

Prevention of Allergy in Infants of Allergic Mothers by Probiotic *Escherichia coli*

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Key Words

Allergy in infants · Allergy prevention · After-birth oral colonization · Probiotic *E. coli*

Abstract

Background: The objective is to study the effect of after-birth oral colonization by a probiotic *Escherichia coli* strain in infants of allergic mothers to reduce occurrence of allergy later in life. **Methods:** In a controlled clinical trial, 158 infants were randomly divided into groups of (i) 56 colonized infants of allergic mothers, (ii) 57 control infants of allergic mothers, and (iii) 45 control infants of healthy mothers. Incidence rates of bacterial pathogens in stool and levels of anti-*E. coli* immunoglobulins and some cytokines in serum were determined, and secretory IgA was monitored in stool filtrates and maternal milk. Clinical check-ups of infants aged 4 days, 3 and 6 months, 2, 3 and 5 years were carried out and clinical symptoms of allergy were monitored. One milliliter of the probiotic *E. coli* strain was administered to infants of allergic mothers at first within 48 h after birth and subsequently 3 times a week over a period of 4 weeks. Control infants of allergic and healthy mothers were monitored in these intervals as well. **Results:** Presence of the *E. coli* strain was monitored in stool samples throughout the study. At the conclusion of the study, allergy symptoms were found in 14 infants of control allergic mothers, 7 infants of healthy mothers, and

in 2 colonized infants of allergic mothers. Colonization affected levels of several cytokines and specific anti-*E. coli* antibodies. **Conclusions:** After birth, targeted colonization of the intestine by a probiotic *E. coli* strain can be an effective means of allergy prevention in infants of allergic mothers.

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Introduction

Physiological intestinal flora is an important modulator of intestinal and other immune functions [1]. Changes in intestinal microbial composition have been observed in infants with allergy and in those who developed allergies later in life [2–5]. Significant differences in cytokine levels IL-4, IL-10, IL-13 and IFN- γ have been found in healthy and allergic groups of mothers and their children [6].

Attempts have been made to substitute randomly acquired intestinal flora by intentional colonization using probiotic bacterial strains. Lactobacilli and *Bifidus* bacteria have been used worldwide with no adverse effect but providing only limited short-term, or no protection. Administration of *Lactobacillus acidophilus* had no effect on food allergy [7] and asthma [8], supplementation of *Bifidobacterium lactis* did not affect intestinal microbiota in preterm infants [9], and there is no reported effect of

Table 1. Characteristics of the test groups

Group (n)	Weeks of gestation	Birth length cm	Birth weight, g	Breastfeeding, weeks		Allergic fathers	Allergic siblings	Smoking mothers	Pets
				range	median				
H (45)	39 (38–42)	50 (48–53)	3,200 (2,800–3,900)	3–72	29	6 (15%)	5 (12%)	10%	38%
A (57)	38 (37–42)	51 (47–53)	3,300 (2,990–4,000)	2–80	28	21 (46%)	35 (62%)	12%	46%
AC (56)	39 (38–42)	50 (48–52)	3,100 (2,880–3,900)	6–88	31	28 (50%)	15 (27%)	8%	42%

H = Healthy mothers; A = allergic mothers; AC = allergic mothers of colonized children.

treatment of infantile colic using *Lactobacillus reuteri* [10]. *Lactobacillus* GG had no effect on primary prevention of sensitization in infants [11]. A significant reduction of infant eczema has been reported by Kalliomaki et al. [12], Isolauri et al. [13] and Abrahamson et al. [14]. Osborn and Sinn [15] and Kukkonen et al. [16] describe an insignificant effect of lactobacilli administration on overall allergy incidence rates in infants. Several studies describe positive effects of infant intestinal colonization by non-pathogenic *E. coli* strains. Schulze and Sonnenborn's group [17] emphasize the renaissance of the use of probiotics and Poisson et al. [18] and Lari et al. [19] recommend their use in intensive care units and in newborn and premature infants. The results of Cukrowska et al. [20] of colonizing premature infants by *E. coli* Nissle show a specific proliferative and antibody response.

Our previously published results show that the unique probiotic *E. coli* strain, administered orally after birth, remains dominant in the intestine over several months. Its prolonged presence in the gut stimulates production of serum and local antibodies [21] and reduces the presence of bacterial pathogens in the gut and on other mucous surfaces, and, according to results of a questionnaire, reduces the incidence of nosocomial infections and allergies 10 and 20 years after its administration [22]. These favorable properties of the *E. coli* strain, particularly the prevention of nosocomial infections in high-risk infants, have been recognized in clinical practice [23].

This study shows the effect of after-birth colonization of infants of allergic mothers by a probiotic *E. coli* (Colinfant®) on: (1) the incidence rate of allergies within the first 5 years of life; (2) IgE antibodies against common allergens in sera of colonized and non-colonized infants and their mothers; (3) cytokine levels of IL-4, IL-5, IL-6, IL-13, IFN- γ , TGF- β and anti-*E. coli* immunoglobulins in serum, and (4) secretory IgA levels in stool filtrates and milk.

Test Groups and Methods

A total of 158 studied full-term infants (average birth weight 3,050 g, birth length 50 cm) was randomly divided into groups of: (1) colonized infants of allergic mothers, the 'allergic colonized' group AC (n = 56); (2) non-colonized control infants of allergic mothers, the 'allergic' group A (n = 57), and (3) control infants of healthy mothers, 'healthy' group H (n = 45).

In an open, controlled, randomized, clinical study, the average age of mothers was 30 years in group H, 32 years in group A, and 33 years in group AC. All infants were full-term and born vaginally. Characteristics of studied test groups are given in table 1.

Conditions for Inclusion of Allergic Mothers in the Study

The following criteria were applied in the selection of allergic mothers: (a) absence of other illnesses; (b) clinical manifestation of allergy for more than 24 months; (c) monitoring of allergy by an allergologist (via a written report); (d) positive skin test, positive specific IgE, and (e) positive reaction to antiallergic treatment. Informed and signed consent was obtained from all participating mothers.

Probiotic Treatment of Infants

The *E. coli* strain, patented in 1992 (No. 264572) and registered in 1997 in the Czech Republic as Colinfant, is produced by Dyntec Co. (Terezín, Czech Republic). One dose, containing 0.8×10^9 of lyophilized *E. coli* in 1 ml, was administered orally within 48 h after birth, and subsequently 3 times weekly over a period of 4 weeks.

Characteristics of the Strain

The strain was obtained from the international collection of *E. coli* and *Klebsiella* strains in Copenhagen, Denmark. According to its biochemical properties it belongs to normal human intestinal *E. coli* strains, serotype O83:K24:H31, and occurs very rarely as a human spontaneous type. It is sensitive to most antibiotics and has no plasmid. Its detailed characteristics are described in our previous studies [21, 23]. The strain has not changed any of its properties over 30 years, including sensitivity to antibiotics, absence of plasmid and genetic characteristics.

Test Samples

Samples of maternal blood, cord blood, infant venous blood and infant stool were taken prior to administration of the vaccine and at the ages of 3–4 days, 3 and 6 months, 1, 2, 3 and 5 years.

Table 2. Detection of *E. coli* vaccine strain in colonized infants

	3 days (n = 56)	3 months (n = 52)	6 months (n = 51)	1 year (n = 35)	2 years (n = 22)	3 years (n = 17)	5 years (n = 16)
O83+	22 (39%)	34 (65%)	27 (53%)	17 (49%)	6 (27%)	3 (18%)	2 (13%)
O83	18 (32%)	13 (25%)	7 (14%)	5 (14%)	3 (14%)	3 (18%)	2 (13%)
Total	71%	90%	67%	63%	41%	36%	26%

O83+ = *E. coli* O83 vaccine strain with other *E. coli* strains; O83 = *E. coli* O83 vaccine strain only.

Table 3. Incidence of allergy during the follow-up periods

Group (n)	3 months	6 months	1 year	2 years	3 years	5 years
H (45)	4% (2/45)	11% (5/45)	15% (7/45)	16% (7/42)	15% (7/44)	16% (7/42)
A (57)	9% (5/57)	14% (8/57)	21% (12/57)	28% (14/50)*	28% (14/49)*	31% (14/45)*
AC (56)	2% (1/56)	2% (1/56)	2% (1/56)	2% (1/50)	2% (1/48)	4% (2/46)

* Significant differences relative to group AC are expressed as $p < 0.05$.

Colostrum and milk samples were collected during the breast-feeding period. Blood sera, colostrum and milk samples (after centrifugation and fat removal) and children's stool filtrates were stored at -20°C .

Infants' stool filtrates were prepared as 1 g of stool suspended in 1 ml of PBS with 1 mM phenylmethylsulfonyl fluoride, centrifuged at 8,000 g, and supernatants used for analysis. The *E. coli* strain was detected on blood agar, Endo agar and MPA agar in stool of colonized infants. The specificity of the strain was confirmed by agglutination with specific rabbit antiserum. Levels of IL-4, IL-5, IL-6, IL-10, IL-13, IFN- γ and TGF- β were quantified by ELISA on high-adsorption polystyrene microtitration plates (Nunc). For paired antibodies where the second antibody was biotinylated, a R&D System (Wiesbaden, Germany) was used according to the manufacturer's recommendations. Results were read from the calibration curves of the recombinant cytokine R&D System using Genesis software. Detection limits for 1:10 diluted samples were 10 pg/ml for IL-4, IL-5, IL-6 and IFN- γ , and 30 pg/ml for IL-10, IL-13 and TGF- β . Specific IgE was estimated immunoenzymically by RISA (Ring-Immuno-Sorbent Assay) using enzyme allergosorbent test (Doverton) kits and Dynex allergen rings (FX-4) with a mixture of the most common food allergens (egg white, cow's milk, flower, peanut and soya bean antigens), or Dynx-1 with the most common respiratory allergens (timothy grass, ray, birch wormwood, mite, hair and mould antigens). Results were obtained from the calibration curve and expressed as kU/l and RAST classes. Totals of sIgA in colostrum, milk and stool filtrates were determined by radial immunodiffusion using binding site kits.

Statistics

Overall distribution of duration of allergy incidence was compared between studied groups using Kaplan-Meier analysis and

Table 4. Types of allergy

Group	Mothers					Infants			
	n	respiratory	skin	both	others	n	respiratory	skin	food
H	45	–	–	–	–	7	2	6	1
A	57	26	5	66	2	14	2	10	3
AC	56	34	2	78	–	2	1	2	1

Mantel-Haenszel (log-rank) test. Differences in distribution of cytokines among groups were compared using the Mann-Whitney U test (Wilcoxon rank sum test). $p < 0.05$ was considered significant. Results are shown as time-varying medians and percentiles 25–75%.

Results

All infants were born by vaginal delivery and all were breastfed. Differences of duration of breastfeeding among groups were statistically insignificant (table 1). The *E. coli* strain O83:K24:H31 was never found in infant stool samples before its administration. The strain was detected in some colonized infants during the entire span of the study, starting on day 3 (table 2). At the time of conclusion of the study, results of the Kaplan-Meier analysis show

Table 5. Serum cytokine levels in infants until the age of 3 months (percentage values in parentheses)

Cytokines	Group H			p H vs. A	Group A			p H vs. AC	Group AC		
	median	25th–27th percentile	positive samples total		median	25th–27th percentile	positive samples total		median	25th–27th percentile	positive samples total
<i>Cord blood</i>											
IL-4	221	169–367	23 (55)/42	0.6635	248	172–545	36 (65)/55	0.4672	294	193–700	45 (78)/58
IL-5	143	116–195	16 (38)/42	0.1176	189	144–251	29 (52)/56	0.4030	196	162–238	42 (72)/58
IL-6	211	131–1,817	14 (33)/42	0.3594	179	144–200	15 (27)/55	0.4644	181	157–212	23 (40)/58
IL-10	306	210–464	20 (56)/36	0.8943	321	197–448	43 (83)/52	0.2187	373	227–566	39 (70)/56
IL-13	943	289–2,609	29 (76)/38	0.1666	2,011	556–3,521	43 (80)/54	0.2744	1,391	703–2,727	48 (86)/56
IFN- γ	1,068	614–2,178	20 (49)/41	0.0355	486	195–854	22 (40)/55	0.9156	432	209–860	42 (72)/58
TGF- β	70,008	39,911–103,040	42 (98)/43	0.1358	42,356	2,412–88,889	54 (100)/64	0.3218	67,752	24,353–102,374	55 (100)/55
<i>3 days</i>											
IL-4	197	146–252	20 (50)/40	0.0167	268	177–715	45 (67)/67	0.8808	305	162–696	48 (75)/64
IL-5	172	128–287	17 (42)/40	0.9220	202	159–234	36 (54)/67	0.0604	225	163–304	46 (72)/64
IL-6	224	167–1,705	14 (33)/42	0.1669	197	160–235	20 (30)/67	0.4647	176	127–241	26 (41)/64
IL-10	314	209–640	19 (51)/37	0.9571	363	221–480	50 (78)/64	0.3944	341	234–939	39 (63)/62
IL-13	598	444–1,286	26 (72)/36	0.2920	945	431–2,529	53 (80)/66	0.8803	1,229	319–2,925	52 (84)/62
IFN- γ	622	318–1,835	18 (43)/42	0.1304	467	143–1,029	40 (62)/64	0.5370	403	180–1,236	47 (73)/64
TGF- β	67,707	35,775–109,968	43 (100)/43	0.2515	53,736	28,922–82,148	64 (100)/64	0.6407	54,006	29,382–89,181	61 (100)/61
<i>3 months</i>											
IL-4	173	132–199	20 (47)/43	0.0021	314	188–478	45 (70)/64	0.9577	295	159–702	49 (78)/63
IL-5	199	158–321	16 (37)/43	0.4263	179	121–311	38 (59)/64	0.2532	204	161–311	38 (60)/63
IL-6	165	144–718	12 (28)/43	0.3664	164	128–245	22 (34)/64	0.4147	154	129–185	18 (29)/63
IL-10	368	287–504	22 (47)/47	0.3831	297	201–623	41 (67)/61	0.4293	248	196–459	37 (29)/61
IL-13	1,020	341–2,100	32 (82)/39	0.0436	2,020	597–3,253	48 (76)/63	0.1031	1,170	413–2,891	48 (79)/61
IFN- γ	509	220–1,056	28 (65)/43	0.0226	476	191–1,070	30 (51)/59	0.9347	495	264–1,039	37 (62)/60
TGF- β	71,880	39,540–116,323	44 (100)/44	0.0277	42,077	15,030–85,892	62 (98)/63	0.0725	64,245	23,773–109,559	62 (100)/62

reduction of allergy incidence in group AC as compared to group A. Clinical signs of allergies were found in 7 (16%) infants of healthy mothers (group H), 14 (31%) in group A (allergic controls), but only in 2 (4%) infants of the colonized group (AC). This confirms significant differences between groups A and AC ($p < 0.001$). The allergy incidence reduction in group AC compared to group H was not significant. A summary of allergy incidence within specified intervals and types of allergies is shown in tables 3 and 4. Laboratory data do not fully reflect the clinical situation. Differences in total secretory IgA between groups in stool and mother milk's were not statistically significant.

Specific IgE levels in serum were elevated in both groups of allergic mothers (88% in group AC and 92% in group A). Milk samples showed increased levels of IgE in 18% of group AC and 22% of the allergic groups of mothers. Infant serum of allergic mothers revealed increased levels of IgE in 7% of colonized and 20% of non-colonized controls at 1 year of age. Correlation with clinical signs of

allergy was proven only in 3 infants of allergic control mothers.

Comparison of cytokine levels among groups was difficult due to great individual differences. Samples with levels under detection limits were considered as negative. Slightly higher levels of IL-4 and significantly higher levels of IL-5 ($p = 0.0071$) were detected in sera of allergic mothers as compared with serum levels of healthy mothers. Values in children's sera of up to 3 months are shown in table 5. Levels of IL-4 and IL-13 were higher in infants of allergic mothers, and IFN- γ and TGF- β were higher in infants of healthy mothers compared to allergic mothers. The allergic phenotype can be recognized at 3 months of age and no substantial differences were found later.

Colonization with the Colinfant vaccine did not substantially influence cytokine levels in serum, infant stool filtrates and mother's milk. Serum IgA and IgG were significantly higher in group AC than in group H at 3 months, 6 months and 1 year of age ($p < 0.001$). Levels of IgM were higher in group AC only at the age of 3 months.

Discussion

The *E. coli* strain used in this study has been studied in our former work since 1965. Colonization of the intestine was effective for prevention of nosocomial infections in premature infants and decreased the numbers of pathogens and the necessity to use of antibiotics. Our understanding of mechanisms responsible for positive effects of colonization with probiotic *E. coli* strains is incomplete. The presence of type 1 fimbriae and its excellent colonizing ability are its favorable features. The first colonizer also benefits from quantitative dominance. However, the overall mechanism is rather complex.

In developed countries, slow colonization of the intestine with enterobacteria may reduce exposure of the developing immune system to lipopolysaccharides. Lipopolysaccharide deficiency may impede the ability of the infant's immune system to distinguish between harmful and harmless antigens (e.g. lack of development of tolerance against food antigens). Probiotics can modify the immune development [5, 26, 27]. Details of intestinal colonization with *E. coli* administered orally to infants can be found in our earlier studies [21, 23]. Comparison of the effect of *E. coli* and lactobacilli shows that *Lactobacilli* administration resulted only in a significant reduction of atopic eczema [13], but no other differences in occurrence of allergic diseases were found [15].

This study covers a period between birth and the age of 5 years. All infants were full-term, born by vaginal delivery. During the 5-year period, clinical symptoms of allergy were found only in 2 infants of allergic mothers colonized after birth with the probiotic *E. coli* strain compared to 14 infants of allergic control mothers. The difference is statistically highly significant. Seven cases of allergy occurred in infants of healthy mothers. It is evident that colonization with probiotic *E. coli* suppresses development of allergy.

The used *E. coli* strain had additional positive effects on studied infants. Levels of pathogenic and potentially pathogenic bacteria (*Klebsiella*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*) found in stool samples of colonized infants of allergic mothers were significantly lower during the first 3 months of age than in infants of allergic and healthy control mothers. These pathogens are some of the most dangerous in the first months of life [24].

Numerous studies report on values of cytokines in serum and mother's milk of healthy and allergic mothers and in their infant serum. Results are rather controversial. It seems that levels of IL-13 and IL-5 have greater

significance and reflect a better Th2 polarization than IL-4. Both increased and decreased production of IFN- γ was described to be associated with allergic phenotype [27]. In our study it was difficult to compare cytokine levels among the infants studied due to the magnitude of observed differences and a large number of observations found under the detection limit. Allergic phenotype, higher IL-4 and IL-13 and lower IFN- γ and TGF- β , dominated in the allergic group but the values observed were not quantitatively different. The allergic phenotype is evident in the first 6 months of life, later there are no substantial differences between children of healthy and allergic mothers. Cytokine levels do not reflect the pronounced clinical effect of colonization with probiotic *E. coli* used in our trial. The clinical effect of the Colinfant strain is complex and bacterial colonization of the intestine does not influence levels of mucosal cytokines in a significant way. Higher levels of IgA in serum of colonized infants can increase the defense ability of the infants against enteral infections due to possible cross-reactions between the antibodies.

Conclusions

To conclude: (1) Oral administration of probiotic *E. coli* after birth resulted in effective colonization of the infant's intestine and maintained dominant presence in the stool over several weeks. (2) Early colonization of the intestine decreased significantly clinical symptoms of allergy in infants of allergic mothers. Cytokine levels in several body fluids are not markedly influenced by colonization. (3) The replacement of natural, incidental and at times pathogenic colonization of the intestine by controlled oral administration of *E. coli* after birth may be a therapeutic option to reduce and possibly prevent allergies later in life.

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References

- 1 Fanaro S, Chierici R, Guerini P, Vigi V: Intestinal microflora in early infancy: composition and development. *Acta Paediatr* 2003; 441(suppl):48–55.
- ▶ 2 Kirjavainen PV, Arvola T, Salminen SJ, Isolauri E: Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy and weaning. *Gut* 2002;51: 51–55.
- ▶ 3 Björkstén B, Sepp E, Julge K, Voor T, Mikkelsen M: Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;108:516–520.
- ▶ 4 Noverr MC, Huffnagle GB: 'The microflora hypothesis' of allergic diseases. *Clin Exp Allergy* 2005;35:1511–1520.
- ▶ 5 Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E: Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001;107:129–134.
- ▶ 6 Prokešová L, Lodinová-Žádníková R, Žižka J, Kocourková I, Novotná O, Petrásková P, Šterzl I: Cytokine levels in healthy and allergic mothers and their children during the first year of life. *Pediatr Allergy Immunol* 2006;17:175–183.
- 7 Majamaa H, Isolauri E: Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997;99:179–185.
- ▶ 8 Rautava S, Kalliomäki M, Isolauri E: New therapeutic strategy for combining the increasing burden of allergic diseases: Probiotics – Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group Report. *Allergy Clin Immunol* 2005;116:3–7.
- ▶ 9 Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M: Effect of *Bifidobacterium lactis* Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled randomized study. *J Clin Microbiol* 2006;44:4025–4031.
- ▶ 10 Savino F, Pelle E, Palumeri E, Oggero R, Miniero R: *Lactobacillus reuteri* (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. *Pediatrics* 2007;119:e124–e130.
- ▶ 11 Kopp MV, Hennemuth I, Heinzmann A, Urbánek R: Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of *Lactobacillus GG* supplementation. *Pediatrics* 2008; 121:850–856.
- ▶ 12 Kalliomäki M, Salminen S, Pousta T, Arvilommi H, Isolauri E: Probiotics and prevention of atopic disease: four-year follow-up of a randomized placebo-controlled trial. *Lancet* 2003;361:1869–1871.
- ▶ 13 Isolauri E, Arvola T, Sütas Y, Salminen S: Probiotics in management of atopic eczema. *Clin Exp Allergy* 2000;30:1605–1610.
- ▶ 14 Abrahamsson TR, Jakobsson T, Böttcher MF, Fredrikson M, Jenmalm MC, Björkstén B, Oldaeus G: Probiotics in prevention of IgE-associated eczema: a double-blind, randomized placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:1174–1180.
- 15 Osborn DA, Sinn JHK: Probiotics in infants for prevention of allergic disease and food hypersensitivity. *The Cochrane Collaboration*. New York, Wiley, 2009, vol 1, pp 1–109.
- ▶ 16 Kukkonen K, Nieminen T, Poussa T, Savilahti E, Kuitunen M: Effect of probiotics on vaccine antibody responses in infancy – a randomized placebo-controlled double-blind trial. *Pediatr Allergy Immunol* 2006; 17:416–421.
- 17 Schulze J, Sonnenborn U, Olschläger T, Kruis W: Das probiotische Konzept; in: *Probiotika*. Stuttgart, Hippokrates, 2008, pp 41–48.
- ▶ 18 Poisson DM, Borderon JC, Amorim-Sena JC, Laugier J: Evolution of the barrier effects against an exogenous drug-sensitive *Escherichia coli* strain after single or repeated oral administration to newborns and infants aged up to three months admitted to an intensive care unit. *Biol Neonate* 1986;49:1–7.
- ▶ 19 Lari AR, Gold F, Borderon JC, Laugier J, Lafont P: Implantation and in vivo antagonistic effects of antibiotic-susceptible *Escherichia coli* strain administered to premature newborns. *Biol Neonate* 1990;58:73–78.
- ▶ 20 Cukrowská B, Lodinová-Žádníková R, Enders C, Sonnenborn U, Schulze J, Tlaskalová-Hogenová H: Specific proliferative and antibody response of premature infants to intestinal colonization with non-pathogenic probiotic *E. coli* strain Nissle 1917. *Scand J Immunol* 2002;55:204–209.
- ▶ 21 Lodinová-Žádníková R, Slavíková M, Tlaskalová-Hogenová H, Adlerberth I, Hanson LA, Wold A: The antibody response in breast-fed and non-breast-fed infants after artificial colonization of the intestine with *E. coli* O83. *Pediatr Res* 1991;29:396–399.
- ▶ 22 Lodinová-Žádníková R, Cukrowská B, Tlaskalová-Hogenová H: Oral administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). *Int Arch Allergy Immunol* 2003;131:209–211.
- 23 Lodinová-Žádníková R, Tlaskalová H, Korych B, Bartáková Z: The antibody responses in infants after oral administration of inactivated and living *E. coli* vaccines and their protective effect against nosocomial infections; in Mestecky J (ed): *Advances of Mucosal Immunol*. New York, Plenum Press, 1995, pp 1434–1438.
- ▶ 24 Kocourková I, Žádníková R, Rosová V: Effect of oral colonization with a probiotic *E. coli* strain on the intestinal microflora of children of allergic mothers during the first year of life. *Folia Microbiol* 2007;52:189–193.
- 25 Isolauri E, Stas Y, Kankaanpää P, Arvilommi H, Salminen S: Probiotics: effects of immunity. *Am J Clin Nutr* 2001;73(suppl):444S–450S.
- ▶ 26 Rautava S: Potential use of probiotics in the neonate. *Semin Fetal Neonatal Med* 2006;12: 45–53.
- 27 Smart JM, Kemp AS: Increased Th1 and Th2 allergen-induced cytokine response in children with atopic disease. *Clin Exp Allergy* 2002;32:795–802.