

Probiotics in the Management and Prevention of Atopy

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Introduction

In the last few decades, industrialized countries have witnessed a disturbing rise in the prevalence of atopy. Because this epidemiological trend has emerged during an era of unparalleled hygiene and successful control of infection, researchers believe that the waxing of atopy is aetiologically linked to waning exposure to microbial agents. It has been convincingly demonstrated that infection can modulate the incidence of allergic disorders, possibly through the elicitation of Th1-polarized phenomena that subvert the Th2 character of atopy. Intestinal flora, both pathogenic and commensal, show a similar ability to modulate the local immunological environment, and this local modulation can influence systemic immunological events. The mechanisms by which this immune conditioning occurs have not yet been elucidated, but the clinical implications seem clear: probiotics, which exploit the prophylactic effects of live microbial food ingredients, are now being explored with growing interest as a bona fide therapeutic option in the management—and even primary prevention—of allergic diseases.

The Hygiene Hypothesis

The advancing credibility of probiotics has been fueled in part by the hygiene hypothesis—the proposition that the dramatic rise in the

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prevalence of atopy in the Western world over the last 20 yr is aetiologically linked to the relatively aseptic Western lifestyle. Indeed, there is evidence that the increase may be a result of changes in levels of allergen exposure (1,2); it has been repeatedly demonstrated that factors which predispose children to infection may confer protection against the subsequent development of atopic disease.

Probiotics and Atopy

One important factor associated with elevated rates of childhood infection is large family size, and a number of studies have reported diminished rates of atopy or allergic symptoms among children from larger households. For example, a study of 2111 Scottish school children (3) found a significant protective effect of large sibship with respect to several manifestations of allergic disease, although the actual impact of previous incidents of infection was equivocal. A similar, inverse correlation between the number of siblings and the prevalence of disease has been found specifically for hay fever (4). In a population of more than 7500 German children, the likelihood of atopic sensitization, as assessed by skin-prick test, was found to decrease linearly with the number of siblings (5), and similar trends were found among 11,371 candidates for enrollment in the Italian military (6). Moreover, a prospective study of 1035 infants followed from birth and monitored for the incidence of asthma and the prevalence of frequent wheezing showed that regular exposure to other children, either at home or at day care during the first 6 mo of life, protected against the development of asthma (7). Early attendance at nursery facilities, may, like large sibship, enhance the chance of infection and therefore reduce the risk of atopy, and this finding has been corroborated elsewhere (7,8). For example, a German study found that children from families of up to three people were more likely to have positive skin-prick tests if they were enrolled in childcare outside the home at a later age; for those from larger families, the age of enrollment had no effect on atopy (8).

Other infection-related factors appear to be operative as well. Exclusion from some conventional immunization programs, for example, seems to have a protective effect, possibly because some vaccines preferentially activate Th2-type cells, as has been shown in adults who received measles inoculum (9). To this end, 23 New Zealand children who avoided immunization experienced no asthma or other allergic illness before age 10, and the remainder of the 1265-child cohort, who had been immunized, experienced asthma and allergy at rates between 20% and 30% (10). In a population of 1206 children aged 7 and 8 yr, early life use of antibiotics by children of parents with hay fever was significantly related to the subsequent development of asthma, hay

fever, and eczema, and—although the statistical significance was borderline—skin-test positivity in these children (11).

Importantly, this inferential evidence for the hygiene hypothesis, *vis-à-vis* lifestyle and environmental considerations, has been confirmed by more direct associations between infection history and subsequent protection against the development of atopy. Admittedly, some respiratory infections have been associated with susceptibility to asthma; infection with respiratory syncytial virus increases sensitization to aeroallergens in the first year of life (12) and augments the risk of later presentation of allergic disease (13), as does respiratory exposure to a number of other infectious agents (14,15). However, it would generally seem that the exuberance of one's *infection pedigree*—i.e., one's history of Th1-polarized immunity, may predict resistance to Th2-polarized phenomena. In a study of 1659 young Italian men, seropositivity for Hepatitis A was associated with a significantly reduced incidence of atopy and sensitization to specific aeroallergens and allergic disease (16). Another study by the same group showed that exposure to some orofecal and food-borne pathogens—specifically Hepatitis A, *Toxoplasma gondii*, and *Helicobacter pylori*—was inversely correlated with respiratory allergy (17). There is further evidence from Guinea-Bissau that measles infection and early exposure to BCG may protect children from the development of atopy (18,19). Likewise, infantile exposure to bacterial endotoxin, which elicits a predominately type 1 response, has also been associated with reduced allergic sensitization (20). Notably, these epidemiological findings have been verified experimentally: adenoviral infection, which induces a Th1-polarized response, inhibits the development of allergic airway inflammation in a murine model (21).

If involuntary exposure to foreign organisms can promote or prevent atopy, allergic sensitization, and allergic disease, it stands to reason that deliberate exposure may be therapeutic. One possible site for such intervention is the gut. Examination of the intestinal microflora of infants from Sweden and Estonia has revealed differences in the pattern of microbial colonization between the two countries, and has shown that that Estonian infants have a microfloral profile similar to that characteristic of Western European children in the 1960s (22). This finding indicates a shift in the intestinal microflora of infants in highly industrialized countries concomitant with the dramatic rise in atopy. The knowledge that the establishment and maintenance of gut flora throughout life, and during infancy in particular, has profound implications for the homeostasis of the immune system has therefore prompted researchers to consider the role of the gut's bacterial microenvironment in the development and treatment of atopy (23,24).

Microorganisms and the Gut

The intestinal epithelium acts as a barrier between the luminal contents and the internal milieu (25). This barrier, however, is incomplete. The intestinal contents are generally compartmentalized, yet the epithelium is constantly sampling a variety of molecules through absorptive processes, as well as particulates such as bacteria, through specific phagocytic and pinocytotic activity. Specialized follicle-associated epithelia (or M cells) overlie the organized lymphoid tissue, which includes Peyer's patches (PP) (26); this epithelium transports both molecules and organisms to the immune system contained therein.

The epithelium is constantly being formed in the crypts so that the cells at the apex of villi are sloughed off approximately every 72 h. Thus, under normal circumstances, the barrier is incomplete. This characteristic of gut epithelium is further borne out by baseline studies of intestinal permeability which show that absorptive mechanisms are constantly engaged, and that the tight junctions which seal the potential gaps between epithelial cells are also not consistently impermeable. Increased permeability to small and large molecules, as well as to microorganisms (via translocation), has been reported in a variety of human diseases. For example, increased permeability is invariably found in gastrointestinal allergic conditions (27), and has been documented in response to nonsteroidal anti-inflammatory drugs, in inflammatory bowel disease, and in atopic eczema.

Thus, the epithelium plays an active role in absorption and secretion, and also acts as a barrier. Notably, a dynamic cross-communication between microflora and mucosal epithelial cells of the intestine has been understood for some time (28,29). Bacteria have developed a variety of physical and molecular strategies to facilitate attachment to intestinal epithelium (30). Once attached, organisms can colonize, and can also inject genes, proteins, and other molecules into the cells to which they have adhered (31,32). A cardinal illustration of this interaction is provided by pathogenic strains of *E. coli*, which bind readily to intestinal epithelium (33); they translocate the receptor for intimin into the host cell, which then expresses it on the cell surface, thus promoting further attachment. Another pathogen, the cholera vibrio, secretes an exotoxin whose B subunit binds to a specific epithelial receptor identified as GM1 ganglioside (34); the α subunit then activates the cell to secrete ions and water, resulting in severe diarrhea.

The interaction of the epithelium with potentially injurious and even invasive pathogenic organisms has been the subject of considerable investigation in the last decade. Intestinal bacteria have been documented to induce the production of a broad and strain-dependent spectrum of pro-inflammatory and anti-inflammatory cytokines both

in vitro and in vivo (35-38). The epithelium is known to be capable of synthesizing a variety of molecules involved in inflammation. These include cytokines and chemokines (IL-1, -6, -8, TNF- α , MCP-1, GM-CSF, RANTES), prostaglandins, and other products of arachidonic-acid metabolism, mucins, carbohydrates, and others (25). It has become manifest through systematic study that the epithelium represents at least one class of cells stimulated by pathogenic microorganisms to synthesize many of these molecules. Indeed, epithelial production of IL-6 and IL-8 is activated upon interaction with *Salmonella* or pathogenic *E. coli* (31,35,39); pathogenic organisms elicit the synthesis and secretion of IL-8 by epithelial cells, and also upregulate the expression of chemokine receptors to orchestrate the migration of neutrophils to the luminal surface (40). Although the precise identity of the signals passing between the organism and the epithelium varies according to the strain, this interaction generally requires epithelial attachment of the microbe or the synthesis of molecules that facilitate indirect interaction.

Lying immediately below the epithelial surface is a panoply of immune-inflammatory cells, including epithelial lymphocytes, dendritic cells, and mast cells. Factors secreted by the epithelium can then interact with these cells and modulate their activity, either promoting or inhibiting inflammation as well as innate or adaptive immune responses. Since these immune and inflammatory cells are motile, arriving from the bone marrow via the bloodstream, they can also migrate to distal sites, such as lymph nodes, where they may exert additional modulatory effects, either directly or indirectly (see Fig. 1).

However, despite the enormous burden of potentially pathogenic organisms with which it is constantly interacting, the intestine is maintained in a homeostatic state of regulated inflammation. Information on the effects of commensal bacteria upon the epithelium is scarce. Some symbiotic gut bacteria can induce intestinal epithelial cells to express carbohydrate moieties, which can then be used as a metabolic source of energy (32). Recent studies, using a co-culture system with bacteria and human colonic epithelial cells (T84), have suggested that nonpathogenic organisms may also exert a regulatory effect upon the epithelium. Neish et al. (41), for instance, have shown that a nonpathogenic organism, *S. pullorum*, can inhibit the effect of a variety of agents capable of stimulating the synthesis of IL-8, a potent neutrophil chemoattractant; prior incubation of live *S. pullorum* with T84 cells inhibited pro-inflammatory phenomena, including synthesis of IL-8, following stimulation with a pathogenic *Salmonella* strain (*typhimurium*), TNF α , phorbol myristic acetate, or carbachol. This suppressive effect was not observed with killed organisms or conditioned medium from bacterial cultures or even with bacterial lysates, and was

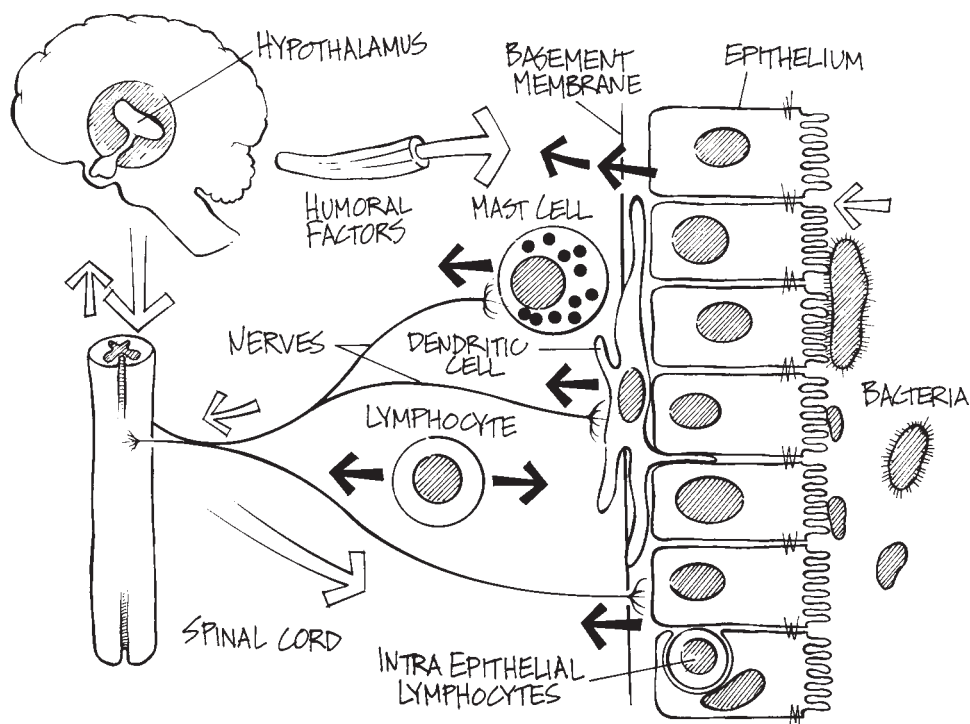


Fig. 1. Schematic diagram of the intestine depicting bacteria, epithelium, and mucosal inflammatory and immune cells and nerves. The text describes how luminal bacteria, through their interaction with the epithelium, can induce the latter to produce an entire range of mediators, including products of arachidonic-acid metabolism and cytokines. These, in turn, can influence the surrounding environment, which contains such important potentially regulatory and inflammatory cells as dendritic cells, lymphocytes, and mast cells. These in turn are stimulated, and release further factors which promote enhancement or inhibition of the immune and inflammatory processes. Also depicted in this diagram, but not described in the text, are nerves which play a very important role in the regulation of the integrity of the mucosa. Because no information exists on the role of probiotics in this regard, the authors have chosen to omit reference to the nervous system in the text, but have included it here so that the reader can appreciate its potential role and involvement in the beneficial effects induced by probiotics.

shown to involve arrest of the NF κ B pathway through inhibition of I κ B degradation. The authors went on to suggest that nonpathogenic commensals may employ this mechanism to downregulate the immune response in the normal human intestine. Whether this pathway is activated by probiotic organisms in general, or is only activated by certain nonpathogenic bacteria, is not yet clear.

In the context of probiotic therapy, it has been hypothesized that prophylactic microflora elicit an immune response that modulates

Th2-affiliated cytokine production or privileges the induction of tolerance, possibly through the production of IL-10 (42,43). Although the source of IL-10 is unknown, it is interesting that IL-10 has been shown to be synthesized by epithelial cells upon ligation of CD1d, an MHC-related molecule involved in the presentation of bacterial glycolipids (44). Feeding of *lactobacilli* to human infants with atopic eczema increases IL-10 in serum and enhances IL-10 production by mitogen-stimulated peripheral-blood mononuclear cells in vitro, pointing to an elegant, if preliminary, mechanistic explanation for the beneficial effects of microbial exposure in atopic disease (45). However, experiments in which *lactobacilli* were fed to IL-10-deficient mice (46)—which invariably develop colitis under conventional conditions involving exposure to normal bacterial flora (47)—suggest that the effects of *lactobacilli* are not necessarily dependent upon IL-10. In this case, colonization of the intestine by *lactobacilli*, even in the absence of endogenous IL-10 production, diminished the intestinal inflammatory response; similarly, oral administration of lactulose, which promotes the growth of *lactobacilli*, inhibited the onset of colitis.

Regardless of the equivocal role of IL-10, it is evident that microorganisms may play a role in the downmodulation of Th2 processes. Sütas et al. have documented both reduced proliferation (48) and diminished IL-4 production (49) in lymphocytes exposed to prototypic food antigens previously hydrolyzed by *Lactobacillus* GG-derived enzymes. Moreover, although the absence of gut flora had no effect on the induction of oral tolerance to ovalbumin in mice (50), confirming the existence of complementary immune-regulatory mechanisms, antibiotic treatment of neonatal and adult mice did result in a distinct Th2 profile. Indeed, the abrogation of gut flora enhanced total IgG1 and IgE, attenuated IgG2a production in serum, and promoted the expression of a Th2-polarized cytokine profile by splenocytes stimulated in vitro with immobilized anti-CD3 antibody (51). Collectively, these data imply that intestinal microbes evoke the maturation of an immunologic phenotype that subverts the development or persistence of robust Th2 phenomena.

Another possibility, advanced in a double-blind, crossover study from Peltola et al. (52), is that microbes mitigate the activation of innate immunity. Concurrent administration of *Lactobacillus* GG during milk challenge prevented the upregulation of phagocytic receptors by neutrophils and monocytes in milk-hypersensitive adults, but somewhat paradoxically was immunostimulatory in health subjects.

Therapy

The mechanistic details explaining the role of the microbial and associated cytokinetic microenvironment in the gut have not yet been

elucidated. However, the documented immunological repercussions to changes in bacterial flora support the idea that deliberate intervention in this area can be used to modulate immune-inflammatory responses. In some published studies by Isolauri and colleagues, for example, probiotic therapy with *lactobacilli* in humans was accompanied by decreased intestinal permeability to macromolecules (27), and benefits have been demonstrated in such local conditions as inflammatory bowel disease (53) and infection by intestinal pathogens (54). Reports of more generalized systemic effects of probiotics are more difficult to explain, but we are beginning to understand that commensal organisms can transmit signals, through the effects on intestinal epithelium, directly or indirectly, to the rest of the body beyond the local environment in the intestine. Among the non-gastrointestinal disorders to show a promising response to probiotic treatment are bacterial vaginosis (55), urinary tract infection (56), and even a particular murine model of cancer (57).

A number of recent studies have also documented a compelling link between intestinal microbial colonization and resistance to the development of allergy and asthma. Alm et al. (58) compared Swedish children reared in anthroposophic communities (which advocate the very judicious use of antibiotics and vaccination, and whose diet typically includes live *lactobacilli*) with those accustomed to a more conventional Western lifestyle. Anthroposophic children had a lower prevalence of atopy, and the risk of atopy was inversely related to how closely the children observed anthroposophic customs. Corroborating these findings, analysis of cultured fecal samples from 2-yr-old children in Sweden and Estonia indicated a lower rate of colonization by *lactobacilli* in allergic than nonallergic subjects, although the proportion of aerobic bacteria, especially coliforms and *Staphylococcus aureus*, was elevated in the intestinal flora of allergic children (59). Moreover, lower titers of microbial short-chain fatty acids, with the exception of *Clostridium difficile*-associated I-caproic acid, have been measured in the feces of allergic infants (60). Building on these findings, Kalliomäki et al. (61) analyzed the population of intestinal microflora in infants at high risk of atopic disease. They found that differences in the bacterial fatty-acid profile in fecal samples from infants as young as 3 wk of age could predict the development of atopic sensitization at 12 mo. A reduced ratio of bifidobacteria to clostridia was characteristic of subjects in whom atopy was developing. This study in particular argues persuasively for the important neonatal role of indigenous intestinal bacteria in the elaboration of an atopy-prohibitive or -permissive immunologic environment.

This experimental and epidemiological evidence evinces the intriguing possibility that manipulation of gut flora represents a viable

therapeutic option for the management of atopic diseases. Data concerning the prophylactic effects of live-culture yogurt consumption for allergy and asthma remain equivocal (62,63). However, Majamaa and Isolauri (27) have shown significant amelioration of clinical symptoms and immunologic parameters in a randomized, placebo-controlled trial of probiotic therapy in infants with atopic eczema and cow's-milk allergy. Indeed, addition of live *Lactobacillus* GG to the hydrolyzed whey formula fed to patients on a strict cow's-milk elimination diet accelerated the resolution of eczema and dramatically reduced evidence of intestinal inflammation. A subsequent study has correlated these improvements in clinical score with changes related specifically to allergic inflammation, including a reduction in the concentration of soluble CD4 in serum and eosinophil-derived protein X in urine following probiotic intervention (64).

Perhaps the most dramatic demonstration of the potential for probiotics in a clinical context is a recent double-blind, randomized, placebo-controlled trial in which *lactobacilli* were given prenatally to mothers with high risk for atopic eczema, allergic rhinitis, or asthma, and then postnatally for 6 mo to their infants; the frequency of atopic eczema in the probiotic group was one-half that of the placebo. In addition, and most importantly, the incidence of asthma and allergic rhinitis was considerably lower than predicted. Again, this suggests that the effect of colonization of the intestine by a particular set of commensal organisms may have profound systemic effects on several of the manifestations of allergy and asthma (65).

Regardless of inconclusive mechanistic details, probiotics represent a therapeutic paradigm that, unlike conventional modalities, attends to the epidemiology, and possibly the aetiology, of atopy. For this reason, probiotic intervention during infantile microbial colonization of the gut—and the articulation of an intrinsic immunologic “disposition” antithetical to Th2 polarization—may be a realistic consideration for the primary prevention of allergy and asthma.

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