

Probiotics in the management of atopic eczema

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Summary

Background Over the last two decades the incidence of allergic diseases has increased in industrialized countries, and consequently new approaches have to be explored.

Objective The potential of probiotics to control allergic inflammation at an early age was assessed in a randomized double-blind placebo-controlled study.

Methods A total of 27 infants, mean age 4.6 months, who manifested atopic eczema during exclusive breast-feeding and who have had no exposure to any infant or substitute formula were weaned to probiotic-supplemented, *Bifidobacterium lactis* Bb-12 or *Lactobacillus* strain GG (ATCC 53103), extensively hydrolysed whey formulas or to the same formula without probiotics. The extent and severity of atopic eczema, the growth and nutrition of infants, and concentrations of circulating cytokines/chemokines and soluble cell surface adhesion molecules in serum and methyl-histamine and eosinophilic protein X in urine were determined.

Results The SCORAD score reflecting the extent and severity of atopic eczema was 16 (7–25) during breast-feeding, median (interquartile range). After 2 months, a significant improvement in skin condition occurred in patients given probiotic-supplemented formulas, as compared to the unsupplemented group; $\chi^2 = 12.27$, $P = 0.002$. SCORAD decreased in the *Bifidobacterium lactis* Bb-12 group to 0 (0–3.8), and in the *Lactobacillus* GG group to 1 (0.1–8.7), vs unsupplemented 13.4 (4.5–18.2), median (interquartile range), in parallel with a reduction in the concentration of soluble CD4 in serum and eosinophilic protein X in urine.

Conclusion The results provide the first clinical demonstration of specific probiotic strains modifying the changes related to allergic inflammation. The data further indicate that probiotics may counteract inflammatory responses beyond the intestinal milieu. The combined effects of these probiotic strains will guide infants through the weaning period, when sensitization to newly encountered antigens is initiated. The probiotic approach may thus offer a new direction in the search for future foods for allergy treatment and prevention strategies.

Keywords: allergic inflammation, atopy, breast-feeding, food allergy, infants, probiotics

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Introduction

Probiotics, defined as live microbial food ingredients beneficial to health [1], are normal commensal bacteria of the healthy human gut microflora. The most frequently used

genera are lactobacilli and bifidobacteria, and the best-documented current therapeutic application is in the prevention and treatment of diarrhoeal diseases [2,3]. The clinical effects have been explained by promotion of gut barrier functions: normalization of increased intestinal permeability [4] and altered gut microecology [5] and enhancement of intestinal IgA responses [6]. The strains selected for the study, *Bifidobacterium lactis* Bb-12 and *Lactobacillus*

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strain GG (ATCC 53103), have both proved safe at an early age [2,3].

Recent epidemiological and experimental studies have demonstrated an inverse association between infections and atopy and their cross-regulatory properties on T-helper responder phenotype [7,8]. Beneficial interactions between microbes and allergic host may also arise from the control of allergic inflammation, a decisive factor in the propagation of sensitization to atopic disease [9,10]. Confrontation with microbial antigens begins in the gastrointestinal tract with the establishment of the indigenous microflora. Normal microflora impacting on the mucosal surface has profound effects on the immunological development of the host [11]; such early and constant microbial stimulus may outweigh that of occasional infections. Indeed, the potential of specific strains of microflora to modulate the immune responses to dietary antigens and to balance the generation of pro-inflammatory and anti-inflammatory cytokines has been demonstrated *in vitro* [12–14].

Gut microflora as an endogenous defence mechanism may be amenable to therapeutic exploitation to affect allergic inflammation particularly during the weaning period, when sensitization to newly encountered antigens is initiated. Therefore, to evaluate whether the documented immunomodulatory effects of probiotics provide clinical benefit, we assessed in a randomised double-blind placebo-controlled trial the effects of probiotic bacteria in young infants with atopic eczema, frequently the initial manifestation of allergy.

Methods

Subjects

We selected 27 infants from a group of 100 with early onset atopic disease. They fulfilled the Hanifin criteria for atopic eczema in children [15]. Additional inclusion criteria were that the eczema had begun during exclusive breast-feeding and that the patients had had no exposure to any infant formula or substitute formula before enrolment, and that they tolerated the assigned formulas. In selected infants, atopic eczema began at 1.6 (1.3–2.0) months of age, mean (95% confidence interval, CI). Gastrointestinal symptoms, including vomiting, loose stools and diarrhoea, occurred additionally in 15 (56%) cases. In most cases (82%) there was a positive history of atopic diseases in first-degree family members. They were enrolled at weaning, at a mean age of 4.6 months, when they were still fully breast-fed.

At enrolment, serum total IgE concentration (Phadebas IgE Prist, Pharmacia, Uppsala, Sweden) was 7 (IQR 0–20) kU/L. A positive (≥ 0.4 kU/L) radioallergosorbent assay (Pharmacia) for egg, cow milk and wheat was demonstrated

in 16%, 11% and 4% of the patients, respectively, and skin prick test reactivity [16] to egg, cow milk and wheat in 15%, 8% and 15%, respectively.

All families gave written informed consent to participate. The Tampere University Hospital Committee on Ethical Practice had approved the study.

Design

In this randomized double-blind study, the patients were divided into three groups; two were weaned to a probiotic-supplemented extensively hydrolysed whey formula (Peptid-Tutteli, Valio Ltd, Helsinki, Finland) and one to the same formula without probiotics. The strains, *Bifidobacterium lactis* Bb-12 (Christian Hansen A/S, Hørsholm, Denmark) and *Lactobacillus* GG (Valio Ltd, Helsinki, Finland), were selected in view of their documented safety and efficacy in controlling acute infantile diarrhoea. On the basis of previous clinical [2,3] and colonization studies demonstrating colonization even in patients with diarrhoeal disease [5,17], the concentrations of probiotic bacteria in the formulas ranged from 3×10^8 (*Lactobacillus* GG) to 1×10^9 (*Bifidobacterium lactis* Bb-12) colony-forming units (cfu)/g.

An independent microbiologist controlled the formulas for the respective bacteria using the standard plate counts method.

Outcome measures

Primary outcome measures were the extent, severity and subjective symptoms (pruritus and sleep loss) of atopic eczema. Secondary measures were the serum concentrations of soluble cell surface molecules and cytokines/chemokines and urinary concentrations of methyl-histamine and eosinophilic protein X (EPX).

Of these markers, granulocyte macrophage-colony stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), tumour necrosis factor- α (TNF α) were selected to evaluate the inflammatory state, and soluble CD4 (sCD4), soluble CD8 (sCD8), IL-2 soluble receptor α (IL-2 sR α) to reflect T-cell-related inflammatory state [18–21], and RANTES (Regulated upon Activation, Normal T-cell Expressed and presumably Secreted) and monocyte chemotactic protein-1 (MCP-1) to evaluate mast cell activity in circulation. Transforming growth factor- β 1 (TGF- β 1) and IL-1 receptor antagonist (IL-1ra) were assessed in serum samples to evaluate the reactive anti-inflammatory state in the circulation. Urinary EPX has been purported to be a marker of eosinophilic inflammatory activity and the urinary metabolite of histamine, methyl-histamine, a marker of early allergen-induced responses [22,23].

Evaluation of atopic eczema

The severity of atopic eczema was assessed by the SCORAD method, established by the European Task Force on Atopic Dermatitis [24], prior to introduction of the randomly assigned formula, and 2 months and 6 months later. The extent was estimated using the rule of nines, the intensity being the sum of the scores for erythema, oedema and/or papules, excoriation, lichenification and dryness. The subjective manifestations, including pruritus and sleep loss, were assessed from parents' estimations. SCORAD was obtained with the calculation: $\text{Extent}/5 + 3.5 \times \text{intensity} + \text{subjective score}$.

Immunological evaluation

Venous blood and urine samples were taken prior to introduction of the randomly assigned formula and 2 months later and kept at -70°C until analysis. An enzyme-linked immunosorbent assay method was applied using commercial kits of sCD4, sCD8, IL-2sR α , sICAM-1, IL-1ra, RANTES, MCP-1; purchased from RD Systems, Minneapolis, MN, USA, and TNF- α , GM-CSF, TGF- β 1; purchased from Genzyme, Cambridge, MA, USA. Methyl-histamine and EPX concentrations in diluted urine samples were measured by radioimmunoassay using reagents from Pharmacia & Upjohn Diagnostics, Uppsala, Sweden. Results are expressed as μg of methyl-histamine or EPX per mmol of creatinine in the urine sample.

Growth and nutrition

The nutrient intake was evaluated as described elsewhere [25]. In brief, parents kept home records of nutrient intake for 3 consecutive days before and after the intervention, quantities of solid and liquid ingested being estimated with household measures. Calculations of nutrient intake were made with the AIVO computer program, compiled from Finnish nutrient databases [26] and manufacturers' data. Weighing the infant quantified breast milk intake. Length and weight were measured with a recumbent infant length board and an electronic scale before introduction of the extensively hydrolysed formulas, and 2 and 6 months later. For further characterization of nutritional status, serum concentrations of albumin, pre-albumin, urea and alkaline phosphatase were determined during follow-up.

Statistical analysis

Data are given as means with 95% CI or medians with interquartile range (IQR). The Kruskal–Wallis test compared the SCORAD scores in the study groups. To avoid

repeated pairwise testing, this test was also used for comparison between the groups of changes from baseline in immunological variables. In a case of statistical significance, Wilcoxon signed-rank test was used in pairwise comparisons. ANOVA was used to compare formula intake and nutritional variables between the groups and the χ^2 test for comparison of the proportions of infants with clinical improvement in the three groups. Spearman's rank correlation coefficient was used to determine the degree and significance of association between urinary methyl-histamine and EPX concentrations and the severity of atopic eczema (SCORAD scores).

Results

The effect of probiotics on atopic eczema

SCORAD during breast-feeding was 16 (7–25), median (IQR). After weaning to a tolerated extensively hydrolysed formula, the scores decreased, reflecting an improvement in skin condition in terms of extent, intensity and subjective assessment, but the response differed between the study groups (Fig. 1). The improvements were confined to the probiotic-supplemented groups, where a significant change in the SCORAD scores was seen at 2 months' evaluation in 9/9 of the patients receiving *Bifidobacterium lactis* Bb-12, 9/9 in the *Lactobacillus* GG group as compared to 4/9 in patients receiving the unsupplemented extensively hydrolysed formula; $P = 0.002$. At this stage, the SCORAD scores in the *Bifidobacterium lactis* Bb-12 group had decreased to 0 (0–3.8), and in the *Lactobacillus* GG group to 1 (0.1–8.7), vs 13.4 (4.5–18.2) in the unsupplemented group; $P = 0.01$. After 6 months the median (IQR) SCORAD score was 0 (0–6.6) in all groups alike.

The effect of probiotics on immunological variables

In patients receiving *Bifidobacterium lactis* Bb-12 and *Lactobacillus* GG, but not in the unsupplemented group, the concentration of serum sCD4 decreased after 2 months' intervention (Table 1); the Kruskal–Wallis test demonstrated a statistically significant difference in the changes from baseline between the three study groups ($P = 0.005$). The concentrations of sCD8 and IL2sR α in sera tended to decline in all three groups after consumption of the assigned formulas. Serum TGF- β 1 concentration was again modified by probiotics ($P = 0.007$, Kruskal–Wallis test), but the response diverged in patients given a probiotic strain (Table 1): it decreased in those receiving *Bifidobacterium lactis* Bb-12 ($P = 0.04$), whereas it tended to increase with *Lactobacillus* GG ($P = 0.07$). The serum concentrations of IL-1ra, TNF α , GM-CSF, sICAM-1, RANTES and MCP-1 α were not modified by probiotics.

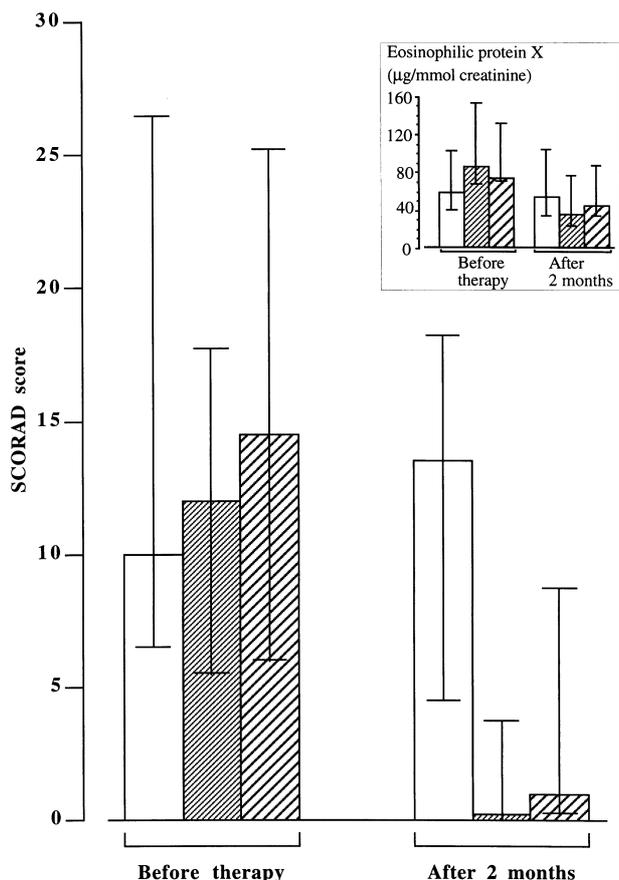


Fig. 1. The SCORAD score, median (interquartile range), evaluation of the extent and severity of atopic eczema and the symptoms (pruritus and sleep loss) of the patients before and after 2 months' consumption of the randomly assigned extensively hydrolysed whey formula, unsupplemented (open bars) or supplemented with *Bifidobacterium lactis Bb-12* (densely hatched bars) or with *Lactobacillus strain GG* (hatched bars). Insert: the concentration of eosinophilic protein X in urine before and after 2 months' consumption of the respective formulas.

Before intervention, the concentration of EPX in urine correlated significantly with the SCORAD scores during breastfeeding ($\rho = 0.53$, $P = 0.01$) but that of methyl-histamine showed no correlation ($\rho = 0.24$, $P = 0.27$). A significant reduction in the urinary EPX was detected in the probiotic-supplemented groups, *Bifidobacterium lactis Bb-12* ($P = 0.01$) and *Lactobacillus GG* ($P = 0.04$); Fig. 1 insert.

Growth and nutrition

At enrolment, breast milk comprised the source of nutrition; 108 (97–120) mL/kg, mean (95% CI). After 2 months, the groups were comparable with respect to formula intake, mean (95% CI): in patients receiving the unsupplemented

extensively hydrolysed formula 75 (63–88) mL/kg, in patients receiving *Bifidobacterium lactis Bb-12* 73 (63–88) mL/kg and in patients receiving *Lactobacillus GG* 76 (70–82) mL/kg; $P = 0.96$. The consumption equalled 53 (38–68)%, 52 (42–62)% and 56 (50–62)% of the total energy intake of the infants in the respective treatment groups; $P = 0.85$. After 6 months, consumption of formulas had decreased to 63 (56–69) mL/kg, i.e. 42 (37–47) kcal/kg [177 (156–199) kJ/kg]. A parallel increase in solid food intake was recorded: 64 (59–70) kcal/kg [270 (245–294) kJ/kg], this comprising 60 (56–65)% of total energy intake.

During the 6 months' follow-up, the mean intake of probiotics was $3\text{--}8 \times 10^{10}$ cfu/d. No significant decreases were observed in the numbers of the bacteria in the test formulas during storage.

The growth of the patients in all study groups was normal during the entire follow-up period. The mean (95% CI) length-for-age standard deviation scores before, after 2 months' and after 6 months' intervention were -0.04 ($-0.41\text{--}0.33$), -0.11 ($-0.46\text{--}0.22$) and 0.0 ($-0.43\text{--}0.42$), respectively. Before intervention, the mean (95% CI) serum albumin, pre-albumin, alkaline phosphatase concentrations were within the normal range: 41 (40–42) g/L, 0.14 (0.14–0.15) g/L and 672 (564–780) U/L, respectively, and no differences were observed between the groups during follow-up. The serum urea concentration tended to be low during breastfeeding, 1.98 (1.79–2.16) mmol/L, mean (95% CI); normal range 2.0–6.0 mmol/L. Probiotic supplementation was shown at the 2 months' evaluation to have benefited this nutritional variable: in patients receiving *Bifidobacterium lactis Bb-12* 4.1 (3.4–4.7), *Lactobacillus GG* 3.3 (2.7–3.7) vs. unsupplemented 2.9 (2.1–3.7) mmol/L, mean (95% CI); $P = 0.02$. At 6 months, the concentration was normal, 4.0 (3.5–4.4) mmol/L, in all study groups alike.

Discussion

The human intestinal microflora is a complex and metabolically active ecosystem. Its establishment is a systematic process, which continues through the first years of life [27]. This is a critical period, when immune responsiveness to ubiquitous antigens is consolidated. The notion that the microflora, and thus probiotics, may have a role in immune priming of the infant to the extrauterine world is based on the recent demonstration in experimental animals that the gut-associated lymphoid tissue evolves through bacterial colonization [28]. The capacity to produce IgA-secreting cells increases progressively in response to intestinal antigenic stimulation, particularly from the gut microflora [29]. Importantly, bacterial colonization appears mandatory for the generation of oral tolerance instead of sensitization to dietary antigens [30]. Thus qualitative

Table 1. The concentrations, median (interquartile range), of soluble CD4 (sCD4), soluble CD8 (sCD8), IL-2 soluble receptor α (IL-2sR α) and transforming growth factor- β 1 (TGF- β 1) in sera of atopic patients before (before) and after 2 months' (after) consumption of the randomly assigned extensively hydrolysed whey formula (EHF), unsupplemented or supplemented with *Bifidobacterium lactis* Bb-12 or with *Lactobacillus* strain GG.

	EHF		EHF +		EHF +	
			<i>Bifidobacterium lactis</i> Bb-12		<i>Lactobacillus</i> strain GG	
	Before	After	Before	After	Before	After
sCD4	5118	4456	5601	1404*	5540	4458*
pg/mL	(4735–5555)	(3482–5235)	(5092–6189)	(1021–1635)	(5151–6484)	(3507–4601)
sCD8	15	10	12	11	11	8
ng/mL	(10–17)	(9–12)	(11–17)	(9–12)	(8–13)	(7–11)
IL-2sR α	4307	3605	3605	3251*	4618	3739
pg/mL	(3789–4429)	(2927–3981)	(2927–4885)	(3019–3387)	(3605–5554)	(3527–5161)
TGF- β 1	75	75	76	46*	54	68
ng/mL	(63–82)	(67–87)	(75–82)	(39–55)	(50–62)	(56–82)

* $P < 0.05$, in comparisons to the baseline measurements (in case of overall statistically significant difference between groups in Kruskal–Wallis test).

differences in the composition of the gut microflora affect the immunological homeostasis of the host.

The criteria for a probiotic include that the strain be of human origin, be safe in human use and stable to acid and bile, and that it adhere to the intestinal mucosa [1]. The genera used in the present study, *lactobacilli* and *bifidobacteria*, evince a natural association with the human mucosal surfaces of the mouth, gastrointestinal tract and genitourinary tract.

Probiotic bacteria have been shown to promote the intestine's endogenous host defence mechanisms [1–6], which may be amenable to therapeutic exploitation to control early allergic inflammation, a key to preventing the progression of atopic symptomatology. Indeed, central to the relevance of our approach was that this extreme population is at highest risk of multiple food allergy [25] and asthma [31], while concomitantly not yet having developed the relevant mature immunological memory [7]. Our strategy, based on the consumption of specific microorganisms of the healthy gut microflora, is an important extension of the observation that there is counterregulation of atopic-type immune responses by specific microbial antigens [7,8] and of the recent epidemiological demonstration of an inverse association between infections at an early age and atopy later in life [32,33].

Probiotics were here shown to reduce the extent, severity and subjective symptoms of atopic eczema in young infants. In unsupplemented group, control of atopic eczema was achieved by 6 months, while in the probiotics-supplemented after 2 months' therapy, indicating earlier control of allergic

inflammation. In sensitized infants, dietary antigens evoke inflammatory reaction, which impairs the intestine's barrier function, leading to aberrant antigen uptake, imbalance of the intestinal microflora and release of pro-inflammatory cytokines, which further perpetuate the inflammatory response and barrier dysfunction [34]. In unbalanced microflora, pathogens are abundantly present, leading to abrogation of the interaction maintained in health between the microflora and the immune system; strong inflammatory response may then also be directed to the microfloral bacteria [35]. Probiotics have been shown to alleviate intestinal inflammation, as measured by faecal TNF α [36], and to promote the gut barrier functions, as directly evaluated by macromolecular absorption [4], and to normalize altered gut microecology [1]. As indicated here, probiotics may counteract inflammatory changes beyond the intestinal milieu.

In addition to diminishing clinical signs and symptoms of atopic eczema, alleviation of allergic inflammation was supported by the reduction of sCD4 in serum and EPX in urine of patients receiving *Bifidobacterium lactis* Bb-12 and *Lactobacillus* GG. Soluble CD4 has been found to be elevated in several diseases associated with chronic inflammation [19], while urinary EPX has been shown to reflect the activity of allergic inflammation in childhood asthma [23]. The distinct probiotic effects on TGF- β 1 indicate that probiotic bacteria may exert their anti-inflammatory effects via several pathways. The generation of TGF- β 1 has been shown to be crucial in reversing mucosal allergic inflammation [37], and thereby it may alleviate the subjective

symptoms particularly in atopic patients with eosinophilic esophagitis and gastroesophageal reflux disease. Indeed, as both processes, reduction of soluble CD4, a marker of T-cell activation, and increase of TGF- β 1, mediating IgA production and low-dose oral tolerance induction [38,39], are involved in suppression of inflammatory response, it is not surprising that the clinical outcome was indistinguishable.

Our results constitute the first clinically documented demonstration of a possible role for specific microbes in controlling allergic inflammation. Investigations are in progress to determine the interaction between the gut microflora and the immunological homeostasis of the host. An improved understanding of the immunomodulatory mechanisms accredited to different microbes, their combinations and growth factors may offer novel disease-specific probiotic functional foods, also carrying implications for future allergy prevention and treatment strategies.

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References

- Salminen S, Bouley C, Boutron-Ruault MC et al. Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998; 80:S147–71.
- Isolauri E, Juntunen M, Rautanen T, Sillanauke P, Koivula T. A human *Lactobacillus* strain (*Lactobacillus* GG) promotes recovery from acute diarrhea in children. *Pediatrics* 1989; 88:90–7.
- Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994; 344:1046–9.
- Isolauri E, Majamaa H, Arvola T, Rantala I, Virtanen E, Arvilommi H. *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 1993; 105:1643–50.
- Isolauri E, Kaila M, Mykkänen H, Ling WH, Salminen S. Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci* 1994; 39:2595–600.
- Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human lactobacillus strain. *Pediatr Res* 1992; 32:141–4.
- Cookson WOCM, Moffatt MF. Asthma: an epidemic in the absence of infection? *Science* 1997; 275:41–2.
- Erb KJ. Atopic disorders: a default pathway in the absence of infection. *Immunol Today* 1999; 20:317–22.
- Baraniuk JN. Pathogenesis of allergic rhinitis. *J Allergy Clin Immunol* 1997; 99:S763–72.
- Leung DY. Atopic dermatitis: immunobiology and treatment with immune modulators. *Clin Exp Immunol* 1997; 27 (Suppl. 1):25–30.
- Cebra JJ. Influences of microbiota on intestinal immune system development. *Am J Clin Nutr* 1999; 69:1046S–51S.
- 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; 98:216–24.
- 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; 43:687–9.
- Pessi T, Sütas Y, Saxelin M, Kallioinen H, Isolauri E. Anti-proliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microb* 1999; 65:4725–8.
- Hanifin JM. Atopic dermatitis in children. *Pediatr Clin N Am* 1991; 38:763–89.
- Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol* 1995; 97:9–15.
- Kaila M, Isolauri E, Sepp E, Mikelsaar M, Salminen S. Fecal recovery of a human *Lactobacillus* strain (ATCC 53103) during dietary therapy of rotavirus diarrhea in infants. *Biosci Microflora* 1998; 17:149–51.
- Wegner CD, Gundel RH, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990; 248:456–9.
- Goto H, Gidlund M. Soluble CD4: a link between specific immune mechanisms and non-specific inflammatory responses? *Scand J Immunol* 1996; 43:690–2.
- Nikolaus S, Bauditz J, Gionchetti P, Witt C, Lochs H, Schreider S. Increased secretion of pro-inflammatory cytokines by circulating polymorphonuclear neutrophils and regulation by interleukin 10 during intestinal inflammation. *Gut* 1998; 42:470–6.
- Wang C-H, Hsieh W-Y, Shih L-Y et al. Increased progenitor cell proliferation in the peripheral blood of patients with bronchial asthma: the role of nitric oxide. *J Allergy Clin Immunol* 1999; 104:803–10.
- O'Sullivan S, Roquet A, Dahlén B, Dahlén SE, Kumlin M. Urinary excretion of inflammatory mediators during allergen-induced early and late phase asthmatic reactions. *Clin Exp Allergy* 1998; 28:1332–9.
- Koller DY, Halmerbauer G, Frischer T, Roithner B. Assessment of eosinophil granule proteins in various body fluids: is there a relation to clinical variables in childhood asthma? *Clin Exp Allergy* 1999; 29:786–93.
- European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis. The SCORAD Index. *Dermatology* 1993; 186:23–31.
- Isolauri E, Tahvanainen A, Peltola T, Arvola T. Breast-feeding of allergic infants. *J Pediatr* 1999; 134:27–32.

- 26 Rastas M, Seppänen R, Knuuts LR, Karveti RL, Varo P. Nutrient components of foods. Helsinki: Publication of the Social Insurance Institution, 1993.
- 27 Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999; 69:1035S–45S.
- 28 Shroff KE, Meslin K, Cebra JJ. Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. *Infect Immun* 1995; 63:3904–13.
- 29 Moreau MC, Ducluzeau R, Guy-Grand D, Muller MC. Increase in the population of duodenal IgA plasmocytes in axenic mice monoassociated with different living or dead bacterial strains of intestinal origin. *Infect Immun* 1978; 21:532–9.
- 30 Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–45.
- 31 Bergmann RL, Edenharter G, Bergmann KE et al. Atopic dermatitis in early infancy predicts allergy airway disease at 5 years. *Clin Exp Allergy* 1998; 28:965–70.
- 32 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–60.
- 33 von Mutius E, Fritsch C, Weiland SK, Röhl G, Magnussen H. Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. *BMJ* 1992; 305:1395–9.
- 34 Perdue MH, McKay DM. Integrative immunophysiology in the intestinal mucosa. *Am J Physiol* 1994; 30:G151–65.
- 35 Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Büschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* 1995; 102:448–55.
- 36 Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997; 99:179–86.
- 37 Hansen G, McIntire JJ, Yeung VP, Berry G, Thorbecke GJ, Chen L, DeKruyff RH, Umetsu DT. CD4⁺ T helper cells engineered to produce latent TGF- β 1 reverse allergen-induced airway hyperreactivity and inflammation. *J Clin Invest* 2000; 105:61–70.
- 38 Letterio JJ, Roberts AB. Regulation of immune responses by TGF- β . *Annu Rev Immunol* 1998; 16:137–61.
- 39 Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor beta in breastmilk: a potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 1999; 104:1251–7.40.