are potentially harmful to the host. From an evolutionary point of view it is more likely that non-pathogenic microorganisms that have been present through the evolution

of our immune system drive the process. The gut microbiota is an obvious place to search for such microorganisms.

GUT MICROBIOTA AND ALLERGY

The wide variations in allergy prevalence between different countries and the fact that the prevalence has increased considerably over the last 40-50 years and the role of the microflora in the development of immunity in infants, have led to the suggestion that variations in patterns of microbial colonisation of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in allergy prevalence throughout the world [16]. Over the past few years, differences have been documented in the composition of the intestinal microflora between healthy infants in countries with a low and a high prevalence of allergy [21-23] and between allergic and non-allergic infants in both environments [24]. These studies indicate that an imbalance in the gut flora and differences in the indigenous intestinal flora might affect the development and priming of the immune system in early childhood. This in turn could affect the risk for allergy. The fact that differences are present before any clinically manifest disease [24, 25] would indicate that the differences between allergic and non-allergic children are not secondary phenomena. Although all the studies conducted so far confirm differences in the composition of the gut flora, no particular protective or potentially harmful bacterial species can yet be identified. This is not surprising, given the enormous number of microbial strains and the complicated ecology of the gut flora.

Italian studies in military personnel have lent support to a possible role of the intestinal microflora in the pathogenesis of allergic disease. Matricardi *et al.* studied the prevalence of allergic disease and sensitisation to inhalant allergens among military personnel in relation to serological markers of previous infections [26, 27]. Interestingly, there was an inverse relationship between respiratory allergy and the presence of antibodies against infections transmitted through the oro-faecal route, such as toxoplasmosis, hepatitis A and *H. pylori*, but no relationship with antibodies against agents transmitted through the respiratory tract, i.e. measles, mumps, rubella, chickenpox, cytomegalovirus, and herpes simplex virus.

There are at least three recent studies in which differences in the composition of the gut microbiota were observed between allergic and non-allergic infants. In one study, the counts of *S. aureus* were higher and the prevalence of *Bacteroides* and *Bifidobacteria* were lower in allergic children at two years [24]. This was confirmed in a subsequent study, which in addition showed that the allergic infants had higher counts of clostridia up to 3 weeks, but not later [25]. A higher prevalence of colonisation with *Clostridium difficile* in allergic infants at 12 months was originally suggested in a considerably larger study, using an indirect method [28]. In that study, various microbial metabolic products were determined in stool specimens from healthy and allergic infants. The advantage with this approach over conventional bacteriological methods in that information is obtained about metabolism and ecology.

When analysing the levels of short chain fatty acids, which are products of microbial metabolism, isocaproic acid was only detected in stool samples from allergic infants, while the levels of isobutyric acid were higher in healthy children. The former fatty acid strongly indicates colonisation with *Clostridium difficile* and the later findings indicates the presence of a lactic acid producing gut flora.

Observed differences in the composition of the gut microbiota between allergic and healthy infants are not limited to species. The composition of the *Bifidobacterium* [29] and *Lactobacillus* [30] flora also differ between the two groups. Although the significance of these differences is unknown, they are interesting as these species are consistently linked to probiotic properties and differences in the *Bifidobacterium* flora between allergic and non allergic infants is the only consistent finding in all clinical studies so far.

It is not conclusively known whether the differences between allergic and non-allergic infants are primary or secondary to disease. In two recent prospective studies, however, less bifidobacteria were detected during the first weeks of life in babies who developed allergy during infancy [25, 31]. Furthermore, colonisation intensity during the first week of life was lower in babies who developed allergy during the first year of life [24] and microflora- associated characteristics such as the levels of short chain fatty acids differed between the two groups at three weeks (but not later during infancy) [25].

Recently, it was shown in a population based cohort comprising 3000 children and their mothers that vaginal colonization with Staphylococci during mid- pregnancy was associated with asthma during the 5th year of life in the children (OR, 2.2; 95% CI, 1.4-3.4) [32]. *Staphylococci* are not part of the normal vaginal flora, which is normally dominated by lactic acid bacteria. The findings are interesting, as the vaginal flora is the first microbial environment that the infant comes in contact with during the birth process.

In this respect, the current controversy regarding breast feeding and the development of asthma and allergy is of interest. While a protective effect was reported in several earlier studies [33], most recent studies have reported negative effects of breastfeeding on allergic diseases [34], although it reduces wheezing in infants [35], which is mostly caused by infections. This variation over time could be due to variation in selection bias, as the recommendation to breastfeed in order to prevent allergy may have altered the characteristics of the women who breastfeed, alternatively that breastfeeding may have become a risk factor for allergic diseases in recent years because it prevents infections early in life [36, 37]. Furthermore, there were previously pronounced differences in the composition of the gut microbiota between breast fed and formula fed infants [38]. These differences are not nearly as prominent with the use of modern humanised infant formulae [22]. Thus, any protective effect of breast feeding in the past may have been a consequence of an ecological effect on the gut microbiota.

As the differences in the composition in the intestinal microbiota between allergic and non-allergic infants were present already before any manifestations of disease, perhaps

even in the microenvironment during birth, the studies indicate that they are primary. It should be remembered, however, that the studies do not prove that any particular bacteria are either beneficial or harmful, as the observed differences could be a consequence of other, unknown, differences.

PROBIOTICS AND ALLERGY

Probiotics are non-pathogenic microorganisms which, when ingested, exert a positive influence on the health or physiology of the host, beyond its nutritional value. The term "prebiotic" is used for eaten compounds that promote a microflora that is beneficial to health. Although used for many years, it is only recently that the mechanisms of action and effects of pre- and probiotics have been studied using the same pharmacological approach as for drugs. They have been tried in a wide range of clinical situations, including prevention or treatment of antibiotic-associated disorders, infectious gastroenteritis and diarrhoea, lactose intolerance, intestinal infections and colonization by pathogenic bacteria, traveller's diarrhoea, irritable bowel syndrome, inflammatory bowel disease, colonic cancer, urogenital infections and tumours, allergy, responses to vaccination, and reduction of serum cholesterol levels. Only some of the studies for a limit number of disorders have been performed in children.

In the search for probiotics bacteria, industry has mainly focussed on various strains of lactobacilli, rather than on bifidobacteria. There is so far no conclusive evidence that a certain strain would be superior to other strains, as most studies so far have been limited to comparing a single strain with placebo or dead microorganisms, rather than other live bacteria.

Probiotics are increasingly marketed and used as health promoting agents. Most of them are lactic acid producers, mostly *Lactobacilli* and *Bifidobacteria*. These species are commensal bacteria common to the gut of all mammals studied, as well as various non-mammalian vertebrates [39]. The safety in infants, including newborn babies, and in healthy and immunocompromised people is verified by numerous clinical studies, in which up to 10¹¹ were given [40]. Needless to say, formal documentation of safety is limited to such bacteria that are used as probiotics and marketed commercially.

Recent clinical trials lend support to the concept that the intestinal microflora may actually have an important role for the immune regulation to allergens and not merely be coincidentally associated with allergic manifestations. Studies even suggest that live microorganisms could in the future be used for the treatment of allergy, or even the prevention of disease. Unfortunately, none of the studies are conclusive, as the study groups were mostly small and the observation period short and important controls were missing. Ongoing and future studies, meeting the same criteria as those used for documenting efficacy of pharmaceutical drugs, will help defining the role of probiotics in clinical routine practice.

The first report was published in 1997 and showed a modest reduction of skin symptoms in infants with atopic dermatitis after one month of treatment with a strain of *L. rhamnosus* [41]. A reduction in the levels of the

inflammatory markers TNF α and α 1-AT in faeces was noted in the 13 infants receiving lactobacilli in addition to an extensively hydrolysed formula, but not in the 14 babies receiving only hydrolysed formula. The observation period was only one month, however, and there was no effect on systemic immune responses.

Since then, at least three other treatment studies have been conducted with this and other probiotic strains. In a small controlled study from the same Finnish group as the initial study, 27 infants who manifested atopic eczema during exclusive breast-feeding and who had no exposure to any infant or substitute formula were weaned to extensively hydrolysed whey formula with or without lactic acid producing bacteria [42]. After 2 months, a significant improvement in skin condition occurred in patients given a *Bifidobacterium lactis* or *Lactobacillus* strain with the hydrolysed formula. The severity of the eczema was modest at the onset however, the study groups were small and the follow-up period was short, making it difficult to draw any definite conclusions of the study.

Supplementation with a *Bifidobacterium* strain was assessed in a third randomised study from Finland, comprising 21 infants with early onset atopic eczema. Although a beneficial effect on symptoms was not proven in the study, the data indicate that bifidobacteria supplementation may modify the gut microbiota [43].

In a Danish double-blind placebo controlled crossover study, two *Lactobacillus* strains (*L. rhamnosus* and *L. reuteri*) were given in combination for 6 weeks to children with AD [44]. The mean age of these children was over 5 years, i.e. they were considerably older than the infants reported in the other three studies. After active treatment, 56% of the patients improved, as compared to 15% in the control group ($p=0.001$). Although the extent of the eczema decreased significantly, this was the case for the total SCORAD index only in atopic children with elevated IgE levels and a positive skin prick test. Furthermore, in the latter group of patients, the levels of ECP decreased among those treated with lactobacilli.

Although all the four studies summarised above indicate a beneficial effect of lactic acid producing bacteria, the question of clinical efficacy remains open, since the study groups were small and besides the modest effect on eczema, the clinical and immunological outcomes differed. In particular, the studies diverged with regard to outcome in patients with defined IgE mediated disease.

Although there is yet little documentation of the superiority of certain strains of lactic acid bacteria that are marketed as probiotics over other similar, wild strains, there is presently no data to suggest that other strains effective. However, it seems reasonable to assume that live microorganisms are more effective than killed bacteria of the same species. The efficacy of oral supplementation of viable and heat-inactivated probiotic bacteria in the management of atopic disease and their effects on the composition of the gut microbiota was studied in 35 infants with atopic eczema and allergy to cow's milk, who received extensively hydrolysed whey formula with or without viable or heat-inactivated *Lactobacilli* [45]. The treatment with heat-inactivated lactobacilli was associated with adverse gastrointestinal

symptoms and diarrhoea, while the decrease in the symptom scores among infants treated with viable lactobacilli tended to be greater than within the placebo group, although not significantly so.

The potentially allergy preventive effects of probiotics was assessed in a double blind, placebo controlled study comprising 132 infants with a family history of allergy [46]. Mothers were given lactobacilli or placebo, starting before birth and the treatment was then continued to the mothers while breast feeding and to the babies when weaned. At two years, the cumulative incidence of atopic dermatitis was reduced by about 50% in the treatment group, although there was no effect on sensitisation or serum IgE levels [46], nor on the incidence of cow's milk allergy as verified by challenge (6/54 in the placebo group and 11/53 among those receiving *Lactobacilli*) [47]. Despite that, the authors concluded that the treatment prevented early "atopic disease", even when the bacteria were given to the mothers while breast feeding and not to their babies.

At 4 years, atopic eczema was defined as relapsing or chronic itchy eczematous lesions with typical localisation. The 12-month prevalence was 14/53 children receiving lactobacilli, versus 25/54 in the group, which received placebo during infancy (relative risk 0.57, 95% CI 0.33-0.97) [47]. In contrast, seasonal allergic rhinitis at 4 years was more common among the *Lactobacillus*-treated children, although not significantly so (10/53 versus 5/54). Furthermore, the prevalence of positive skin prick tests was similar in the two groups. Blinding in the clinical assessment was less than perfect, however, and the prevalence of eczema at this age was high.

As summarised above, the hitherto published studies on lactic acid bacteria in the treatment and prevention of allergy have yielded inconclusive results, although they are encouraging. In a very recent Position paper issued by the European Academy of Allergy and Clinical Immunology, it was concluded that evidence supporting the use of probiotics in the prevention or treatment of allergy is still preliminary [48]. Several ongoing studies will hopefully better define the potential of currently marketed lactic acid bacteria in the treatment or prevention of asthma and allergy. If the efficacy is confirmed, it is reasonable to suspect that this may be more obvious in, or even limited to infancy and early childhood, i.e. before the immune responses to allergens and immune regulatory networks have been established.

CONCLUDING REMARKS

Although it is still unknown what environmental factors in modern industrialised societies that are responsible for the high and increasing prevalence of asthma and other allergic diseases, epidemiological, clinical and experimental research indicates an important, or even critical role of the intestinal microflora. Epidemiological studies have reported serological evidence of oro-faecal infections to be associated with fewer allergies, differences in the composition of the gut microbiota in infants living in children with a high and a low prevalence of allergies, as well as between allergic and healthy infants and children. Such differences can be seen also between healthy infants who later will or will not develop allergic manifestations. Treatment studies suggest

that eczema in infants and children might improve by the addition of lactic acid producing bacteria to the diet. Furthermore, prospective intervention studies suggest that altering the gut microflora in early infancy may in fact reduce the incidence of subsequently developing atopic eczema. It is still too early, however to recommend lactic acid producing bacteria or other microbial treatment for allergy treatment or prevention, as studies published are inconclusive with regard to which type of patients that would benefit from the treatment, if there are strain differences with regard to efficacy and whether in fact it is indicated in IgE mediated disease. The results of the clinical studies so far therefore need to be confirmed and extended. In the meantime, the gut microbiota and host-microbe interactions in infancy remain among the most interesting targets for research into the environmental factors influencing the development of asthma and allergy.

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