

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Probiotics in primary prevention of allergic disease – follow-up at 8–9 years of age

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To cite this article: West CE, Hammarström M-L, Hernell O. Probiotics in primary prevention of allergic disease – follow-up at 8–9 years of age. *Allergy* 2013; DOI: 10.1111/all.12191.

Keywords

allergy prevention; asthma; eczema;
Lactobacillus paracasei ssp *paracasei* F19;
nitric oxide.

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Accepted for publication 25 April 2013

DOI:10.1111/all.12191

Edited by: Bodo Niggemann

Abstract

Background: Long-term effects of probiotics in primary prevention of allergic disease need further evaluation. We previously reported a reduced cumulative incidence of infant eczema by feeding *Lactobacillus paracasei* ssp *paracasei* F19 (LF19) during weaning. Therefore, we assessed effects of LF19 on the prevalence of allergic disease at school age.

Methods: In a double-blind placebo-controlled trial infants were randomized to daily intake of cereals with ($n = 89$) or without LF19 10^8 CFU ($n = 90$) from 4–13 months of age. At age 8–9, we evaluated the prevalence of allergic disease (eczema, allergic rhinitis, asthma, and food allergy) by clinical examination and validated questionnaires. IgE sensitization was assessed by skin prick test (inhalant allergens) and specific IgE levels (food allergens). Lung function was evaluated by a spirometry reversibility test. Fractional exhaled nitric oxide (FE_{NO}) was measured.

Results: Of 171 children that completed the intervention, 121 were assessed at age 8–9. In the probiotic group, 15/59 (25%) were diagnosed with any allergic disease vs 22/62 (35%) in the placebo group [OR (95% CI) 0.62 (0.28–1.36)]. Corresponding numbers for IgE-associated allergic disease were 9/53 (17%) vs 12/59 (20%) [0.80 (0.31–2.09)]. Median (25th–75th percentile) FE_{NO} was 9 (8–12) in the probiotic vs 8 (7–12) ppb in the placebo group ($P > 0.05$). There was no effect of LF19 on lung function measures ($P > 0.05$).

Conclusions: There was no long-term effect of LF19 on any diagnosed allergic disease, airway inflammation or IgE sensitization. This suggests delayed eczema onset but to fully examine long-term benefits a larger study population had been needed.

The increase in allergic disease has been attributed to environmental factors including disturbances in early gut colonization patterns (1, 2) and reduced gut microbial diversity (3–5) preceding the development of allergic disease. With the recognized role of gut microbiota in immune development and regulation (6, 7), there has been interest in shaping gut microbiota establishment with probiotics. Probiotics may, directly or indirectly, modulate the developing immune system and have been variously shown to have immune-stimulating effects (8). While both antenatal and postnatal factors may influence susceptibility to immune dysregulation (1), oral tolerance is established in the gut postnatally (9). The gut-associated lymphoid tissue (GALT) accounts for about 70% of the immune system, reflecting the huge immunological challenge conferred by the intestinal luminal contents. Gut microbiota are proposed to convey immune-regulating effects via complex pathways within and further than the GALT (6, 7, 9). During weaning, the maturing mucosal immune system is exposed to an increasing array of dietary antigens and a more complex gut microbiota. Therefore, we hypothesized that feeding the probiotic *Lactobacillus paracasei* ssp *paracasei* F19 (LF19) during weaning could be an effective tool in primary prevention of allergic disease. In our previous reports, LF19 reduced the risk of infant eczema (10) in conjunction with effects on adaptive

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T-cell development, whereas there was no effect on IgE sensitization (10, 11).

To date, clinical trials have assessed probiotics in primary prevention of allergic disease (namely eczema) with conflicting results (12, 13) but long-term follow-up of these study cohorts is not yet fully evaluated. Specifically, long-term follow-up (≥ 5 years of age) of respiratory allergic disease is limited to three reports that showed no benefit (14–16) although none of these studies evaluated lung function. Others have reported that synbiotics prevent asthma-like symptoms in children at high-risk of allergic disease (17) and that some probiotics lessen the severity of established asthma and reduce symptoms of allergic rhinitis in children (18).

Therefore, we assessed the long-term preventative effect of feeding LF19 during weaning on development of allergic disease, including respiratory allergies and airway inflammation, at school age. We also investigated if LF19 persisted in the gut microbiota.

Methods

The baseline study was conducted during 2000–2003 in Umeå, Sweden. The study was registered at www.clinicaltrials.gov (NCT 00894816). The design has previously been described in detail (10, 19). Briefly, 179 healthy, term infants with no prior allergic manifestations were randomized to daily intake of infant cereals with ($n = 89$) or without ($n = 90$) addition of LF19 1×10^8 CFU per serving from 4 to 13 months of age. Exclusion criteria were cesarean delivery and medications or supplements that could have affected the gut microbiota. The cereals were gradually introduced to all infants from 4 months, the recommended age for introducing complementary feeds at that time. By 6 months of age, the majority of infants had reached the target dose 1×10^8 CFU daily. Parents were asked not to give any other products containing probiotics during this period. No other dietary advice was given. The cereals contained cow's milk protein and were rice based from 4 to 6 months and wheat based from 6 to 13 months. Of 179 included infants, 171 completed the intervention phase.

The follow-up was conducted during 2009–2011 when children were 8–9 years old. Written and oral information was provided before enrollment, and written consent was signed by parents. Approval of the trial was obtained from the local ethics committee of Umeå University. Neither the study personnel nor the children or their parents were aware of group assignment. The unblinding was performed after the study was completed.

Clinical assessment

All children were assessed by one of two trained study pediatricians (Dr C. E. West or Dr M. Borgström) including an inspection of the eyes, ears, nose and skin, palpation of the abdomen, auscultation of the heart and lungs. Parents completed a modified questionnaire used in the Obstructive Lung disease in Northern Sweden (OLIN) pediatric cohort study, based on the ISAAC core questions with added questions on symptoms of wheeze, eczema, and other allergic conditions

(20). A skin prick test was carried out on the volar forearm. Allergens tested included *Dermatophagoides pteronyssinus* Derp1 and *Dermatophagoides farinae*, cladosporium, alternaria, birch, timothy, mugwort, dog, cat, horse, guinea pig, and rabbit (ALK-Abello, Hørsholm, Denmark). A test was considered positive if a wheal ≥ 3 mm was observed in response to any of the allergens in the presence of an appropriate response to the positive control (10 mg/mL histamine dihydrochloride; ALK-Abello) and no response to the negative control (allergen diluent; ALK-Abello). Specific IgE to cow's milk, egg white, wheat, codfish, and peanut was analyzed using ImmunoCap, Thermo Fisher scientific Phadia, Uppsala, Sweden, according to the manufacturer's instructions. Specific IgE > 0.35 kU/L was considered positive.

Lung functions tests

Spirometry was performed by the same trained allergy nurse in standardized manner (21, 22) with the exception that a nose clip was not used. Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured with a Spirare spirometer, using a Spirare SPS310 ultrasound sensor, Diagnostica AS, Oslo, Norway. Fifteen minutes after inhalation of 1.0 mg of terbutalin (Bricanyl, AstraZeneca, London, UK), the spirometry was repeated. Fractional exhaled nitric oxide (FE_{NO}) was measured using a NIOX Mino device, Aerocrine, Stockholm, Sweden. If there was an ongoing respiratory infection, tests were postponed until resolution of infection.

Diagnosis of allergic disease

Diagnoses were based on information from clinical assessments, questionnaires, and medical records. Current eczema was diagnosed in children with typical skin lesions responsive to topical steroids. The severity was assessed by the SCORing Atopic Dermatitis (SCORAD) index (23). Current asthma was diagnosed if the child had a physicians' diagnosis of asthma with wheeze that responded to bronchodilator therapy and/or a clinical history of wheeze and an increase in FEV₁ $> 12\%$ from baseline following terbutalin inhalation. Current allergic rhinitis was diagnosed if the child had a clinical history of symptoms suggestive of allergic rhinitis plus evidence of sensitization to inhalant allergens. Current IgE-mediated food allergy was diagnosed if there was a history of immediate (< 60 min) symptoms after contact with and/or consumption of food plus specific IgE levels > 0.35 kU/L to the implicated food. A child was classified as having current allergic disease if there was a diagnosis of eczema, asthma, allergic rhinitis, and/or food allergy. Current IgE-associated allergic disease included the former criteria plus confirmed IgE-association.

Randomly amplified polymorphic DNA-PCR (RAPD-PCR) for verification of LF19 in stool

Stool was collected by a spatula into a sterile tube and stored at home in the freezer before transportation to the hospital where samples were stored frozen at -70°C until analysis.

Stool samples were serially diluted and placed on Rogosa agar. Lactobacilli with colony and Gram-staining morphology similar to that of LF19 were analyzed by RAPD-PCR for verification (24).

Statistics

The sample size was restricted to children available for follow-up. Logistic regression was used to determine the odds ratio (OR) of developing each binary allergic outcome in the probiotic compared with the placebo group. Logistic regression was also performed adjusting for potential confounders (gestational age and family history of allergic disease). Questionnaire data were analyzed by the chi-squared test. Continuous data that were normally distributed were assessed by Student's *t*-test and by Mann–Whitney U-test when data were non-normally distributed. A *P*-value < 0.05 was considered statistically significant. Data were analyzed with SPSS Software version 20.0 (SPSS Institute, Inc, Chicago).

Results

Of 171 children that completed the baseline study, 121 (70.8%) entered the follow-up study at age 8–9 (Fig. 1). The proportion of children who had received probiotics (*n* = 59; 48.8%), and placebo (*n* = 62; 51.2%) was comparable (*P* > 0.05). As displayed in Table 1, there were no statistically significant differences between the groups in demographic characteristics except for mean gestation.

Clinical outcome measures

At age 8–9, there were no statistically significant differences between the groups in any diagnosed allergic disease (Table 2). In agreement with our previous report, fewer

children were diagnosed with current eczema (10) and current IgE-associated eczema in the probiotic compared with the placebo group although the difference was not statistically significant. All children had mild–moderate eczema (SCORAD <40). The reported frequency of ‘ever eczema’ defined as ‘an itchy rash persisting for at least 6 months affecting the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes’ was comparable: 16/59 (27.1%) in the probiotic and 19/62 (30.6%) in the placebo group (*P* = 0.67). In the probiotic group, 8/59 (13.6%) reported ‘ever food hypersensitivity’ vs 8/62 (12.9%) in the placebo group (*P* = 0.92). The corresponding numbers for ‘ever food allergy’ was 5/59 (8.5%) vs 6/62 (9.7%) (*P* = 0.82). More parents reported ‘ever wheeze’ (ever wheezing or whistling in the chest) in the probiotic 22/59 (37.3%) compared with 14/62 (22.6%) the placebo group, and this was borderline significant (*P* = 0.08). Conversely, there was no difference in the frequency of children ever diagnosed with asthma: 12/59 (20.3%) in the probiotic and 12/62 (19.4%) in the placebo group (*P* = 0.89). Further, there was no difference in the frequency of children that had ever had rhinitis symptoms (i.e., suffered from sneezing, runny nose or nose blocking in the absence of a cold): 8/58 (13.8%) in the probiotic vs 6/62 (9.7%) in the placebo group (*P* = 0.48). At age 8–9, there was no difference in the frequency of diagnosed respiratory allergic disease between the groups (Table 2). Adjusting for additional potential confounders (maternal or bi-parental allergy, adherence to treatment, antibiotic use, regular probiotic use) did not substantially affect the clinical outcomes (data not shown).

Lung function measures

Mean (SD) FVC at baseline was comparable: 2.2 (0.4) and 2.2 (0.3) L in the probiotic and placebo groups, respectively

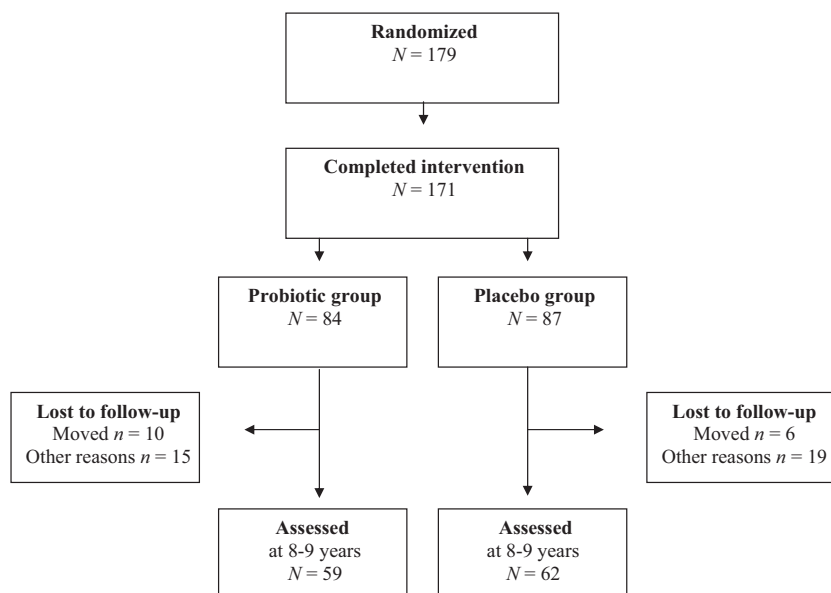


Figure 1 Flow chart of the study participants.

($P = 0.90$). The corresponding number for FEV₁ was 1.8 (0.3) L in both groups ($P = 0.95$). In the probiotic group, 1/58 (2%) of the children had a postbronchodilator change

>12% vs 5/59 (9%) in the placebo group ($P = 0.27$). Next, we evaluated FE_{NO} as a marker of airway inflammation. Median (25th–75th percentile) FE_{NO} was 9 (8–12) in the probiotic vs 8 (7–12) ppb in the placebo group ($P = 0.26$).

Table 1 Characteristics of the study population

Characteristic	Probiotic (<i>n</i> = 59)	Placebo (<i>n</i> = 62)	<i>P</i> -value
Female	61.0%	53.2%	0.39
Birth weight (g), mean (SD)	3780 (516)	3640 (484)	0.13
Gestational age (weeks), mean (SD)	40.5 (1.2)	40.0 (1.3)	0.02
Maternal allergic disease	45.8%	40.3%	0.55
Paternal allergic disease	34.5%*	43.5%	0.31
Both parents allergic disease	19.0%*	22.6%	0.63
Sibling with allergic disease	40.7%	48.4%	0.39
Any first grade relative with allergic disease	72.9%	75.8%	0.71
Mother university degree	66.1%	69.4%	0.70
Father university degree	44.8%*	51.6%	0.46
Maternal smoking	6.8%	9.7%	0.74
Paternal smoking	5.3%†	6.6%†	1
Never lived on a farm	93.2%	96.8	0.43
Furred pets at home at present	52.5%	53.2%	0.94
Never furred pets at home	28.8%	30.6%	0.83
Firstborn	54.2%	48.4%	0.52
Siblings	96.6%	91.9%	0.44
Ever breastfed	94.9%	95.2%	1
Total breastfeeding (months), mean (SD)	7.9 (3.9)	7.9 (4.2)	0.97
Antibiotics during intervention	16.9%	32.3%	0.05
Current intake of probiotics	28.8%	21.0%	0.32

*Missing information on one father.

†Missing information on two and one fathers in the probiotic and placebo group, respectively.

Categorical data were assessed by chi-squared test or Fisher's exact test. Continuous data were assessed by Student's *t*-test. Bold font indicates statistically significant relationship.

LF19 is a transient colonizer

Lactobacilli were cultivated in 49/50 (98%) of the samples in the probiotic compared with 54/54 (100%) in the placebo group. LF19 was not identified in any sample, despite plates displaying colonies with a morphology typical of LF19. The molecular confirmation of these colonies showed that the DNA fingerprint was not identical to LF19.

Discussion

The preventative effect of LF19 on infant eczema was not sustained at school age, and there was no long-term benefit on any diagnosed allergic or IgE-associated allergic disease. Specifically, there was no effect of LF19 on respiratory allergic disease, lung function measures, or FE_{NO}. We also investigated whether LF19 had persisted in the gut microbiota but LF19 was not isolated in any analyzed stool sample. Collectively, this suggests delayed onset of eczema but no long-term benefit of LF19 and supports the view that probiotics are transient colonizers, also when administered before gut microbiota are fully established.

In agreement with our previous report, fewer children were diagnosed with eczema (10) and IgE-associated eczema at school age in the probiotic compared with the placebo group, although the difference was no longer statistically significant. The frequency was lower than anticipated as the 12-month prevalence of reported eczema in this age group was 26% in an unselected Swedish cohort (25). However, the reported frequency of children with 'ever eczema' was comparable which suggests that the previously observed benefit of LF19 was delayed onset of eczema. In agreement, Niers et al. (26) reported a reduced cumulative incidence of eczema at 3 months in infants receiving a probiotic combination

Table 2 Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the main clinical outcomes in children assessed at age 8–9 years

Clinical outcome	Probiotic	Placebo	OR (95% CI)	<i>P</i> -value	aOR* (95% CI)	<i>P</i> -value
Allergic disease	15/59 (25%)	22/62 (35%)	0.62 (0.28–1.36)	0.23	0.71 (0.31–1.60)	0.41
IgE-associated allergic disease	9/53 (17%)	12/59 (20%)	0.80 (0.31–2.09)	0.65	0.96 (0.35–2.63)	0.94
Eczema	7/59 (12%)	11/61 (18%)	0.61 (0.22–1.70)	0.35	0.71 (0.25–2.06)	0.53
IgE-associated eczema	4/53 (8%)	7/59 (12%)	0.61 (0.17–2.20)	0.45	0.74 (0.20–2.84)	0.67
Asthma	7/59 (12%)	7/62 (11%)	1.06 (0.35–3.22)	0.9	0.98 (0.31–3.09)	0.98
IgE-associated rhinitis	6/57 (11%)	5/60 (8%)	1.62 (0.44–5.91)	0.47	1.62 (0.44–5.91)	0.47
IgE-associated rhinitis and/or asthma	6/57 (11%)	7/60 (12%)	0.89 (0.28–2.83)	0.85	0.96 (0.29–3.12)	0.94
IgE-associated food allergy	2/54 (4%)	2/60 (3%)	1.05 (0.14–7.73)	0.96	NC†	
Sensitization (any)‡	17/53 (32%)	16/59 (27%)	1.27 (0.56–2.86)	0.57	1.52 (0.64–3.61)	0.34
Sensitization (foods)	9/54 (17%)	10/60 (17%)	1.00 (0.37–2.68)	1.00	1.16 (0.41–3.22)	0.78
Sensitization (inhalants)	15/58 (26%)	14/60 (23%)	1.15 (0.50–2.65)	0.75	1.33 (0.55–3.22)	0.52

*Allergic heredity (any first grade relative) and gestational age were included in the logistic regression model.

†NC, Not calculated because of few cases.

‡Refers to children who were tested for sensitization to both food (specific IgE > 0.35 kU/L and inhalant allergens (skin prick test positive).

perinatally compared with placebo. This benefit was not continual at 1 and 2 years of age.

Probiotics for primary prevention of allergy (namely eczema) have been investigated in clinical trials with differing results (12, 13). To date, long-term follow-up ≥ 5 years of age of these cohorts are limited to three reports; Kalliomäki et al. (14) reported a sustained effect of perinatal administration of *L. rhamnosus* GG (LGG) on eczema at 7 years, with no effect on respiratory allergies, while Jensen et al. observed no benefit of postnatal administration of a *L. acidophilus* strain at 5 years (15). Prenatal administration is sometimes argued as most efficacious (12, 13) but despite pre- and postnatal administration of probiotics plus galactooligosaccharides, there was no long-term benefit at age 5 in the large-scale trial by Kuitunen et al. (16). However, children born by cesarean section had decreased risk of IgE-associated allergic disease, suggesting that this subgroup of infants with altered gut colonization (27) and increased risk of respiratory allergic disease (28) might benefit in a long-term perspective. Thus far, no other clinical trial has evaluated LF19 for allergy prevention or evaluated if a prenatal component would have added to the benefit. For comparison, colonization with a group of lactobacilli (*L. casei*, *L. paracasei* and *L. rhamnosus*) during the first months of life was associated with reduced risk of allergic disease at age 5 in a Swedish observational study (29). In another birth cohort study, early colonization with *L. paracasei*, but not *L. rhamnosus* or *L. acidophilus*, was associated with reduced risk of eczema at age 2 years but not IgE sensitization (30).

Although a causal relationship has not been established, higher frequencies of wheeze (31) and trends toward higher frequencies of asthma and rhinitis in LGG-treated children (14, 32) have been reported. We observed a trend that 'ever wheeze' was more frequent in the probiotic group, with no difference between the groups in diagnosed asthma. It is of note that we observed neither benefit nor harm of probiotics on lung function measures or airway inflammation. In agreement (33), there was no long-term effect of synbiotics on airway inflammation at age 5.

During the intervention, the presence of LF19 in stool was confirmed in the majority of infants ingesting LF19 (19). Lactobacilli are transient colonizers but permanent establishment has been suggested if administered early in life. As LF19 was not detected in any of the analyzed samples at age 8–9, this supports the view that LF19 is a transient colonizer. In the baseline study, we observed less antibiotic use in the probiotic group (19). Whether this had an impact on the developing gut microbiome, with possible links to the reduced risk of infant eczema (10), is under study.

There are potential limitations to this study. A loss to follow-up with reassessment of 70% of the original study population might have biased the results. Also, the majority of reported clinical trials evaluating probiotics in primary prevention of allergic disease have been small-scale studies (13) including this study and may therefore not be sufficiently powered to detect long-term effects. To be able to examine if the lower frequency of the primary outcome 'any allergic disease' (25% in the probiotic vs 35% in the placebo

group) is an effect of the intervention, a sample size of 350 per group would have been needed ($\alpha = 5\%$ and power 80%). Consequently, conservative interpretation of the study findings is needed. Nevertheless, it remains important to report long-term outcomes of these already initiated cohorts. A recent report suggested long-term benefit (at 4 years) not only on eczema but also on rhinoconjunctivitis in children treated perinatally with a *L. rhamnosus* but not a *Bifidobacterium* strain (34). Firstly, this suggests that effects are species dependent, and secondly, that the primary preventative effects may go beyond eczema.

In summary, there was no long-term benefit of LF19 on any diagnosed allergic disease, IgE-associated allergic disease, or airway inflammation. Despite these findings and the inconsistent results in allergy prevention studies (12, 13), we infer that the probiotic concept needs further evaluation. We need to define optimal strains or combination of strains, timing and subpopulations most likely to benefit (12, 13, 35). For this, we need sufficiently powered clinical trials with predefined outcomes.

Acknowledgments

We wish to acknowledge the participating families; RN Åsa Sundström for excellent assistance in the follow-up clinics; Allergy nurse Birgitta Domeij for excellent assistance with lung function and skin prick testing; Dr Malin Borgström for valuable help in the follow-up clinics; Dr Janet Håkansson for administering the RAPD-PCR analyses and Dr Maria Hedberg and Ms Elisabet Granström for cultivation of lactobacilli; Ms Michelle Norman for help with data entry; Ass professor Hans Stenlund for statistical advice. This study was supported by Arla Foods AB, Sweden, through regional agreement between Umeå University and Västerbotten county council on cooperation in the field of Medicine; European Union's Seventh Frame Work Programme under grant agreement no 222720; Swedish Society for Medical research; Swedish Asthma and Allergy Association; the Ekhaga and Oskar foundations; Insamlingsstiftelsen at Umeå University. Sponsors had no involvement in the study design, collection, analysis, and interpretation of data, in the writing of the report or in the decision to submit the paper for publication.

Author contributions

Christina E. West designed, coordinated, and supervised the study, performed the analysis, and prepared the manuscript. Olle Hernell was involved in the design of the study and provided intellectual input. Marie-Louise Hammarström provided intellectual input.

Conflicts of interest

Dr West has received funding and speaker honorarium from Arla Foods; speaker honorarium and travel assistance to attend a conference from Nestlé Nutrition. Prof. Hernell has received funding from Semper AB and Arla Foods. Prof. Hammarström declares no conflict of interest.

References

- West CE, D'Vaz N, Prescott SL. Dietary immunomodulatory factors in the development of immune tolerance. *Curr Allergy Asthma Rep* 2011;**11**:325–333.
- Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. *Curr Opin Pediatr* 2009;**21**:794–800.
- Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012;**129**:434–440, 40 e1–2.
- Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. *J Allergy Clin Immunol* 2008;**121**:129–134.
- Bisgaard H, Li N, Bonnelykke K, Chawes BL, Skov T, Paludan-Müller G et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol* 2011;**128**:646–652. e1–5.
- Brandtzaeg P. The gut as communicator between environment and host: immunological consequences. *Eur J Pharmacol* 2011;**668** (Suppl 1):S16–S32.
- Tsuji NM, Kosaka A. Oral tolerance: intestinal homeostasis and antigen-specific regulatory T cells. *Trends Immunol* 2008;**29**:532–540.
- Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. *J Allergy Clin Immunol* 2007;**120**:255–262.
- Verhasselt V. Oral tolerance in neonates: from basics to potential prevention of allergic disease. *Mucosal Immunol* 2010;**3**:326–333.
- West CE, Hammarström ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol* 2009;**20**:430–437.
- West CE, Hernell O, Andersson Y, Sjöstedt M, Hammarström ML. Probiotic effects on T-cell maturation in infants during weaning. *Clin Exp Allergy* 2012;**42**:540–549.
- Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R et al. Clinical use of probiotics in pediatric allergy (CUPPA): a World Allergy Organization position paper. *World Allergy Organ J* 2012;**5**:148–167.
- West CE, Prescott SL. Prebiotics and probiotics in prevention of allergic disease. In: Basedow D. (editor), *UptoDate*, Waltham, MA, 2013. Available at: www.uptodate.com. Last accessed 10 January 2013.
- Kalliomäki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007;**119**:1019–1021.
- Jensen MP, Meldrum S, Taylor AL, Dunstan JA, Prescott SL. Early probiotic supplementation for allergy prevention: Long-term outcomes. *J Allergy Clin Immunol* 2012;**130**:1209–1211.
- Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;**123**:335–341.
- van der Aa LB, van Aalderen WM, Heymans HS, Henk Sillevs Smitt J, Nauta AJ, Knippels LM et al. Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis. *Allergy* 2011;**66**:170–177.
- Chen YS, Jan RL, Lin YL, Chen HH, Wang JY. Randomized placebo-controlled trial of lactobacillus on asthmatic children with allergic rhinitis. *Pediatr Pulmonol* 2010;**45**:1111–1120.
- West CE, Gotheffors L, Granström M, Käyhty H, Hammarström ML, Hernell O. Effects of feeding probiotics during weaning on infections and antibody responses to diphtheria, tetanus and Hib vaccines. *Pediatr Allergy Immunol* 2008;**19**:53–60.
- Rönmark E, Lundbäck B, Jonsson E, Platts-Mills T. Asthma, type-1 allergy and related conditions in 7- and 8-year-old children in northern Sweden: prevalence rates and risk factor pattern. *Respir Med* 1998;**92**:316–324.
- Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R et al. General considerations for lung function testing. *Eur Respir J* 2005;**26**:153–161.
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al. Standardisation of spirometry. *Eur Respir J* 2005;**26**:319–338.
- Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993;**186**:23–31.
- Björneholm S, Eklöv A, Saarela M, Mättö J. Enumeration and identification of Lactobacillus F19. *Microb Ecol Health Dis* 2002;**14**(Suppl 3):7–13.
- Rönmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundbäck B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol* 2009;**124**:357–363, 63 e1–15.
- Niers L, Martin R, Rijkers G, Sengers F, Timmerman H, van Uden N et al. The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy* 2009;**64**:1349–1358.
- Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006;**118**:511–521.
- Håkansson S, Källen K. Caesarean section increases the risk of hospital care in childhood for asthma and gastroenteritis. *Clin Exp Allergy* 2003;**33**:757–764.
- Johansson MA, Sjögren YM, Persson JO, Nilsson C, Sverremark-Ekström E. Early colonization with a group of Lactobacilli decreases the risk for allergy at five years of age despite allergic heredity. *PLoS ONE* 2011;**6**:e23031.
- Penders J, Thijs C, Mommers M, Stobberingh EE, Dompeling E, Reijmerink NE et al. Intestinal lactobacilli and the DC-SIGN gene for their recognition by dendritic cells play a role in the aetiology of allergic manifestations. *Microbiology* 2010;**156**:3298–3305.
- Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics* 2008;**121**:e850–e856.
- Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;**361**:1869–1871.
- Kukkonen AK, Kuitunen M, Savilahti E, Pelkonen A, Malmberg P, Mäkelä M. Airway inflammation in probiotic-treated children at 5 years. *Pediatr Allergy Immunol* 2011;**22**:249–251.
- Wickens K, Black P, Stanley TV, Mitchell E, Barthow C, Fitzharris P et al. A protective effect of Lactobacillus rhamnosus HN001 against eczema in the first 2 years of life persists to age 4 years. *Clin Exp Allergy* 2012;**42**:1071–1079.
- Hernell O, West CE. Clinical effects of probiotics: scientific evidence from a paediatric perspective. *Br J Nutr* 2013;**109** (Suppl 2):S70–S75.