The Development of Gut Immune Responses and Gut Microbiota: Effects of Probiotics in Prevention and Treatment of Allergic Disease

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Abstract

The infant’s immature intestinal immune system develops as it comes into contact with dietary and microbial antigens in the gut. The evolving indigenous intestinal microbiota have a significant impact on the developing immune system and there is accumulating evidence indicating that an intimate interaction between gut microbiota and host defence mechanisms is mandatory for the development and maintenance of a balance between tolerance to innocuous antigens and capability of mounting an inflammatory response towards potential pathogens. Disturbances in the mucosal immune system are reflected in the composition of the gut microbiota and vice versa.

Distinctive alterations in the composition of the gut microbiota appear to precede the manifestation of atopic disease, which suggests a role for the interaction between the intestinal immune system and specific strains of the microbiota in the pathogenesis of allergic disorders. The administration of probiotics, strains of bacteria from the healthy human gut microbiota, have been shown to stimulate anti-inflammatory, tolerogenic immune responses, the lack of which has been implied in the development of atopic disorders. Thus probiotics may prove beneficial in the prevention and alleviation of allergic disease.

Introduction

At birth the infant’s immune system is inexperienced and immature, yet it must be able to distinguish between potentially pathogenic microbial invaders on the one hand and harmless environmental antigens on the other. The immune system gathers experience as it is continuously faced with a myriad of antigens. Quantitatively, as well as qualitatively, the most important site of this interaction is the intestine. Encounter alone does not guarantee maturation, however. The intestinal immune system develops in intimate connection with the indigenous microbiota of the gut and has distinct features, the most prominent of which being the ability to launch suppressive, tolerogenic responses essential to maintaining a disease-free state in the gut as well as systemically.

In this paper we discuss the maturation of host defence from the point of view of the intestinal immune system in relation to the indigenous gut microbiota. Aberrant immune responsiveness with an imbalance in the composition of microbiota in the gut is characteristic to the pathogenesis of many inflammatory conditions. The development of atopic diseases in particular appears to be associated with defective tolerogenic responsiveness in the gut and alterations in the gut microbiota. The rise in incidence of atopic diseases in the industrialised world during the last decades, attributed to changes in lifestyle and improved hygiene, may thus be partly explained by altered gut microecology. This conception opens new avenues for prevention and treatment of atopic diseases by probiotic supplementation.

The Development of the Immune System in Early Infancy and the Atopic Type of Immune Responsiveness

Helper cells have paradigmatically been labelled either T helper (Th)1 or Th2 cells depending on the cytokines they produce (Mosmann et al., 1986; Del Prete et al., 1991). Th1 cells produce predominantly interferon (IFN-γ) and play a central role in immune defence against intracellular pathogens. In contrast, Th2 cells produce cytokines such as interleukin (IL-)4, 5 and 13 and are implicated in responses to helminthic infections. Both Th1- and Th2-type responses have been recognised in the pathogenesis of human disease as well (Romagnani, 1996): Inflammatory processes propagated by Th1 cells are central to the development of autoimmune diseases such as diabetes and rheumatoid arthritis, whereas Th2 type responses are the hallmark of atopic disease, as IL-4 and IL-5 lead to IgE production and eosinophilia, respectively (Jabara et al., 1988; Pene et al., 1988). It is well established that there is a counter-regulatory balance between Th1 and Th2 responses, but recently distinct mechanisms effecting both Th1 and Th2 cells have been discovered. Suppressive cytokines, such as transforming growth factor (TGF)-β and IL-10, secreted by gut-derived regulatory T cells named Th3 and Tr1 cells, respectively, provide important suppressive balance and protection from disease (Nagler-Anderson, 2000).

Notwithstanding the ample evidence indicating the involvement of Th2-skewed immune responsiveness in fully established atopic disease, the events leading to the establishment of such pathological skewness early in life remain less well understood. It is of note that due to the
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immunological balance prevailing in utero the immune responses early in life are physiologically predominantly of the Th2-type (Piccinni et al., 1998). Recently, it has been observed that neonatal CD8 T cells produce large amounts of IL-13 which may account for the Th2 bias in the infant (Ribeiro-do-Couto et al., 2001). Interestingly, however, a previous study indicated that infants who later develop atopic disease exhibit impaired IL-13 production at birth (Williams et al., 2000). The same phenomenon has been demonstrated with regard to IL-4 as well, as neonatal IL-4 responses were lower in infants who developed atopic disease as compared to those who remained healthy (Prescott et al., 1999). Consequently, a significant overlap in the concentrations of Th2 cytokines, such as IL-4, and IgE antibodies prevails between atopics and non-atopics at an early age (Kulig et al., 1999; Prescott et al., 1999). Thus the concentration of IgE antibodies in cord blood, thought to reflect sensitisation in utero, predicts poorly the development of atopic disease (Bergmann et al., 1997).

An age-dependent decline in Th2 responses during the early postnatal period in non-atopic children and a converse pattern in atopic children has been observed (Prescott et al., 1999), suggesting that infants who develop atopic disease may exhibit defective suppression of Th2-type responsiveness. Recent observations have lead to the conclusion that such suppressive mechanisms at least in part originate in the gut as a result of stimulation by dietary and microbial antigens.

The prevalence of atopic diseases, including food allergy, atopic eczema, allergic rhinoconjunctivitis and asthma, has increased significantly throughout the industrialised world over the last decades. Whilst there is a strong hereditary component in the development of atopic disease (Bergmann et al., 1997), the rapid increase in morbidity can only be explained by environmental factors. The hygiene hypothesis of allergy, first introduced by Strachan (Strachan, 1989), suggests a causal relationship between reduced exposure to microbes at an early age and the increase in the prevalence of atopic disease. There is epidemiological evidence indicating that infectious diseases early in life may protect the individual from atopy. In a prospective study of over 329 children, the risk of atopic eczema was reduced if the infant encountered the first pathogen in utero (Kiili et al., 2002). Similar associations have been made with orofaecal infections, such as those caused by the hepatitis A virus or Toxoplasma gondii (Matricardi et al., 2000). These phenomena have been explained by the reciprocity of the Th1-type immune responses elicited against intracellular pathogens and the Th2-type responses central in atopic disease. It is assumed that infectious disease in early infancy directs the developing immune system in a Th1-skewed direction which down-regulates Th2-responsiveness. However, as it has recently been pointed out (Monteleone et al., 2001), the most important source of tolerogenic stimuli may be the indigenous microbiota of the intestine. The immunomodulatory events produced by the interaction between the intestinal immune system and microbiota as well as the induction and maintenance of oral tolerance are complex and surpass the Th1/Th2 paradigm.

The Gut as an Organ of Immune Defence and the Development of Oral Tolerance

The intestinal immune system must be able to discriminate between potentially pathogenic microbial antigens and the non-pathogenic dietary and indigenous microbial antigens in order to avoid both invasive infections and chronic inflammatory conditions. The unresponsiveness to dietary proteins and indigenous microbiota, known as oral tolerance, is the result of several distinct processes. Furthermore, there is growing evidence suggesting that resident microbiota provide the intestinal immune system with endogenous stimuli which are essential for its normal maturation and function (Toms et al., 2001).

There are somewhat contradictory data on the type of immune responsiveness preferentially elicited by the intestinal immune system although it is apparent that T lymphocytes are the main effector cells in responses to luminal antigens. Results obtained from animal models suggest that mucosal T cell responses are favourably of the Th2 type (Xu-Amano et al., 1992). This Th2-bias is thought to originate in utero and as a result, transient sensitisation to dietary antigens is common at an early age. On the other hand, however, Th2-type responsiveness, such as IgE antibody production seems to be most readily susceptible to suppression (Nagler-Anderson, 2000). Furthermore, dietary antigens appear to induce Th1-type responsiveness in the human intestine (MacDonald et al., 2001), which may down-regulate Th2-type responsiveness.

Oral tolerance has traditionally been defined as the induction of peripheral unresponsiveness as the result of oral administration of soluble protein antigens (Nagler-Anderson, 2000). It is well documented that there are distinct mechanisms, including clonal deletion, clonal anergy and active suppression, by which tolerance, defined recently not only as unresponsiveness but more broadly as ‘any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a noninjurious class of immune response’ (Weiner, 2001), is induced and maintained in the intestinal mucosa. The relative contributions of these mechanisms appear to be dependent on the dose of the antigen: High doses of oral antigen result in clonal anergy/deletion, a phenomenon often explained by small amounts of antigen bypassing the intestinal immune system, gaining access to circulation and thus inducing anergy due to the lack of sufficient costimulatory signalling (Weiner, 2001).

Oral administration of antigen leads to the generation of a unique subset of T cells named Th3 cells in both animal models (Chen et al., 1996) and humans (Fukaura et al., 1996). Th3 cells produce predominantly TGF-β and are thought to be pivotal in the active suppression leading to oral tolerance after low doses of oral antigen. After the priming of Th3 cells has taken place in the Peyer’s patches, the gut-originating cells migrate to the periphery and are capable of suppressing inflammatory responses upon reactivation thus mediating tolerance in sites other than the gut as well. (Weiner, 2001)

In addition to down-regulating both Th1- and Th2 type responses, TGF-β elicits immunomodulatory effects on antigen-presenting cells (Takeuchi et al., 1998).
Furthermore, TGF-β may favour the development of IL-10-secreting regulatory Tr1 cells (Toms et al., 2001), another subset of suppressive T cells characteristic of the intestinal immune system. IL-10 has a central role in maintaining intestinal homeostasis, especially by suppressing inflammatory responses towards intestinal microbial agents (discussed below).

There is an intimate interplay between different subsets of T cells and antigen-presenting cells, such as dendritic cells, in the intestine. In murine models, high basal levels of IL-4, IL-10 and TGF-β expression have been detected in the intestinal mucosa (Weiner, 2001) and this cytokine milieu may be crucial for the induction of Th2 and Th3 type responsiveness. However, as it has recently been pointed out (MacDonald, 2001), there may be major differences between species in mucosal immune responses. In fact, there are data on record indicating a Th1-skewed cytokine profile as a constant finding in the intestine of humans (Nagata et al., 2000). A transient induction of IFN-γ producing Th1 cells has been detected in the early phases of oral tolerance formation (Mowat et al., 1999). Even though both Th1 and Th2 cytokines regulate the function of Th3 cells, neither are essential for the induction of peripheral tolerance in a murine model (Garside et al., 1995; Mowat et al., 1999; Shi et al., 1999). The individual role of each functional subset of T cells in the inductive phase of oral tolerance thus remains to be elucidated, but it is evident that Th3 cells provide tolerogenic suppression both in the intestine and in other target organs.

The Development of the Indigenous Gut Microbiota

The indigenous microbiota of the gut are an essential part of the intestine’s defence and homeostasis mechanisms. Bacteria are present throughout the intestine, but the major concentration of microbes is found in the large intestine (Benno et al., 1986; Salminen et al., 1998). The gastrointestinal tract is sterile at birth and microbial colonisation commences immediately after birth (Benno et al., 1986). The establishment of the indigenous gut microbiota is a systematic process which continues through the first years of life (Benno et al., 1986; Salminen et al., 1998).

The maternal vaginal and intestinal microbes constitute a source of bacteria colonising the intestine of the newborn. Colonisation is also determined by contact with the surrounding environment. Facultative gram-positive cocci (staphylococci, streptococci and enterococci) and enterobacteria are the first bacteria to colonise the intestine, followed by anaerobic colonisation during the second day of life. Bifidobacteria then become the predominant species in the intestinal flora in breast-fed infants. Judging from culture-based data it is thought that at least 500 different microbial species coexist, although on a quantitative basis 10-20 genera predominate, among them Bacteroides, Lactobacillus, Clostridium, Fusobacterium, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Escherichia, and Veillonella.

The diet may exert a major effect on the composition and activity of the gut microbiota. In infants, it is thought that those who are breast-fed have a natural predominance of bifidobacteria, while the formula-fed have a profile more complex and similar to the adult indigenous microbiota, with enterobacteria, lactobacilli, bacteroides, clostridia, bifidobacteria and streptococci (Harmsen et al., 2000). After weaning, the composition of the microbiota gradually alters to resemble that of the adult.

Immune Responses to Gut Microbiota and Gut Barrier Function

The indigenous gut microbiota provide the intestinal immune system with stimulation essential to its maturation and homeostasis. Experimental models in which animals are kept in germ-free conditions show impaired development of the intestinal immune system, failure in oral tolerance formation and chronic inflammatory processes resembling inflammatory bowel disease. In a murine model, germ-free animals maintained the tendency to elicit a systemic immune response with IgE production upon oral antigen administration, whereas control animals did not (Sudo et al., 1997). The aberrant IgE responsiveness could be corrected by reconstitution of the gut microbiota at the neonatal stage, but not at a later stage. In humans, recent studies have shown significant differences in the constitution of intestinal microbiota between infants born vaginally and by caesarian section (Grönlund et al., 1999). Colonisation was associated with the maturation of humoral immune mechanisms, particularly of circulating IgA- and IgM-secreting cells (Grönlund et al., 2000). IgA antibodies contribute to antigen exclusion in the gut and thus take part in the formation of oral tolerance. TGF-β is a pivotal factor in IgA production (Stavnezer, 1995; Petitprez et al., 1999), and even though the conception that orally induced unresponsiveness is accompanied by local IgA responsiveness has recently been criticised (Nagler-Anderson, 2000), it is likely that the IgA response elicited in response to colonisation is TGF-β-mediated.

In physiological circumstances tolerance towards the indigenous intestinal microbiota is established and maintained. There is evidence indicating that IL-10, a tolerogenic cytokine, is produced in response microbial stimuli: Mice with defective IL-10 production infected with Helicobacter hepaticus developed Th1-type intestinal inflammation, whereas normal mice produced IL-10 and remained healthy (Kullberg et al., 1998). Interestingly, IL-10 deficient mice have decreased levels of resident lactobacilli in the neonatal period and normalising the amount of these bacteria in the colon prevented the development of intestinal inflammation (Madsen et al., 1999a).

The gastrointestinal barrier is essential in controlling antigen transport in the gut. Both luminal and mucosal factors maintain the integrity of the barrier by restricting colonisation by pathogens, eliminating foreign antigens that have penetrated the mucosa, and regulating the antigen-specific immune responses (Sanderson et al., 1993). At an early age the gastrointestinal barrier is immature with increased permeability leading to excessive antigen transfer. This explains partly the proneness of infants to inflammatory reactions to dietary antigens.
It is clear that proteases of the resident microbes take part in processing dietary antigens already before they come into contact with the mucosal surface. It has been demonstrated that oral tolerance is not achieved without intraluminal degradation of antigenic structures (Barone et al., 2000). However, the indigenous microbiota appear to have an even more profound impact on the barrier function as in the absence of intestinal microbes antigen transport is increased (Isolauri et al., 2001). Tolerance to resident microbiota is abrogated in individuals with inflammatory bowel disease (Duchmann et al., 1995), a condition associated with increased intestinal permeability that correlates with mucosal injury (Gitter et al., 2001). In a study conducted with a murine model, Madsen and co-workers demonstrated that IL-10 deficient mice develop an intestinal permeability defect due to a dysregulated Th1-type immune response to normal enteric microbes and that the increased permeability preceded the development of mucosal inflammation (Madsen et al., 1999b).

The complex interplay between the indigenous microbiota and the intestinal immune system is reflected in tolerance formation and immune responses in other organs as well. Recently, a novel concept of cytokine fields was introduced by Kourilsky and Truffa-Bachi (Kourilsky et al., 2001): A cytokine field is defined by the predominant pattern of cytokines in a given space at a given time. The cytokine field is created by the cytokine-producing cells and, reciprocally, the cytokine field determines the mediators produced by the cells. Thus cells arriving at the site are confronted to the cytokine field, and, more importantly, cells migrating to other sites may spread a particular cytokine field. The induction and maintenance of oral tolerance can be understood as establishing a cytokine field by the intestinal immune system, which is spread into the target organs by migrating effector cells. There are empirical data on record in concordance with this conception, as oral administration of antigen induces antigen-specific tolerogenic, TGF-ß secreting Th3 cells. The induction and maintenance of oral tolerance can be understood as establishing a cytokine field by the intestinal immune system, which is spread into the target organs by migrating effector cells. There are empirical data on record in concordance with this conception, as oral administration of antigen induces antigen-specific tolerogenic, TGF-ß secreting Th3 cells which can be detected in the systemic circulation (Fukaura et al., 1996).

Probiotics in Balancing Gut Microecology and the Intestinal Immune System

Probiotic therapy is based on the concept of normal, healthy composition of indigenous microbiota in the gut. Clinical use of probiotics, defined as live microbial food supplements or components of bacteria which have been demonstrated to have beneficial effects on human health (Salminen et al., 1998), aims in normalising increased intestinal permeability, improving intestinal barrier functions and alleviating intestinal inflammatory responses by restoring the normal composition of gut microbiota and providing immunomodulatory stimuli for the intestinal immune system. Hence clinical conditions involving impaired mucosal barrier functions and inflammation in association with pathological changes in the gut microbiota offer suitable targets for probiotic therapy. An imbalance of the gut microbiota has been reported in association with numerous inflammatory and infectious conditions, including rheumatoid arthritis (Malin et al., 1996a) and rotavirus diarrhoea (Isolauri et al., 1994), as well as atopic disease (discussed below).

The use of probiotics, mainly lactobacilli and bifidobacteria, has been extensively investigated by controlled clinical studies in the prevention and treatment of various gastrointestinal infectious and inflammatory conditions, of which acute infantile diarrhoea is the most thoroughly studied. *Lactobacillus* strain GG (ATCC 53103) supplementation significantly reduces the duration of diarrhoea (Isolauri et al., 1991; Majamaa et al., 1995; Pant et al., 1996) and the duration of rotavirus shedding in rotavirus diarrhoea (Guarino et al., 1997) compared to placebo. The same probiotics stabilise the gut microbiota (Isolauri et al., 1994) and improve increased intestinal permeability caused by rotavirus infection (Isolauri et al., 1993). Furthermore, a significant increase in anti-rotavirus IgA-secreting cells has been reported in response to probiotic therapy (Kaila et al., 1992; Majamaa et al., 1995). Probiotics have also been demonstrated to be effective in prevention of acute infantile diarrhoea (Saavedra et al., 1994; Oberhelman et al., 1999; Szajewska et al., 2001).

Extending work on animal models of inflammatory bowel disease (Madsen et al., 1999a), oral bacteriotherapy with *Lactobacillus* GG has been shown to increase the gut IgA response and thereby promote the immunological barrier of the intestine in patients with Crohn’s disease (Malin et al., 1996b). Pouchitis, a non-specific inflammation of the ileal reservoir, is associated with reduced amounts of lactobacilli and bifidobacteria and an increased amount of *Clostridium perfringens* in the reservoir microbiota (Ruseler-van-Embden et al., 1994). The effect of a combination of probiotics has been demonstrated in prevention of recurrence in chronic pouchitis (Gionchetti et al., 2000). According to recent observations, administration of a probiotic mixture resulted in a significant increase in the concentration of IL-10 in biopsies from patients with pouchitis over a period of 9 months (Ulisse et al., 2001). This suggests the production of anti-inflammatory cytokines as the mechanism by which probiotics exert their effect.

Probiotics in the Prevention and Treatment of Atopic Disease – Mechanisms of Action

There is accumulating evidence associating alterations in the gut microbiota with the development of atopy and atopic disease. The altered composition of intestinal microbiota appears to precede the development of atopy: Infants who develop atopy have more clostridia and fewer bifidobacteria and enterococci in their stools as compared with infants who remain healthy (Björkstén et al., 2001, Kalliomäki et al., 2001a). Furthermore, recent observations indicate that infants who suffer from atopic disease harbour a distinct pattern of bifidobacteria comprising mostly of adult-like strains, as compared to healthy infants with a typical infant pattern consisting mainly of *Bifidobacterium bifidum* (Ouwehand et al., 2001). Children with manifest atopic disease at two years of age are less often colonized with lactobacilli and harbour larger amounts of coliforms and *Staphylococcus aureus* as assessed by faecal cultures in comparison with children without atopic disease (Björkstén et al., 1999b).
As the changes in gut microbiota associated with atopic disease precede the clinical manifestations, alteration of gut microecology with probiotic supplementation may offer a means of preventing the disorder. Indeed, in a double-blind, placebo-controlled trial Lactobacillus GG administered before birth and subsequently during the 3 first months of life reduced significantly the incidence of atopic eczema in high-risk infants (Kalliomäki et al., 2001b).

It is of note that on the one hand the gut microbiota of infants without atopic disease resembles that of breast-fed infants and, on the other hand, infants with atopic disease host an adult-like composition of bacteria, resembling the gut microbiota of formula-fed infants. This suggests that breast milk might contain factors which favour the development of infant-type microbiota which may in turn protect from the development of atopic disease, albeit the evidence on the protective value of breastfeeding per se is less than conclusive. Supporting this, the concentration of anti-inflammatory TGF-ß was lower in the breast milk of mothers whose infants developed atopic eczema during exclusive breastfeeding as compared to mothers whose infants remained healthy during this period (Kalliomäki et al., 1999). Conversely, breast milk from allergic mothers contains higher amounts of IL-4 as compared to non-allergic mothers (Bottcher et al., 2000).

Maternal probiotic supplementation before delivery and during pregnancy increases the amount of TGF-ß in breast milk and is associated with reduced incidence of atopic eczema (Rautava et al., 2002). This demonstrates both the tolerogenic axis between the intestine and the mammary gland and the dependence of early immunological development on exogenous stimuli.

Probiotics have immunoregulatory properties in fully established atopic disease as well. Bovine caseins hydrolysed with Lactobacillus GG-derived enzymes suppresses lymphocyte proliferation (Sütas et al., 1996a) and IL-4 production (Sütas et al., 1996b) in vitro. In clinical studies, probiotics of the same strain have been shown to alleviate symptoms associated with atopic eczema in infants and suppress inflammatory responsiveness in the gut (Majamaa et al., 1997) as well as systemically (Isolauri et al., 2000). The immunomodulatory effects obtained by probiotic therapy are at least in part mediated by enhanced production of suppressive cytokines, namely IL-10 (Pessi et al., 2000) and TGF-ß (Isolauri et al., 2000).

Probiotic therapy has also been studied in adults suffering from adverse reactions to cow’s milk. The indigenous gut microbiota in milk-hypersensitive adults does not differ from healthy individuals. However, administration of Lactobacillus GG resulted in a significant increase in the number of bifidobacteria in healthy individuals but not in milk-hypersensitive subjects (Apostolou et al., 2001). Probiotic therapy alleviated the symptoms and suppressed the inflammatory responsiveness associated with the disorder (Pelto et al., 1998). Probiotic therapy may thus have a more complex effect on gut microecology than simply promoting or colonising the intestine with the strain administered by reinforcing a healthy composition of indigenous microbiota.

Conclusions

The intestinal indigenous microbiota may be considered a yet unexplored organ of host defence with a pivotal role in normal maturation and function of the intestinal immune system. The gut microbiota take part in maintaining normal intestinal barrier function partly by degrading dietary antigens and preventing colonisation by pathogens. Furthermore, microbial stimuli are essential in establishing tolerogenic immunological responsiveness, elicited by Th3 and Tr1 cells, which suppresses the production of inflammatory mediators. The establishment of healthy intestinal microbiota early in life plays a key role in acquiring a disease-free state in the gut as well as systematically, as human disorders with inflammation of the Th1 or Th2 type are accompanied with disturbances in the composition of the gut microbiota. In particular atopic diseases, the incidence of which has increased in the industrialised world, are associated with characteristic changes in the microbiota. Changes in lifestyle, including diet and exposure to microbes, may explain aberrations in intestinal colonisation thus expanding the hygiene hypothesis of allergy to emphasise the importance of the indigenous microbiota.

Probiotic therapy is based on the concept of healthy gut microbiota. The targets for probiotic supplementation in prevention and treatment of atopic diseases are restoration to normal of increased intestinal permeability and unbalanced gut microecology, improvement of the intestine’s immunological barrier functions, alleviation of the intestinal inflammatory response, and reduced generation of pro-inflammatory mediators by enhancement of tolerogenic immune responsiveness.

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