# Probiotics: A novel approach in the management of food allergy

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**Background:** *The gastrointestinal microflora is an important constituent of the gut mucosal defense barrier. We have previously shown that a human intestinal floral strain,* Lactobacillus *GG (ATCC 53103), promotes local antigen-specific immune responses (particularly in the IgA class), prevents permeability defects, and confers controlled antigen absorption.*

**Objective:** *The aim of this study was to evaluate the clinical and immunologic effects of cow's milk elimination without* (n = *14) and with* (n = *13) the addition of* Lactobacillus *GG (5 × $10^8$ colony-forming units/gm formula) in an extensively hydrolyzed whey formula in infants with atopic eczema and cow's milk allergy. The second part of the study involved 10 breast-fed infants who had atopic eczema and cow's milk allergy. In this group* Lactobacillus *GG was given to nursing mothers.*

**Methods:** *The severity of atopic eczema was assessed by clinical scoring. The concentrations of fecal $\alpha_1$-antitrypsin, tumor necrosis factor-α, and eosinophil cationic protein were determined as markers of intestinal inflammation before and after dietary intervention.*

**Results:** *The clinical score of atopic dermatitis improved significantly during the 1-month study period in infants treated with the extensively hydrolyzed whey formula fortified with* Lactobacillus *GG. The concentration of $\alpha_1$-antitrypsin decreased significantly in this group* (p = *0.03) but not in the group receiving the whey formula without* Lactobacillus *GG* (p = *0.68). In parallel, the median (lower quartile to upper quartile) concentration of fecal tumor necrosis factor-α decreased significantly in this group, from 709 pg/gm (91 to 1131 pg/gm) to 34 pg/gm (19 to 103 pg/gm)* (p = *0.003), but not in those receiving the extensively hydrolyzed whey formula only* (p = *0.38). The concentration of fecal eosinophil cationic protein remained unaltered during therapy.*

**Conclusion:** *These results suggest that probiotic bacteria may promote endogenous barrier mechanisms in patients with atopic dermatitis and food allergy, and by alleviating intestinal inflammation, may act as a useful tool in the treatment of food allergy. (J Allergy Clin Immunol 1997;99:179-85.)*

***Key words:*** *Atopic eczema, food allergy, tumor necrosis factor-α, $\alpha_1$-antitrypsin, eosinophil cationic protein*

The mucosae represent a first line in host defense.[1] Human beings are initially exposed to numerous environmental antigens during infancy, particularly through food. The intestinal mucosa is efficient in assimilating antigens encountered by the enteric route,[2, 3] but high-level antigen exposure during the first few months of life may predispose individuals to allergic sensitization.[4] Intestinal inflammation seems to be a predisposing factor in the increased sensitization of a subject.[5, 6]

The intestinal microflora is an important constituent of the gut mucosal barrier.[7, 8] In the absence of intestinal microflora, antigen transport is increased,[9] and the induction of oral tolerance may be abrogated.[10, 11] Intact rather than intestinally processed cow's milk proteins have been shown to stimulate peripheral blood mononuclear cells to release proinflammatory cytokines in patients with cow's milk allergy.[12] In a like manner, it has been shown that cow's milk proteins degraded by lactobacilli, but not those degraded by trypsin and pepsin, may generate tolerogenic peptides from the native protein.[13] These findings tend to substantiate the hypothesis that specific strains of intestinal microflora may aid in host protection against allergic sensitization.

The current approach in the management of food allergy is complete avoidance of foods proven to cause symptoms. In infants with cow's milk allergy, extensively hydrolyzed formulas are used to eliminate cow's milk antigens from the diet. However, even extensive pepsin-

*Abbreviations used*

| | |
|---|---|
| cfu: | Colony-forming units |
| CI: | Confidence interval |
| ECP: | Eosinophil cationic protein |
| IFN-γ: | Interferon-γ |
| TNF-α | Tumor necrosis factor-α |

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trypsin hydrolysis does not render the formula nonantigenic,[14] and even minute quantities of immunoreactive components of the native protein are capable of eliciting an allergic reaction.[15] We hypothesize that oral introduction of probiotics, defined as a live microbial feed supplement that beneficially affects the host by improving the intestinal microbial balance,[16] may prove to be a useful tool for the treatment of food allergy by alleviating intestinal inflammation. We therefore evaluated the clinical and immunologic effects of cow's milk elimination without and with the addition of lactobacilli in an extensively hydrolyzed whey formula in infants with atopic eczema and cow's milk allergy. The second part of the study involved breast-fed infants who had atopic eczema and cow's milk allergy. In this group *Lactobacillus* GG was given to nursing mothers. The severity of atopic eczema was assessed by clinical scoring,[17] and the intestinal inflammation was monitored by determining the concentrations of fecal $\alpha_1$-antitrypsin, eosinophil cationic protein (ECP), and tumor necrosis factor-α (TNF-α). Systemic immune response was evaluated by determining the concentration of ECP in sera and by determining the cytokine production (IL-4, interferon-γ [IFN-γ], TNF-α) of peripheral blood mononuclear cells.

## METHODS

### Patients and study design

In the first part of the study 31 infants, aged 2.5 to 15.7 months, fulfilling the Hanifin criteria[18] for atopic eczema in infants, were evaluated. They had been referred to a pediatric clinic because of atopic eczema and a clinical history suggestive of cow's milk allergy. In addition to atopic eczema, gastrointestinal disturbances such as loose stools, vomiting, or diarrhea were seen in six (19%) patients. A positive family history of atopic diseases (asthma, atopic eczema, and allergic rhinitis) or food allergy in first-degree relatives was noted in 26 patients (84%). The eczematous lesions were treated with emollients and topical corticosteroids. No patient was receiving systemic corticosteroid therapy. The patients were put on a cow's milk elimination diet and participated in a randomized double-blind study. Another group (group Wh, $n = 16$) received extensively hydrolyzed whey formula (Peptidi-Tutteli; Valio Ltd., Helsinki, Finland), and another group (group WhGG, $n = 15$) received the same formula fortified with *Lactobacillus* GG $5 \times 10^8$ colony-forming units (cfu)/gm formula (supplied by Valio Ltd., Helsinki, Finland). *Lactobacillus* GG (ATCC 53103) is a human strain with the ability to survive passage through the gastrointestinal tract.[19] The patients were randomized at presentation because we hypothesized that the addition of *Lactobacillus* GG in an extensively hydrolyzed formula could hasten recovery and alleviation of intestinal inflammation. The patients received the allocated formula for 1 month (study period) and were then clinically examined (by HM). Thereafter, all patients ($n = 31$) received the extensively hydrolyzed whey formula (without *Lactobacillus* GG), and at 2 months, were examined by the same doctor. The amount of formula infants received varied between 500 and 1000 ml, depending on the age of the child. Otherwise, the diet was normal for the age including foods such as potato, berries, vegetables, meat, and cereals.

In the second part of the study, 11 breast-fed infants (group M-GG), aged 0.6 to 8.5 months (mean age, 4.4 months) who had atopic eczema, were evaluated. In addition to atopic eczema, gastrointestinal disturbances were seen in six (55%) patients. In this group *Lactobacillus* GG was given to nursing mothers, because it has previously been shown that administration of probiotic bacteria may enhance antigen-specific IgA antibody production in the mammary gland.[20] Before commencement of *Lactobacillus* GG treatment, nine mothers had reduced their intake of cow's milk, and six mothers had eliminated cereals (wheat, barley, rye, and oats) from their diets without any significant improvement of eczema in the infants. Breast-feeding was continued, and a dose of $2 \times 10^{10}$ cfu *Lactobacillus* GG was given twice daily for 1 month to the nursing mothers of atopic infants. The mothers remained on a restricted diet during the 1-month study period. Thereafter, the children were examined (by HM). A positive family history of atopic diseases or food allergy in first-degree relatives was noted in all patients.

After the study periods, the patients were allocated to double-blind, placebo-controlled cow's milk challenge or open cow's milk challenge as previously described.[21] Only those having a positive reaction to cow's milk challenge were included in the final study population. Cow's milk challenge was positive in 27 of 31 patients in the first part of the study and in 10 of 11 patients in the second part of the study.

Blood and fecal samples were collected from nine healthy age-matched control subjects to determine the same inflammatory parameters as those from atopic children.

### Samples

Blood and fecal samples were collected before the commencement of the treatments and 1 month (after the study period) and 2 months later from the patients in the first part of the study and 1 month later from the patients in the second part. All fecal samples were handled in the same way: immediately after spontaneous defecation, the sample was cooled and stored at +6° C for transportation. Within a maximum of 12 hours, the sample was delivered in a cold-transport box, frozen, and stored at −70° C until analysis.

Breast milk samples were collected before the commencement of the mothers' *Lactobacillus* GG treatment and 1 month later. The samples were collected in sterile, covered glass jars and promptly frozen until further processing. The concentration of β-lactoglobulin in formula and breast milk specimens was determined as previously described.[22] β-Lactoglobulin was found in 68% of breast-milk specimens. The concentrations varied from 0.2 to 8.9 ng/ml. The concentration of β-lactoglobulin in the extensively hydrolyzed whey formulas was less than 0.05 ng/mg, which is in keeping with that previously described.[23] Total breast-milk IgA was measured by radial immunodiffusion (LC-Partigen-IgA; Behringwerke AG, Marburg, Germany), as instructed by the manufacturer. There was little interindividual variation in total IgA concentrations in breast milk. The concentrations varied from 0.06 to 0.45 gm/L. Total breast-milk IgA remained unaltered during the *Lactobacillus* GG treatment.

### Skin prick tests and determination of serum total IgE and RAST

The serum total IgE concentration (Phadebas RAST; Pharmacia, Uppsala, Sweden), cow's milk-specific IgE (RAST, Pharmacia), and skin prick test responses were determined in all patients. Prick testing was done as previously described.[21] Antihistamine medication was discontinued for periods of 3 days to 6 weeks before skin testing, depending on the drug's duration of action. Prick testing was done on the volar aspect of

the forearm by using a commercially available cow's milk allergen (ALK; Allergologisk Laboratorium, Horsholm, Denmark) and histamine dihydrochloride, 10 mg/ml (ALK), as positive control. Reactions were read after 15 minutes, and a response half of the histamine reaction size or more was recorded as positive if the mean diameter of the wheal was at least 3 mm and the negative control was 0 mm at the same time.

### Determination of the colonization of *Lactobacillus* GG

To ensure that *Lactobacillus* GG colonizes the gut of atopic infants, the presence of *Lactobacillus* GG was determined in fecal samples before the intervention studies in a preliminary study. The samples were collected before administration of *Lactobacillus* GG and 1 week and 1 month after the commencement of *Lactobacillus* GG administration. *Lactobacillus* GG was analyzed in fecal samples by plating a homogenized and diluted sample on de Man Rogosa Sharpe agar (Amersham, Bury, U.K.), which was incubated anaerobically for 3 days at 37° C as described earlier.[24] Typical white, large, creamy colonies were enumerated; and their cell structure and lactose fermentation were determined (gram-positive, uniform rods in chains, lactose fermentation negative).

In children receiving *Lactobacillus* GG, the content of *Lactobacillus* GG in feces after 1 week of administration varied between $9.0 \times 10^5$ and $6.5 \times 10^7$ cfu/gm, and after 1 month, between $8.8 \times 10^4$ and $6.7 \times 10^5$ cfu/gm. In those infants in whom *Lactobacillus* GG was administered to nursing mothers, the content of *Lactobacillus* GG in feces after 1 week of administration varied between $4.0 \times 10^7$ and $7.2 \times 10^7$ cfu/gm and, after 1 month, between $10^3$ and $8.8 \times 10^7$.

### Determination of the severity of atopic dermatitis

The severity of atopic dermatitis was scored according to the SCORAD method established by the European Task Force on Atopic Dermatitis.[17] Briefly, the extent (score A) of the dermatitis was estimated by using the rule of nines. The intensity (score B) of the dermatitis was the sum of the individual scores (0 to 3) for erythema, edema, and/or papules, excoriation, lichenification, and dryness. The subjective (score C) manifestations (scored 1 to 10), including pruritus and sleep loss, were assessed from parents' estimations. SCORAD was obtained by the calculation: $A \div 5 + 3.5 \times B + C$.

### Determination of ECP in sera

The quantitative determination of ECP in serum was performed by radioimmunoassay (Pharmacia ECP RIA), as instructed by the manufacturer. The radioactivity was determined by using a 1470 Wizard gamma counter (Wallac Ltd., Turku, Finland), and the ECP concentrations were read from a standard curve obtained with standards from 1 to 200 μg/L.

### Determination of $\alpha_1$-antitrypsin in feces

Frozen fecal specimens were thawed at room temperature and homogenized. Approximately 1 gm was transferred to a glass tube and lyophilized. The resulting dry material was ground, and 50 mg was transferred to an Eppendorf tube (Kartell, Milan, Italy). One milliliter of 0.15 mol/L NaCl solution was added, and $\alpha_1$-antitrypsin was extracted by vigorous mixing in a Vortex mixer for 20 minutes at room temperature. The resulting suspension was centrifuged at 25,000 *g* for 10 minutes to remove debris, and the supernatant was used for the determination of $\alpha_1$-antitrypsin with a Behring BNA nephelometer according to the manufacturer's instructions. The results are given as milligrams per gram of dry weight of lyophilized feces.

### Determination of ECP and TNF-α in feces

Deep-frozen fecal specimens were thawed at room temperature, suspended 1:1 (wt/vol) in physiologic saline solution, and allowed to sediment. Of the supernatant, 0.5 to 1.0 ml was transferred to an Eppendorf tube and centrifuged at 25,000 *g* for 10 minutes. The supernatant was then used for the determination of ECP and TNF-α. Determination of fecal ECP was performed as described above for serum specimens. A commercial enzyme immunoassay (Human TNF-α ELISA Kit; Endogen Inc., Boston, Mass.) was used, as instructed for serum specimens, for the determinations of fecal TNF-α.

### Determination of TNF-α, IL-4, and IFN-γ production during lymphocyte induction

Mononuclear cells containing mainly lymphocytes were isolated by Ficoll-Paque (Pharmacia) gradient centrifugation at 400 *g* for 30 minutes at 20° C. To induce lymphocytes, $6.25 \times 10^5$ isolated cells in 1 ml of RPMI-1640 medium containing antibiotics, glutamine, and 10% human AB serum were cultured in a humidified 5% $CO_2$ atmosphere at 37° C for 48 hours together with concanavalin A (Pharmacia) at a final concentration of 50 μg/ml and cow's milk protein solution (skim milk powder; Valio Finnish Co-operative Dairies' Association, Helsinki, Finland) at a final protein concentration of 1 mg/ml. A control cell population was generated with RPMI-1640 medium alone. The supernatants were collected and stored frozen at −70° C until the determination of cytokines was carried out. Commercial enzyme immunoassays were used (Human TNF-α ELISA Kit [Endogen Inc.], IL-4 [CLB, Amsterdam, Netherlands], IFN-γ [Endogen Inc.]) as instructed by the manufacturers. The results from different runs were equalized by using comparison of standard curves.

### Statistics

By reason of the skewed distributions of serum IgE concentrations, logarithmic (ln) transformation was used, and data are presented as means with 95% confidence intervals (CIs). The concentrations of inflammatory parameters are presented with medians with lower and upper quartiles. The Wilcoxon signed-rank test and the Mann-Whitney U test were used in statistical comparisons.

### Ethics

The study protocol was approved by the Ethical Review Committee of Tampere University Hospital. Informed consent was obtained from the parents.

## RESULTS

### Clinical data

The mean (95% CI) age at onset of symptoms of atopic eczema was 2.4 months (1.4 to 3.3 months) in patients in the first part of the study ($n = 27$). The durations of exclusive and total breast-feeding were 2.8 months (2.1 to 3.5 months) and 5.9 months (4.5 to 7.2 months), respectively. The mean (95% CI) serum total IgE was 31 kU/L (15 to 61 kU/L). RAST for cow's milk was positive (>0.4 kU/L) in 10 patients (37%). The result of skin prick test with cow's milk was positive in eight patients (30%).

The severity of atopic dermatitis in each patient

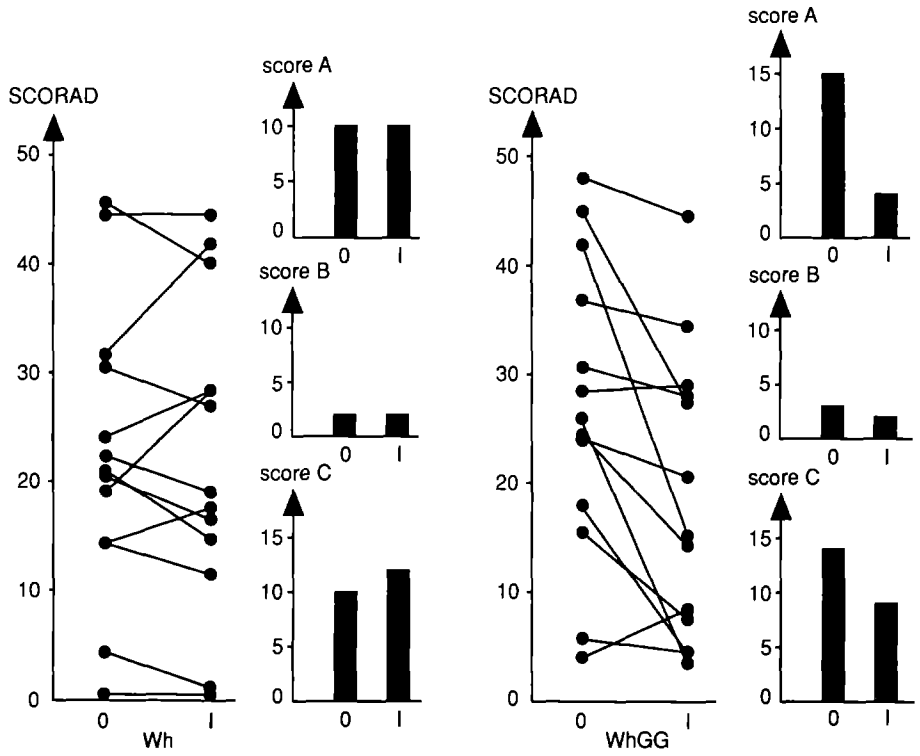


**FIG. 1.** Clinical score of atopic dermatitis (SCORAD) in each patient and median score for extent *(A)*, intensity *(B)*, and subjective score *(C)* for atopic dermatitis before treatment *(0)* and 1 month later *(I)* in infants receiving extensively hydrolyzed whey formula without *(Wh)* or with *Lactobacillus* GG *(WhGG)*.

before the commencement of the treatments and 1 month later (i.e., after the study period) are presented in Fig. 1. The severity of atopic dermatitis was comparable between the groups before treatment. The median (lower quartile to upper quartile) SCORAD score in group Wh was 21 (14 to 31) and in group WhGG 26 (17 to 38) before treatment ($p = 0.33$). There was a significant improvement of SCORAD score after 1 month's intervention in those receiving *Lactobacillus* GG ($p = 0.008$) but not in those receiving extensively hydrolyzed formula without *Lactobacillus* GG ($p = 0.89$). The SCORAD score was then 19 (13 to 31) in group Wh and 15 (7 to 28) in group WhGG. The decrease in the SCORAD score in group WhGG was due to the reduction of the extent (score A, $p = 0.004$), intensity (score B, $p = 0.05$), and subjective score (score C, $p = 0.01$) for atopic dermatitis (Fig. 1). The improvement in SCORAD score was achieved by 2 months in the Wh group, and in group WhGG it remained unchanged after cessation of *Lactobacillus* GG administration. At 2 months, the median (lower quartile to upper quartile) SCORAD score in group Wh was 14 (2 to 38), and in group WhGG, 16 (6 to 25).

The mean age at onset of symptoms of atopic eczema was 1.2 months (0.6 to 1.8 months) in infants in the second part of the study (group M-GG, $n = 10$). The mean (95% CI) serum total IgE was 17 kU/L (5 to 56 kU/L). RAST for cow's milk was positive (>0.4 kU/L) in three patients (30%), and skin prick test response to cow's milk was positive in three patients (30%). The median (lower quartile to upper quartile) SCORAD score in group M-GG was 26 (20 to 36) before treatment and 11 (0 to 25) 1 month later ($p = 0.007$).

### The concentration of ECP in sera

The concentration of ECP in sera in healthy control subjects ($n = 9$) was significantly lower, 3.3 μg/L (2.1 to 6.6 μg/L), than in atopic children ($p = 0.001$). The concentration of ECP in sera was 13.8 μg/L (6.4 to 24.0 μg/L) in group Wh and 11.4 μg/L (5.8 to 18.9 μg/L) in group WhGG before treatment. There was a trend toward a decreased concentration of ECP at 1 month in both groups alike. After the 1-month study period, the concentrations were 11.3 μg/L (6.3 to 13.8 μg/L) in group Wh and 10.2 μg/L (5.7 to 11.6 μg/L) in group WhGG. At 2 months, the concentration of ECP was 5.3 μg/L (3.6 to 8.8 μg/L) in group Wh and 6.0 μg/L (4.5 to 10.7 μg/L) in group WhGG. The concentration of ECP in sera in group M-GG was 5.2 μg/L (2.5 to 10.8 μg/L) before treatment and 8.3 μg/L (6.3 to 10.6 μg/L) 1 month later.

### Concentrations of $\alpha_1$-antitrypsin, TNF-α, and ECP in feces

In healthy control subjects ($n = 9$), the median (lower quartile to upper quartile) concentration of $\alpha_1$-antityp-

**TABLE I.** Concentrations of fecal $\alpha_1$-antitrypsin, TNF-α, and ECP before treatment (0) and 1 month later (I) in infants receiving extensively hydrolyzed whey formula without (Wh) or with *Lactobacillus* GG (WhGG) and in infants whose mothers were receiving *Lactobacillus* GG (M-GG)

| | Wh group ($n$ = 14) | WhGG group ($n$ = 13) | M-GG group ($n$ = 10) |
|---|---|---|---|
| $\alpha_1$-Antitrypsin 0 (mg/gm) | 1.7 (1.5-2.3) | 1.4 (0.5-1.9) | 1.7 (0.7-3.1) |
| $\alpha_1$-Antitrypsin I | 1.7 (1.1-2.8) | 0.5 (0.5-1.0) | 1.6 (1.1-3.1) |
| TNF-α 0 (pg/gm) | 632 (126-1880) | 709 (91-1131) | 2 (0-40) |
| TNF-α I | 494 (147-1009) | 34 (19-103) | 0 (0-141) |
| ECP 0 (ng/gm) | 77 (30-131) | 71 (59-122) | 60 (34-163) |
| ECP I | 47 (22-103) | 48 (15-99) | 44 (8-56) |

Data denote median (lower quartile to upper quartile).

sin was 0.5 mg/gm (0.5 to 1.7 mg/gm). The concentration of $\alpha_1$-antitrypsin was comparable between groups Wh and WhGG before treatment ($p$ = 0.22). As indicated in Table I, the concentration of $\alpha_1$-antitrypsin decreased significantly in group WhGG ($p$ = 0.03), but not in group Wh ($p$ = 0.68), during the 1-month study period. At 2 months, the concentration of $\alpha_1$-antitrypsin was 1.2 mg/gm (0.5 to 1.6 mg/gm) in group Wh and 0.5 mg/gm (0.5 to 0.7 mg/gm) in group WhGG. The concentration of $\alpha_1$-antitrypsin remained unaltered during the treatment in infants whose mothers were receiving *Lactobacillus* GG.

The concentration of fecal TNF-α was 0 pg/gm (0 to 0.8 pg/gm) in healthy control subjects. The concentration of fecal TNF-α was significantly higher in atopic children ($p < 0.0001$, Table I). The concentration of TNF-α was comparable between groups Wh and WhGG before treatment ($p$ = 0.57). The concentration of fecal TNF-α decreased significantly in group WhGG ($p$ = 0.003) but not in group Wh ($p$ = 0.38) during the 1-month study period (Table I). A reduction in TNF-α concentration was achieved by 2 months in group Wh; whereas in the subjects in group WhGG, who were also given the extensively hydrolyzed formula without *Lactobacillus* GG, a trend toward increased TNF-α was detected. The concentration of TNF-α was then 84 pg/gm (25 to 129 pg/gm) in group Wh and 144 pg/gm (20 to 338 pg/gm) in group WhGG. In group M-GG the concentration of TNF-α before treatment was at a significantly lower level than that in groups Wh and WhGG (Table I).

The concentration of fecal ECP was 44.9 ng/gm (33.8 to 127.7 ng/gm) in healthy control subjects. The concentration of ECP was comparable between the study groups before treatment ($p$ = 0.83). The concentration of fecal ECP remained unaltered during the treatment in group Wh ($p$ = 0.86) and in group WhGG ($p$ = 0.46). At 2 months, the concentration of fecal ECP was 38 ng/gm (17 to 111 ng/gm) in group Wh and 22 ng/gm (7 to 83 ng/gm) in group WhGG. There was a trend toward a decreased concentration of fecal ECP ($p$ = 0.06) after 1 month of treatment in group M-GG (Table I).

### Cytokine release in peripheral blood mononuclear cell culture supernatants

The concentrations of IL-4 in RPMI, concanavalin A, and cow's milk–induced culture supernatants before treatment were 0.1 pg/ml (0.05 to 0.15 pg/ml), 1.1 pg/ml (0.6 to 2.9 pg/ml), and 0.1 pg/ml (0.07 to 0.22 pg/ml), respectively, in infants receiving extensively hydrolyzed whey formula with or without *Lactobacillus* GG. The concentrations of IFN-γ in RPMI, concanavalin A, and cow's milk–induced culture supernatants before treatment were 5.9 pg/ml (3.7 to 7.7 pg/ml), 7.6 pg/ml (5.2 to 11.3 pg/ml), and 6.7 pg/ml (5.7 to 10.7 pg/ml), respectively. Those for TNF-α were 90.6 pg/ml (49.8 to 180.7 pg/ml), 135.0 pg/ml (37.1 to 479.8 pg/ml), and 172.6 pg/ml (75.4 to 281.7 pg/ml), respectively. The concentrations of these cytokines remained unaltered during the study period, and there were no differences between groups Wh and WhGG after 1 month of treatment with extensively hydrolyzed whey formula with or without *Lactobacillus* GG.

## DISCUSSION

Antigen processing in the gut is associated with the generation of oral tolerance.[25] There is evidence that during the process of absorption across the intestinal mucosa, antigens are subtly altered into tolerogenic form.[26] Newborns lack many specific and nonspecific intestinal features that are necessary for protecting them from environmental antigens.[1] In the immature gut, because of an immature mucosal barrier, antigen transfer in intact form is increased,[27] an important prerequisite for the development of food allergy.[1, 3]

The hitherto imperfect understanding of the role of food allergy in atopic dermatitis has fueled a constant debate on the optimal treatment of infants with atopic eczema and food allergy. However, well-controlled studies suggest that dietary antigens do contribute to the exacerbation of atopic dermatitis at least in a subset of patients.[21, 28, 29] In these patients, as also seen in this study, an elimination diet is associated with an alleviation of clinical symptoms of atopic eczema and reversal of some disturbances in immune responses to dietary antigens.[30, 31] We have previously

shown that antigen transfer is increased in patients with atopic eczema.[32] One explanation for the altered antigen transfer in these patients could be the lack of or reduced secretion of IFN-γ, which has been held to be a primary component of the atopic state.[33] IFN-γ has been shown to have an effect on antigen transport and presentation.[34] Impairment of the intestine's barrier against antigen uptake in atopic eczema[32] may be the key determinant in exaggerated immune responses to common dietary and environmental antigens, and consequently, a link between food allergy and atopic eczema.

In this study intestinal inflammation in the patients with atopic eczema and cow's milk allergy was demonstrated particularly in increased concentrations of TNF-α before treatment. In a recent study we demonstrated enhanced TNF-α concentrations, particularly after cow's milk challenge.[35] Intestinal inflammation is considered to be a predisposing factor for increased sensitization of a subject.[5, 6] Interestingly, in this study the TNF-α concentration was low in infants who were breast-fed. Antiinflammatory components in breast milk may have an effect on the concentration of TNF-α.[36]

Using animal models, we have previously shown that lactobacilli may promote gut barrier function and counteract the permeability disorder associated with food allergy.[27] Generally, the use of lactic acid bacteria is based on the premise that the preparations can reinforce the gut microflora.[7] Our study is the first to examine the contribution of antigen elimination with probiotic treatment to restoration of intestinal barrier function and to evaluate the effects of this treatment on the clinical outcome of the patients. An important prerequisite for an effective probiotic strain is the ability to survive passage through the gastrointestinal tract.[7] The strain chosen for this study, *Lactobacillus* GG, has been shown to fulfill this criterion and transiently colonize the gut.[19, 24] Oral bacteriotherapy resulted in alleviation of the intestinal inflammation in our patients, which was seen as a decrease in fecal $\alpha_1$-antitrypsin and TNF-α concentrations in infants receiving *Lactobacillus* GG. Simultaneously, a significant clinical improvement in the extent, intensity, and subjective score for atopic dermatitis was demonstrated in these infants. It has recently been shown that *Lactobacillus* GG–derived enzymes generate peptides with suppressive effects on lymphocyte proliferation,[13] and bovine caseins degraded by enzymes of *Lactobacillus* GG downregulate IL-4–producing activity of peripheral blood mononuclear cells.[37] Therefore antigen elimination and reinforcement of the normal intestinal flora together may preserve the intestinal barrier and aid in silencing the hypersensitivity reactions.

It has previously been shown that *Lactobacillus* GG promotes antigen-specific immune responses, particularly in the IgA class,[27, 38] and targets antigen transport across Peyer's patches.[27] The uptake of antigens by Peyer's patches appears to be an important event in the generation of local secretory immune response.[39] Antigens and IgA form complexes in the gut, which are readily entrapped in mucus, and their clearance is thereby facilitated.[40] One explanation for the favorable effect of *Lactobacillus* GG could thus be an improvement in antigen elimination by the gut mucosal barrier. On the other hand, the targeting of antigen transport across Peyer's patches may be of importance. Although the site of oral tolerance induction is still unclear, there is evidence to indicate that Peyer's patches play an important role.[41] Moreover, lactobacilli have been shown to potentiate the production of IFN-γ by isolated T cells.[42] Recently, IFN-γ has been shown to inhibit TNF-α release of intestinal mucosal mast cells.[43] Increased IFN-γ secretion might prevent detrimental effects of TNF-α[44] and consequently have favorable effects, particularly on the reduction of allergic inflammation.

Taken together, the results of this study suggest that probiotic bacteria may downregulate hypersensitivity reactions and intestinal inflammation in patients with atopic eczema and food allergy. By promoting endogenous barrier mechanisms, probiotic bacteria might have a role in the treatment of food allergy.

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