

Role of probiotics in food hypersensitivity

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The definition of probiotics has evolved concomitant with a resurgence of research interest in host-microbe crosstalk. The original definition stated that the live active culture beneficially affects the host by improving its intestinal microbial balance, while current conceptions are based on target- and site-specific effects of clearly defined strains. The establishment of normal microbiota in the intestine represents a key process whereby the intestinal milieu is kept disease-free as it performs its dual function: mounting an inflammatory response to pathogens and maintaining hyporesponsiveness to innocuous antigens. Probiotic therapy is based on this concept of a healthy well-balanced gut microbiota. The probiotic performance of strains differs, however. Different bacteria have clearly defined adherence sites and immunological effects and divergent effects in the healthy versus inflamed mucosa. Hence, notwithstanding recent demonstrations of the important immunoregulatory potential of the healthy well-balanced gut microbiota, current probiotic research is directed towards identification of specific strains with anti-allergenic potential.

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Abbreviations

TGF transforming growth factor
Th T-helper (cell)

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Introduction

The dilemma of host immune defence is to generate an effective response to pathogens maintaining unresponsiveness to antigens in food and the normal host microbiota. The phenomenon termed 'oral tolerance' is crucial in avoiding local and peripheral inflammatory responses to ubiquitous antigens [1]. Could allergies be ascribed to a lack of such physiological anti-inflammatory processes?

There are data to suggest that the prevalence of atopic diseases (i.e. atopic eczema, allergic rhinoconjunctivitis and asthma) has increased over recent decades throughout the industrialized world [2]. These conditions are associated with the generation of T-helper (Th)2-type cytokines, including interleukin-4, interleukin-5 and interleukin-13, which promote immunoglobulin E production and eosinophilia [3]. The Th2-skewed immune type may be balanced by cytokines that are secreted by Th1, Th3 and T regulatory cell 1 cells, partly as a result of stimulation by the gut microbiota [4,5,6–9].

In general, the 'hygiene hypothesis' of allergy implies that recent advances such as improved sanitation and lifestyle changes, including the declining family size, in Western societies have reduced the extent of microbial exposure [10]. Infectious disease in early childhood may in fact prevent allergic disease. An important criterion for such a beneficial sequel is that the infection also be prevalent in developed countries, because the majority of infants, even those genetically predisposed to atopy, are still spared from atopic diseases. Moreover, exposure should occur very early, because the first expression of the atopic immune responder type frequently occurs within the first few months of life [11]. The establishment of the gut microbiota in providing an initial and massive source of microbial stimuli may be a good candidate 'infection'. Delayed compositional development of the normal intestinal microbiota has indeed been shown to hinder the maturation of host defence mechanisms in the gut in early infancy, when exposure to novel allergenic challenges in diet and environment continues, thereby carrying a potential risk for atopic disease [12,13].

The rationale underlying probiotic therapy in allergic diseases is, in the simplest terms, to provide a microbial stimulus for the host immune system by means of cultures of beneficial live micro-organisms that are characteristic of the healthy infant gut microbiota.

However, there are more unresolved issues than resolved ones in probiotic therapy. How is food allergy related to the emergence of the atopic responder phenotype? What are the targets of probiotic therapy in the allergic/at-risk host? What are probiotics to date and what are the specific probiotics of the future?

Exposure, sensitization and hypersensitivity: still an open sequence of events

The increased prevalence of allergic disease in industrialized countries cannot be explained by novel emerging allergens sensitizing the host in addition to the traditional dietary allergens and aeroallergens. Rather, it would appear that environmental changes more directly shape the immune responder type of the host during a critical period of life [14•]. The ubiquitous allergens may thus act as recipients of the altered immunological responses, and not as their cause.

Data accumulating from recent research corroborate for such a view. First, exposure to allergens that sensitize the host immune system may not necessarily induce allergic disease. For dust mite allergens, earlier data indicated a dose–response link between exposure and clinical symptoms [15]. Such a connection is no longer obvious, although differences between allergens clearly exist. Lau *et al.* [16••] from the Multicentre Allergy Study Group in Germany evaluated pulmonary function and bronchial hyperresponsiveness in 645 of a cohort of 1314 children followed from birth. Sensitization to indoor allergens such as house dust mites (as assessed by specific immunoglobulin E), but not the level of exposure to these allergens, was shown to be associated with asthma, wheeze and increased bronchial responsiveness. Second, Platts-Mills *et al.* [17] identified a modified Th2-type response, characterized as an immunoglobulin G and immunoglobulin G₄ antibody response (which does not confer increased risk for asthma), on exposure to cat allergen but not on exposure to mite allergen. Similarly, production of anti-inflammatory interleukin-10 (another modified Th2-type function) consistently accompanies infection with helminth parasites, and infected children manifest less atopy [18]. These data imply that eradication of allergens from the early environment may not suffice to resolve the increasing problem of allergic disease. More important may be promoting factors that reinforce an anti-inflammatory, potentially tolerogenic type of immune response in order to assimilate potentially allergenic challenges. Such factors would need to exert their effects at an early age.

Physiologically, immune responses early in life are predominantly of the Th2-type because of the immunological balance that prevails *in utero* [19]. According to a recent study [20] this Th2 bias may, at least in part, be due to the large amounts of interleukin-13 produced by

neonatal CD8⁺ T cells. Interestingly, however, it has been observed that neonatal Th2 responses, including the production of interleukin-13 [21] and interleukin-4 [22], are actually lower in infants who develop atopic disease than in those who remain healthy. It has been suggested that healthy infants exhibit an age-dependent decline in Th2 responses during the early postnatal period, whereas infants who develop atopic disease with initially lower Th2 responsiveness are characterized by a converse pattern [22]. It would thus appear that increased production of Th2 cytokines is the result of defective immune regulation associated with the development of atopic disease, rather than a direct cause of such development.

Probiotics: extending the hygiene hypothesis

New approaches in the fight against allergies are clearly called for, the target being the persistence of the allergic responder pattern beyond infancy and not given allergens in the environment. Establishment of the gut microbiota commences immediately after birth [23,24], thus providing timely counter-regulatory signals against sustained Th2-skewed immunity.

The predominant faecal organisms by the end of the first week of life have been reported to comprise bifidobacteria, bacteroides, clostridia, enterobacteria and streptococci. Breast-fed infants harbour a natural predominance of bifidobacteria, with specific strains present, whereas formula-fed infants have a profile that is more complex and similar to the adult microbiota, with enterobacteria, lactobacilli, bacteroides, clostridia, bifidobacteria and streptococci [25]. After weaning, the composition gradually develops to resemble that of the adult. In adults, anaerobes dominate with high numbers of bacteroides, eubacteria, peptostreptococci and often bifidobacteria and with totals of 10¹¹–10¹² colony-forming units/g faeces [24]. With further ageing the numbers of lecithinase-negative clostridia and peptostreptococci further increase and those of bifidobacteria decrease [24]. New methodology may reveal greater numbers of microbes and as yet unidentified microbes in different parts of the gastrointestinal tract.

The indigenous bacteria have sometimes been classified as potentially harmful or health promoting (Table 1). Probiotics have been selected from members of the normal healthy intestinal microbiota, most of them belonging to genera *Lactobacillus* or *Bifidobacterium*, but new probiotic microbes from other species and genera have recently been introduced.

Scientific validation of probiotics: from colonization to specific mechanisms

The original definition of ‘probiotic’ stated that the live active culture beneficially affects the host by improving

Table 1. The important characteristics of intestinal colonization

Characteristic	Comment
Normal intestinal biota	The complex collection and balance of micro-organisms that normally inhabit the healthy gastrointestinal tract, fulfilling a role in host nutrition, physiology and control of the immune system
Establishment of gut microbiota	Establishment of the gut microbiota is a stepwise, well controlled process that occurs during the first years of life
Balance between harm and benefit	An individual balance exists between potentially harmful and health-promoting strains (an individually optimized healthy gut microbiota)
Balance between normal microbiota and immune system	A critical balance exists between the normal microbiota and the immune system of the host; tolerance to the healthy balanced gut microbiota is generated and maintained

its intestinal microbial balance, while current conceptions are based on target- and site-specific effects of clearly defined strains.

Quality control of probiotics

Selection of probiotics is from the healthy human intestinal or mucosal microbiota as the primary source, with added emphasis on survival in the gut, acid and bile stability, and temporary colonization of the mucosal surfaces in the intestinal tract. Quality control measures include strain identification and purity assessment using modern biotechnological tools. Adhesion must be assessed using at least two independent test systems, for instance adhesion to human intestinal mucus and human intestinal tissues [26,27]. Other tests include adhesion to Caco-2 cells, platelet aggregation, and comparison with other dairy and human strains [26,28]. Some probiotic strains adhere better to the small intestine, whereas some bind specifically to different parts of the large intestine [26]. It is likely that strains also adhere differently in healthy versus damaged mucosa [29], and even closely related probiotics have been shown to possess different in-vitro properties [26]; this may also explain differences in clinical effects.

The criteria applied in probiotic selection have formed the basis for safety assessments and evaluation of clinical efficacy, and these properties therefore must be preserved during all stages of manufacturing and processing [30]. However, it has recently been demonstrated [31] that strains with lower total in-vitro binding capacity may still effect highly competitive exclusion of pathogens or harmful bacteria *in vivo*, indicating a need for further characterization of adhesion properties to develop preclinical selection methodologies for candidate probiotic strains.

Colonization is not mandatory for probiotic effect

Adherence and colonization are considered important for probiotic action, but long-term intestinal colonization may only be possible during very early infancy and immediately after birth [32]. No proof of a need for colonization has been reported and probiotics mostly reside only transiently in faecal samples from humans

following dietary intake. Transient colonization may be important in the case of, for example, acute diarrhoea because unbalanced intestinal microbiota in such conditions can be corrected by brief probiotic intervention [33].

In general, many probiotic effects are unlikely to be related to the intestinal microbiota changes indicated by faecal cultures. Micro-organisms that directly interact with the intestinal mucosa are obscured by faecal flora and thus are poorly characterized. Therefore, efficacy verified in human clinical studies is more important than plain 'colonization'. Detection of ingested probiotics in faeces indicates that the strain is able to survive passage through the intestinal tract, but is no indication of adherence or colonization [34]. For immune regulation, adherence properties may be required [35]. In fact, colonization and the immunomodulatory effects of probiotics may be mutually exclusive; sporadically intruding micro-organisms may elicit specific responses in the gut-associated lymphoid tissues [36]. Once adopted by the normal microbiota, their potential to stimulate an immune response may be abolished, because oral tolerance mechanisms have been shown to apply to the resident microbiota as well [37]. This hypothesis is supported by earlier findings on the clinical efficacy of inactive probiotics without any immune-enhancing effect [38,39].

What are the probiotics to date and what are the specific probiotics of the future?

Probiotics have thus far been selected according to very few criteria and the old definition of 'probiotic', whereas the spectrum of probiotic action is in fact rather universal (Table 2) [40–45]. New criteria include local attachment to intestinal mucosa (e.g. in the small intestine) and documented clinical efficacy (e.g. in the treatment and prevention of atopic disease). New probiotics can be produced by exposing the micro-organism to sublethal stress such as acidic conditions or heat, in order to improve survival in the gastrointestinal tract and improve tolerance to stress, and thereby to provide the organism with improved competitiveness against pathogens in the intestinal milieu [46]. Probiotic efficacy may also arise

Table 2. The definition of probiotics: the history behind the current proposition

Definition	Reference
Specific bacteria in yoghurt fermentation balance intestinal microflora	[40]
Substances excreted by one protozoan to stimulate the growth of another	[41]
Substances that have a beneficial effect on animals by contributing to the balance of the intestinal flora	[42]
Live microbial feed supplements that beneficially affect the host animal by improving the intestinal microbial balance	[43]
Mono- or mixed cultures of live micro-organisms that, when applied in humans, affect the host beneficially by improving the properties of the indigenous microflora	[44]
Live microbial food ingredients that are beneficial to health (efficacy and safety scientifically documented)	[45]
Specific live or inactivated microbial cultures that have documented targets in reducing the risk of human disease or in their nutritional management	The present review

from the use of genetically modified bacteria that exhibit improved or added functional properties. These include probiotics engineered to produce anti-inflammatory cytokines [47]. Although no such new probiotics or starter cultures are yet available in food, basic research is active in the field. Consequently, the probiotic concept is rapidly evolving. The updated probiotic definition (Table 2) includes specific probiotic properties to be exploited in the development of disease-specific prophylactic and therapeutic interventions.

Are there targets for probiotic therapy in allergic disease?

Notwithstanding extensive scientific effort, fundamental issues in infant allergic disease, particularly food hypersensitivity, remain unresolved, the result being inconsistent and inadequately supported practices. Probiotics may provide a new treatment and prevention modality for some allergic disorders, but should not be added to the already lengthy list of fashionable, 'omnipotent' cures without scientific evidence. Current probiotic research has improved our understanding of allergic disease, and such work may uncover certain specific processes as targets for probiotic therapy (Fig. 1 provides a summary of targets of probiotic therapy in prevention and treatment of allergic disease). Future probiotics based on such insights will have more thoroughly clarified mechanisms to control specific physiological processes either in the evolution of allergic disease or in their dietary management. Thus far, active research work in experimental animal models and well-documented clinical studies have provided valuable back-up in the pursuit of this objective.

Antigen processing and gut barrier functions

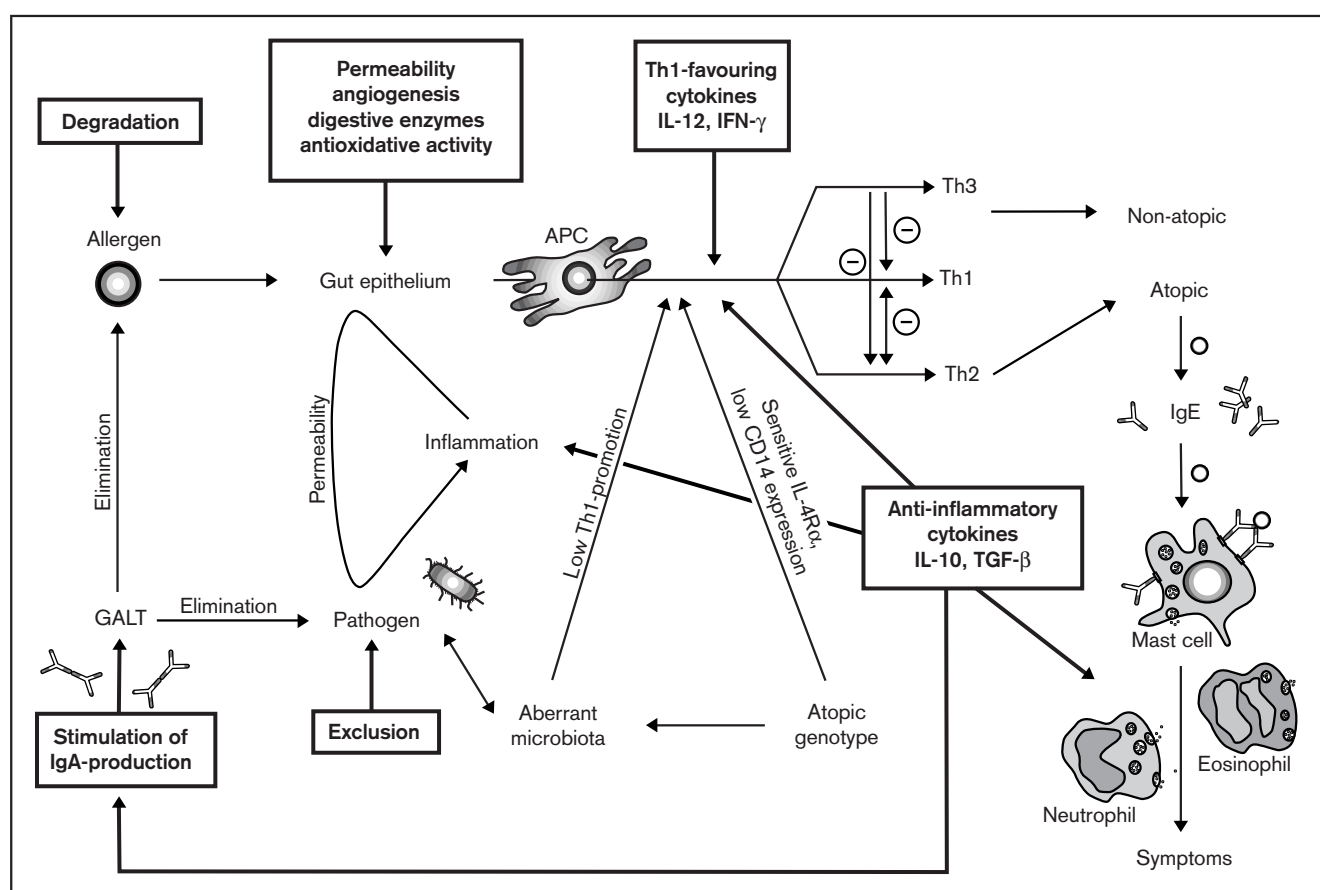
Degradation of antigens is a necessary initial step in controlling inflammatory responsiveness to dietary antigens [48–50]. Most antigens encountered are already processed when they contact the mucosal surface. Proteases of specific strains of the intestinal microbiota contribute to the processing of food antigens in the gut and modify their immunogenicity *in vitro* and *in vivo*. This processing has been linked with the potential to generate peptides with suppressive effects on lympho-

cyte proliferation in healthy persons. Caseins degraded by probiotic bacteria-derived enzymes have further been shown to modulate cytokine production by anti-CD3 antibody-induced peripheral blood mononuclear cells in atopic infants with cow milk allergy [51–53].

The gut microbiota are believed to form an important component of the gut barrier. Such a notion is supported by the demonstration that the capacity to generate immunoglobulin A-producing cells increases progressively in response to intestinal antigenic stimulation, particularly the establishment of the gut microbiota; this also occurs in humans [12,54]. Consequently, the compositional development of the gut microbiota and their impact on the development of atopy and atopic disease has been evaluated. Intestinal microbiota from infants at high risk for atopic diseases were recently analysed by conventional bacterial cultivation and two culture-independent methods [13]: gas–liquid chromatography of bacterial cellular fatty acids and quantitative fluorescence *in-situ* hybridization of bacterial cells. During the neonatal period the bacterial cellular fatty acid profile in faecal samples differed significantly between infants that developed and those that did not develop atopy, and on fluorescence *in-situ* hybridization atopic infants were found to have more clostridia and tended to have fewer bifidobacteria in their stools than did nonatopic infants. The observation was subsequently supported by a binational study that utilized traditional plate culture methods [55], which additionally reported a higher prevalence of lactobacilli and a lower prevalence of enterococci in atopic infants. Those findings indicate that a controlled stepwise colonization process is needed for the maturation of human immunity to a nonatopic mode.

During mucosal dysfunction caused by immaturity, but also secondary to inflammation or hypersensitivity reactions, the normal pattern of antigen handling is impaired; this possibly evokes aberrant immune responses, leading to sensitization [50,56]. Probiotic performance has been shown to manifest in normalization of increased intestinal permeability and improvement in the barrier functions of the intestines [50,57],

Figure 1. Cross-talk between normal gut microbiota and the immune system in the development of allergic disease



The targets (bold boxes and arrows) of probiotic therapy in the prevention or treatment of allergic disease. APC, antigen-presenting cell; GALT, gut-associated lymphoid tissue; IFN, interferon; Ig, immunoglobulin; IL, interleukin; TGF, transforming growth factor; Th, T-helper (cell).

also contributing to the capacity of specific strains to dampen inflammatory responses in the gut.

Anti-inflammatory and antiallergic potential

Initial signals to counter interleukin-4 (and thereby immunoglobulin E and atopy) and interleukin-5 generated eosinophilic inflammation may stem from components of the innate immunity. The Th1-promoting potential of the healthy gastrointestinal microbiota is possibly associated with two structural components of bacteria: the lipopolysaccharide portion of gram-negative bacteria (endotoxin); and a specified CpG motif in bacterial DNA, the latter being 20 times more common in the DNA of bacteria than in higher vertebrates [58,59]. These structures activate immunomodulatory genes via Toll-like receptors that are present, for example, on macrophages, and dendritic and intestinal epithelial cells [60,61•]. Lipopolysaccharide binds to Toll-like receptor 4 on the cell surface, whereas the CpG motif must be taken up into the cell by endocytosis before binding to Toll-like receptor 9 [62,63]. Non-

pathogenic strains of human microbiota have been demonstrated to affect transcription factors (e.g. nuclear factor- κ B), thus modifying gene expression [63,64•,65].

Endotoxin is a potent inducer of interferon- γ and interleukin-12. Higher endotoxin levels have been found in homes where pets are kept. Keeping a dog or a cat, or a whole farm, has been shown to contribute to a lower risk of atopy [66]. Conversely, the homes of allergen-sensitized infants were shown to contain significantly lower concentrations of house dust endotoxin than those of nonsensitized infants [67]. Varying concentrations of endotoxin in house dust may consequently explain the discrepancies between earlier reports on exposure to house dust mite and risk of sensitization and clinical allergy discussed above.

A recent experimental study conducted by Broide *et al.* [68] points to the conclusion that the ability of immunostimulatory bacterial DNA sequences to induce Th1 may not be essential for the antiallergic effect of a

microbial stimulus. The major mechanism by which the gut-associated lymphoid tissue maintains homeostasis is via control of cytokine regulation. The hallmark of an inflammatory response is generation of proinflammatory cytokines, including interleukin-1, interleukin-2, tumour necrosis factor- α and interferon- γ . Even in health, intestinal T cells express activation markers and spontaneously secrete proinflammatory cytokines – phenomena that characterize physiological inflammation [69] – without any adverse health outcome. Healthy homeostasis in the gut milieu may thus be achieved by optimizing the balance of proinflammatory and anti-inflammatory cytokines and other mediators.

Oral tolerance has been defined as the induction of peripheral unresponsiveness as a result of oral administration of soluble protein antigen [4•]. Tolerance may be achieved through several distinct mechanisms (for review [5•]), including generation of a unique subset of T cells designated Th3 cells. These produce predominantly transforming growth factor (TGF)- β , and are believed to be pivotal in the active suppression that leads to tolerance following exposure to low doses of antigen. After 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 on reactivation, thus mediating tolerance systemically [70,71]. With regard to the active immunosuppressive elements involved, the definition of oral tolerance has been extended to embrace '... any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a noninjurious class of immune response' [5•].

Specific strains of the gut microbiota have been shown to contribute to a Th cell population that is amenable to oral tolerance induction [72], and to counter allergy by generation of anti-inflammatory interleukin-10 and TGF- β [5•,7–9]. Normalization of the properties of an unbalanced indigenous microflora by specific strains of the healthy gut microbiota – the rationale underlying probiotic therapy – may thus be seen more broadly as restoration of the immunological milieu in the gut, and not only a colonization resistance-type effect.

As discussed above, in healthy individuals tolerogenic responses are elicited toward the indigenous intestinal microbiota, and a disease-free state in the gut is maintained. Interleukin-10 is produced in response to microbial stimuli in experimental animals, because mice with defective interleukin-10 production infected with *Helicobacter hepaticus* develop Th1-type intestinal inflammation, whereas normal mice produce interleukin-10 and remain healthy [73]. However, the interplay between the gut microbiota and the intestinal immune system is more complex, as demonstrated by the

observation that interleukin-10-deficient mice have reduced levels of resident lactobacilli during the neonatal period; normalizing the number of these bacteria in the colon prevents development of intestinal inflammation [57]. Furthermore, interleukin-10 deficient mice develop an intestinal permeability defect, preceding the development of mucosal inflammation due to a dysregulated Th1-type immune response to normal enteric microbes [74]. Recently, diminished mucosal production of TGF- β with concomitant changes in gut microbiota composition, in particular colonization with *Bacteroides* spp., was detected in a murine model of inflammatory bowel disease [75].

Anti-inflammatory interleukin-10 responses are elicited in response to luminal microbiota in humans as well. In patients with pouchitis (a nonspecific inflammation of the ileal reservoir), reduced numbers of lactobacilli and bifidobacteria, and an increased number of *Clostridium perfringens* in the reservoir microbiota have been observed [76]. Administration of a probiotic mixture resulted in a significant increase in the amount of interleukin-10 in biopsies [77••].

The connection between control of the inflammatory response and the antiallergic potential of specific probiotics is supported by an improvement in the clinical course of atopic eczema in infants with cow milk allergy who were given a probiotic-supplemented elimination diet. In those infants, markers of systemic [8] and intestinal [78] allergic inflammation were significantly decreased, parallel with enhanced production of anti-inflammatory cytokines.

The preventive potential of probiotics in atopic disease has recently been demonstrated in a double-blind, placebo-controlled study [79••]. Probiotics administered prenatally and postnatally for 6 months to children at high risk for atopic disease reduced, at the age of 2 years, the prevalence of atopic eczema to half (23%) as compared with that in infants receiving placebo (46%). The frequency of atopic eczema in the placebo group of that study was within the range reported in another cohort study [80]. Probiotic supplementation in lactating mothers promoted increased levels of TGF- β in breast milk, indicating one mechanism by which the risk for infant atopic eczema may be reduced [81]. The observation is in accord with a previous report that linked a low concentration of this anti-inflammatory cytokine with early atopic eczema in the infant [82]. However, probiotic supplementation had no preventive potential in cow milk allergy. Along the lines of research on respiratory allergy demonstrating that atopic sensitization and the development of asthma may be independent processes [16••], the development of atopy, atopic disease and food allergy may also be regulated by

separate mechanisms, or by a complex cascade of inter-related immunological mechanisms.

Conclusion

The development of probiotic functional foods for the allergic population requires new criteria for strains that are appropriate to specific indications. The epidemiological evidence indicating that early exposure to microbes is associated with a reduced risk for developing atopy and atopic disease is ample, and the epidemic of atopic disease in the industrialized world has been attributed to reduced microbial contact. Probiotic supplementation aims to provide safe but sufficient bacterial exposure in order to avert deviant immune responsiveness and immunoinflammatory disease.

However, our understanding of the cascade of events that are involved in exposure, sensitization and hypersensitivity is by no means satisfactory. A more rigorous understanding of the complex nature of atopy and atopic disease is needed, because it is likely that distinct aetiological factors and pathogenetic mechanisms underlie the heterogeneous manifestations of the disorders. It would thus appear simplistic and erroneous to assume that one mode of prevention or treatment would suffice to counter the plethora of allergic disease. Indeed, experience gained in clinical trials that assessed the effects of probiotics in prevention and treatment of atopic disease is consistent with such a concept; bifidobacteria appear to be effective in reversing the development of atopy, whereas lactobacilli have proved beneficial in prevention of atopic disease but ineffective in protection against cow milk allergy, although both bifidobacteria and lactobacilli appear to be effective in treating the disorder.

In conclusion, rigorous scientific effort is required to elucidate the characteristics of distinct probiotic strains in different manifestations of atopic and food hypersensitivity disorders, because there appears to be no universal solution to these complex phenomena.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- 1 Weiner HL, van Rees EP. Mucosal tolerance. *Immunol Lett* 1999; 69:3–4.
- 2 Downs SH, Marks GB, Sporik R, *et al.* Continued increase in the prevalence of asthma and atopy. *Arch Dis Child* 2001; 84:20–23.
- 3 Pene J, Rousset F, Briere F, *et al.* IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. *J Immunol* 1988; 141:1218–1224.
- 4 Nagler-Anderson C. Tolerance and immunity in the intestinal system. *Crit Rev Immunol* 2000; 20:103–120.

This is an excellent review that ties together current views on oral tolerance.

- 5 Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect* 2001; 3:947–954. A comprehensive review of the current view of mechanisms of oral tolerance is presented.
 - 6 Sanfilippo L, Li CK, Seth R, *et al.* *Bacteroides fragilis* enterotoxin induces the expression of IL-8 and transforming growth factor-beta (TGF-beta) by human colonic epithelial cells. *Clin Exp Immunol* 2000; 119:456–463.
 - 7 Pessi T, Sütas Y, Hurme M, *et al.* Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy* 2000; 30:1804–1808.
 - 8 Isolauri E, Arvola T, Sütas Y, *et al.* Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000; 30:1605–1610.
 - 9 von der Weid T, Bulliard C, Schiffrin E. Induction by a lactic acid bacterium of a population of CD4⁺ T cells with low proliferative capacity that produce transforming growth factor β and interleukin-10. *Clin Diagn Lab Immunol* 2001; 8:695–701.
 - 10 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–1260.
 - 11 Kilpi T, Kero J, Jokinen J, *et al.* Common respiratory infections early in life may reduce the risk of atopic dermatitis. *Clin Infect Dis* 2002; 34:620–626.
 - 12 Grönlund MM, Arvilommi H, Kero P, *et al.* Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. *Arch Dis Child* 2000; 83:F186–F192.
 - 13 Kalliomäki M, Kirjavainen P, Eerola E, *et al.* Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107:129–134.
 - 14 Warner JA, Warner JO. Early life events in allergic sensitisation. *Br Med Bull* 2000; 56:883–893.
- This review outlines prenatal and postnatal factors that possibly explain the recent increase in the prevalence of allergic disease.
- 15 Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house-dust mite allergen (Der p 1) and the development of asthma in childhood: a prospective study. *N Engl J Med* 1990; 323:502–507.
 - 16 Lau S, Illi S, Sommerfeld C, *et al.* Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. *Lancet* 2000; 356:1392–1397.
- This is a clinically important article that challenges current opinions on sensitization and atopic diseases.
- 17 Platts-Mills T, Vaughan J, Squillace S, *et al.* Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357:752–756.
 - 18 van der Biggelaar AHJ, van Ree R, Rodrigues LC, *et al.* Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000; 356:1723–1727.
 - 19 Piccinni MP, Beloni L, Livi C, *et al.* Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998; 4:1020–1024.
 - 20 Ribeiro-do-Couto LM, Boeijs LCM, Kroon JS, *et al.* High IL-13 production by human neonatal T cells: neonate immune system regulator? *Eur J Immunol* 2001; 31:3394–3402.
 - 21 Williams TJ, Jones CA, Miles EA, *et al.* Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. *J Allergy Clin Immunol* 2000; 105:951–959.
 - 22 Prescott SL, Macaubas C, Smallacombe T, *et al.* Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
 - 23 Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol* 1996; 4:430–435.
 - 24 Benno Y, Mitsuoka T. Development of intestinal microflora in humans and animals. *Bifidobacteria Microbi* 1986; 5:13–25.
 - 25 Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, *et al.* Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000; 30:61–67.
 - 26 Kirjavainen PV, Ouwehand AC, Isolauri E, Salminen SJ. The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiol Lett* 1998; 167:185–189.
 - 27 Ouwehand AC, Salminen S, Tolkkio S, *et al.* Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria. *Clin Diagn Lab Immunol* 2002; 9:184–186.

- 28 Kirjavainen PV, Tuomola EM, Crittenden RG, *et al.* In vitro adhesion and platelet aggregation of bacteremia-associated lactobacilli. *Infect Immun* 1999; 67:2653–2655.
- 29 Mao Y, Nobaek S, Kasravi B, *et al.* Effects of *Lactobacillus* strains and oat bran on methotrexate-induced enterocolitis in rats. *Gastroenterology* 1996; 111:334–344.
- 30 Tuomola E, Crittenden R, Playne M, *et al.* Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr* 2001; 73(suppl):393S–398S.
- 31 Lee YK, Lim CY, Teng WL, *et al.* Qualitative approach in the study of adhesion of lactic acid bacteria on intestinal cells and their competition with enterobacteria. *Appl Environ Microbiol* 2000; 66:3692–3697.
- 32 Sepp E, Mikelsaar M, Salminen S. Effect of *Lactobacillus casei* strain GG administration on the gastrointestinal microbiota of newborns. *Microbiol Ecol Health Dis* 1993; 6:309–314.
- 33 Isolauri E, Kaila M, Mykkänen H, *et al.* Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci* 1994; 39:2595–2600.
- 34 Alander M, Satokari R, Korpela R, *et al.* Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. *Appl Environ Microbiol* 1999; 65:351–354.
- 35 van der Heijden, Bianchi ATJ, Dol M, *et al.* Manipulation of intestinal immune responses against ovalbumin by cholera toxin and its B subunit in mice. *Immunology* 1991; 72:89–93.
- 36 Isolauri E, Sütas Y, Kankaanpää P, *et al.* Probiotics: effects on immunity. *Am J Clin Nutr* 2001; 73(Suppl 2):S444–S450.
- 37 Duchmann R, Kaiser I, Hermann E, *et al.* Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* 1995; 102:448–455.
- 38 Majamaa H, Isolauri E, Saxelin M, *et al.* Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* 1995; 20:333–339.
- 39 Simakachorn N, Pichaiat V, Rithipornpaisarn P, *et al.* Clinical evaluation of the addition of lyophilized, heat-killed *Lactobacillus acidophilus* LB to oral rehydration therapy in the treatment of acute diarrhea in children. *J Pediatr Gastroenterol Nutr* 2000; 30:68–72.
- 40 Metchnikoff E. Prolongation of Life. London: William Heinemann; 1907.
- 41 Lilly D, Stillwell E. Probiotics: growth promoting factors produced by microorganisms. *Science* 1965; 147:747–748.
- 42 Parker RB. Probiotics: the other half of the antibiotics story. *Anim Nutr Health* 1974; 29:4–8.
- 43 Fuller R. Probiotic in man and animals. *Appl Bacteriol* 1989; 66:365–378.
- 44 Huis in't Veld J, Havenaar R. Probiotics and health in man and animal. *J Chem Technol Biotechnol* 1991; 51:562–567.
- 45 Salminen S, Bouley C, Boutron-Ruault MC, *et al.* Gastrointestinal physiology and function: targets for functional food development. *Br J Nutr* 1998; 80(suppl):147–171.
- 46 Klaenhammer TR, Kullen MJ. Selection and design of probiotics. *Int J Food Microbiol* 1999; 50:45–57.
- 47 Steidler L, Hans W, Schotte L, *et al.* Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 2000; 289:1352–1355.
- 48 Strober W, Kelsall B, Marth T. Oral tolerance. *J Clin Immunol* 1998; 18:1–30.
- 49 Barone KS, Reilly MR, Flanagan MP, Michael JG. Abrogation of oral tolerance by feeding encapsulated antigen. *Cell Immunol* 2000; 199:65–72.
- 50 Isolauri E, Majamaa H, Arvola T, *et al.* *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 1993; 105:1643–1650.
- 51 Sütas Y, Soppi E, Korhonen H, *et al.* Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. *J Allergy Clin Immunol* 1996; 98:216–224.
- 52 Sütas Y, Hurme M, Isolauri E. Downregulation of antiCD3 antibody-induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. *Scand J Immunol* 1996; 43:687–689.
- 53 Pessi T, Sütas Y, Saxelin M, *et al.* Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microb* 1999; 65:4725–4728.
- 54 Cebra JJ. Influences of microbiota on intestinal immune system development. *Am J Clin Nutr* 1999; 69(suppl):1046–1051.
- 55 Björkstén B, Naaber P, Sepp E, *et al.* The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999; 29:342–346.
- 56 Fargeas MJ, Theodorou V, More J, *et al.* Boosted systemic immune and local responsiveness after intestinal inflammation in orally sensitized guinea pigs. *Gastroenterology* 1995; 109:53–62.
- 57 Madsen KL, Doyle JS, Jewell LD, *et al.* *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; 116:1246–1249.
- 58 Hartmann G, Weiner GJ, Krieg AM. CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. *Proc Natl Acad Sci USA* 1999; 96:9305–9319.
- 59 Kranzer K, Bauer M, Lipford GB, *et al.* CpG-oligodeoxynucleotides enhance T-cell receptor-triggered interferon-gamma production and up-regulation of CD69 via induction of antigen-presenting cell-derived interferon type I and interleukin-12. *Immunology* 2000; 99:170–178.
- 60 Cario E, Rosenberg IM, Brandwein SL, *et al.* Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000; 164:966–972.
- 61 Hemmi H, Takeuchi O, Kawai T, *et al.* A toll-like receptor recognizes bacterial DNA. *Nature* 2000; 408:740–745.
- This study shows that the immune system has evolved a specific receptor that distinguishes bacterial DNA from self-DNA.
- 62 Modlin RL. A Toll for DNA vaccines. *Nature* 2000; 408:659–660.
- 63 Xavier RJ, Podolsky DK. How to get along: friendly microbes in a hostile world. *Science* 2000; 289:1483–1484.
- 64 Neish AS, Gewirtz AT, Zeng H, *et al.* Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000; 289:1560–1563.
- This study demonstrates an intercellular pathway by which nonvirulent enteric organisms modulate gene expression of intestinal epithelial cells.
- 65 Miettinen M, Lehtonen A, Julkunen I, Matikainen S. Lactobacilli and streptococci activate NF-kappa B and STAT signalling pathways in human macrophages. *J Immunol* 2000; 164:3733–3740.
- 66 Heinrich J, Gehring U, Douwes J, *et al.* Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy* 2001; 31:1839–1845.
- 67 Gereda JE, Leung DYM, Thatayatikom A, *et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355:1680–1683.
- 68 Broide DH, Stachnick G, Castaneda D, *et al.* Immunostimulatory DNA mediates inhibition of eosinophilic inflammation and airway hyperreactivity independent of natural killer cells in vivo. *J Allergy Clin Immunol* 2001; 108:759–763.
- 69 O'Farrelly C. Just how inflamed is the normal gut? *Gut* 1998; 42:603–606.
- 70 Fukaura H, Kent SC, Pietrusewitz MJ, *et al.* Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor- β -1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J Clin Invest* 1996; 98:70–77.
- 71 Chen Y, Inobe J, Kuchroo VK, *et al.* Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc Natl Acad Sci USA* 1996; 93:388–391.
- 72 Sudo N, Sawamura S, Tanaka K, *et al.* The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–1745.
- 73 Kullberg MC, Ward JM, Gorelick PL, *et al.* *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10) deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun* 1998; 66:5157–5166.
- 74 Madsen KL, Malfair D, Gray D, *et al.* Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis* 1999; 5:262–270.
- 75 Hata K, Sato AH, Araki Y, *et al.* Sequential changes in luminal microflora and mucosal cytokine expression during developing of colitis in HLA-B27// β 2-microglobulin transgenic rats. *Scand J Gastroenterol* 2001; 36:1185–1192.
- 76 Ruseler-van Embden JGH, Schouren WR, van Lieshout LMC. Pouchitis: result of microbial imbalance? *Gut* 1994; 35:658–664.
- 77 Ulisse S, Gionchetti P, D'Alò, *et al.* Expression of cytokine, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am J Gastroenterol* 2001; 96:2691–2699.
- This important study sheds light on the mechanisms of probiotic anti-inflammatory effects in the human intestine. The results obtained from biopsies clearly indicate the enhancement of interleukin-10 after probiotic therapy *in situ*.
- 78 Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997; 99:179–186.

- 79** Kalliomäki M, Salminen S, Kero P, *et al.* Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076–1079.

This well-controlled study provides the first clinical evidence of probiotic efficacy in the prevention of atopic disease. If the preventive potential extends beyond the first atopic manifestation (i.e. atopic eczema) to asthma, or is confirmed in larger cohort studies in unselected populations, then the observation strongly supports the hygiene hypothesis of allergy.

- 80** Bergmann RL, Bergmann KE, Lau-Schadensdorf S, *et al.* Atopic diseases in infancy. The German multicenter atopy study (MAS-90). *Pediatr Allergy Immunol* 1994; 5(Suppl 6):19–25.

- 81** Rautava S, Kalliomäki M, Isolauri E. Probiotics during pregnancy and breastfeeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; 109:119–121.

- 82** Kalliomäki M, Ouwehand A, Arvilommi H, *et al.* Transforming growth factor beta in breastmilk: a potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 1999; 104:1251–1257.