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The Gut Microbiota and Potential Health Effects of Intervention

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Introduction

The biological and medical communities increasingly realize that the microbiota of the large gut may play 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 already a century ago the Russian scientist, Elia Metchnikoff, indicated the clinical importance of the host colonic microbiota. He also suggested that certain live micro-organisms might promote health. Despite this, for many years there was 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 microbiota of the human gut is an integral component of the host defense system. This has generated considerable interest in the functional food/nutraceutical industry.

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 gut microbiota in the modulation of immune responses to allergens, autoantigens and foods and microbes in the gut is discussed. The potential for preventing or modulating immunologically mediated diseases by pre- and probiotics, as well as their potential role in infections will be discussed.

The Intestinal Microbiota

The intestinal tract performs many different functions. In addition to absorption and digestion, it is also the body's largest organ of host defense.

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 [2]. The total mucosal surface area of the adult human gastrointestinal tract is up to 300 m^2, making it the largest body area interacting with the environment. It is colonized with over 10^{14} micro-organisms, 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. Soon after birth, however, it is colonized by numerous types of micro-organisms. Colonization is complete after approximately 1 week but the numbers and species of bacteria fluctuate markedly during the first 3 months of life. There is a continuous interaction between the microbial flora and the host, comprising a dynamic ecosystem 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 microbiota.

The gut microbiota are 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 development of a balanced immunity [2]. Thus, 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. For example, Th2-mediated immune responses are not susceptible to oral tolerance induction in germ-free mice [3]. Oral tolerance was only induced after the introduction of components of the normal microbiota.

It is also recognized 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) [4].

Rook and Stanford [1] suggested two major syndromes that could be the result of inadequate microbial stimulation early in life. 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 micro-organisms. 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' [5].

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 micro-organisms that have been present through the evolution

Table 1. Definitions

Probiotic	A live microbial food ingredient that is beneficial to health beyond its nutritional properties
Prebiotic	A compound that promotes a microbiota that is beneficial to health

Table 2. Suggested beneficial effects of probiotics in infants and young children

Infectious diarrhea	Confirmed effect
Antibiotic-associated diarrhea	Likely preventive effect
	Unconfirmed therapeutic effect
Lactose intolerance	Confirmed effect
Inflammatory bowel disease	Possible effect
Urogenital infections	Possible effect
Allergy	Possible preventive effect of infant eczema
	Possible therapeutic effect on infant eczema
Type-1 diabetes	Under study

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

Probiotics and Prebiotics

Probiotics are non-pathogenic micro-organisms which, when ingested, exert a positive influence on the health or physiology of the host beyond their nutritional value (table 1). The term 'prebiotic' is used for eaten compounds that promote a microbiota 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 begun to be studied using the same pharmacological approach as for drugs. They have, however, been tried in a wide range of clinical situations, including prevention or treatment of antibiotic-associated disorders, infectious gastroenteritis and diarrhea, lactose intolerance, intestinal infections and colonization by pathogenic bacteria, traveler's diarrhea, irritable bowel syndrome, inflammatory bowel disease, colonic cancer, urogenital infections and tumors, allergy, responses to vaccination, and reduction of serum cholesterol levels (table 2). Only some of the studies for a limit number of disorders have been performed in children. Unfortunately, most of the studies comprise only small study groups and have flaws in their design.

In the search for probiotic bacteria, the industry has mainly focused on various strains of lactobacilli, bifidobacteria and other lactic acid-producing microorganisms. 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 with dead micro-organisms, rather than with other live bacteria.

Lactobacilli and bifidobacteria and other lactic acid producers are commensal bacteria common to the gut of all mammals, as well a various non-mammalian vertebrates. The safety in infants, including newborn babies, and in healthy and immunocompromised people is verified by numerous clinical studies, in which up to 10^{11} were given [6]. However, rare cases of septicemia and endocarditis have been reported caused by some probiotic bacteria, including *Lactobacillus rhamnosus, L. casei, L. paracasei* and *L. plantarum*.

Protection against Diarrhea

Diarrhea is common among children and contributes to pediatric morbidity and mortality worldwide. In developing countries it also contributes to malnutrition. Metchnikoff already suggested that live bacterial cultures, such as those found in yoghurt, might help treat and prevent diarrhea. Three recent meta-analyses concluded that lactobacillus therapy for acute infectious diarrhea in children is safe and effective as a treatment for children with acute infectious diarrhea [7–9]. In one of the studies, the initial search yielded 26 studies of which 9 published met defined quality criteria and were included in the analysis. Strains that had been evaluated in at least one of the double-blinded, placebo-controlled studies included strains of *L. acidophilus, L. reuteri* and *L. rhamnosus,* with doses ranging from 10^9 to 10^{11} bacteria. The former figure corresponds roughly to the number of micro-organisms in a regular portion of yoghurt. Summary point estimates indicated a reduction in diarrhea duration of 0.7 days (95% confidence interval 0.3–1.2 days) in the participants who received lactobacillus as compared with those who received placebo. There was also suggestion of a dose-effect relationship.

In the second meta-analysis 18 studies were included [8]. The analysis suggested that administration of probiotics with standard rehydration therapy reduces the duration of acute diarrhea by approximately 1 day.

A Cochrane Database Systematic Review of probiotics for treating infectious diarrhea, 23 studies with a total of 1,917 participants were analyzed. It was concluded that probiotics reduced the mean duration of diarrhea by 30.5 h [9].

Diarrhea associated with antibiotic intake has also been the subject of several clinical studies. Such studies are logical as it is well known that antibiotic treatment affects the composition of the gut microbiota. The efficacy of probiotics in the prevention and treatment of diarrhea associated with the use of antibiotics was recently assessed in a meta-analysis [10]. Nine randomized double-blind, placebo-controlled studies were recovered, two of which included children. The odds ration in favor of active treatment over placebo in preventing diarrhea associated with the antibiotic treatment was 0.37 (95%

confidence interval 0.26–0.53, $p < 0.001$). It was also concluded, however, that the efficacy of probiotics in treating antibiotic-associated diarrhea remains to be proven.

Microbiota and Allergy

The wide variations in allergy prevalence between different countries, the fact that the prevalence has increased considerably over the last 40–50 years and the role of the microbiota for the development of immunity in infants, have led to the suggestion that variations in patterns of microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in allergy prevalence throughout the world [4]. Over the past few years, differences have been documented in the composition of the intestinal microbiota between healthy infants in countries with a low and a high prevalence of allergy [11] and between allergic and non-allergic infants in both environments [12–15]. The studies indicate an imbalance in the gut flora of allergic infants and could suggest that differences in the indigenous intestinal flora might affect the development and priming of the immune system in early childhood and that the observed differences between allergic and non-allergic children are not secondary phenomena.

Italian studies in military personnel have lent support to a possible role of the intestinal microbiota in the pathogenesis of allergic disease. Matricardi et al. [16] studied the prevalence of allergic disease and sensitization to inhalant allergens among military personnel in relation to serological markers of previous infections. Interestingly, there was an inverse relationship between respiratory allergy and the presence of antibodies against infections transmitted through the oro-fecal route, such as toxoplasmosis, hepatitis A and *Helicobacter 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 four recent studies in which differences in the composition of the gut flora were observed between allergic and non-allergic infants. In one study, the counts of *Staphylococcus aureus* were higher and the prevalence of *Bacteroides* and *Bifidobacteria* were lower in allergic children at 2 years [12]. A higher prevalence of colonization with *Clostridium difficile* in allergic infants at 12 months was suggested in a considerably larger study, using an indirect method [13]. 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 is that information is obtained about microbial metabolism and ecology. When analyzing 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 colonization with *C. difficile* and the later findings indicate the presence of a lactic acid-producing gut flora.

These studies were cross-sectional and did not address the issue whether the differences were primary or secondary to disease. In two prospective studies, however, less bifidobacteria were detected already during the first weeks of life in babies who developed allergy during infancy [17, 18]. These studies demonstrate that differences in the composition in the intestinal flora between allergic and non-allergic infants are present already before any manifestations of disease. Thus, the studies indicate a primary imbalance in the gut flora of infants who develop allergic manifestations.

Observed differences in the composition of the gut flora between allergic and healthy infants are not limited to species. The strains of *Bifidobacterium* [14] and *Lactobacillus* [19] also differ between the 2 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 presence and type of *Bifidobacterium* between allergic and non-allergic infants is the only consistent finding in all clinical studies so far.

Recently, it was shown in a population-based cohort comprising 3,000 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) [20]. Staphylococci are not part of the normal vaginal flora, which is normally dominated by lactic acid bacteria. This is interesting, since the maternal vaginal microbiota is an obvious source of colonization of the infant during the birth process.

The composition of gut microbiota may also be relevant for the controversy regarding breastfeeding and the development of asthma and allergy. While a protective effect was reported in several earlier studies published in the 1970s and 1980s, most recent studies have reported no effects of breastfeeding on allergic diseases, although it does reduce wheezing in infants, which is a symptom caused by infections and not allergy. Previously there were pronounced differences in the composition of the gut microbiota between breastfed and formula-fed infants [21]. These differences are not nearly as prominent with the use of modern humanized infant formulae [11]. Thus, any protective effect of breastfeeding in the past may have been a consequence of an ecological effect on the gut microbial flora.

As the differences in the composition in the intestinal flora 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. Although all the studies conducted so far confirm differences in the composition of the gut microbiota, 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 in the gut.

Probiotics and Allergy

The first double-blind placebo-controlled study of infants with atopic eczema given probiotics showed a modest reduction of skin symptoms after 1 month of treatment with a strain of *L. rhamnosus* [22]. Since then, at least 4 small studies have been conducted with this and other [23] strains. Unfortunately, none of the studies are conclusive, but taken together they suggest that there may be a modest effect on eczema, but only so in infants and young children. None of the studies support any effect on IgE-mediated disease, however.

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 [24]. Mothers were given lactobacilli or placebo, starting before birth and the treatment was then continued to the mothers while breastfeeding and to the babies when weaned. The cumulative incidence of atopic dermatitis was reduced by about 50% in the treatment group, although there was no effect on sensitization. Despite that, the authors concluded that the treatment prevented early 'atopic disease'. Indeed, the 4-year follow-up even suggested a higher incidence of IgE-mediated respiratory symptoms [25].

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, it seems reasonable to assume that live micro-organisms 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 hydrolyzed whey formula with or without viable or heat-inactivated lactobacilli [26]. The treatment with heat-inactivated lactobacilli was associated with adverse gastrointestinal symptoms and diarrhea, 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 hitherto published studies on lactic acid bacteria in the treatment and prevention of allergy have yielded inconclusive results, although they are encouraging. In a 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 [27]. 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.

Other Conditions

It is established clinical experience that many individuals with lactose intolerance can tolerate at least limited amounts of fermented milk products, despite them having a high lactose content. Although not extensively studied in controlled trials, it is reasonable to conclude that probiotics may reduce symptoms of intolerance.

There are also indications that certain strains of lactobacilli and bifidobacteria may possible in the future find a role in pediatric urology, as decreased risk of infection has been reported [28]. This and other claimed positive effects, such as managing inflammatory bowel disease in children [29], remain rather speculative, however.

Concluding Remarks

Although it is still unknown what environmental factors in modern industrialized societies are responsible for the high and increasing prevalence of allergic and autoimmune diseases, epidemiological, clinical and experimental research indicates an important, or even critical role of the intestinal microbiota. Lactic acid-producing bacteria have a confirmed effect on infantile infectious diarrhea but it is still too early to recommend them for allergy treatment or prevention, as the published studies are inconclusive. Thus, the results of the clinical studies so far need to be confirmed and extended.

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Discussion

Dr. Sorensen: Thank you very much for a very nice review. Do you think that the microbiota can influence the response to vaccines given subcutaneously? Let me preface my question with an explanation. In the United States in our clinics for current infections, we are seeing many children who simply fail to respond to lipopolysaccharides, but we also have a large collection of children who have completely failed to respond to four doses of the conjugated vaccine, and they are indistinguishable from children who have never had a vaccine. The reason for my question is that in studies in Brazil, Chile and Columbia no such unresponsiveness is seen, and I always thought that the use of antibiotics could make a big difference because many of the children that we see have been on multiple courses of broad-spectrum antibiotics. So that is why I wonder if antibiotics are having and influence.

Dr. Björkstén: The answer to that is in principle yes, the gut microbiota do modulate systemic immune responses. To answer your question specifically I obviously can't tell you.

Dr. Saavedra: I am fascinated as we look more into the differences in what we call the microbiota which is basically a stool, in other words the distal colonic content, that

we still see the kinds of differences that you are showing. Ultimately that stool is the equilibrium which that particular host reached with whatever bacterial stimulus and food and mucin and everything they produced. When we talk about the approaches that we are taking, as you say not to go back to infecting everybody but potentially stimulating them, one of the big differences, and I speak as a gastroenterologist where we typically say that anything in the small bowel that is above 10^5 or 10^6 constitutes bacterial overgrowth which we actually treat when we deal with patients with this motility, etc. If they have this amount of bacteria we treat them because we think that leads to an inflammatory response which sometimes explains some other symptoms that those patients may have. When we give probiotics, for example, hypothetically if that is going to have an effect, what we are really doing is creating an artificial bacterial overgrowth on a regular basis with low-dose amounts, probably we get to 10^4, 10^6 bacteria, some are lost in the small bowel. Do you think that we may be missing something by not trying better to look at the differential inflammatory immunologic response between the small bowel which is 'sterile' in our modern society versus the colon which has never been sterile?

Dr. Björkstén: Let me just say one thing first. Modern society is not sterile. The difference versus traditional society is the speed by which colonization takes place, and the fact that after the initial colonization there is less exchange with the environment. There was a study from Göteborg some years ago [1] where they compared Pakistani and Swedish infants with sequential analysis of *Escherichia coli* strains and, in the Pakistani infants it was found that, each time a new sample is taken from the baby, the dominating or common strains have changed, while in Sweden once a coli was found, this strain tended to remain. So that is why I don't like the words 'hygiene hypothesis' and 'sterility'. The other point you are raising is important. Stool cultures are probably relevant for what bacteria are present in the colon as you pointed out, while much of the immune system is really in the small intestine. Preferentially the ecology should be studied in the gut just as you indicated. This is why I am interested in microflora-associated characteristics and not only bacterial cultures. On the other hand I would caution against a reductionist approach because it is clearly an interplay between many microbes. But you are right, we may be looking under the lamp where the light is better.

Dr. Klish: I was fascinated by your discussion on the colonization of the intestine between individuals with atopy and normal individuals. Are the differences there because the atopic individual has an upregulated immune system and selects against bacteria or because the bacteria colonize early, downregulate the immune system, and therefore prevent allergy?

Dr. Björkstén: It is the chicken and egg situation. I can say that it is probably not an upregulated or a different immune system in their future allergies because as far as we know there are no such differences at birth. However your point is well taken because my arguments could be turned around by saying that there is a difference in the gut of potentially atopic individuals, some are prone to a certain type of colonization. The only way to prove this is of course in the prospective studies we are doing. We have just completed a 2-year follow-up prospective study comparing 189 newborns whose mothers received lactobacillus or placebo 4 weeks before term, and then the baby for 1 year. We have the immunology and we have the stool samples so we will be able to answer your question. I think though that it is reasonable to suggest that the microbiota are the primary issue.

Dr. Lucas: Just to get that topic that you got near to. One of the major changes in the last century was an order of magnitude increase in the number of cesarean sections so that the babies were born into a sterile rather than in a dirty environment, and of course cesarean section itself is correlated with certain categories of babies, like premature babies, who may have different immunity-related risks. Has this ever been studied?

Dr. Björkstén: Yes, it has. There are several studies showing that cesarean section is associated with an increased risk of allergy development, which would fit into this hypothesis. To answer the question whether the increase in cesarean sections could explain the increase globally, the answer is clearly, no, there is not enough increase to explain this.

Dr. Hanson: You referred to our studies comparing Swedish and Pakistani babies and I think that is very relevant. I would like to add that it is very difficult to colonize an individual with a number of coli or what have you. They may just pass and you never see them or they may be around for a day or two. But the difference I think for a child in Pakistan, for example, is that there is very heavy exposure. They get very large doses and then the bugs can remain and influence the immune system. I think that could be part of the difference with regard to exposure.

Dr. Björkstén: You are right, that is true. On the other hand, Estonia is not a poor hygiene country. Just as I did not grow up under poor hygienic conditions as a child. So this is a relative term. The size of the inoculum matters and in Estonia they had traditional big baby wards rather than the very strict rooming as in Sweden. I should also say that the consistent finding when comparing non-allergic and allergic children, anthroposophic and conventional children, Estonian and Swedish children, is that the diversity of the gut microbiota is larger in the former. They may have the same bacteria as I showed here, but the diversity, the number of strains that you find is larger. And this raises the point that it is the number of hits on the developing immune system rather than any one given bacteria. It is the sort of intensity of the stimulation.

Dr. Laron: Do the gut microbiota influence gastric physiology, and how?

Dr. Björkstén: It is not my field so I can't answer but I think it is well established that it does.

Dr. Laron: I am asking because the majority of gastrointestinal hormones are actually secreted in the stomach.

Dr. Björkstén: That is a very interesting thought, I have no idea.

Dr. Seidman: I was very interested in your parallels with inflammatory bowel disease, and it is interesting that cesarean section has never been shown to be a risk factor for the development of inflammatory bowel disease despite the paradigm of the importance of the gut microbiota in the first trimester of life. As Dr. Lucas mentioned yesterday observational studies may often be due to confounding factors and we have been very interested in looking at the gene environmental reactions and predisposing children to develop inflammatory bowel disease. The story that hepatitis A virus is a marker for a lack of sanitary conditions is probably due to a confounder. Now the polymorphism for the hepatitis A virus, genes have been discovered and people who have polymorphisms for the gene that allow the hepatitis A virus to infect the person are those that are susceptible to atopic disease. It is not because they have been exposed or not exposed to environmental pollutants or to bacterial and viral infections. So hepatitis A is very interesting in that regard and it probably has nothing to do with sanitation but it is due to genetic a factor.

Dr. Björkstén: You are right and that was precisely the point of my slide.

Dr. Wang: Sometimes we suggest that the patients use probiotics, but normally we do not find it very effective for atopic disease in infants. My question is whether probiotics can upregulate the T-regulatory cells? Are there any clinical trials for probiotics in the treatment of asthma? Can the results of your review be explained in that probiotics induce the immune response by blocking the allergen to be absorbed into the body, but the probiotics do not directly regulate the immune response?

Dr. Björkstén: I think that probiotics are probably not going to be the panacea for prevention and the reason is the complexity of the microbiotic interaction. Having said that, to my knowledge there are no studies showing any efficacy of probiotics in the treatment of asthma. The positive results relate to infantile atopic dermatitis.

Dr. Hamburger: To get back to Dr. Seidman's point, how important is the genetics of IgE and allergy in terms of overriding these minute differences that you are attributing to the bacterial flora?

Dr. Björkstén: The genetics is probably trivial in this context and the good news is that not everybody becomes allergic; the bad news it seems that at least 50% of the population has the genetic potential to become allergic. So obviously we have not been able to study the genetics here. I don't think genetics will be very rewarding nor of much interest because what we are looking for in this global epidemiology of immune diseases is clearly not a change in genetic composition of man in one generation. There is clearly an environmental factor operating.

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