Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation

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Clinical and Experimental Allergy

Summary

Background Probiotics are widely studied both in the treatment and prevention of allergic diseases, but their mode of action is poorly known.
Objective Our aim was to examine the effect of probiotic bacteria on in vivo cytokine, antibody, and inflammatory responses in allergy-prone infants.
Methods In a randomized double-blind study, probiotic bacteria or placebo were given for 1 month before delivery to mothers and for 6 months to infants with a family history of allergy. Plasma samples were analysed for C-reactive protein (CRP), total IgA and IgE, food-specific IgA, IgG, and IgE, IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ. We analysed the associations of immunological and inflammatory parameters at age 6 months with probiotic treatment and allergic phenotype at 2 years.
Results Infants receiving probiotic bacteria had higher plasma levels of CRP (P = 0.008), total IgA (P = 0.016), total IgE (P = 0.047), and IL-10 (P = 0.002) than infants in the placebo group. Increased plasma CRP level at age 6 months was associated with a decreased risk of eczema [odds ratio (OR) 0.41 [95% confidence interval (CI) 0.17–0.99], P = 0.046], and with a decreased risk of allergic disease [OR 0.38 (95% CI 0.16–0.87), P = 0.023] at age 2 years, when adjusted with probiotic use.
Conclusion The association of CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the view that chronic, low-grade inflammation protects from eczema. Probiotic-induced low-grade inflammation was characterized by elevation of IgE, IgA, and IL-10, the changes typically observed in helminth infection-associated induction of regulatory mechanisms. The findings emphasize the role of chronic microbial exposure as an immune modulator protecting from allergy.

Keywords atopic, CRP, IgA, IgE, IL-10, oral tolerance, probiotic, regulatory cells

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Introduction

Probiotic bacteria, which are defined as ‘live microorganisms administered in adequate amounts that confer a health effect on the host [1],’ have been suggested as therapeutic agents in atopic and other immune-mediated diseases with failure of tolerance. Despite the positive clinical effects on the prevention and treatment of atopic diseases [2–7], the mechanisms of probiotics are poorly understood. No specific effect on the allergy-associated IgE response has been reported in the prevention or treatment studies. The possible effects of bacterial strains on immune responses are poorly known in vivo.

Several studies, mainly animal experiments, support the hypothesis that exposure to intestinal bacterial strains during infancy stimulates the maturation of the intestinal immune system, induction of oral tolerance being a pivotal part in this process [8, 9]. A reduced number of T cells in gut-associated lymphoid tissue have been reported in germ-free mice compared with specific pathogen-free mice. Furthermore, the low number of T cells was related to the failure of oral tolerance induction, and a recovery in the number of T cells was seen when mice were colonized by Bifidobacteria infantis and Escherichia coli [8, 9].

Mostly based on in vitro studies, probiotic bacteria or their components have been suggested to affect the
stimulation of T-helper type 1 (Th1) or regulatory cytokines, which more specifically control allergic Th2-deviated phenotype. In vitro studies have shown that gram-positive bacteria, such as lactobacilli, activate expression of pro-inflammatory cytokines, such as IL-12, IFN-γ, TNF-α, and IL-6 [10, 11] and regulatory cytokine IL-10 [10] in human peripheral blood mononuclear cell (PBMC) cultures. In allergic infants, dietary supplementation with probiotic bacteria also enhances IFN-γ and IL-4 secretion of stimulated PBMC, but the activation pattern seems to depend on bacterial strain [12]. Some small clinical studies found a decrease in the inflammatory markers, such as fecal TNF-α, soluble serum CD4 and urine eosinophilic protein X, in association with probiotic treatment of allergies [4, 13]. On the other hand, we recently showed that probiotic treatment in infants with atopic eczema induced production of C-reactive protein (CRP) [14], as a marker of non-specific acute-phase response to inflammation. We infer that inflammation may act as the link between probiotics and oral tolerance.

Because the in vitro effects of probiotics are not understood, we aimed to investigate the mechanisms of probiotic preparation consisting of a mixture of bacterial strains and found to protect from eczema when given for 1 month before delivery to mothers and for 6 months to infants with a family history of allergy [15]. We looked at the associations of immunological and inflammatory parameters at age 6 months after probiotic/placebo treatment and allergic phenotype at 2 years.

Materials and methods

Study groups

We studied the in vitro effects of probiotics in a sub-population of 98 randomly selected healthy infants participating in a clinical study on the role of probiotics in the prevention of allergic diseases [15, 16]. A total of 1223 mothers were randomized into this double-blind and placebo-controlled study between November 2000 and March 2003 at the Skin and Allergy Hospital of Helsinki University Central Hospital; 925 infants completed the study. Infants had a high genetic risk of allergy; at least one parent had a doctor-diagnosed allergic disease: asthma, allergic rhinitis (AR), or atopic eczema. Mothers were randomized according to a computer-generated block randomization to receive either two capsules a day of a mixture of four bacterial species [Lactobacillus rhamnosus GG (ATCC 53103) 5 × 10^9 colony-forming units (CFU), L. rhamnosus LC705 5 × 10^9 CFU, Bifidobacterium breve BB9 2 × 10^9 CFU, and Propionibacterium freudenreichii ssp. Shermanii JS 2 × 10^9 CFU in a capsule] or an inert microcrystalline cellulose (placebo). Their infants received the same capsules as the mothers once a day: those given probiotics mixed with sugar syrup containing 0.8 g of prebiotic sugars (galacto-oligosaccharides) and those in the placebo group mixed inert cellulose with sugar syrup only. Mothers received these products for 2–4 weeks before delivery, and their infants for the first 6 months. The products looked, smelled, and tasted the same. The ethics committee of the Hospital for Children and Adolescents, University of Helsinki, approved the study protocol. Written, informed consent was obtained from parents for sample collection and analyses. An external evaluator performed the statistical analyses because the randomization code was not opened in this study.

Clinical follow-up and diagnoses

At 3, 6, and 24 months, a paediatrician blinded to group assignment examined the infants clinically. Any notification by parents during the follow-up period led to clinical examination of infants with symptoms of eczema. Eczema was defined as an itchy skin condition with a dry skin and eczema at typical sites [17, 18]. The severity of eczema was evaluated using the SCORAD score [19].

At 2 years, skin prick tests (SPTs) were performed [20] with egg white, fish (Stallergenes, Antony, France), cat, dog, birch, timothy (ALK-Abello®, Horsholm, Denmark), cow’s milk (CM), and wheat grains (diluted in 0.9% NaCl). Weal diameter > 3 mm greater than the negative control was considered to be positive. At 2 years, a blood sample was drawn and allergen-specific IgE against milk, egg white, birch, timothy, dog, and cat was measured by immunoassay (ImmunoCAP® system, Pharmacia diagnostics, Uppsala, Sweden) with detection limit 0.01 kU/L. Children with a positive SPT to any allergen or any serum allergen-specific IgE concentration of > 0.7 kU/L were considered to be IgE sensitized.

Food allergy was defined as a positive open food challenge after a successful elimination diet [21]. Asthma was defined as two or more doctor-diagnosed wheezy episodes, accompanied by persistent cough or exercise-induced symptoms between the episodes [22]. AR was based on typical symptoms, and a positive SPT to pollens or animal dander or both as in the diagnostic criteria of the International Rhinitis Management Group [23]. Children with eczema, asthma, food allergy, or AR were considered to have allergic disease.

Sample preparation

Blood samples were collected at the age of 6 months (n = 98). Plasma samples of peripheral blood were obtained by centrifugation of the EDTA blood, and were stored at − 50 °C until the analysis.

C-reactive protein

CRP concentrations in plasma samples were measured using human CRP Instant ELISA (Bender MedSystems,
Vienna, Austria) according to the manufacturer’s instructions (detection limit 78 pg/mL).

Cytokine measurements

IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ concentrations were determined by a cytokine bead assay (CBA). The cytokine capture beads were incubated with plasma samples or standards and mixed with the PE-conjugated detection antibodies according to the manufacturer’s instructions (BD Biosciences Pharmingen, San Diego, CA, USA). Cytokine concentrations were measured on a FACScan flow cytometer (CellQuest software, BD Biosciences Pharmingen) with a detection limit of 2.6 pg/mL for IL-2 and IL-4, 3.0 pg/mL for IL-6, 2.8 pg/mL for IL-10 and TNF-α, and 10 pg/mL for IFN-γ. Results were analysed with BD CBA analysis software MAC OS version 9.

Immunoglobulin and antibody measurements

Quantities of CM, α-casein-, and ovalbumin (OVA)-specific IgA antibodies and IgG, and total IgA in plasma samples were measured with ELISA [24, 25]. Concentrations of plasma total IgE, and CM-, and egg white-specific IgE were measured by the Pharmacia UniCAP fluoroenzyme immunoassay (Pharmacia Diagnostics) with a detection limit of 0.35 kU/L.

Statistics

All distributions of plasma CRP and cytokines at the age of 6 months were skewed to the right and were logarithmically (log_{e}) transformed before analysis. Before transformations, the values below the detection limit were replaced by the detection limit divided by 2. The results are given as geometric means with 95% confidence intervals (CIs), and the probiotic group was compared with the placebo group using ANOVA. The comparison was carried out using all infants and in allergic subgroups. The Spearman rank correlations between plasma CRP and cytokines were calculated. The chronological order of measurements was taken into consideration using plasma CRP and cytokines at the age of 6 months as explaining variables and allergic diseases by the age of 2 years as end-points. The plasma CRP and cytokine concentrations were divided into categories (e.g. below vs. above median concentration) and it was analysed whether the level of CRP or cytokine measurements are associated with the diagnosis of allergic diseases. The group with a higher concentration was compared with the group with a lower concentration using logistic regression analysis. The treatment group (probiotic treatment vs. placebo) was included as a categorical covariate. In addition, the analysis was performed including only the placebo group, in order to avoid the possible confounding effect of probiotic treatment. The results are given as odds ratios (OR) with 95% CI. The Mann–Whitney U-test was used for variables with non-normal distributions. Baseline characteristics were analysed using the χ² test or the t-test for independent samples, when appropriate. Statistical analyses were performed using the SPSS for Windows (version 15.0, SPSS Inc., Chicago, IL, USA). Statistical significance was defined as a P-value ≤0.05.

Results

Allergic diseases and atopic sensitization at 2 years

Of the 98 high-risk infants, 41 (42%) were diagnosed with allergic disease and 34 (35%) with eczema, 30 (31%) were IgE-sensitized against at least one allergen, and 46 (47%) had no allergic symptoms or IgE sensitization (non-allergic group). The diagnoses by treatment group were 42% vs. 41% (probiotic vs. placebo) for allergic disease, 31% vs. 39% for eczema, 35% vs. 26% for IgE sensitization and 47% vs. 48% for non-allergy. Among infants with eczema, the SCORAD median score was 14.5 (range: 4–36) in the probiotic group and 17.6 (range: 6–38) in the placebo group (by Mann–Whitney U-test P = 0.564). In the whole study population in the probiotic and placebo groups, abdominal discomfort, vomiting, or excessive crying occurred in, respectively 8% and 8.5% infants. No major side effects were observed. The baseline characteristics between the study groups did not differ (Table 1).

C-reactive protein at age 6 months

Plasma CRP levels were significantly higher in the probiotic than in the placebo group in the whole study population (P = 0.008), in children with any allergic disease (P = 0.006), and in children with eczema at age 2 years (P = 0.008), but not in non-allergic children or in IgE-sensitized children (Table 2). Increased plasma CRP level (> median) at age 6 months was associated with a

| Table 1. Clinical characteristics of study infants in the probiotic (n = 52) and placebo (n = 46) groups |
|---------------------------------------------------|---------------------------------------------------|
| Birth weight (g)*                              | 3586 ± 411                                       | 3590 ± 471                                       |
| Male sex (%)                                     | 29 (56)                                          | 26 (57)                                          |
| Maternal allergy (%)                             | 38 (73)                                          | 36 (78)                                          |
| Paternal allergy (%)                             | 34 (65)                                          | 32 (70)                                          |
| Both parents allergic (%)                        | 20 (39)                                          | 22 (48)                                          |
| Delivery by Caesarean section (%)               | 6 (12)                                           | 7 (15)                                           |
| Any siblings (%)                                 | 26 (50)                                          | 20 (44)                                          |
| Exclusively breastfed (≥ 4 months)              | 15 (29)                                          | 10 (22)                                          |
| Total breastfed (≥ 6 months)                    | 35 (70)                                          | 26 (57)                                          |
| Antibiotics during intervention (%)             | 13 (25)                                          | 18 (40)                                          |

*Mean ± SD.
Cytokine levels at age 6 months

Plasma IL-10 concentrations were higher in the probiotic than in the placebo group in the whole study population ($P = 0.002$) (Table 4), and IL-10 correlated positively with plasma CRP ($r = 0.37$, $P = 0.000$). Plasma IL-10 concentrations were higher in the probiotic than in the placebo group in children who developed any allergic disease ($P = 0.001$) and eczema ($P = 0.001$) and in IgE-sensitized children at age 2 years ($P = 0.007$), whereas no increased IL-10 levels were seen in non-allergic children who received probiotics (Table 4). Plasma IL-10 levels at age 6 months did not show any association with the risk of eczema, allergic disease, or IgE sensitization at age 2 years, when adjusted with probiotic use (Table 3). Neither were plasma IL-10 values at age 6 months in the placebo group associated with the risk of eczema or with the IgE sensitization at age 2 years. In the placebo group with lower IL-10 ($<\text{median}, n = 27$), the prevalence of non-allergic infants was 40.7%, while in the group of higher IL-10 ($>\text{median}, n = 19$) the prevalence was 57.9%. The prevalence of eczema was 44.4% and 31.6% [OR = 0.58 (95% CI 0.17–1.97), $P = 0.577$] and that of IgE sensitization was 29.6% and 21.1% [OR = 0.63 (95% CI 0.16–2.51), $P = 0.516$] in groups with lower and higher IL-10, respectively. Most of the plasma IL-2, IL-4, IL-6, IFN-γ, and TNF-α values were below the detection limits (data not shown).

**Table 2. Plasma CRP levels (mg/L)**

<table>
<thead>
<tr>
<th></th>
<th>Probiotic</th>
<th>Placebo</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All infants</td>
<td>0.19 (0.13–0.27)</td>
<td>0.09 (0.07–0.13)</td>
<td>0.008</td>
</tr>
<tr>
<td>Non-allergic</td>
<td>0.21 (0.12–0.37)</td>
<td>0.14 (0.08–0.23)</td>
<td>0.281</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>0.16 (0.09–0.28)</td>
<td>0.05 (0.03–0.08)</td>
<td>0.006</td>
</tr>
<tr>
<td>Eczema</td>
<td>0.18 (0.09–0.39)</td>
<td>0.06 (0.04–0.09)</td>
<td>0.008</td>
</tr>
<tr>
<td>IgE sensitized</td>
<td>0.19 (0.10–0.36)</td>
<td>0.11 (0.06–0.19)</td>
<td>0.207</td>
</tr>
</tbody>
</table>

$n$ represents the number of samples.

Probiotic preparation vs. placebo in plasma samples at age 6 months. Geometric means, 95% CI.

CI, confidence interval; CRP, C-reactive protein.

Immunoglobulin A, immunoglobulin E, and antibody levels at age 6 months

The total IgA levels in plasma were significantly higher in the probiotic than in the placebo group in the whole study population ($P = 0.016$) (Table 5). The total IgE levels in plasma samples were higher in the probiotic than in the placebo group in the whole study population ($P = 0.047$) (Table 5). This phenomenon was seen among those remaining non-allergic ($P = 0.042$), but not in children developing allergic disease, eczema, or IgE sensitization at age 2 years (Table 5). A higher plasma total IgA level at age 6 months was associated with a higher risk of eczema at age 2 years [OR 3.74 (95% CI 1.48–9.43), $P = 0.005$], but not with a risk of allergic disease or IgE sensitization (Table 3).

The total IgE levels in plasma samples were higher in the probiotic than in the placebo group in the whole study population ($P = 0.000$) (Table 5). Probiotic-induced increase of IgA was seen in the non-allergic group ($P = 0.050$), but not in children with eczema, allergic disease, or with IgE sensitization at age 2 years (Table 5). A higher plasma total IgA level at age 6 months was associated with a decreased risk of eczema at age 2 years [OR 0.38 (95% CI 0.16–0.87), $P = 0.046$], and with a decreased risk of allergic disease [OR 0.38 (95% CI 0.16–0.87), $P = 0.023$] at age 2 years, when adjusted with probiotic use. No association was detected between plasma CRP level and IgE sensitization (Table 3).

When CRP levels were analysed separately in the placebo group, higher levels ($>\text{median}$) were associated with a decreased risk of eczema at age 2 years [OR 0.25 (95% CI 0.07–0.94), $P = 0.041$], but not with the risk of IgE sensitization [OR 1.02 (95% CI 0.27–3.88), $P = 0.976$].

**Discussion**

Our results show that probiotics when given to the infants are not innocent by-standers, but induce inflammation and act as immunomodulators with paradoxical stimulation of a Th2-type response. We showed that a mixture of probiotic bacteria induced in vitro increased plasma levels of CRP, IL-10, total IgA, and total IgE in infants with an allergic predisposition. Increased plasma CRP levels at age 6 months were associated with a decreased risk of eczema
at age 2. The CRP-associated decrease in the risk of eczema was not only seen in children who received probiotics but also in children on placebo. We infer that low-grade inflammation in infancy generates protection from eczema and we emphasize the role of inflammation in the control of tolerance. Probiotics used in the present study had a significant impact on the early development of allergic symptoms as demonstrated in the same study population earlier [15]. In our clinical study, which included 925 children, the cumulative prevalence of eczema and atopic eczema by age 2 years was reduced in the children who received probiotics [15]. Here, we studied a subgroup of 98 children to evaluate the immunological mechanisms of probiotics, and because our study group represents only about 10% of the whole study population it was not possible to demonstrate clinical differences between the treatment groups. It should be emphasized that probiotic bacteria had no adverse effects on infants despite the observed induction of CRP. Plasma CRP levels associated with protection from eczema were far below the clinically relevant values used to screen for bacterial infections.

Our findings in infants at risk of allergy are in good agreement with our previous study of children, who received probiotics for treatment of allergy. In 7.5-month-old infants with IgE-associated atopic eczema, we observed a simultaneous increase in CRP and IL-6 during treatment with *Lactobacillus* GG [14]. Here, the probiotic-induced increase of CRP was seen only in children with the allergic phenotype, but not in the children who remained non-allergic. Compared with those developing allergy, non-allergic children had higher levels of CRP in the placebo group. Thus, an intrinsic deficiency or low production of CRP as a marker of poor inflammatory pressure seems to be a risk factor for eczema.

In the present study, the *in vivo* plasma levels of IL-10 were higher in the probiotic group than in the placebo group. This is in accordance with our earlier study of

### Table 3. Allergic disease, eczema, and IgE sensitization (% of children) at the age of 2 years in groups defined by the level of plasma CRP, IL-10, total IgE, and total IgA at the age of 6 months (n = 98)

| Group | Allergic disease | 95% CI | | | | | Eczema | 95% CI | | | | | IgE sensitization | 95% CI |
|-------|-----------------|--------|---|---|---|---|---|-------|--------|---|---|---|---|
| CRP   | < 0.107 mg/L    | 53.1   | 1.00 | 0.16–0.87| 0.023 | 44.9 | 1.00 | 0.17–0.99| 0.046 | 32.7 | 1.00 | 0.33–1.90| 0.595 |
|       | ≥ 0.107 mg/L    | 30.6   | 0.38 | 0.16–0.87| 0.023 | 24.5 | 0.41 | 0.17–0.99| 0.046 | 29.2 | 0.79 | 0.33–1.90| 0.595 |
| IL-10 | < 4.35 pg/mL    | 40.8   | 1.00 | 0.48–2.44| 0.849 | 34.7 | 1.00 | 0.46–2.48| 0.885 | 27.1 | 1.00 | 0.56–3.24| 0.515 |
|       | ≥ 4.35 pg/mL    | 42.9   | 1.08 | 0.48–2.44| 0.849 | 34.7 | 1.06 | 0.46–2.48| 0.885 | 34.7 | 1.34 | 0.56–3.24| 0.515 |
| Total IgE | < 7.80 kU/L | 31.9 | 1.00 | 0.96–5.24| 0.063 | 25.5 | 1.00 | 0.99–6.01| 0.051 | 17.0 | 1.00 | 1.26–5.75| 0.015 |
|       | ≥ 7.80 kU/L    | 51.0   | 2.24 | 0.96–5.24| 0.063 | 42.9 | 2.45 | 0.99–6.01| 0.051 | 41.7 | 3.32 | 1.26–5.75| 0.015 |
| Total IgA | < 0.142 g/L | 34.0 | 1.00 | 0.84–4.46| 0.121 | 21.3 | 1.00 | 1.48–9.43| 0.005 | 27.7 | 1.00 | 0.48–2.85| 0.739 |
|       | ≥ 0.142 g/L    | 50.0   | 1.94 | 0.84–4.46| 0.121 | 47.9 | 3.74 | 1.48–9.43| 0.005 | 31.9 | 1.16 | 0.48–2.85| 0.739 |

The group with a higher concentration (≥ median) was compared with the group with a lower concentration (< median) using the logistic regression analysis where the treatment group (probiotic treatment vs. placebo) was included as a categorical covariate.

CI, confidence interval; CRP, C-reactive protein; OR, odds ratio.

### Table 4. Plasma IL-10 levels (pg/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Probiotic</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All infants</td>
<td>42/52</td>
<td>26/46</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-allergic</td>
<td>18/24</td>
<td>14/22</td>
<td>0.307</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>18/22</td>
<td>9/19</td>
<td>0.003</td>
</tr>
<tr>
<td>Eczema</td>
<td>14/16</td>
<td>8/18</td>
<td>0.001</td>
</tr>
<tr>
<td>IgE sensitization</td>
<td>16/18</td>
<td>6/12</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Probiotic preparation vs. placebo in plasma samples at age 6 months. Geometric means, 95% CI. n represents the number of samples above the detection limit/total number of samples. CI, confidence interval.
infants with atopic eczema, in whom we showed that treatment with a similar mixture of probiotics as used in the present study resulted in a significant increase of IL-10 in 1 month [14]. The effect on IL-10 was strain specific because the treatment with Lactobacillus GG alone did not induce an increase in IL-10. The probiotic-induced IL-10 response similar to the CRP response was observed only in allergic children, and the levels of IL-10 correlated with CRP concentrations. Accordingly, the IL-10 response reflects probiotic-induced inflammation [14], and an intrinsic deficiency/low production of IL-10 may be a risk factor for allergy. Some studies observed the clinical effect of probiotics only in the group of children with IgE-mediated eczema [5, 6]. Also, we found previously an increase of mitogen-induced IFN-γ secretion by PBMC only in the children who had IgE-mediated eczema and low IFN-γ secretion originally [12]. All these findings indicate that the immunological status of the host is an important determinant for the probiotic-mediated immunological effect.

Unexpectedly, we found higher total IgE levels in the probiotic group than in the placebo group, but food-antigen-specific IgE concentrations did not differ between the groups. The probiotic-induced increase in total IgE was detectable in non-allergic children, but not in allergic children because the increased IgE levels are closely associated with a risk of allergy. As expected, the high plasma total IgE at age 6 months increased the risk of IgE sensitization and tended to increase the risk of eczema at age 2 years in the placebo group. The probiotic-triggered response was characterized by the induction of IL-10 and an increase in total IgE without a change in the allergen-specific IgE. This response resembles the inflammatory changes seen in helminth infections, which, despite their Th2-stimulating properties, confer protection against atopy [26, 27]. Helminth infection-stimulated increase in total IgE and IL-10 is associated with the induction of local regulatory mechanisms, such as TGF-β production in the intestine [28]. The induction of local regulatory mechanisms in the host is important for the survival of the parasite because the down-regulation of immunity prevents the eradication of the parasite. In populations where helminth infections are common, a low prevalence of atopic diseases has been reported [29–32]. In Estonia, the incidence of atopic diseases is low, but total IgE levels increased in children relative to Swedish children [27]. Higher total IgE levels, but less atopic diseases have also been reported in adults in Russia [30]. In populations with a frequent occurrence of parasite infections causing high IgE levels, clinical symptoms of allergy rarely develop, possibly due to development of regulatory mechanisms [32]. Interestingly, antihelminthic treatment of chronically infected children has been reported to result in increased atopic reactivity, which indicates that the protection from atopy is not a permanent effect, but requires continuous stimulation of immune regulation by helminth infections [33]. It is thus possible that the suppression of atopy by probiotics is only achieved during continuous stimulation by the probiotics.

Helminth infestation suppressed allergic airway inflammation in mice and led to an increase in the total IgE level, but did not prevent the development of antigen-specific IgE responses in the sensitized animals [28]. Thus, the induction of IgE as such is not a specific risk for atopic diseases, but is a common phenomenon in helminth infections, which protect from allergies without protecting from IgE sensitization. Similarly, we found that probiotic-induced inflammation detected by CRP protected from atopic diseases but not from IgE sensitization, as also reported by other studies [2, 3]. This suggests, rather, induction of regulatory mechanisms that protect from clinical allergy than the primary prevention of IgE sensitization. Also, experiments in pathogen-free and germ-free mice support the view that exposure to intestinal bacteria induces an increase in IgE levels and oral tolerance. In the pathogen-free animals, immunization

<table>
<thead>
<tr>
<th>N</th>
<th>Total IgE probiotic</th>
<th>Total IgE placebo</th>
<th>Total IgA probiotic</th>
<th>Total IgA placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>All infants</td>
<td>51/51</td>
<td>11.13 (8.05–15.39)</td>
<td>45/45</td>
<td>6.70 (4.51–9.96)*</td>
</tr>
<tr>
<td>Non-allergic</td>
<td>24/24</td>
<td>9.61 (5.71–16.16)</td>
<td>22/22</td>
<td>4.70 (2.91–7.57)**</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>22/22</td>
<td>14.19 (8.71–23.10)</td>
<td>18/18</td>
<td>9.45 (4.40–20.31)</td>
</tr>
<tr>
<td>Eczema</td>
<td>16/16</td>
<td>14.01 (7.62–25.77)</td>
<td>17/17</td>
<td>10.50 (4.81–22.91)</td>
</tr>
<tr>
<td>IgE sensitized</td>
<td>17/17</td>
<td>15.39 (8.93–26.51)</td>
<td>11/11</td>
<td>19.09 (7.47–48.77)</td>
</tr>
</tbody>
</table>

Probiotic preparation vs. placebo in plasma samples at age 6 months. Geometric means, 95% CI.

n represents the number of samples above detection limit/total number of samples.

*P = 0.047;
**P = 0.042;
* = 0.016;
‰ = 0.050; Probiotic preparation in comparison with placebo.

CI, confidence interval.
with OVA resulted in a rapid increase of serum total IgE and the development of oral tolerance, whereas in germ-free animals IgE remained low and oral tolerance was not induced [10].

The suppression of allergic airway inflammation in parasite-infected mice was dependent on the regulatory T cells induced [28]. Mesenteric lymph node cells (MLNC) from infected animals contained elevated numbers of CD4+CD25+Foxp3+ T cells, showed high TGF-β expression, and produced strong IL-10 responses to parasite antigens. Although IL-10 was associated with the induction of intestinal regulatory T cells, MLNC from infected IL-10-deficient animals also transferred the suppression of allergic inflammation in sensitized hosts [28], suggesting that IL-10 is not necessary for the regulation, but is rather a marker of inflammation associated with regulation. Similarly, in our study, although the IL-10 response correlated strongly with the inflammation marker CRP, it was not associated with protection from allergy. Our study design in the infants is limited to the availability of plasma samples and thus the local intestinal changes, such as induction of regulatory T cells or TGF-β, could not be studied in healthy infants on probiotics.

We found increased plasma IgA levels in children who received probiotic bacteria. Immunization studies have shown probiotic bacterial strains to enhance IgA production [34, 35]. Similar to a high total IgE, a high plasma total IgA level at age 6 months was also associated with an increased risk of eczema at age 2 years. High total IgA in allergy could be a result of a compensatory mechanism of an injured immune system, such as high permeability of the gut, which has been reported in atopic dermatitis [36]. Induction of IgA is also a feature typical of parasitic infections [37] and of the exposure to commensal bacteria [38].

Our results of the association of a high CRP value at the age 6 months with protection against eczema at age 2 years confirm the hypothesis that inflammation controls eczema in children. The findings presented here support and extend the ‘hygiene hypothesis’, first introduced by Strachan [39], who suggested a link between microbial load and development of allergic diseases. We show that the inflammation, induced by probiotics with protection against eczema [15], is characterized by an increase in circulating IL-10, IgA, and IgE and thus resembles helminth infection-related immunological consequences. Accordingly, the immune regulation promoted for the survival of the parasite or colonizing bacteria, i.e. probiotics, may benefit the host by development of oral tolerance. The findings provide a novel explanation for the action of probiotics in vivo and emphasize the role of Th2-stimulating microbes able to generate regulatory T cells in the prevention of allergic diseases. This view is important for understanding the hygiene hypothesis because not all infections protect from atopic diseases [40].

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