Eur J Nutr 41 [Suppl 1] (2002) : I/32 – I/37
DOI 10.1007/s00394-002-1105-4

Arthur Ouwehand
Erika Isolauri
Seppo Salminen

The role of the intestinal microflora for the development of the immune system in early childhood

Summary The intestinal tract performs many different functions; in addition to absorption and digestion it is also the body's largest organ of host defence. Part of the intestinal mucosal barrier function is formed by a common mucosal immune system which provides communication between the different mucosal surfaces of the body. The intestine also contains a microbial ecosystem with a large body of microbes, 1–1$^1/_2$ kg in an adult. The microbes and their activity have a major impact on the development and functioning of the intestinal immune system and *vice versa*. This mutual influence also affects the host beyond the intestine.

The intestinal colonisation with a balanced microflora is of main importance for the correct development of the immune system. The importance of the intestinal microflora is most clearly seen in germfree animals, but also diseases like atopy are associated with disturbances in the intestinal microflora. This often manifests itself in a low number of bifidobacteria. The use of probiotics or prebiotics to correct this imbalance and modulate the immune activity has received increasing scientific documentation. The precise mechanisms behind these immune modulatory activities are not well understood and require further investigation.

Key words gastro-intestinal tract – immune system – microflora – probiotics – prebiotics

Arthur Ouwehand (✉) · Seppo Salminen
Department of Biochemistry
and Food Chemistry
University of Turku
20014 Turku, Finland
Tel.: +3 58-2/3 33-68 94
Fax: +3 58-2/3 33-68 84
E-Mail: arthur.ouwehand@utu.fi

Erika Isolauri
Department of Paediatrics
Turku University Central Hospital
20520 Turku, Finland

Introduction

The human intestine performs many diverse functions of which digestion of food and absorption of nutrients are only the beginning. The intestine is the largest immune organ of the body. It produces more antibodies than any other part of the body, 40 mg/kg body weight/day, and contains 80 % of all antibody-producing cells [1]. The intestine is also host to an estimated 10^{14} microbes representing some 400 to 500 different species [2]. In the faeces and proximal part of the colon microbes are present at a density of around 10^{11} organisms per gram wet weight, which represents at least 30 % of the wet faecal mass. The immune system regulates the colonisation of the intestinal microflora by interfering with its ability to bind to the mucosa, while parts of bacterial cells and metabolites modulate the immune systems activity. The intestinal microflora is also an important part of the intestinal mucosal barrier. It is therefore not surprising that the intestinal microflora and the intestinal immune system influence each other and together have an influence on the host, also beyond the intestine.

Intestinal microflora

Diversity

The gastro-intestinal (GI) tract provides a wide range of environments, varying in redox potential, pH, flow rate, nutrient availability, etc. Due to these differences in environment, the composition of the microflora varies throughout the GI tract.

The oral cavity provides many different habitats and

is therefore colonised by a wide range of organisms. During the first few months of life, only mucosal surfaces and the tongue exist as colonisation sites. Upon eruption of teeth, hard non-shedding surfaces appear and also gingival crevices are available for colonisation. The oral cavity maybe colonised by 500 different bacterial species [3]. Streptococci, *Veillonella*, *Neiseria* and *Actinomyces* are the most common genera depending on the habitat. Microorganisms can be present in high levels in saliva, 10^8 cfu/ml, and in dental plaque, up to 10^{11} cfu/g [4].

The oesophagus of humans does not appear to have its own normal residential microflora. The bacteria present usually originate from the oral cavity, upper respiratory tract or from swallowed food [5].

The stomach is characterised by its low pH. In healthy adults the resting pH may be as low as 1 or 2 [3, 6], though in new born infants it may be close to neutral. The low pH restricts the level of colonisation, microflora levels reach at most 10^4 cfu/ml gastric juice [3]. As within the oesophagus, most of the organisms originate from food and the oropharynx [5]. The microorganisms found are usually aciduric species; lactobacilli, streptococci and *Candida albicans*. In addition, a high percentage of people are colonised by *Helicobacter pylori*. Its natural habitat appears to be the mucus-covered non-acid-secreting epithelium of the *antrum*. Many factors combine to induce *H. pylori* cells to change to become pathogenic after many years of being a commensal [7].

Also the duodenum has a sparse microflora (10^4–10^5 cfu/ml), due to the low pH of the digesta released from the stomach, the secretion of pancreatic juice and bile. Also the swift flow of the digesta reduce the chance for colonisation [3]. The composition of the microflora resembles that of the stomach.

In the jejunum, colonisation levels of 10^5–10^7 cfu/ml have been observed. The normal microflora consists of streptococci, lactobacilli, *Haemophilus*, *Veillonella*, *Bacteroides*, *Corynebacterium* and *Actinomyces* [8].

Due to the slower passage of the digesta in the ileum, colonisation occurs, with bacterial populations of 10^7–10^8 cfu/ml [3, 8]. The composition of the microflora resembles that of the colon with facultative anaerobic *Enterobacteriaceae* and obligate anaerobes; *Bacteroides*, *Veillonella*, *Clostridium*, Lactobacilli and enterococci are also present [3].

The colon contains the highest density and diversity of microorganisms in the body. About 10^{10}–10^{11} microorganisms are present per gram colonic contents. More than 40 genera have been identified in faeces using culturing techniques. From faeces 113 species have been isolated; statistical analysis of this suggests the presence of at least 400–500 different species in faeces [2]. This is likely to be an under estimation of the microbial diversity since not all organisms could and can be cultured. In the lumen, anaerobes out number aerobes by a factor of 100–1000. However, on the mucosa this is only a factor of 10, due to leakage of oxygen from tissue. The major genera in the colon are *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Bacillus*, *Peptostreptococcus*, *Fusobacterium* and *Ruminococcus* [3].

Development in health

Upon birth, the intestine is sterile, but it soon becomes colonised by micro-organisms from the environment and the mother's birth canal. Bacteria start to appear in the faeces within hours after birth, and their numbers increase progressively during the first week of life. The first microorganisms to be isolated from the faeces of new born infants are usually facultative anaerobic organisms; *E. coli* and other enterobacteria, staphylococci and streptococci. These organisms change the initially aerobic GI tract to an anaerobic environment which is suitable for colonisation by obligate anaerobic organisms. Once the intestine has become anaerobic bifidobacteria, clostridia and *Bacteroides* spp. appear in the faeces.

The method of birth has been observed to have a pronounced influence on the colonisation of the infant's intestine. The faecal colonisation of infants born by caesarean section has been found to be delayed compared to vaginally born infants. Also the composition of the faecal microflora was different after caesarean section [9, 10].

The effect of the type of feeding, breast or formula, on the colonisation is currently much debated. The traditional view holds that breast fed infants are colonised mainly by bifidobacteria, while formula fed infants have a mixed microflora not particularly high in bifidobacteria. However, in recent reports on the composition of the microflora of infants, such a difference could often not be observed. This has been attributed to an improved composition of infant formulae, current highly hygienic obstetric practices and improved bacteriological methodologies [11]. Upon the introduction of solid foods, the composition of the intestinal microflora gradually becomes more complex. At two years of age, the microflora resembles that of an adult [10].

The environment in which people live and the food they consume have also been observed to influence the composition of the intestinal microflora [12]. How these differences in microflora composition may affect the health status is not known.

Composition during disease

Disease can have a dramatic influence on the composition of the normal intestinal microflora. This is very clear in the case of infectious diarrhoea where a change

European Journal of Nutrition, Vol. 41, Supplement 1 (2002)

in the composition of the microflora, the presence of a pathogenic microorganism, causes disease. However, other immune system related diseases also involve changes in the composition of the normal microflora. In some cases this change in composition is caused by the disease, but in other cases it may be the cause of disease.

Infants with allergy have been observed to have reduced faecal colonisation of *Bifidobacterium* and *Lactobacillus* sp. [13], while atopics have a reduced ratio of bifidobacteria to clostridia [14]. Also the composition of the *Bifidobacterium* flora has been observed to be different between atopic and healthy infants. Atopic infants are mainly colonised by *B. adolescentis* while healthy infants have a typical *Bifidobacterium* flora with *B. bifidum*, *B. breve* and *B. infantis* [15].

During active periods of Crohn's disease and ulcerative colitis reduced levels of obligate and facultative anaerobes have been observed in the faeces of patients. Especially bifidobacteria and lactobacilli were reduced as compared to patients with inactive disease and healthy controls [16, 17]. Thus, in general it appears that *Bifidobacterium* levels are reduced during disease [18].

Intestinal immune system

General

The intestinal immune system is the major immune organ of the body. The principle antibody in the intestine is immunoglobulin (Ig) A, which usually is present in a dimeric form. IgA is well suited for its function in the intestine. It is relatively resistant against proteolysis, in particular IgA2, which is important considering the environment in the intestine. In contrast to IgG, the major systemic immunoglobulin, IgA does not elicit an inflammatory reaction. IgA can thus bind antigens and exclude them from the intestinal mucosa without causing inflammation [1].

The predominant site of antigen sampling in the intestine are the Peyer's patches. These parts of the gut-associated lymphoid tissue (GALT) are covered with specialised membrane (M) cells. M cells specifically sample the contents of the gut and transfer antigens to antigen presenting cells which present the antigen to B and T-cells. Naive T-cells can develop into T helper 1 (Th1) or T helper 2 (Th2) cells. Th1 cells will direct the differentiation of B-cells to IgA producing cells while Th2 cells direct B-cell differentiation towards IgE producing cells. Interestingly, M cells have a preference for the uptake of IgA-complexed antigens thus further stimulating the production of IgA.

The major functions of the intestinal immune system are exclusion of antigens and to provide tolerance to antigens, since all food components and the normal intestinal microflora are in principle antigens. This oral tolerance is provided through suppression of Th1 cells by interleukin (IL)-4, IL-10 and transforming growth factor-β when exposed to low concentrations of antigens. High doses cause clonal anergy; T cells are in a state of cellular unresponsiveness which makes them incapable of secreting IL-2 or of proliferating [19].

Maturation

At birth, the intestinal immune system is immature with very few IgA producing B cells present in peripheral blood of new borns. Consequently, the presence of IgA is low and the major initial antibody type is IgM. After 1–2 months, IgA becomes the dominant antibody in the intestine and reaches adult levels at the end of the first year of life. However, under heavily exposed conditions these levels can be reached within a few weeks, indicating the importance of microbial exposure. The number of Peyer's patches is initially low and increases upon maturation of the immune system [20]. Also other environmental factors, like pollution, have been suggested to affect the development of the immune system [21].

Microbes and the intestinal immune system

The normal microflora

The most dramatic example of the importance of the intestinal microflora for the development of the immune system comes from studies with germfree animals. In the absence of microbes, a mammal has a reduced number of Peyer's patches and less then one tenth of the number of IgA producing B cells compared to a conventional animal [20]. Upon exposure to a normal microflora, ex-germfree animals develop a immune system very much like conventional animals. This indicates the importance of the intestinal microflora for the development of the immune system.

As mentioned earlier, infants are also born germfree. The acquisition of the normal microflora therefore plays an important role in the development of the immune system and the presence of an unbalanced microflora is associated with disease.

Hygiene

Over the past few decades, an increase in the prevalence of allergy has been observed in industrialised countries [22]. It has been hypothesised that this increase relates to a reduced exposure to microbial antigens as a consequence of increased hygiene and vaccination. This causes a reduced stimulation of the intestinal immune system with bacterial antigens. Bacterial antigens stim-

ulate the production of Th1 cytokines IL-6, IL-12, IL-18 and interferon (IFN)-γ, directing the immune system away from a Th2-mediated immune response (Fig. 1).

It is obvious that for public health safety reasons it is undesirable to reduce the level of hygiene or abandon vaccination. Avoiding all possible allergens is not feasible; thus other approaches need to be considered.

Probiotics

The options for increasing the microbial exposure without increasing the health risk are the use of prebiotics and/or probiotics.

Probiotics have been defined in many different ways. One of the current definitions is *microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being of the host* [23]. Examples of probiotics are *Lactobacillus rhamnosus* GG, *L. casei* Shirota, *L. johnsonii* La1 and *Bifidobacterium lactis* Bb12. Many health effects have been reported for probiotics and some, like immune modulation are well established [24]. Several mechanisms of how probiotics can modulate the immune system have been proposed.

By modulating the composition and/or activity of the intestinal microflora the exposure to dietary antigens can be changed. Selected lactobacilli and bifidobacteria have been shown to be able to enhance the production of IgA [25, 26]. Reduction in the production of IgE has also been observed in mice [27].

Different parts of the probiotic cell have been observed to be able to modulate the immune system. Cell wall material, peptidoglycan [28] and teichoic acids [29] but also cytoplasmic contents [30, 31] have been suggested to elicit immune reactions. The latter could relate to specific bacterial DNA sequences that have been observed to affect the immune system [32]. However, the precise mechanisms behind the immune modulation by probiotics is still largely unknown, although adhesion to the intestinal mucosa is thought to be of importance [29]. Close contact of the probiotics with the intestinal mucosa and possibly some benign translocation may lead to an enhanced interaction of the probiotics and the intestinal immune system. This interaction will stimulate naive T cells to differentiate to Th1 cells under the influence of IFN-γ, IL-2 and IL-12, while the development of Th2 cells is down regulated under the influence of IL-4. The result of this shift in T-cell differentiation from Th2 to Th1 is a reduced production of IgE and an increased secretion of IgA [33], which leads to a reduced allergic response (Fig. 1).

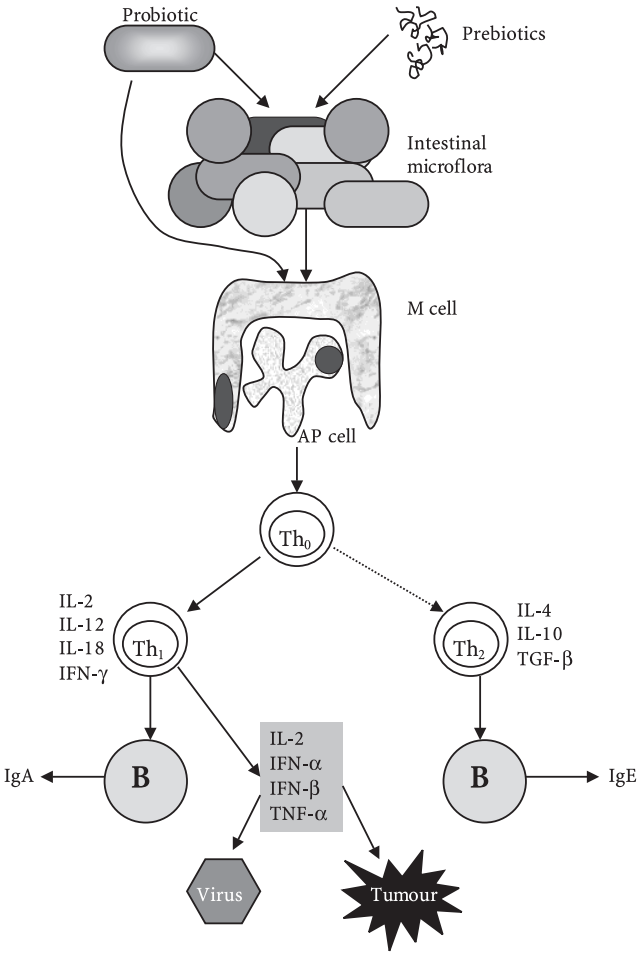

Fig. 1 Probiotics and prebiotics can directly or indirectly influence the intestinal immune system, through active uptake by M cells and transfer to antigen presenting cells. This may shift the Th1/Th2 balance in favour of the former, leading to an increase in IgA producing B-cells and a concomitant reduction in IgE producing cells. This, in turn, leads to a reduced allergic response

Prebiotics

Prebiotics are *nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health* [34]. Examples of prebiotics are fructo-oligosaccharides, galacto-oligosaccharides and lactulose. In most cases prebiotics will stimulate the growth and activity of bifidobacteria and lactobacilli. This is a desirable effect since some diseases, as described previously, are associated with reduced numbers of bifidobacteria. The health effects of prebiotics are less well established than those for probiotics and their effect on the immune system is still unknown [35]. However, because prebiotics influence the composition and activity of the normal microflora and the microflora is known to have a major effect on the immune system, it can be anticipated

European Journal of Nutrition, Vol. 41, Supplement 1 (2002)

that also prebiotics indirectly modulate the immune system.

Developments for the future

The use of probiotics and prebiotics to modulate the composition and/or activity of the intestinal microflora is likely to be more widely applied. Prebiotics have a long history of safe use and only mild side effects as diarrhoea and flatulence are associated with overdosing. It is well established that many selected prebiotics have the ability to influence the intestinal microflora [36]. Because of this, it could be expected that prebiotics would also indirectly affect the immune system. Unfortunately this has, until now, received little attention [35].

Selected probiotics have been shown to function as adjuvants for oral immunisation [37], the properties of prebiotics in this respect are not known and deserve further investigation.

Probiotic lactic acid bacteria have no known side effects and their safety record is excellent [38]. This makes them good candidates for influencing the immune system in adults, children and infants. Alternative applications of probiotics should be considered in the future. The use of non-viable probiotics would have great advantages in terms of storage, handling and shelf life. Non-viable probiotics have been observed to produce certain health effects, among others immunological effects [39]. Also the use of parts of probiotic cells should be considered; both cytoplasmic and cell wall fractions have been shown to modulate the immune system *in vitro*. In relation to this, the feeding of relatively large amounts of probiotic bacteria also implicates the feeding of large amounts of nucleic acids, since bacteria can consist of up to 50% nucleic acids by dry weight. Nucleotide fortified infant formula has been shown to result in higher specific antibody titres compared to unfortified formula [40]. Even the trace element content of probiotic bacteria could provide a positive influence on the host's immune system [41]. These and other aspects of probiotics deserve further investigation in order to understand the mechanisms behind the immune modulatory effects of probiotics.

Conclusion

It has been observed that the intestinal microflora has a major influence on the development and functioning of the immune system. Modulation of the intestinal microflora with probiotics or prebiotics may provide a means for improving the immune status in infants, adults and elderly. Selected probiotics have been shown to modulate the immune response. For prebiotics this has not been sufficiently investigated. However, it could be anticipated that some immune effects may be observed and should therefore be investigated in more detail. Both approaches, pre- and probiotic administration, could provide safe means of improving the immunological development and functioning.

References

1. Helgeland L, Brandtzaeg P (1999) Development and function of intestinal B and T cells. Microb Ecol Health Dis 12 (Suppl 2):110–127
2. Moore WEC, Holdeman LV (1974) Human faecal flora: the normal flora of 20 Japanese-Hawaiians. Appl Microbiol 27:961–979
3. Tannock G (1995) Normal microflora: an introduction to microbes inhabiting the human body. Chapman & Hall, London, pp 14–21
4. Gibbons RJ, van Houte J (1975) Bacterial adherence in oral microbial ecology. Ann Rev Microbiol 29:19–44
5. Sjöstedt S (1989) The upper gastrointestinal microflora in relation to gastric disease and gastric surgery. Acta Chir Scand (Suppl 551):1–57
6. Sanford PA (1992) Digestive System Physiology. Edward Arnold, London, pp 46–72
7. Lee A, Hazell S (1993) Pathogenicity of *Helicobacter pylori*: a perspective. Infect Immun 61:1601–1610
8. Justesen T, Nielsen OH, Hjelt K, Krasilnikoff PA (1984) Normal cultivable microflora in upper jejunal fluid in children without gastrointestinal disorders. J Pediatr Gastroenterol Nutr 3: 683–686
9. Grönlund M-M, Lehtonen O-P, Eerola E, Kero P (1999) Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarian section. J Pediatr Gastroenterol Nutr 28:19–25
10. Kleesen B, Bezirtzoglou E, Mättö J (2000) Culture-based knowledge on biodiversity, development and stability of human gastrointestinal microflora. Microb Ecol Health Dis (Suppl 2):53–63
11. Heavy PM, Rowland IR (1999) The gut microflora of the developing infant: microbiology and metabolism. Microb Ecol Health Dis 11:75–83
12. Benno Y, Endo K, Mizutani T, Namba Y, Komori T, Mitsuoka T (1989) Comparison of faecal microflora of elderly persons in rural and urban areas of Japan. Appl Environ Microbiol 55:1100–1105
13. Björkstén B, Naaber P, Sepp E, Mikelsaar M (1999) The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy 29: 342–346
14. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants developing or not developing atopy. J Allergy Clin Immunol 107:129–134
15. He F, Ouwehand AC, Isolauri E, Hashimoto H, Benno Y, Salminen S (2001) Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. FEMS Immunol Med Microbiol 30:43–47
16. Giaffer MH, Holdsworth CD, Duerden BI (1991) The assessment of faecal flora in patients with inflammatory bowel disease by a simplified bacteriological technique. J Med Microbiol 35:238–243

17. Hartley MG, Hudson MJ, Swarbrick ET, Hill MJ, Gent AE, Hellier MD, Grace RH (1992) The rectal mucosa-associated microflora in patients with ulcerative colitis. J Med Microbiol 36:96–103
18. Shimoyama T, Hori S, Tamura K, Yamamura M, Tanaka M, Yamazaki K (1984) Microflora of patients with stool abnormality. Bifidobacteria Microflora 3: 35–42
19. Spiekermann GM, Walker WA (2001) Oral tolerance and its role in clinical disease. J Pediatr Gastroenterol Nutr 32: 237–255
20. Hanson LÅ, Dahlman-Höglund A, Karlsson M, Lundin S, Dahlgren U, Telemo E (1999) Normal microbial flora of the gut and the immune system. In: Hanson LÅ, Yolken RH (eds) Nestlé Workshop Series, Vol. 42. Vevey/Lippincott-Raven Publishers, Philadelphia, pp 217–228
21. Nicolai T (1997) Epidemiology of pollution-induced airway disease: urban/rural differences in East and West Germany. Allergy 52:35–36
22. Holgate ST (1999) The epidemic of allergy and asthma. Nature 402:B2–B4
23. Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci Technol 10:107–110
24. Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau M-C, Roberfroid M, Rowland I (1998) Functional food science and gastrointestinal physiology and function. Br J Nutr 80:S147–S171
25. Majamaa H, Isolauri E, Saxelin M, Veskari T (1995) Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 20:333–338
26. Yasui H, Nagaoka N, Mike A, Hayakawa K, Ohwaki M (1992) Detection of *Bifidobacterium* strains that induce large quantities of IgA. Microb Ecol Health Dis 5:155–162
27. Matsuzaki T, Yamazaki R, Hashimoto S, Yokokura T (1998) The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. J Dairy Sci 81:48–53
28. Stewart-Tull DES (1980) The immunological activities of bacterial peptidoglycans. Ann Rev Microbiol 34:311–340
29. Morata de Ambrosini V, Gonzales S, de Ruiz Holgado AP, Oliver G (1998) Study of the morphology of the cell walls of some strains of lactic acid bacteria and related species. J Food Pro 61:557–562
30. Pessi T, Sütas Y, Saxelin M, Kalloinen H, Isolauri E (1999) Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. Appl Environ Microbiol 65:4725–4728
31. Tejada-Simon MV, Pestka JJ (1999) Proinflammatory cytokine and nitric oxide induction in murine macrophages by cell wall and cytoplasmic extracts of lactic acid bacteria. J Food Prot 62:1435–1444
32. Klinman DM, Yi A-K, Beaucage SL, Conover J, Krieg AM (1996) CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12 and interferon γ. Proc Natl Acad Sci 93:2879–2883
33. Kirjavainen PV, Apostolou E, Salminen SJ, Isolauri E (1999) New aspects of probiotics – a novel approach in the management of food allergy. Allergy 54: 909–915
34. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412
35. Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 71:1682S–1687S
36. Gibson GR (1999) Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. J Nutr 129:1438S–1441S
37. Pouwels PH, Leer RJ, Boersma WJA (1996) The potential of *Lactobacillus* as a carrier for oral immunization: development and preliminary characterization of vector systems for target delivery of antigens. J Biotechnol 44:183–192
38. O'Brien J, Crittenden R, Ouwehand AC, Salminen S (1999) Safety evaluation of probiotics. Trends Food Sci Technol 10:418–424
39. Ouwehand AC, Salminen SJ (1998) The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8:749–758
40. Pickering LK, Granoff DM, Reed Erickson J, Masor ML, Cordle CT, Schaller JP, Winship TR, Paule CL, Hilty MD (1998) Modulation of the immune system by human milk and infant formula containing nucleotides. Pediatrics 101: 242–249
41. Vuorinen A, Mantere-alhonen S (1982) On the trace elements in *Propionibacterium freudenreichii*-mass. Meijeritieteellinen Aikauskirja 40:53–59