

Anti-allergic effects of probiotic Dahi through modulation of the gut immune system

Dahi probiyotiğinin barsak immün sisteminin düzenlenmesi yoluyla anti-allerjik etkileri

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Background/aims: The alarming increase in allergy in the last few decades demands the development of new anti-allergic prevention strategies, and consumption of functional foods (i.e. probiotic Dahi, which has already been proven to enhance immunity by modulation of the gut mucosal immune system) may be one of them. In the present study, we evaluated anti-allergic effects of a Dahi (yogurt) containing probiotic *Lactobacillus acidophilus*, *L. casei* and normal Dahi culture *Lactococcus lactis* biovar *diacetylactis* (named probiotic Dahi) on ovalbumin induced allergy in mice. **Methods:** Allergy was induced by injecting (i.p.) ovalbumin at 0 and 14 days. Animals were fed with standard diet (control), milk, control Dahi or probiotic Dahi for 21 days. Total and ovalbumin-specific IgE, cytokines and lymphocyte proliferation index were examined after 7, 14 and 21 days. **Results:** Feeding of probiotic Dahi completely suppressed the elevation of total and ovalbumin-specific IgE in the serum of ovalbumin-injected mice. Similarly, splenocytes collected from mice fed with probiotic Dahi entirely lost the total and ovalbumin-specific IgE production property during in-vitro culture. Production of T helper (Th)-1 cell-specific cytokines, i.e. interferon - γ and interleukin (IL)-2, increased, while Th2-specific cytokines, i.e. IL-4 and IL-6, decreased in the supernatant of cultured splenocytes collected from mice fed with probiotic Dahi compared to the other groups. Moreover, ovalbumin-stimulated lymphocyte proliferation was strongly suppressed by feeding of probiotic Dahi in comparison to milk and control Dahi. **Conclusions:** Results of the present study indicate that probiotic Dahi suppressed ovalbumin-induced allergic consequences characterized by decreasing levels of total and ovalbumin-specific IgE and lymphocyte proliferation and skewed ovalbumin-induced Th2-specific immune response towards Th1-specific response.

Key words: Probiotic Dahi, allergy, immunoglobulin E, cytokines, cell proliferation

Amaç: Son dekatlarda artarak, alarm vermeye başlayan allerjik hastalıklar, aralarında fonksiyonel gıdaların (ör. Dahi probiyotiğinin barsak mukozal immün sistemini düzenleyerek bağışıklığı iyileştirdiği daha önce kanıtlanmıştır), tüketimi gibi yeni anti-allerjik korunma stratejileri geliştirilmesini gerekli kılmaktadır. Bu çalışmada, probiyotik olarak *Lactobacillus acidophilus*, *L. casei* ve normal Dahi kültürü *Lactococcus lactis* biovar *diacetylactis* (ismen Dahi probiyotiği) içeren Dahiyle (yoğurt) beslenen, farelerde ovalbumin ile indüklenen allerji durumundaki etkinliği araştırılmıştır. **Yöntem:** Alerji 0 ve 14. günlerde ovalbumin (ip) enjeksiyonuyla indüklendikten sonra hayvanlar, standart diyet (kontrol), süt, kontrol Dahi ve probiyotik Dahi ile 21 gün beslendiler. 7, 14 ve 21 gün sonra total ve ovalbumin-spesifik IgE, sitokinler ve lenfosit proliferasyon indeksleri saptandı. **Bulgular:** Ovalbumin-enjekte edilen farelerin probiyotik Dahi ile beslenmesi, serumlarında ovalbumin-spesifik ve total IgE düzeylerini tamamiyle baskılamıştı. Benzer şekilde, probiyotik Dahiyle beslenen farelerden toplanan splenositlerin, in-vitro kültürde total ve ovalbumin-spesifik IgE üretiminin tamamen yok olduğu görülmüştür. Probiyotik Dahiyle beslenen farelerden toplanan ve kültürlen splenositlerin süpernatantında, diğerlerine göre T helper (Th)-1 spesifik sitokinler (ör. IFN- γ ve IL-2) artmış, Th2 spesifik sitokinler ise (ör. IL-4 ve IL-6) azalmıştır. Dahası, probiyotik Dahiyle beslenmeye, ovalbumin-uyarılmış lenfosit çoğalması, süt ve kontrol Dahiyle karşılaştırıldığında kuvvetle baskılanmıştı. **Sonuçlar:** Bu çalışmanın sonucunda görülmüştür ki, probiyotik Dahi ovalbumin ile indüklenen allerjik durumları, total ve ovalbumin-spesifik IgE seviyelerini azaltarak, lenfosit proliferasyonu ve ovalbumine bağlı gelişen immün yanıtı Th2 özgülünden Th1'e özgüle döndürmek suretiyle baskılamaktadır.

Anahtar kelimeler: Probiyotik Dahi, alerji, immunoglobulin E, sitokinler, hücre çoğalması

INTRODUCTION

The increase in the prevalence of allergic disorders over the past few decades is challenging the

scientific community and medicare professionals. Allergy is a result of IgE-dependent and -independ-

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dent hypersensitivity reactions (1). Approximately 1 in 5 or 18.4% of people in the United States suffer from allergic diseases, and the prevalence is increasing daily, because to date, no curable therapy is available on the market for the treatment of allergic consequences. Hence, this demands the development of new prevention strategies to inhibit the rapidly increasing prevalence of allergic diseases. Use of probiotics as an alternative medicine for the treatment of allergy has emerged recently and has attracted the attention of many scientists worldwide (2). Probiotics can be defined as 'live microorganisms that upon ingestion give several health benefits and considered as a generally recognized as safe (GRAS)' (3). Various strains of lactic acid bacteria (LAB) especially lactobacilli and bifidobacteria are considered as probiotics and have been reported to suppress the allergic reactions in different animal models (4-6) as well as in human subjects (7). Matsuzaki et al. (5) reported that feeding of the heat-killed *Lactobacillus casei* strain Shirota suppressed total IgE production in ovalbumin (OVA)-sensitized mice. Pohjavuori et al. (8) also showed that oral administration of *L. rhamnosus* GG elevated interferon (IFN)- γ production by peripheral blood mononuclear cells of infants with cow's milk allergy and IgE-associated dermatitis. It has been noticed that the anti-allergic effects of various strains of lactobacilli are strictly strain-dependent (9); thus, searching new, safer and more easily available probiotic LAB strains is an important area of present research in food science and nutrition.

Although various LAB have been reported for anti-allergic effects, few of them are available on the market in the form of tablets or powder. However, these commercially available products have various issues such as survivability, loss of efficiency to establish into the gut and psychological illness of patients. Thus, the addition of probiotic LAB with food, i.e. milk products, may overcome these limitations and provide nutritional benefits along with health benefits. It has been known that LAB can easily grow in dairy products, i.e. yogurt or Dahi, as they have a good buffering environment and essential nutrients for their growth. Considering all these facts, we developed a Dahi (yogurt) by incorporating two newly established probiotic strains (*L. acidophilus* and *L. casei*) along with Dahi cultures (named probiotic Dahi), with various technological modifications (10). Preliminary studies have shown that ingestion of probiotic Dahi enhanced

innate immune function in terms of increased production of sIgA and improved phagocytic activity of macrophages in mice (11). On the basis of these results, we hypothesized that ingestion of probiotic Dahi may also strengthen the immune response against OVA-induced allergic consequences. Hence, in the present study, we made an attempt to examine the anti-allergic effects of probiotic Dahi on OVA-induced allergic mice.

MATERIALS AND METHODS

Bacterial Strains and Preparation of Dahi(s)

Probiotic strains *L. acidophilus* NCDC14, *L. casei* NCDC19 and Dahi starter culture *Lactococcus lactis* biovar *diacetylactis* NCDC-60 (DRC-1) were procured from the National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, India. Probiotic Dahi was prepared as described elsewhere (10). In brief, standardized milk was inoculated with *L. acidophilus* ($\sim 10^7$ CFU/ml), *L. casei* ($\sim 10^4$ CFU/ml) along with DRC-1 ($\sim 10^6$ CFU/ml) for preparation of probiotic Dahi and incubated at 37°C for 12-14 h. DRC-1 control Dahi was prepared by inoculating DRC-1 culture ($\sim 10^7$ CFU/ml) and then incubating at 30°C for 12-14 h.

Animals and Feeding Schedule

Swiss albino mice (6-8 weeks old and 25-31 g body weight) were obtained from the small animal house of the institute and housed in the polypropylene cages (2-3 animals per cage) with 12-12 h light/dark cycle at 20-25°C ambient temperature. Animals were divided into 4 groups (n=15), i.e. 1. *allergic control*: fed with synthetic diet (SD), 2. *milk-fed group*: fed with non-fermented milk containing 2.5% fat (20% water solution) along with SD, 3. *DRC-1 Dahi-fed group*: fed with DRC-1 control Dahi along with SD, and 4. *probiotic Dahi-fed group*: fed with probiotic Dahi along with SD for 21 days. SD contained 38.5% starch, 20% casein, 25% sucrose, 10% refined oil, 1% vitamin mixture, 5% mineral mixture, 0.2% choline chloride, and 0.3% methionine. Vitamin and mineral mixtures were prepared and mixed according to Association of Official Agricultural Chemists (12). Food and water intakes were recorded daily. Allergic response was induced by injecting OVA (20 μ g of OVA and 2 mg of Al(OH)₃ at a total volume of 200 μ l) intraperitoneally on days 0 and 14, and animals (n=5) were sacrificed after 7, 14 and 21 days. The study protocol was approved by the Institutional

Animal Ethics Committee, and animals were maintained as per the rules of animal handling and care of the National Institute of Nutrition, Hyderabad, India.

Preparation of Serum Samples

Blood was drawn from orbital venous plexus in a vial at 7, 14 and 21 days of experimental periods, and serum was separated by centrifuging blood samples at 5000 x g for 10 min and then frozen at -80°C till further use.

Isolation and Culture of Splenocytes

Mice were sacrificed by cervical dislocation with brief anesthesia. The spleen was removed aseptically and suspended in RPMI 1640 culture medium supplemented with 10% fetal calf serum (FCS; heat inactivated at 56°C for 30 min), sodium bicarbonate, sodium pyruvate, HEPES, penicillin, and streptomycin. Splenocytes were prepared by gently teasing splenic tissues using sterile needle and forceps. Suspension was allowed to stand for 2 min to sediment tissue clumps. The splenocytes-enriched upper portion of the medium was transferred to a 15 ml sterile centrifuge tube and centrifuged at 1000 x g for 5 min. Cells were washed with RPMI medium and red blood cells (RBCs) lysed by 1 ml of erythrocyte lysis buffer (10 ml Tris HCl 0.17M and 90 ml of NH₄Cl 0.6M, pH-7.2). Erythrocyte lysis buffer was washed out by adding 5 ml of culture medium and centrifuging at 1000 x g for 5 min. After washing twice, splenocytes were suspended in 1 ml of RPMI 1640 containing 10% FCS and cells were counted from a small aliquot of cell suspension. Finally, cells were plated on a 96-well plate containing 1 µg/well OVA in 100 µg of RPMI with 10% FCS (n=4 per animal sample) and allowed to grow for 48 h at 37°C in CO₂ incubator. After incubation, supernatant media was harvested from half of the samples (n=2 from each animal sample) and used for determination of IgE and cytokines, and the remaining wells were used for determination of lymphocyte proliferation index.

Measurement of Total IgE

Total IgE in serum and supernatant of cultured splenocytes was determined by using ELISA kit (Bethyl Laboratories, Montgomery, TX, USA). Operating procedures strictly followed the manufacturer's instructions. The principle of the ELISA kit was to employ the quantitative sandwich enzyme immunoassay.

Measurement of OVA-Specific IgE

OVA-specific IgE in serum and supernatant of cultured splenocytes was determined by using indirect ELISA (13). In brief, 100 µl of antigen (OVA; 100 µg/ml) in 0.06 M carbonate buffer was coated in test wells and 100 µg of carbonate buffer alone in control wells by incubating overnight at 37°C. After drying, 200 µl of 70% methanol was added in each well and left for 20 min to fix antigen and dried by incubating at 37°C for 10 min (14). Free binding sites were blocked by adding 350 µl of blocking solution and incubated at room temperature for 1 h with occasional shaking. Blocking solution was removed and wells were washed three times with PBS/T. Serum (100 µl) was added to each well and the plate was incubated for 1 h at 37°C with occasional shaking. After washing, goat anti-mouse IgE-HRP conjugate secondary antibody was added. After washing, TMB substrate solution was added for color development and the reaction stopped with 100 µl of 4N H₂SO₄. Optical density (OD) was measured at 450 nm using ELISA plate recorder (Model ETY-119, Oriental Instruments Ltd, Tokyo, Japan), and OVA-specific IgE concentration was expressed as OD/100 µl.

Estimation of Cytokine Levels

Cytokines in serum and supernatant of culture medium were measured by ELISA kits (IFN-γ, interleukin (IL)-4 and IL-6 [Bender Medsystem, Delhi, India] and IL-2 [Genetix Asia Pvt. Ltd., Delhi, India]). Assays were performed following the instructions of the manufacturers.

Cell Proliferation Assay

Proliferative response of splenocytes was estimated using the colorimetric MTT assay (15). Briefly, 10 µl fresh filtered MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; 5 mg/ml dissolved in RPMI-1640] was added in 48 h cultured lymphocytes and the plate was incubated for 4 h at 37°C. During this period, formazan crystals were formed at the bottom of each well. Spent media along with suspension of cultured cells was pipetted out, and 100 µl of acid isopropanol (0.1 N HCl in anhydrous isopropanol) was mixed to dissolve crystals. After a few minutes at room temperature, OD was measured by using a plate analyzer in dual wavelength (i.e. test wavelength of 540 nm and reference wavelength of 630 nm). Plates were normally read within 1 h of adding acid isopropanol. Cell proliferation was expressed as proliferation index (A540 with OVA/A540 without OVA).

Statistical Analysis

Results were expressed as mean $\pm$ standard error of mean. Statistical significance was tested by employing analysis of variance (ANOVA) and comparison between means was calculated by either Duncan's multiple range test (DMRT). Differences of $p < 0.05$ were considered statistically significant.

RESULTS

Effect on Total IgE Production

Total serum IgE levels in normal mice at basal level were 150.12 ng/ml, which increased significantly and reached 535 ng/ml after 21 days of OVA-immunization in the control group of mice (Figure 1). Interestingly, elevation of IgE levels in serum were almost completely suppressed in animals fed with probiotic Dahi and levels were maintained similar to basal during the entire experimental period. While a moderate suppressive effect on increased IgE levels was also observed in DRC-1 (control) Dahi-fed animals, it was abstractedly mild compared to probiotic Dahi (Figure 1A). No significant suppressive effect was observed on serum IgE levels in milk-fed animals during the entire experimental period.

Furthermore, the suppressive effect of probiotic Dahi on total IgE in OVA-stimulated cultured splenocytes collected from different experimental groups was also confirmed (Figure 1B), and results showed that the production of total IgE was also fully suppressed by splenocytes collected from probiotic Dahi-fed animals during the entire experimental period (21 days).

Effect on OVA-Specific IgE Production

Figure 2 clearly shows that OVA-specific IgE was significantly increased in serum as well as supernatant of OVA-challenged splenocytes, indicating that the increased total IgE was due to increased OVA-specific IgE during the entire experimental period. Further, the feeding of probiotic Dahi significantly suppressed the elevation of OVA-specific IgE in serum as well as supernatant of OVA-stimulated splenocytes, while feeding of control Dahi and milk were unable to restrict elevation of these antibodies in both serum as well as supernatant of cultured splenocytes.

Effects on Cytokine Production

The circulatory levels of cytokines, i.e. IFN- γ , IL-2, IL-4 and IL-6 were unchanged in the serum of mice in all groups, except for a slight increase in

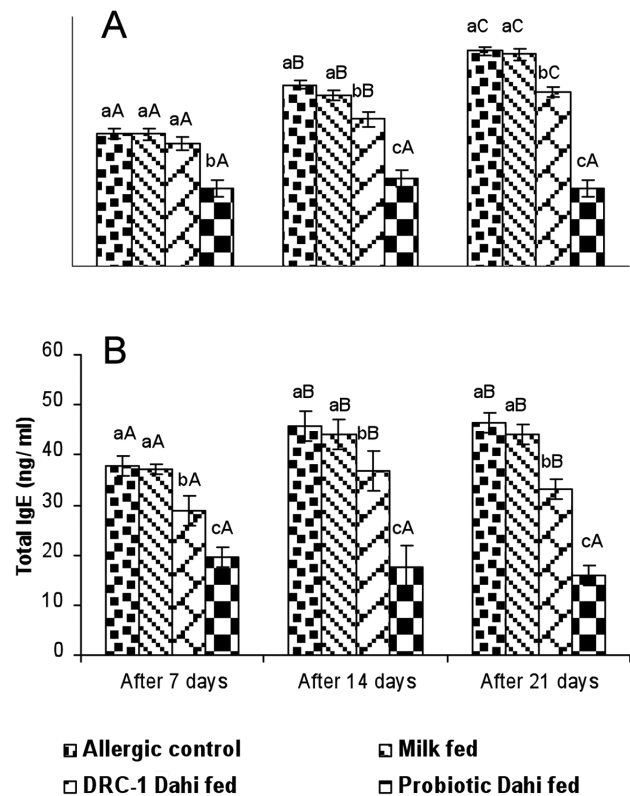


Figure 1. Total IgE levels in serum (A) and in the supernatant of cultured splenocytes (B) collected from OVA-induced allergic mice fed with SD (allergic control), skim milk (milk-fed), control Dahi (DRC-1 Dahi-fed) and probiotic Dahi.

a,b,c,d Values with different symbols between various groups at a particular time are significantly different at the level of $p < 0.05$. A,B,C Values with different symbols at various time points (7, 14 and 21 days) in a particular group are significantly different at the level of $p < 0.05$.

IFN- γ and slight decrease in IL-4 levels in the probiotic Dahi-fed animals (data not shown). However, when we examined the production of these cytokines in the supernatant of OVA-stimulated cultured splenocytes collected from all experimental groups, it was seen that the levels of IFN- γ and IL-2 were significantly decreased and levels of IL-4 and IL-6 were significantly increased in the supernatant of OVA-stimulated cultured splenocytes collected from control mice. Feeding of probiotic Dahi reversed this effect by significantly increasing production of IFN- γ and IL-2 and decreasing production of IL-4 and IL-6 in the supernatant of splenocytes collected after the first OVA-injection (after 7 days) and also remained similar after the second OVA-injection on day 14 (Tables 1 and 2). However, levels of IFN- γ and IL-2 also increased slightly in the supernatant of cultured splenocytes collected from mice fed with DRC-1 (control) Dahi

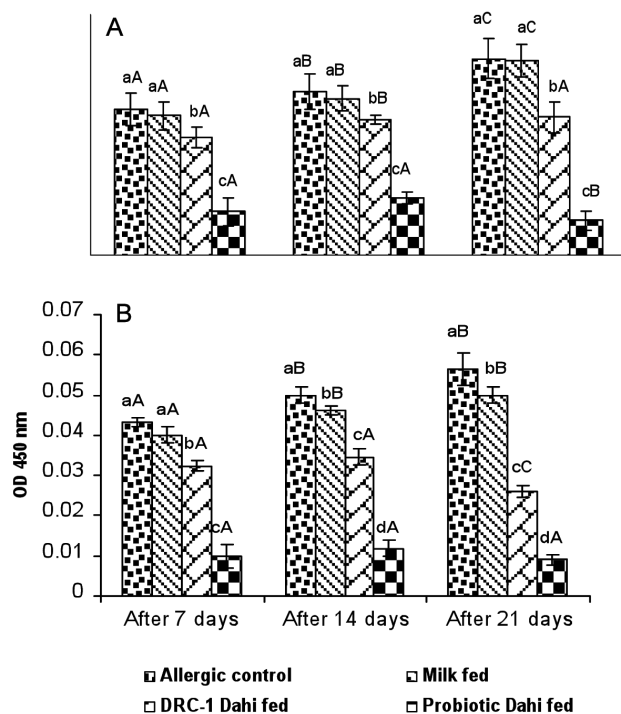


Figure 2. OVA-IgE levels in serum (A) and in the supernatant of cultured splenocytes (B) collected from OVA-induced allergic mice fed with SD (allergic control), skim milk (milk-fed), control Dahi (DRC-1 Dahi-fed) and probiotic Dahi.

a,b,c,d Values with different symbols between various groups at a particular time are significantly different at the level of $p < 0.05$. A,B,C Values with different symbols at various time points (7, 14 and 21 days) in a particular group are significantly different at the level of $p < 0.05$.

and milk, but values were distantly lower than with probiotic Dahi.

Effect on Cell Proliferation of Cultured Splenocytes

Data in Figure 3 show that splenocyte proliferation in terms of proliferation index was continuously increased after challenging with OVA in mice (control

group) up to day 21 of the experimental period. While feeding of probiotic Dahi completely suppressed the OVA-induced proliferation rate of splenocytes, feeding of control Dahi also exhibited inhibitory effects on proliferation of splenocytes, but it was vaguely effective compared to probiotic Dahi. No effect on suppression of OVA-stimulated splenocyte proliferation was observed in milk-fed animals.

DISCUSSION

In the present study, we observed that feeding of probiotic Dahi is remarkably effective in suppressing OVA-induced allergic consequences in mice. This effect of probiotic Dahi was distantly dominant compared to control Dahi and milk feeding. As the allergy is a health threat with increasing prevalence, it demands the development of prevention strategies (16). Consumption of functional foods might be one such strategy that can prevent the rapidly increasing prevalence of allergy. OVA-induced allergy in mice is a good model for studying various food allergic consequences and validating new treatment strategies (17). OVA is an allergen that induces allergy when injected (i.v./i.p./i.n.) into the body of experimental animals. During induction of OVA-mediated allergy, various common characteristics of human food allergy such as diarrhea and anxiety have been observed in mice models (18). In the present study, we did not observe diarrhea or vomiting among any of the mice groups, which indicates that the selected dose of OVA was not chronic, which can disrupt the normal immunomodulatory function in mice.

Moreover, OVA-induced allergy is a common model to study *in-vitro* and *in-vivo* IgE-mediated immunological consequences. Various strains of LAB have been reported to reduce OVA-induced allergic consequences in cell culture and animal models (4-6, 19). In the present study, feeding of pro-

Table 1. Effect of feeding of different experimental diets on the level of IFN- γ and IL-2 cytokines in the supernatant of cultured splenocytes¹

Groups	IFN- γ (pg/ml)			IL-2 (pg/ml)		
	Experimental period (days)			Experimental period (days)		
	7	14	21	7	14	21
Allergic control	207.1 $\pm$ 11.02 ^{aA}	199.2 $\pm$ 12.34 ^{aA}	190.4 $\pm$ 14.32 ^{aA}	146.6 $\pm$ 7.43 ^{aA}	108.4 $\pm$ 5.44 ^{aA}	111.2 $\pm$ 7.43 ^{bA}
Milk-fed	215.5 $\pm$ 9.83 ^{aA}	219.5 $\pm$ 16.21 ^{aB}	208.9 $\pm$ 15.23 ^{aB}	160.3 $\pm$ 5.23 ^{aB}	139.2 $\pm$ 7.33 ^{aA}	134.0 $\pm$ 5.21 ^{bB}
DRC-1 dahi-fed	272.6 $\pm$ 8.99 ^{aB}	247.4 $\pm$ 12.43 ^{bC}	231.9 $\pm$ 16.93 ^{bB}	167.3 $\pm$ 6.11 ^{aB}	162.5 $\pm$ 5.12 ^{aB}	158.7 $\pm$ 9.01 ^{aB}
Probiotic dahi-fed	493.9 $\pm$ 11.28 ^{aE}	557.7 $\pm$ 20.21 ^{bE}	598.4 $\pm$ 23.32 ^{bC}	220.9 $\pm$ 9.21 ^{aD}	223.9 $\pm$ 5.48 ^{aD}	284.5 $\pm$ 10.76 ^{aD}

¹ Values are mean $\pm$ SEM of 5 animals in each group.

a,b,c Values with different superscripts in a row for a particular parameter are significantly different at the level of $p < 0.05$.

A,B,C Values with different superscripts in a column for a particular parameter are significantly different at the level of $p < 0.05$.

Table 2. Effect of feeding of different experimental diets on the level of IL-4 and IL-6 cytokines in the supernatant of cultured splenocytes ¹

Groups	IL-4 (pg/ml)			IL-6 (pg/ml)		
	Experimental period (days)			Experimental period (days)		
	7	14	21	7	14	21
Allergic control	91.3±7.11 ^{aA}	104.56±6.23 ^{bA}	114.50±9.23 ^{aA}	103.4±7.23 ^{aA}	96.2±7.38 ^{aA}	120.4±6.11 ^{bA}
Milk-fed	86.6±9.33 ^{aB}	75.23±5.64 ^{aB}	80.23±8.12 ^{aB}	90.1±5.55 ^{aB}	85.6±5.34 ^{aA}	94.2±6.98 ^{aB}
DRC-1 dahi-fed	60.2±2.41 ^{aC}	76.20±7.21 ^{bB}	78.90±8.88 ^{bB}	79.7±6.77 ^{aB}	80.1±3.49 ^{aA}	90.1±6.21 ^{aB}
Probiotic dahi-fed	33.0±7.83 ^{aE}	29.645±6.09 ^{aD}	27.24±6.39 ^{aC}	56.8±4.11 ^{aD}	54.9±7.44 ^{aC}	50.2±9.01 ^{aC}

¹ Values are mean ± SEM of 5 animals in each group.

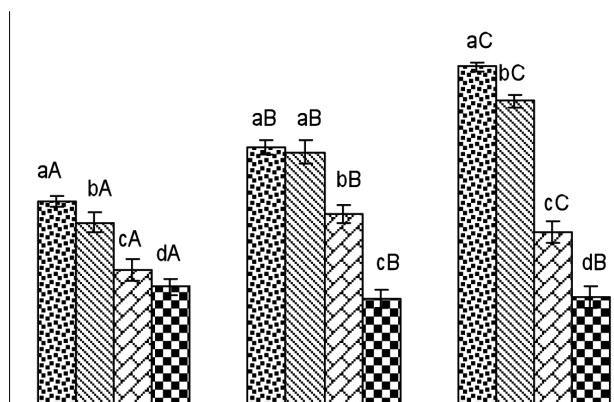
a,b,c Values with different superscripts in a row for a particular parameter are significantly different at the level of p<0.05.

A,B,C Values with different superscripts in a column for a particular parameter are significantly different at the level of p<0.05.

biotic Dahi significantly suppressed the elevation of serum levels of total and OVA-specific IgE in OVA-injected mice. Similarly, splenocytes collected from mice fed with probiotic Dahi completely lost the capacity of OVA-stimulated production of total and OVA-specific IgE. This indicates that probiotic Dahi inhibited the OVA-mediated allergic response in the immune cells to produce IgE antibodies. Similar results were also found in several previous studies demonstrating that ingestion of LAB decreased total and OVA-specific IgE levels in cell culture and mice models (13, 20-22). However, in these studies, pure LAB cultures and/or their extracts were used, while in the present study, we prepared a fermented milk product (Dahi) that may be used for direct human consumption as a safe, easily consumed and palatable functional food with anti-allergic effects.

Moreover, IgE-induced allergy is reported to be caused by a skew in the balance between Th1 and Th2 immune cell response (23, 24). One of the main mechanisms behind the regulation of IgE production by the probiotic Dahi might be preservation of balance between Th1 and Th2 response. The results of the present study proved that feeding of probiotic Dahi suppressed the production of Th2-specific cytokines (IL-4 and IL-6) and increased production of Th1-specific cytokines (IL-2 and IFN-γ). It indicates that probiotic Dahi may skew the OVA-induced Th2 immune response towards Th1 immune response, and hence might be beneficial in preventing IgE-mediated allergy. The regulation of allergic response depends on the proliferation and regulation of immune cells such as B, T and natural killer -cells (23, 24). In the present study, we observed that probiotic Dahi feeding significantly suppressed the OVA-stimulated splenocyte proliferation. These findings indicate that probiotic Dahi has the potential to induce immune cells in a beneficial manner for regulating the proliferation as well as functional activity in response to an allergen.

While the exact mechanism of action of probiotic Dahi is not known, our previous results showed that probiotic Dahi consumption strengthens the innate immune response in mice. Moreover, it can be clearly seen from the results of present study that anti-allergic effects of probiotic Dahi were remarkably higher than of control Dahi and milk, which indicates that the anti-allergic potential of probiotic Dahi increased due to addition of probiotic bacteria. The increased biological efficacy of probiotic Dahi compared to control Dahi might be due to the higher content of bio-constituents such as conjugated linolenic acid (CLA), free fatty acid, complex oligosaccharides, and bioactive peptides in probiotic Dahi. Recent studies done in our laboratory demonstrated that probiotic Dahi contains

**Figure 3.** Stimulation index of splenocytes collected from OVA-induced allergic mice fed with SD (allergic control), skim milk (milk-fed), control Dahi (DRC-1 Dahi-fed) and probiotic dahi.

a,b,c,d Values with different symbols between various groups at a particular time are significantly different at the level of p<0.05.

A,B,C Values with different symbols at various time points (7, 14 and 21 days) in a particular group are significantly different at the level of p<0.05.

a higher amount of CLA, free fatty acids (25), oligosaccharides (26) (prebiotics), and bioactive peptides (27, 28), which are well known for their immunomodulatory functions (29-32). This further proves the higher biological efficacy of probiotic Dahi than milk and control Dahi, especially with regard to suppression of OVA-induced allergic consequences. Although probiotic Dahi was found dominantly effective in suppressing OVA-induced allergic consequences, further investigations are

required to know the detailed mechanism of action of probiotic Dahi and/or its bio-constituents.

In conclusion, probiotic Dahi can be put forward as a new alternative therapeutic agent for prevention of allergic diseases to benefit the millions of people suffering from food allergy. Despite these observations, however, much research is still needed to determine the true efficacy and safer use of probiotic Dahi for human consumption in preventing food allergy.

REFERENCES

- Kay AB. Allergy and allergic diseases. *N Engl J Med* 2001; 344: 30-7.
- Shimojo N, Suzuki S, Campos E, et al. Effects of probiotics on the immune system and allergic diseases. *Allergol Int* 2005; 54: 515-20.
- Fuller R. Probiotics in human medicine. *Gut* 1991; 32: 439-42.
- Ishida Y, Bandou I, Kanzato H, et al. Decrease in ovalbumin specific IgE of mice serum after oral uptake of lactic acid bacteria. *Biosci Biotechnol Biochem* 2003; 67: 951-7.
- Matsuzaki T, Yamazaki R, Hashimoto S, et al. The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J Dairy Sci* 1998; 81: 48-53.
- Ohno H, Tsunemine S, Isa Y, et al. Oral administration of *Bifidobacterium bifidum* G9-1 suppresses total and antigen specific immunoglobulin E production in mice. *Biol Pharm Bull* 2005; 28: 1462-6.
- Morita H, He F, Kawase M, et al. Preliminary human study for possible alteration of serum immunoglobulin E production in perennial allergic rhinitis with fermented milk prepared with *Lactobacillus gasseri* TMC0356. *Microbiol Immunol* 2006; 50: 701-6.
- Pohjavuori E, Viljanen M, Korpela R, et al. Lactobacillus GG effect in increasing IFN gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol* 2004; 114: 131-6.
- Ezendam J, van Loveren H. Probiotics: immunomodulation and evaluation of safety and efficacy. *Nutr Rev* 2006; 64: 1-14.
- Yadav H, Jain S, Sinha PR. Preparation of low fat probiotic. *J Dairying Food Home Sci* 2005; 24: 172-7.
- Jain S, Yadav H, Sinha PR. Stimulation of innate immunity by oral administration of Dahi containing probiotic *Lactobacillus casei* in mice. *J Med Food* 2008; 11: 652-6.
- AOAC. Official methods of analysis (14th ed). Washington, DC: Association of Official Agricultural Chemists, 1984.
- Ohno H, Tsunemine S, Isa Y, et al. Oral administration of *Bifidobacterium bifidum* G9-1 suppresses total and antigen specific immunoglobulin E production in mice. *Biol Pharm Bull* 2005; 28: 1462-6.
- Vorhchoor JA, Meiring MI, Wyngaardt S, et al. Polystyrene, poly-L-Lysine and nylon as adsorptive surfaces for the binding of whole cells of mycobacterium tuberculosis H37 RV of ELISA plates. *J Immunoassay* 1990; 11: 413-28.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* 1983; 65: 55-63.
- Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol* 2008; 121: 1331-6.
- Bochner BS, Hamid Q. Advances in mechanisms of allergy. *J Allergy Clin Immunol* 2003; 111: S819-S823.
- Saldanha JC, Gargiulo DL, Silva SS, et al. A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice. *Braz J Med Biol Res* 2004; 37: 809-16.
- Murosaki S, Yamamoto Y, Ito K, et al. Heat killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen specific IgE production by stimulation of IL-12 production in mice. *J Allergy Clin Immunol* 1998; 102: 57-64.
- Sunada Y, Nakamura S, Kamei C. Effects of *Lactobacillus acidophilus* strain l-55 on experimental allergic rhinitis in BALB/c mice. *Biol Pharm Bull* 2007; 30: 2163-6.
- Peng S, Lin JY, Lin MY. Antiallergic effect of milk fermented with lactic acid bacteria in a murine animal model. *J Agric Food Chem* 2007; 55: 5092-6.
- Flinterman AE, Knol EF, van Ieperen-van Dijk AG, et al. Probiotics have a different immunomodulatory potential in vitro versus ex vivo upon oral administration in children with food allergy. *Int Arch Allergy Immunol* 2007; 143: 237-44.
- Botturi K, Vervloet D, Magnan A. T cells and allergens relationships: are they that specific? *Clin Exp Allergy* 2007; 37: 1121-3.
- Fujiwara D, Inoue S, Wakabayashi H, et al. The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. *Int Arch Allergy Immunol* 2004; 135: 205-15.
- Yadav H, Jain S, Sinha PR. Production of free fatty acids and conjugated linoleic acid in probiotic Dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* during fermentation and storage. *Int Dairy J* 2007; 60: 205-10.
- Yadav H, Jain S, Sinha PR. Study of oligosaccharide formation during storage of skim milk fermented with Lactococci and Lactobacilli bacteria. *J Dairy Res* 2007; 74: 154-9.
- Jain S, Yadav H, Sinha PR. Probiotic Dahi containing *Lactobacillus casei* protects against Salmonella enteritidis infection and modulates immune response in mice. *J Med Food* 2009; 12: 576-83.
- Jain S, Yadav H, Sinha PR, Marotta F. Modulation of cytokine gene expression in spleen and Peyer's patches by feeding Dahi containing probiotic *Lactobacillus casei* in mice. *J Dig Dis* 2009; 10: 49-54.
- O'Shea M, Bassaganya-Riera J, Mohede IC. Immunomodulatory properties of conjugated linoleic acid. *Am J Clin Nutr* 2004; 79: 1199S-1206S.
- Stulnig TM. Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. *Int Arch Allergy Immunol* 2003; 132: 310-21.
- Metugriachuk Y, Tsuchiya J, Marotta F, et al. Effect of a prebiotic-enriched phytocompound in improving ovalbumin allergenicity. *Chin J Dig Dis* 2006; 7: 206-10.
- Silva SV, Malcata FX. Caseins as source of bioactive peptides. *Int Dairy J* 2005; 15: 1-15.