The effects of probiotics supplementation timing on an ovalbumin-sensitized rat model

Juan Huang¹, Yan Zhong², Wei Cai¹, Hongbo Zhang¹, Wenjing Tang² & Bing Chen²

¹Department of Pediatric Surgery, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; and ²Department of Nutrition, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Correspondence: Wei Cai, Department of Pediatric Surgery, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200092, China. Tel./fax: +86 021 6501 1627; e-mail: caiw204@yahoo.com.cn

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Abstract

The aim of the present study was to investigate the effect of oral probiotic bacteria administration at different times on ovalbumin-sensitized rats. Brown-Norway (BN) rats were orally sensitized with ovalbumin for 6 weeks. Probiotics were administered before the initial sensitization (prevention group) or at the end of sensitization period (treatment group). In whole-course intervention group, probiotics were administered 2 weeks before the initial sensitization until 1 week after the end of sensitization period. Ovalbumin-immunoglobulin E (IgE) level, intestinal barrier function and immune responses were analyzed. The positive control group had a significantly increased ovalbumin-IgE level (P < 0.05), impaired intestinal barrier function and skewed T-helper 1 (Th1)/Th2 cytokine balance compared with the negative control group. In probiotics prevention and whole-course intervention groups, the infiltration of inflammatory cells (eosinophil and mast cells) in small intestinal mucosa was significantly lower (P < 0.05), and the ratio of cytokine interferon-γ/interleukin-4 produced by spleen and mesenteric lymph nodes significantly higher (P < 0.05) than in the positive control group, which suggested a cytokine profile inclined to Th1. Both probiotics prevention and prebiotics treatment could attenuate food allergic response. Probiotics prevention tends to modulate the immune response, whereas probiotics treatment has a more obvious effect in enhancing intestinal integrity.

Introduction

The prevalence of food allergy has been increasing in recent decades worldwide and it has been recognized as the most common cause of anaphylaxis (Shek & Lee, 2006; Almqvist et al., 2008). Food allergy, which is triggered by an aberrant immune response elicited by orally ingested food allergens, is generated through complicated mechanisms which are not yet fully understood.

The immune system of an infant at birth is not fully developed and tends to be directed towards a T-helper 2 (Th2) phenotype. The Th2 phenotype leads to the stimulated production of immunoglobulin E (IgE) by B cells, thus increasing the risk of allergic reactions through activation of mast cells. Based on the hygiene hypothesis, early childhood is the critical period for the establishment of Th1/Th2 balance, and a skewed Th1/Th2 balance is linked with the reduced microbial exposure during childhood (Seiskari et al., 2007). It has been shown that early exposure to microbial stimuli can shift the Th1/Th2 balance towards favoring the Th1 side, which might be the immune basis for preventing or treating the occurrence of food allergy.

Besides the Th2-biased immune response, the increased intestinal inflammation in food allergy could lead to abdominal symptoms such as abdominal pain, diarrhea and malnutrition, and aggravate the illness in allergic patients. The intestinal inflammation in food allergy appears to be the culmination of both local epithelial dysfunction and a more generalized immune dysregulation that results in allergic predisposition (Ko & Mayer, 2005). The intestinal epithelium theoretically acts as a barrier restricting macromolecule penetration, and in normal conditions, whole food proteins cannot cross the epithelium. But if the integrity of intestinal barrier is damaged, a small portion of food allergens could cross the intestinal barrier to gain access and activate effector cells (Liu et al., 2006); this is related to
the severity of the food allergic reaction. It has been found that food antigen permeability through the intestinal mucous membrane is increased in allergic patients (Fujitani et al., 2007). Therefore, it is conceivable that maintaining the intestinal barrier function and decreasing the inflammatory reaction would be practical and efficient in preventing or treating food allergy.

The functions of probiotics, especially lactic-acid bacteria, have been studied in a range of human diseases including cancer, infectious diseases, gastrointestinal disorders and allergies (Adel-Patient et al., 2005; Lemberg et al., 2007), and most of the effects are related with modulating immune activity and enhancing intestinal barrier function. Intervention studies with probiotics in atopic diseases have found controversial results. Probiotics supplementation to pregnant mothers (2–4 weeks before the delivery) and infants (6 months after birth) tended to reduce IgE-associated diseases such as atopic eczema in the infants (Abrahamsson et al., 2007). However, probiotics supplementation to 3–12-month-old infants with atopic dermatitis for 12 weeks seemed to have no effect on improving allergic symptoms (Viljanen et al., 2005). Some studies have shown that the probiotics strain used appears to be related to the final results (Maassen & Claassen, 2008), and the timing of probiotics administration has been suggested to influence the development of allergy (Kukkonen et al., 2007). At present, a pivotal question that needs to be answered in clinical practice is whether probiotics should be used as a preventive measure for high-risk populations or as a treatment measure for patients diagnosed with food allergy, but there are very few data on the relation between the timing of probiotics administration and the effect on food allergy, and the underlying mechanisms have not been elucidated.

Animal models are widely used to test the effectiveness of preventive or treatment methods, and to identify the mechanisms of these methods in food allergy. Peritoneal injection of ovalbumin has been used to induce food allergy in many studies, but the shortcoming of this sensitization method is that it is not a natural way to induce allergy reaction, and the use of adjuvant might prevent the immune response in the models. In our study, the food allergy model was induced by oral gavaged ovalbumin, which is similar to natural allergen sensitization in humans (Kim et al., 2008). In Knippels’ study, it was shown that Brown-Norway (BN) rats may provide a suitable animal model for inducing specific IgG and IgE responses as well as specific T cell-mediated hypersensitivity (DTH) to ovalbumin upon exposure via the enteral route without the use of adjuvants (Knippels et al., 1998). In our preliminary study, the same high level of ovalbumin-specific IgE was produced by oral sensitization of ovalbumin as by peritoneal injection of ovalbumin, which suggests that the food allergy model was induced successfully (Huang et al., 2009).

In this study, we compared the effect of probiotics administration for prevention and treatment on alleviating food allergic response and investigated the related mechanisms in ovalbumin-sensitized rats.

Materials and methods

Animals

Fifty 3-week-old female BN rats, weighing between 45 and 50 g, were obtained from the Animal Center of the Chinese Academy of Medical Sciences (Beijing, China). The rats were housed in an animal room maintained at 23 ± 3 °C with a 12-h light/dark cycle and a relative humidity of 50–70%, and free access to ovalbumin-free rodent diet and water. The rats were allowed to acclimatize for 7 days before the initiation of the study. All animal studies were approved by an independent ethics committee of Shanghai Jiao Tong University.

Probiotics

The probiotics administered were a mixture of living Lactobacillus rhamnosus GG (American Type Culture Collection 53103) and Bifidobacterium longum BB536 (kindly given by the Institute of Biology and Pharmacology of Shanghai Jiao Tong University) in a weight ratio of 1:1. The mixed probiotics powder was resuspended in phosphate-buffered saline (PBS; pH 7.2) at a concentration of 1 × 10^9 CFU mL⁻¹ before supplementation, and the final concentration for each probiotic was 0.5 × 10^9 CFU mL⁻¹. The administered dosage was 1.0 mL day⁻¹. Identification of the strains was confirmed by 16S rRNA gene nucleotide sequences.

Food allergy induction and probiotics supplementation regimen

The animals were randomly divided into five groups: negative control, positive control, probiotics prevention, probiotics treatment and whole-course intervention group (n = 10 in each group). The animals in the negative control group were orally gavaged with 1 mL PBS daily for 6 weeks. The animals in the positive control group and the three probiotics administration groups were given 1 mg ovalbumin (1 mg mL⁻¹ per os) (purity, > 95%; Sigma-Aldrich, St. Louis, MO) daily for 6 weeks and the animals were deprived of food for 2 h before the oral sensitization. Ovalbumin and probiotics were gavaged separately, with an interval of 8 h. The animals not receiving probiotics or ovalbumin were given PBS instead. In the probiotics prevention group, 1 mL of probiotics solution was given daily for 2 weeks before sensitization was initiated. In the probiotics treatment group, probiotics solution was given as a supplement to animals for 2 weeks, starting 1 week before the end of...
sensitization period. In the whole-course intervention group, probiotics administration was started 2 weeks before the initial sensitization until 1 week after the end of sensitization period. Before sacrifice, the rats were challenged orally with 100 mg ovalbumin, and 1.5 h later, blood and tissue samples were collected (Fig. 1).

**Measurement of ovalbumin-specific IgE in serum**

Serum ovalbumin-specific IgE level was analyzed by enzyme-linked immunosorbent assay (ELISA). Plates were coated for 6 h at 37°C with 0.1 M NaHCO₃ containing 100 μg/mL ovalbumin. The plates were blocked for 2 h at 37°C with 200 μL 3% bovine serum albumin in PBS. Plates were washed, and 100 μL of diluted serum was applied and kept overnight at 4°C. The amount of bound antibody was analyzed using biotinylated anti-mouse IgE, biotinylated anti-mouse IgG2a and avidin–horseradish peroxidase conjugate (BD Pharmingen, San Diego, CA). Plates were read in a microplate autoreader (Wako Pure Chemical Industries Ltd, Osaka, Japan) at 450 nm. Serum titers for ovalbumin-specific IgE were expressed as relative ELISA units, referring to a laboratory standard sera pool.

**Determination of intestinal mucosal permeability**

Intestinal permeability was assessed by oral administration of fluorescein isothiocyanate (FITC)-conjugated dextran 4000 (FITC–dextran, Sigma-Aldrich). All animals were gavaged orally with FITC–dextran (20 mg 100 g⁻¹ body weight) 30 min before blood samples were collected. Whole blood was obtained by cardiac puncture and was allowed to clot at 4°C for 30 min and centrifuged (1500g) for 10 min. Serum FITC–dextran concentration was determined by fluorescence spectrophotometry (Hitachi model F-4010, Tokyo, Japan) at an excitation wavelength of 495 nm and an emission wavelength of 512 nm using a standard curve performed in duplicate.

**Detection of secretory IgA (sIgA) in intestinal content**

Small intestinal tissues from the ligament of Treitz to the ileocecal valve were excised. The luminal contents were gathered by flushing with 10 mL of ice-cold PBS (pH 7.4) and kept at −80°C until analysis. The luminal contents were diluted to 20 mL in PBS and an aliquot of 1 mL was centrifuged at 10 000g for 20 min to obtain a clear supernatant, which was used for sIgA measurement. The level of sIgA was quantified with a specific rat-IgA ELISA kit according to the manufacturer’s instructions (Bethyl Laboratories Inc., Montgomery, TX). Samples were diluted to the testing range of the kit and the sIgA concentration in the original sample was corrected with a dilution factor.

**Determination of colonic microbial communities**

Fecal samples were collected directly from colon when the animals were sacrificed and stored at −80°C until analysis. Total bacterial DNA was extracted from each fecal sample by a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) and was then used as a template for quantitative real-time

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**Fig. 1.** Experimental protocol shows different probiotic supplementation regimen in different intervention groups.
PCR. The genus-specific 16S rRNA gene-targeted primer sequences used for bifidobacteria were as follows: the forward primer Lm26-f (5'-GATTCTGCTGCTCCCTATGTA ACTG-3') and the reverse primer Lm3-r (5'-CGGGTGCTCCCACTTTCATG-3'). The primers for lactobacilli were as follows: the forward primer L159-f (5'-GGAACACTGATGTA ATACCG-3') and the reverse primer L677-r (5'-CACCCGC TACACATGGAG-3'). These primers sets were previously reported by Chen et al. (2007). Bifidobacteria genus-specific primers Lm26-f and Lm3-r have been validated for bifidobacterial specificity by Kaufmann et al. (1997) and Satokari et al. (2001), respectively. Lactobacillus genus-specific primers L159-f and L677-r have been validated for Lactobacillus specificity by Heilig et al. (2002). To check for specificity, the sequences of the selected PCR primers were compared with the sequences available at the BLAST database search program (http://www.ncbi.nlm.nih.gov/BLAST). Quantitative real-time PCR was performed with the Mx3000P QPCR System (Stratagene, La Jolla, CA). The copy number of 16S rRNA gene of the target bacteria in the template DNA solution was estimated using standard curves from the known concentrations of the corresponding DNA fragment. The standard curves were prepared in the same PCR assay as used for samples. The real-time PCRs were performed in triplicate and average values were used for enumeration. In all assays, the amplification efficiency was > 70%, and the standard curve showed a linear range across at least 5 logs of DNA concentrations, with a correlation coefficient > 0.99.

Histological analysis
Small intestinal tissue specimens were fixed in 10% formalin, embedded in paraffin wax on the oriented edge, and cut into 5-μm-thick sections for hematoxylin and eosin staining. The villus height and crypt depth were determined in longitudinal sections. The measurements were made in 10 villi and 10 crypts for each segment. The villus height was determined from the crypt opening to the top of the villus, and the crypt depth from the base of the crypt to its opening, using ocular micrometers. Multiple 4-μm sections were stained with o-toluidine blue for mast cell counting. Individual eosinophil cells were counted in a blinded fashion at 15–20 high-power fields (HPF) at × 40 and expressed as cells per HPF. Mast cell counting was done at × 100 magnification. The slides were analyzed with a Leitz Dialux 20 EB microscope (Wetzlar, Germany).

Measurement of cytokines in cell culture of mesenteric lymph nodes (MLNs) and spleen
The spleen and the MLNs were collected, and single cell suspension was prepared and resuspended in RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Sigma-Aldrich), 2 mM glutamine, 50 μM 2-mercaptoethanol, 50 IU/mL penicillin and 50 μg/mL streptomycin (complete media). Cells were incubated at 1 × 10⁶ cells per well in 24-well plates in a final volume of 200 μL with 100 μg mL⁻¹ ovalbumin at 37 °C in 5% CO₂ atmosphere. Culture supernatant was harvested 48 h later, and the concentrations of interferon-γ (IFN-γ), interleukin-4 (IL-4) and IL-10 were determined by ELISA according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). All procedures were performed using sterile technique. The Th1/Th2 balance was assessed as the IFN-γ/IL-4 ratio according to the description of Shahid et al. (2002). The IFN-γ/IL-4 ratio of each sample was calculated, and then compared among groups.

Statistical analysis
The results were expressed as mean ± SD. Data were analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL). The bacterial results in fecal samples were expressed in log₁₀ copy numbers g⁻¹ wet weight. The differences of all the parameters among groups were first compared by Kruskal–Wallis ANOVA; when a significant difference among groups was found, then the differences between the two groups were determined using the Mann–Whitney U-test. A P-value < 0.05 was considered to be significant.

Results
Serum ovalbumin-specific IgE antibody level
Serum ovalbumin-specific IgE level was significantly higher in the positive control group than in the negative control (P < 0.05). In the probiotics prevention, treatment and whole-course intervention groups, the ovalbumin-specific IgE levels were significantly lower compared with the positive control group (P < 0.05) (Fig. 2).

Intestinal permeability
Intestinal permeability to FITC-dextran 4000 was significantly increased in the positive control group compared with the negative control group (P < 0.01). In all three probiotics administration groups, FITC-dextran 4000 concentration in serum was significantly lower than in the positive control group (P < 0.01), with the lowest value being observed in the treatment group (Fig. 3).

sIgA concentration in intestinal content
There was no significant difference in the concentration of sIgA in the intestine between positive and negative control groups. The sIgA level increased significantly in all probiotics administration groups compared with the levels in the positive and negative control groups (P < 0.05), with the
Colonial microbial communities

The logarithm number of bifidobacteria and lactobacilli in fecal samples were used for comparison. The number of bifidobacteria and lactobacilli genera in the positive control group decreased compared with the negative control group, but the difference was not significant. In the three probiotics administration groups, the numbers increased compared with the positive control group, but the difference was not significant (Table 1).

Morphology of small intestinal tissues

As shown by histological staining, the mucosal tissues of small intestine were shorter and disrupted in the positive control rats, whereas the mucosa was intact in the negative control group. Villus height and crypt depth were significantly lower in the positive control than in the negative control group \((P < 0.01)\), and this trend was reversed by probiotics administration in the three intervention groups (Table 2). More eosinophilic and mast cell infiltration was found in small intestinal mucosa in the positive control group than in the negative control group \((P < 0.05)\), whereas in the three probiotics administration groups (prevention, treatment and whole-course intervention), the eosinophilic and mast cell infiltration was significantly reduced compared with the positive control group \((P < 0.01\) and < 0.05, respectively) (Fig. 5a and b).

Cytokine production by splenic and MLN cells

To evaluate the effects of probiotics administration on host systemic and local intestinal immune responses, cytokine production was tested in the culture supernatant of isolated cells from spleen and MLNs. Th2 cytokines IL-4 (Fig. 6a) and IL-10 (Fig. 6b) from spleen and MLN cell culture had a similar profile. The levels of both IL-4 and IL-10 were significantly higher in the positive control group than in the negative control group \((P < 0.05)\), and the levels were significantly decreased in the three probiotics administration groups when compared with the positive control group \((P < 0.01)\). For the level of IFN-\(\gamma\) (Fig. 6c), the results for the spleen and MLNs in the probiotics prevention and whole-course intervention groups, and the result for the spleen in the probiotics treatment group were significantly reduced compared with the corresponding results in the
positive control group ($P < 0.05$). To determine the change of Th1/Th2 balance, we analyzed the IFN-γ/IL-4 ratio, which was significantly decreased in the positive control group compared with the negative control group ($P < 0.05$). The ratios for spleen and MLNs in the probiotics prevention and whole-course intervention groups were significantly higher than in the positive control group ($P < 0.05$), which suggests that the Th1/Th2 balance was shifted towards the Th1 side, whereas probiotics administration in the treatment group did little to shift the balance (Fig. 6d).

**Discussion**

The results of this study show that probiotics administration by prevention, treatment and whole-course intervention could attenuate the allergic response to allergen ovalbumin by decreasing the ovalbumin-specific IgE level. The underlying mechanism might be related to the improved intestinal barrier function, the decreased inflammatory cell infiltration in intestine and the shifted Th1/Th2 cytokine balance in the local and systemic immune response.

Based on the hygiene hypothesis for allergic diseases, it is feasible to prevent or treat food allergy by exposure to the microorganism, especially by probiotics supplementation. In human trials, both probiotics and prebiotics supplementation can modulate the immune function and reduce the risk of allergic diseases, but the results have not been consistent (Prescott & Bjorksten, 2007). Several factors such as the host status, the supplemented probiotics species/strain and the administered dosage are suggested to account for the different results. Recently, the timing of probiotics supplementation was suggested to influence the effect of probiotics (Kopp & Salfeld, 2009). In our study, we used a mixture of *L. rhamnosus* GG and *B. longum* BB536, both of which were previously proved to be promising probiotics strains against allergic diseases (Grüber et al., 2007; Odamaki et al., 2007), to compare the effects of probiotics supplementation in alleviating food allergic reaction when administered...
at different times. The ovalbumin-specific IgE level, which characterizes allergic reactions, is crucial for the allergic symptoms, and in this study, a significantly increased ovalbumin-IgE level was found after ovalbumin sensitization in the positive control group compared with the negative control group, which suggests that the food allergy model was induced successfully.

The comparable protective effects were observed in the three probiotics intervention groups, suggesting that short-term probiotics interventions can achieve the same effects as long-term intervention. The modulation of colonic bacteria is thought to be the most important and basic effect of probiotics supplementation. We found a decreasing trend for the number of bifidobacteria and Lactobacillus in the positive control group compared with the negative control group. The administration of probiotics increased the number of bifidobacteria in the three probiotic intervention groups, whereas the number of Lactobacillus was only increased obviously in the whole-course intervention group. The results suggest that bifidobacteria might be more vulnerable during ovalbumin sensitization and more easily modulated by probiotics supplementation. It has been shown that the extraneous administered probiotics cannot survive in the intestine for long periods of time, and in our study, the fecal samples were collected at least 1 week after probiotics administration was terminated. Therefore the number of bifidobacteria or Lactobacillus might decline to the basal level, which could partly explain why no significant difference was observed between probiotics administration groups and the positive control group.

An increased plasma concentration of FITC–dextran 4000 was found in the positive control group, which suggests that the gastrointestinal mucosal barrier was impaired due to the disrupted paracellular tight junction after ovalbumin sensitization, leading to the increased permeation of large molecule food antigens into the intestinal mucosa. In contrast, in the three probiotics administration groups, the concentration of FITC–dextran 4000 was significantly reduced. Studies have proved that probiotics supplementation could maintain the intestinal integrity in conditions such as necrotizing enterocolitis and during gastrointestinal surgery (Yang et al., 2006). Our results also suggest that administration of probiotics is likely to decrease the allergic reaction to ovalbumin, at least in part, by decreasing the paracellular permeability of ovalbumin in intestinal mucosa.

Gut microbiota is essential for the initiation of an infant’s own IgA production (Bottcher et al., 2002) and could influence the development of the entire IgA system (Sjogren et al., 2009). Our results show no significant difference of sIgA level between the positive and the negative control group; this is not in agreement with most other studies, which show that lower sIgA and selective IgA deficiency are
associated with allergic manifestations (Frossard et al., 2004). However, in a study performed by Perrier et al. (2010) a significantly higher ovalbumin-specific IgA level was found in food allergy BALB-C mice, similar to our result, and they used the same oral sensitization route as we did. Those authors speculated that the difference between their study and other studies might be due to the different genetic susceptibilities to the allergen. The different sensitization route might be associated with the different Th1 cytokine reaction. The level of sIgA was significantly increased in the three probiotics administration groups compared with the positive and negative control groups. This indicates that probiotics administration can increase sIgA production in intestine. sIgA is the most plentiful immunoglobulin on the mucosal surface, where it neutralizes harmless food antigens and prevents them from penetrating the epithelium (Silvey et al., 2001). Because a different subtype of IgA exists in the intestine, ovalbumin sensitization might induce ovalbumin-specific IgA production, and probiotic supplementation might induce other specific IgAs to act as a protective factor. Therefore, to identify the response of different subtype of IgE to OVA and probiotics stimulation, it will be necessary to analyze the subtype-specific sIgA in the future study.

By histological analysis, we found that in the positive control group the intestinal mucosal villi were damaged, and mast cell and eosinophil infiltration in the intestinal mucosa was increased. However, intestinal mucosal tissue samples from the three probiotics administration groups had significantly decreased numbers of mast cells and eosinophil infiltration compared with the positive control group. This effect can be attributed to the increased number of bifidobacteria in the intestine (Brandt et al., 2003; Shimada et al., 2008), and the modulated local and systemic immune response. Villous height was significantly increased only in the probiotics treatment and whole-course intervention groups. The results indicate that the intestinal trophic function of probiotics may not be sustained for a long time after cessation of probiotics administration, and preventative supplementation of probiotics cannot prevent the intestinal villus damage caused by the allergic reaction afterward.

The results of the study also provide evidence for the role of probiotics administration in immune development in model infant animals. Probiotics preventative supplementation and whole-course intervention changes the immune response profile from an allergy-prone (Th2) to a Th1 profile during the sensitization period.

Most previous studies have shown that the Th2 cytokine (IL-4) level was increased and Th1 cytokine (IFN-γ) decreased in allergy-prone murine models or patients compared with the negative control group (Okunuki et al., 2000; Shahid et al., 2002; Lee & Burks, 2006). In contrast, in our study the level of both IL-4 and IFN-γ in the ovalbumin sensitization positive control group was increased. In Perrier’s study (Perrier et al., 2010), a similar result was obtained, which suggests that IFN-γ may not counteract the production of Th2 cytokines and prevent the development of allergic reactions in food allergy-induced animals. Moreover, human studies demonstrated that allergen-stimulated IFN-γ cells are present in allergic individuals (Ng et al., 2002; Tay et al., 2007). The groups supplemented with probiotics had a decreased level of Th2-like cytokines of both IL-4 (from spleen) and IFN-γ (from spleen and MLNs) in all probiotics supplementation groups. The IFN-γ/IL-4 ratio shows that the Th1/Th2 balance was significantly decreased in the positive control rats and increased in the probiotics prevention and whole-course intervention groups. No significant difference was observed between the probiotics treatment and the positive control group. This suggests that probiotics supplementation has the potential to downregulate the cellular response to food antigens and may reverse the immune response profile from a Th2- to a Th1-like response in infant animals, this effect occurring only when probiotics are given at an early stage before or during allergen sensitization.

On the other hand, the probiotics supplementation resulted in a decrease of IL-10, evidence that IL-10 may be involved in promoting appropriate immune priming to food antigens. In this study, we only evaluated the effects of probiotics on the immune system by comparing the Th1/Th2 ratio, but this may not be enough. To determine the exact mechanism of probiotics action on the immune system and its effect on a decreased ovalbumin-specific IgE level in food allergy rats, more studies will be needed, including studies of CD4⁺CD25⁺Foxp³⁺ T regular cells and B-cell proliferation activity.

In conclusion, in the present work we demonstrate the significant contribution of oral administration of probiotics to alleviating food allergic reaction by improving immune and intestinal barrier function in animal models. Probiotics administration by either prevention or treatment for a short time is effective, which may suggest that long-term probiotics supplementation is unnecessary and uneconomical. More importantly, we found that preventive probiotics administration exerts its effect mainly by alleviating the inflammation in intestine and modulating allergic immune response profile towards the Th1 side, whereas short-term probiotics treatment can decrease allergic reaction by maintaining the intestinal barrier function with its trophic effects on intestine. But as the data are so conflicting, more research needs to be done before clinical trials are undertaken.

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