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Randomized Placebo-Controlled Trial of Lactobacillus on Asthmatic Children With Allergic Rhinitis

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Summary. Previous studies have suggested that probiotic administration may have therapeutic and/or preventive effects on atopic dermatitis in infants; however, its role in allergic airway diseases remains controversial. To determine whether daily supplementation with specific *Lactobacillus gasseri* A5 for 8 weeks can improve the clinical symptoms and immunoregulatory changes in school children suffering from asthma and allergic rhinitis (AR). We conducted a randomized, double-blind, placebo-controlled study on school children (age, 6–12 years) with asthma and AR. The eligible study subjects received either *L. gasseri* A5 (n = 49) or a placebo (n = 56) daily for 2 months. Pulmonary function tests were performed, and the clinical severity of asthma and AR was evaluated by the attending physicians in the study period. Diary cards with records of the day- and nighttime peak expiratory flow rates (PEFR), symptoms of asthma, and AR scores of the patients were used for measuring the outcome of the treatment. Immunological parameters such as the total IgE and cytokine production by the peripheral blood mononuclear cells (PBMCs) were determined before and after the probiotic treatments. Our results showed the pulmonary function and PEFR increased significantly, and the clinical symptom scores for asthma and AR decreased in the probiotic-treated patients as compared to the controls. Further, there was a significant reduction in the TNF-α, IFN-γ, IL-12, and IL-13 production by the PBMCs following the probiotic treatment. In conclusion, probiotic supplementation may have clinical benefits for school children suffering from allergic airway diseases such as asthma and AR. **Pediatr Pulmonol. 2010; 45:1111–1120.** © 2010 Wiley-Liss, Inc.

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INTRODUCTION

Allergic diseases such as atopic eczema, urticaria, perennial allergic rhinitis (AR), and allergic asthma are becoming most common chronic diseases in developing as well as developed countries.[1] One of the explanations for the increased prevalence of these diseases lies in the well-known ''hygiene hypothesis,'' which postulates that the increase in allergic diseases reflects a decrease in infections during childhood.[2–4]

Probiotics are live organisms which exert a beneficial effect in the prevention as well as treatment of allergic diseases through modification of immune system of host via gut ecosystem.[5,6] Intestinal microbiota differs in infants who later develop allergic diseases,[7,8] and feeding probiotics to infants at risk has been shown to reduce their rate of developing eczema.[9,10] Clinical trials also suggest that the exposure to microbes through the gastrointestinal tract powerfully shapes immune function.[6] In particular, lactobacilli are considered to induce reactions involving Th1 cells and to improve allergic diseases.[11,12] Orally administered heat-treated *Lactobacillus casei* (strain *Shirota*) was found to inhibit IgE production induced by ovalbumin in mouse serum.[13] Moreover, heat-treated *L.*

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plantarum L-137 injected intraperitoneally was demonstrated to suppress IgE production in response to casein allergy in mice.[14] Oral administration of lysed *Enterococcus faecalis* FK-23 resulted in a decrease in the peritoneal accumulation of eosinophils induced by ragweed pollen.[15] In humans, *L. rhamnosus* strain GG (LGG) administered perinatally reduced the incidence of atopic eczema in children at risk during the first 2 years of life[9] and beyond infancy.[10] Thus, the effects of probiotic supplementation are likely to be most substantial in gastrointestinal disorders or related allergic diseases such as atopic eczema associated with food allergy.

In contrast, only a few studies have examined the effects of probiotics on allergic airway diseases such as rhinoconjunctivitis and asthma, and the results of these studies have been controversial.[16–18] However, it has been shown that *L. paracasei* might improve the quality of life of adolescents with perennial allergic rhinitis (AR),[16,17] and Hatakka et al.[19] studied the long-term consumption of probiotic milk and suggested that lactobacilli could be beneficial for the respiratory tract. Another recent report indicated that probiotic supplementation can protect infants receiving childcare against episodes of diarrheal illness and fever but not against respiratory illness.[20] Further, LGG did not alleviate the symptoms of patients with birch pollen allergy.[21]

Since there have been only a few clinical trials, insufficient information is available regarding the significance of lactobacilli in allergic disorders, particularly in allergic airway diseases.[22] Therefore, in this randomized, double-blind, placebo-controlled clinical trial, we aim to investigate the effect of *L. gasseri* strain PM-A0005 (A5; ProMD Biotech Co. Ltd., Tainan, Taiwan) supplementation on the clinical symptoms and immunological profiles of school children with AR and asthma.

MATERIALS AND METHODS

Patients

This study enrolled asthmatic children (age, 6–12 years) with a history of mild to moderate persistent asthma for at least 1 year (GINA steps 2–3) accompanied by persistent AR. All the participants or their guardians received detailed information regarding the study protocol and subsequently signed consent forms and answered a modified Chinese version of the "International Study of Asthma and Allergic Disease in Childhood" (ISAAC-C) questionnaire as well as additional questions pertaining to the diagnosis and assessment of asthma and AR.[23] This study protocol was approved by the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital. The criteria for diagnosing asthma was based on a history of 2 or more episodes of wheezing within the last 6 months, and/or a bronchodilator-test confirmation of the positive response of a 12% increase in the forced expiratory volume (FEV1) in 1 sec. Persistent AR was diagnosed based on the presence of one or more of the following six signs or symptoms for four or more days per week and for more than 4 weeks prior to the recruitment of the patients: pharyngeal pruritus, sneezing, watery rhinorrhea, itchy nose and eyes, and nasal obstruction.[24] Patients who had previously been treated with immunotherapy, oral or parenteral corticosteroids administered for more than 15 consecutive days, depot steroids, inhaled corticosteroids in doses greater than 1,000 μg/day (beclomethasone dipropionate), and inhaled β2-agonists more than 4 times a day and those suffering from other respiratory diseases, such as an anatomical abnormality of the upper respiratory tract, and congenital cardiovascular diseases were excluded.

Study Design

This clinical trial was a double-blind, randomized, placebo-controlled study conducted in the allergy and asthma clinic of the Department of Pediatrics at the National Cheng Kung University Hospital between October 2006 and March 2007. The participants were first evaluated over a 2-week run-in period (baseline, V0) during which they were assessed to determine their eligibility for the follow-up protocol. Further, the participants' symptoms were recorded daily; the peak expiratory flow rate (PEFR) was determined twice a day; written informed consent was obtained from the parents; and intervention was initiated (Fig. 1). During this period, the comprehensive medical and allergy histories of the enrolled subjects were taken, particularly the history of AR and the medication used for its control. Other evaluations included skin-prick tests for responsiveness to eight common aeroallergens, a differential blood count (including total eosinophil count), and measurements of total serum IgE as well as IgE specific to house dust and mixed pollen by using the Unicap system (Pharmacia Diagnostics, Uppsala, Sweden). A positive skin test was defined as the presence of $\geq$1 positive reaction with a weal diameter of $\geq$5 mm. Total serum IgE was measured by performing a solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). The allergen sensitization profiles were Der p (n = 87, 72.5%), Der f (n = 81, 67.5%), Cockroach (n = 46, 38.3%), Dog (n = 27, 22.5%), fungus mix (n = 38, 31.6%), pollen mix (n = 18, 15.0%), Shrimp (n = 24, 20.0%), and Milk (n = 8,) in all study patients (n = 120). All patients who met the eligibility criteria were randomized into either the probiotic-treated group or the control group (Fig. 1). A computerized randomization schedule was prepared by the hospital biostatistician with allocation by the pharmacy department. Patients in the probiotic-treated group were prescribed 1 capsule of *L. gasseri* PM-A0005 (A5; 2×10^9 cells/capsule) twice a day, while those in the control group were prescribed

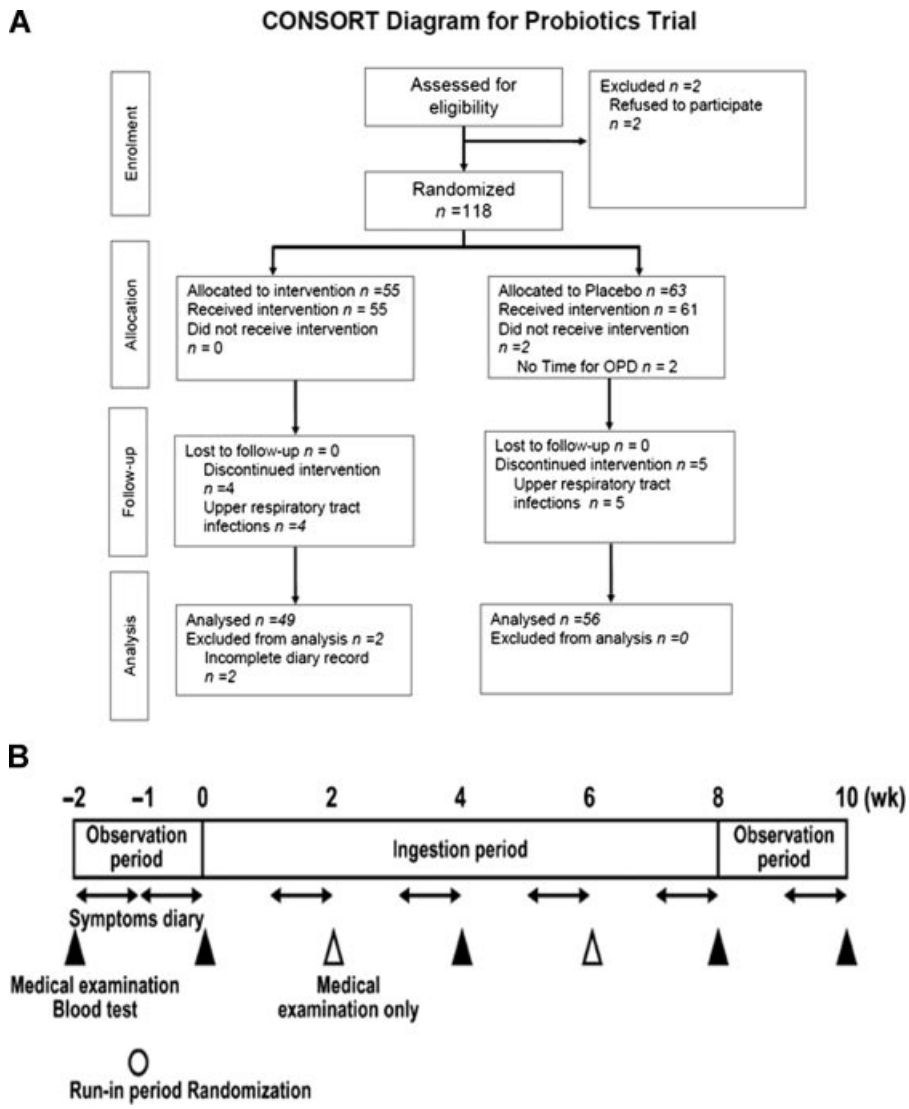

Fig. 1. Study protocol for the probiotic clinical trials. A: CONSORT diagram. B: Flow chart. The study was carried out between October 2006 and March 2007.

the placebo capsule, which contained only milk powder, at the same dosage for 8 weeks.

Preparation of the Lactobacillus and Placebo Capsules

First, *L. gasseri* A5, which exhibits probiotic characteristics, was isolated from the intestinal tract of normal, healthy humans at ProMD Biotech Co. Ltd. by using the IFN high-throughput detecting (IHTD) system. A high-density culture system was used to produce a freeze-dried powder of *L. gasseri* A5 at concentrations of more than 10^{11} colony-forming units (CFU)/g. Each capsule of both variants contained more than 2×10^9 CFU, while the placebo capsule contained only starch powder made from rice. All the capsules were supplied by ProMD Biotech Co. Ltd., which has current good manufacturing practice (cGMP) facilities, and were stored below 4°C. Stability tests demonstrated that the cell viability could be maintained at room temperature for up to 3 months. An acute oral toxicity study on test mice (FR-AC00235), conducted by the Development Center for Biotechnology (Taipei, Taiwan), for the highest dose of 5,000 mg/kg *L. gasseri* A5 administered orally for 14 days revealed that the treatment had no side effects on the mice.

Assessments

The participants underwent clinical assessments at 2-week intervals (V1–V3) up to the end of intervention week 8 (V4) and a final assessment at week 10 (V5). During this period, a physician examined each patient's

clinical symptoms, and blood samples were collected before and after administering the *Lactobacillus* supplement. The patients were instructed to maintain a symptom and medication diary. During each visit, demographic, clinical, and symptomatic outcomes and the requirement of β2-agonists, rescue medication such as oral prednisolone, and unscheduled visits to the clinic or emergency unit were recorded. Childhood asthma control test (CACT) in Chinese version[25] was evaluated in each study subject before and after treatment.

Diary Cards

The patients' parents were provided diary cards on which they were required to record the asthmatic scores, medication consumption, and PEFR of the patients throughout the run-in and treatment periods. The day- and nighttime symptoms of asthma were recorded on a 4-point scale (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms). The sum of the day- and nighttime symptom scores represented the daily asthma scores. During the trial, all patients were received same controllers in the whole study period according to individual asthmatic status and allowed to take the following rescue medications if required: inhaled β2-agonists (terbutaline aerosol), and oral corticosteroids (prednisolone, 5 mg). The number of puffs inhaled and/or tablets ingested was recorded. The PEFR was measured daily, in the morning and night; three measurements were recorded on each occasion, and the best one was used in the subsequent calculations.

Pulmonary Function Test

The physician measured the forced vital capacity (FVC), FEV_1, forced expiratory volume in a vital capacity range of 25–75% (FEF_{25-75}), and PEFR prior to and following bronchodilator inhalation by performing spirometry during the visits in V0, V3, and V5.

Assessment of Alterations in Cytokine Production

Peripheral blood mononuclear cells (PBMCs) at a concentration of 1×10^6 cells/ml were collected from the patients assigned to the probiotic-treated groups prior to and following the treatment (n = 41) and were then incubated with phytohemagglutinin (PHA; 10 μg/ml), *Dermatophagoides pteronyssinus* (Der p; 1 μg/ml), or Der p (1 μg/ml) supplemented with *L. gasseri* A5 (bacteria/cell ratio, 1:1) for 72 h. Production of the cytokines TNF-α, IFN-γ, IL-10, IL-12p40, and IL-13 in the culture supernatants was assayed using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc. Minneapolis, MN). The detection limit for these cytokines was >5 ρg/ml.

Statistical Methods

Two-way analysis of variance (ANOVA) was performed with fixed factors as continuous variables in the model for comparison between the treatment and control groups. The paired *t*-test was used to assess the changes from baseline within each group. The *P*-value below 0.05 was considered to be significant. Cochran–Mantel–Haenszel statistics for categorical variables was used to compare the differences between groups.

RESULTS

Study Population

Of 120 pediatric patients enrolled, 105 were randomly assigned to receive either probiotics (49 patients) or placebo therapy (56 patients). Intention-to-treat analysis was carried out, and Figure 1B shows the relevant patient flow chart. Table 1 presents the descriptive characteristics of the 105 subjects who fulfilled the inclusion criteria. As shown, the randomization process ensured good comparability between the probiotic-treated and control groups. No statistically significant difference was observed for any of the demographic, clinical, and functional variables. Further, the clinical severity of asthma and AR was also comparable between the two groups. No significant differences were observed in the total serum IgE, rate of sensitization to various aeroallergens, PEFR, and pulmonary function test results between the groups in the evaluation at baseline. Further, the childhood asthma control test (CACT) evaluation for asthma control performed over 4 weeks prior to the study was not significantly different between these two groups. Taking into account only the predicted values for the prebronchodilator-inhalation FEV_1, most of the patients in the two groups suffered from moderate persistent asthma. Moderate persistent AR also predominated among the patients investigated. There were six patients with one or more protocol deviations, with no difference between treatments. However, none of the protocol deviations justified exclusion of the data from the analysis, and all the available data were included.

Changes in Pulmonary Function

Table 2 shows the changes in the parameters assessed in the spirometry assay for pulmonary function performed during each clinical visit. As compared to the patients in the control group, those who received probiotic treatment exhibited significant improvements in the FEV_1, FVC, FEV_1/FVC(%), and MEF_{25-75} after the study period. Moreover, the response to bronchodilator dilatation test decreased significantly in the probiotic-treated group as compared to the control group ($P < 0.001$); this suggested that probiotic treatment may decrease bronchial hyperreactivity (BHR) in asthmatic children.

TABLE 1—Baseline Characteristics of Participants

	Probiotic-treated	Placebo
Number	49	56
Height (cm)[1]	132.0 ± 16.1	134.1 ± 16.9
Age (years)	8.1 ± 3.0	9.4 ± 4.1
Sex (male %)	57.1	57.1
Total IgE (IU/ml)	937.4 ± 1157.0	1052.6 ± 2005.8
Positive skin test		
More than one common aeroallergen (%)	11 (22.4)	16 (28.5)
Mite (Der p) allergen only (%)	38 (77.6)	40 (71.5)
Severity score[2]		
Asthma (AS)	2.0 ± 0.9	2.1 ± 1.0
Allergic rhinitis (AR)	2.2 ± 0.8	2.3 ± 1.0
Child asthma control test[3]	22.0 ± 4.3	21.3 ± 5.4
FEV_1 (% of the predicted value)	91.34 ± 8.90	89.07 ± 1.21
Number of study subjects with FEV_1 reversibility (%)[4]	38 (77.6)	41 (73.2)

[1]The data presented as mean ± SD.
[2]The coding of severity of asthma was according to GINA (Global Initiative For Asthma, http://www.ginasthma.com/) as score 1, intermittent asthma; 2, mild persistent asthma; 3, moderate persistent asthma; and 4, severe persistent asthma. The coding of severity of allergic rhinitis (AR) was according to ARIA (Allergic Rhinitis and its Impact on Asthma, http://www.whiar.org/) as score 1, mild intermittent AR; 2, moderate to severe intermittent AR; 3, mild persistent AR; 4, moderate to severe persistent AR.
[3]The score of Child Asthma Control Test was according to our previous report[25] and web site (http://www.asthmacontrol.com/child.html).
[4]The criteria for the presence of FEV_1 reversibility was the percentage increase more than 12 % in FEV_1 after bronchodilator inhalation as compares with the pre-bronchodilator.

Changes in the PEFR

On each visit to the clinic, the patients were given a symptom diary card for maintaining daily records of their best day- and nighttime PEFR scores, day- and nighttime asthmatic symptom scores, and AR symptom scores over a 2-week period. In addition to daily self-performed PEFR measurements, the patients were instructed to measure the PEFR during each clinical visit with the assistance of a specialized nurse. Figure 2 shows the PEFR values of the patients recorded during each clinical visit (Fig. 2A) and the changes in the day- and nighttime values recorded in the patients' diary cards (Fig. 2B). Comparison of the PEFR values recorded at each clinical visit during the study period revealed a significant increase in the values of the probiotic-treated patients as compared to the controls ($P < 0.05$). The two groups did not differ significantly with regard to the increase in the daytime PEFR values; however, the nighttime PEFR values increased significantly during the first 2 weeks (V1; $P < 0.05$) and at the end of the study period (V4; $P < 0.05$) in the probiotic-treated groups as compared to the placebo control group.

Changes in the Asthma and AR Symptoms

By reviewing the asthmatic symptoms and daily records of the asthmatic children with AR, we noted a steady improvement in the day- and nighttime asthmatic symptoms in the probiotic-treated (daytime scores: 1.2 ± 1.0 vs. 0.7 ± 0.6 at baseline, $P < 0.01$; nighttime scores: 1.3 ± 1.1 vs. 1.0 ± 0.9 at baseline, $P < 0.05$) and control groups (daytime scores: 1.3 ± 1.0 vs. 0.9 ± 0.8 at baseline, $P < 0.05$; nighttime scores: 1.2 ± 1.0 vs. 0.9 ± 0.9 at baseline, $P < 0.05$) during the study period. Although, there is no significantly different of improvement rate in the asthmatic and AR symptoms between probiotic-treated and placebo group, with regard to the number of study patients within group who showed an improvement in terms of a reduction in the symptom scores from the beginning (V0) to the end of the study (V4; Fig. 3), there was a statistically significant improvement rate in the probiotic-treated group on the daytime asthmatic symptoms (37/49, 75.5%, $P = 0.01$; Fig. 3A) and on the AR symptoms (29/41, 70.7%, $P = 0.01$; Fig. 3C), in contrast, there is no significantly different of improvement rate in the placebo group on the daytime asthmatic symptoms (35/56, 62.5%; $P = 0.06$; Fig. 3A) and on the AR symptoms (25/47, 53.2%; $P = 0.66$; Fig. 3C). There was no difference in the number of patients who showed an improvement in the nighttime asthmatic symptoms between these two groups (Fig. 3B).

Changes in the Severity of Asthma and AR and in the Asthma Control Test Results

The physicians (J.R.L. and C.S.H.) who were blinded to the assignment of patients into groups evaluated them for the severity of asthma and AR during each clinical visit. As shown in Figure 4A,B, the severity of asthma and AR among the patients in both the probiotic-treated and control groups continued to improve in each successive

TABLE 2—Changes in the Parameters of Pulmonary Function Test

	Probiotic-treated group					Placebo group					*P* value[1]		
	V0	V3	V5	V3 − V0 (%)	V5 − V0 (%)	V0	V3	V5	V3 − V0 (%)	V5 − V0 (%)	V3 − V0	V5 − V0
FEV_1	1.21 ± 0.33	1.23 ± 0.35	1.52 ± 0.40	1.98 ± 0.36	9.05 ± 2.25	1.37 ± 0.40	1.36 ± 0.43	1.42 ± 0.42	0.48 ± 0.51	1.80 ± 0.61	0.522	**0.028**
FVC	1.29 ± 0.33	1.29 ± 0.37	1.39 ± 0.43	−0.01 ± 0.10	8.14 ± 1.85	1.45 ± 0.47	1.43 ± 0.47	1.46 ± 0.48	−0.46 ± 0.31	0.77 ± 1.71	0.589	**0.035**
PEFR	204.93 ± 57.98	219.45 ± 64.31	222.98 ± 61.92	7.08 ± 9.52	8.80 ± 5.85	221.28 ± 50.93	240.59 ± 56.41	238.45 ± 54.17	8.73 ± 7.61	7.76 ± 3.16	0.879	0.754
FEV_1/FVC (%)	94.52 ± 5.97	96.17 ± 4.59	95.48 ± 4.71	1.74 ± 0.53	1.01 ± 0.43	95.79 ± 4.53	95.6 ± 4.69	94.73 ± 5.85	−0.15 ± 0.12	−1.1 ± 0.23	0.317	**0.007**
MEF_{25-75}	2.01 ± 0.76	2.11 ± 0.70	2.04 ± 0.57	4.63 ± 1.33	1.39 ± 0.91	2.08 ± 0.52	2.18 ± 0.67	2.11 ± 0.61	4.78 ± 1.61	1.74 ± 0.41	0.572	0.211
Post-bronchodilator FEV_1 change (%)	9.83 ± 10.21	8.23 ± 8.52	3.89 ± 2.42	−16.28 ± 14.31	−60.46 ± 21.61	7.42 ± 13.41	4.16 ± 5.25	8.67 ± 9.25	−33.91 ± 21.61	16.85 ± 10.61	0.511	**<0.001**

[1]Denote the *P* value of the difference of change in V3−V0 and V5−V0 between probiotics-treated and placebo group using Mann−Whitney test.

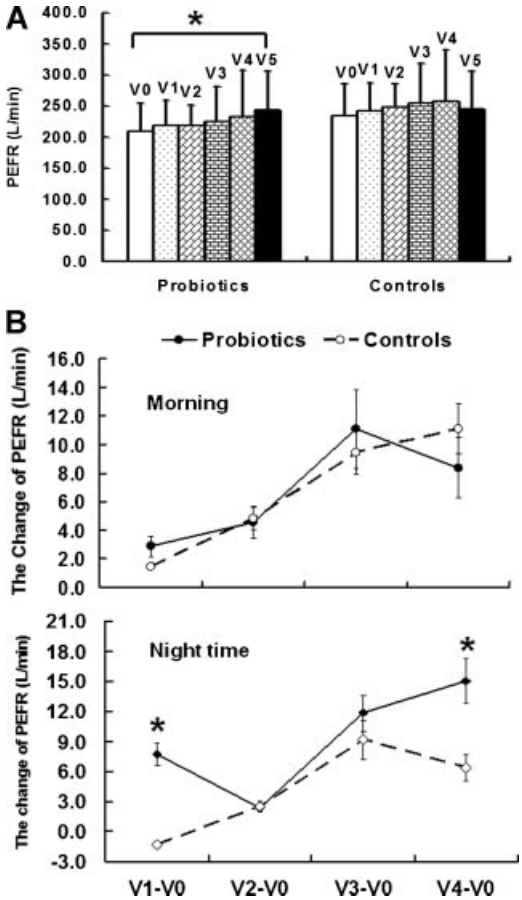

Fig. 2. The peak expiratory flow rate (PEFR) evaluated during clinical visits (A) and records in the diary cards of the study subjects (B). Asterisk (*) denotes $P < 0.05$ for comparison between V0 and V5 (A) or between the probiotic-treated and control groups (B).

clinic visit and differed significantly from that observed during the evaluation at baseline (V0; $P < 0.001$). The study subjects were evaluated for asthma control by using a Chinese version of the CACT at baseline and at the end of the study period. The results revealed a significant increase in the asthma control levels in both the probiotic-treated and control groups ($P < 0.001$; Fig. 4C). Moreover, the number of patients who showed an improvement in the CACT score was higher in the probiotic-treated group (33/49, 67.3%) than in the control group (33/56, 58.9%; $P < 0.05$).

Changes in the Serum Total IgE and Cytokine Production

Although the serum levels of total IgE decreased slightly in both the probiotic-treated (937.4 ± 1157.0 vs. 853.2 ± 1103.2 IU at baseline) and control groups (1052.6 ± 1005.7 vs. 843.2 ± 1397.2 IU at baseline) at the end of the study period, the difference was not

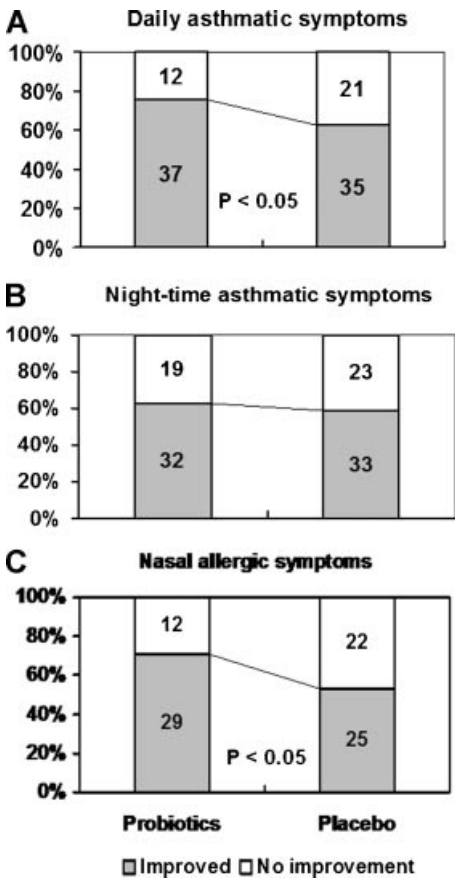

Fig. 3. The number of patients in the probiotic-treated and control groups who demonstrated an improvement or no improvement in the day- (A) and nighttime (B) asthmatic symptoms and in the allergic rhinitis (AR) symptoms (C) at the end of the study period. P value denotes the statistically comparison of improvement rate within probiotics-treated or placebo group.

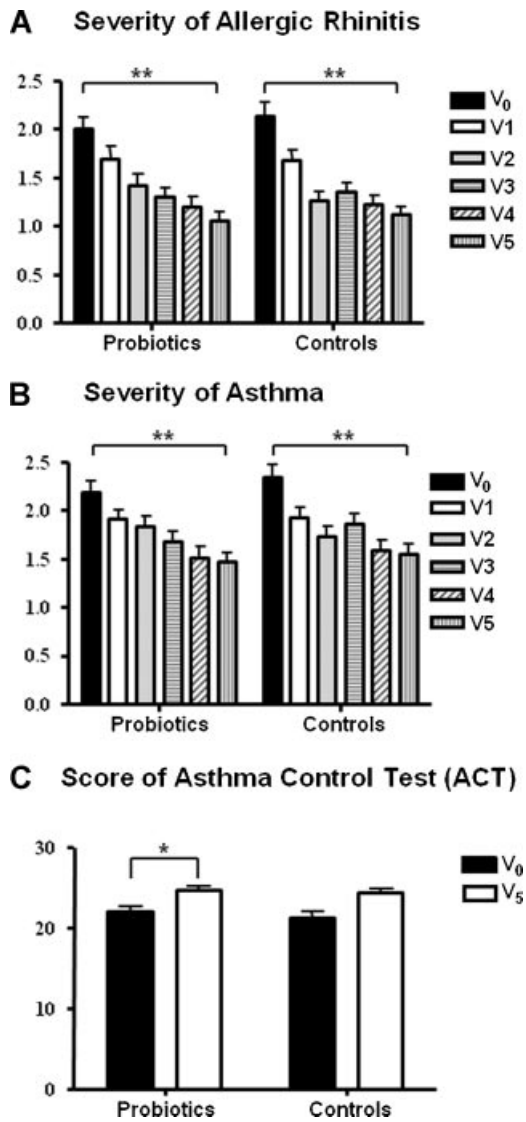

Fig. 4. Changes in the clinical severity of AR (A) and asthma (B) and the CACT test scores (C) evaluated by the attending physicians. Asterisk (*) denotes $P < 0.05$ and double asterisk (**) denotes $P < 0.01$ for comparison between V1 and V5.

statistically significant. Figure 5 shows the production of the inflammatory cytokine, TNF-α, and Th2 cytokine, IL-10, and IL-13 in the supernatants of PHA- or Der p allergen-stimulated PBMCs collected from the study patients before (V0) and after treatment (V5). We observed a significant decrease in the TNF-α and IL-13 production by the PBMCs that were incubated with medium alone and by the Der p-stimulated PBMCs (for TNF-α), and by the PHA- and Der p-stimulated PBMCs (for IL-13), following the ingestion of *L. gasseri* capsules for 8 weeks as compared to those of control group ($P < 0.05$). Although IL-10 production in medium alone and by Der p-stimulated PBMCs was lower in the probiotic-treated group after treatment, but not to a significantly level as compared to control group. To our unexpected findings, in Figure 6, we also observed that after probiotic treatment, the production of Th1 cytokines, such as IFN-γ and IL-12, production by the PBMCs stimulated with PHA or Der p allergen decreased significantly as compared to those of control group ($P < 0.05$). An assay was also performed for other Th2 and regulatory cytokines, such as IL-4, IL-5, and TGF-β produced by the PBMCs under various conditions; however, their levels were below the detectable limits.

DISCUSSION

It is well known that probiotic bacteria, which improve the intestinal microbial balance, may facilitate the modulation of immune responses that are beneficial for the prevention of atopic eczema in infants.[5,12,13] However, whether these bacteria have any clinical applications in the prevention or treatment of established allergic airway diseases remains controversial and requires more

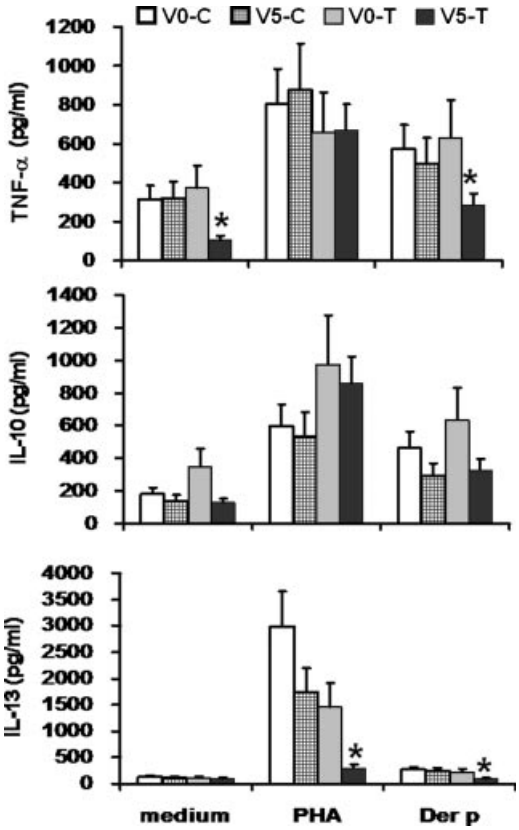

Fig. 5. TNF-α, IL-10, and IL-13 production by PBMCs (1×10^6 cells/ml) that were collected from the probiotic-treated patients (T; N = 41) and control group (C; n = 38) before (V0) and after (V5) the treatment and were subsequently stimulated with PHA (10 μg/ml), Der p (10 μg/ml), or Der p (10 μg/ml) supplemented with *L. gasseri* A5 (1×10^6 cells/ml). Asterisk (*) denotes $P < 0.05$ for comparison between the levels recorded before and after probiotic treatment.

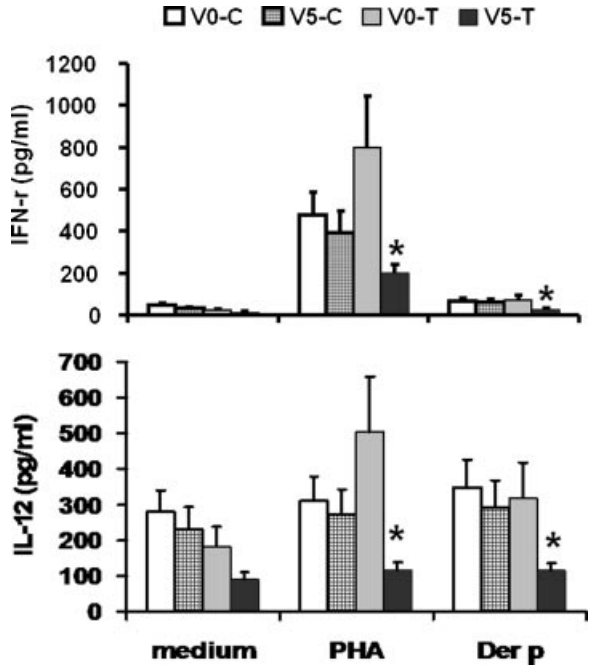

Fig. 6. INF-γ, IL-12p40 production by PBMCs (1×10^6 cells/ml) that were collected from the probiotic-treated patients (T; N = 41) and control group (C; n = 38) before (V0) and after (V5) the treatment and were subsequently stimulated with PHA (10 μg/ml), Der p (10 μg/ml), or Der p (10 μg/ml) supplemented with *L. gasseri* A5 (1×10^6 cells/ml). Asterisk (*) denotes $P < 0.05$ for comparison between the levels recorded before and after probiotic treatment.

comprehensive clinical trails. In this study, we found that daily ingestion of *L. gasseri* A5 for 8 weeks as a supplement to the other medications taken by school children for the control of asthma and AR could significantly increase their pulmonary function and PEFR and decrease their clinical symptom scores for asthma and AR. More importantly, the global assessment of *Lactobacillus* supplementation performed by physicians for the patients participating in this trial yielded satisfactory results with no major or minor adverse reactions occurring throughout study period. Previously, only a few clinical trials have explored the clinical effects of probiotic administration on AR in children[15,16] and adults.[17–19] Our findings revealed that the subjective asthmatic symptoms (daytime) and objective airway function measurements improved significantly in allergic asthmatic children who received probiotic supplementation.

The underlying mechanism for this beneficial effect of probiotics for asthmatic children is presently unknown. There are at least three known hypotheses regarding the antiallergic effects of probiotics:[26] probiotics may alter the Th2 cytokine profile in atopic conditions due to their Th1-adjuvant effect;[27,28] probiotics may induce a specific subset(s) of regulatory T lymphocytes (Treg), such as Th3, Th1, and natural Treg, and thus suppress the allergen-induced inflammatory response;[29–31] and probiotics may interact with professional antigen-presenting cells such as macrophages and dendritic cells (DCs) in the gastrointestinal mucosa and thus achieve systemic immunological tolerance.[31–34] In this study, we found no significant difference in the serum total IgE level and the degree of allergen sensitization before and after probiotic treatment; further, there was no significant elevation in the Th1 cytokines, INF-ρ and IL-12 productions by stimulated and unstimulated PBMCs derived from the probiotic-treated subjects (Fig. 6). These results appear to rule out the possibility that probiotics function as Th1 adjuvants that counterbalance the status of predominant Th2 cytokines in atopic children. On the other hand, production of the cytokine IL-10, mainly by the inducible Treg, decreased instead of increasing following probiotic treatment (Fig. 5). Furthermore, we also found that the percentage of $CD4^+CD25^{high}Foxp3^+$ T lymphocytes (natural Treg) among the PBMCs before ($0.42 \pm 0.39\%$) and after

($0.41 \pm 0.37\%$) the probiotic treatment did not differ significantly, although the natural Treg function improved after the treatment (data not shown); this may have been due to the decreased TNF-α production by the PBMCs of treated patients, as we have reported previously.[35] Therefore, it appears that probiotics are unlikely to induce natural or inducible Treg in treated subjects in order to suppress allergen-induced airway inflammation in asthma and AR.

Although we did not obtain direct evidence to demonstrate that lactobacilli modulated the immunological functions of DCs in the probiotic-treated subjects, recently, several studies have shown that probiotics directly enhance the activity of human DCs populations[31–34] to promote Th1 differentiation.[33,34] The mechanisms underlying such immune modulations are unknown. However, it has been demonstrated that the cell wall of lactobacilli contains immunomodulatory components such as cell-surface molecules and peptidoglycan that may play an important role in activating immunocompetent cells in the intestine.[32] For example, *Lactobacillus* spp. were found to bind the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)[30] or toll-like receptors (TLRs) 2, 4, and 9[35] that prime monocyte-derived DCs to become tolerogenic and drive the development of Treg, which produce increased levels of IL-10 and are capable of inhibiting the proliferation of bystander T cells.[29–31] The detailed mechanisms underlying the interactions occurring between probiotics and DCs to achieve systemic immunological tolerance against allergic disease require further investigation.

There are several limitations in this clinical trial for evaluating the effects of probiotics on asthma and AR. In this study, there exists a significant interference (baseline noise) in the placebo effect on the control group, and this makes it difficult to compare clinical responses to *Lactobacillus* supplementation. This interference is mainly due to the concomitant use of pharmacological agents for controlling the symptoms of asthma and AR by both groups during the study period. Although we have included the medication score records for the concomitant use of medications by daily controllers and emergent relievers in this study, the medication scores decreased significantly in both the probiotic-treated and control groups with no significant difference between the groups. The mechanism for this psychological expectation into placebo effect of allergic symptoms improved has been relatively common in several clinical trials for asthma and other allergic diseases.[36] Another issue that remains unresolved in this study is the long-term benefit of *Lactobacillus* in reducing asthma exacerbation and conferring immunological tolerance to allergens. More importantly, studies on the effects of probiotics in allergic diseases have had varied results, which may due to several factors, including host factors (e.g., genetic differences in microbial responses and allergic predisposition) and environmental factors such as general microbial composition, individual microbiota, diet (including the consumption of probiotic substances), and antibiotic treatment.[26] Therefore, our findings in this clinical trail may have been influenced by these factors that limit its applications for the general population.

In summary, we have found that the airway function, clinical symptoms, and immunoregulatory cytokine production improved significantly in school children with asthma and AR who received *L. gasseri* A5 as a daily supplement for 8 weeks when compared with the control group. As discussed earlier, the antiallergic effects of *Lactobacillus* remain unclear, and its clinical effectiveness in the treatment of asthma and AR requires further investigation to facilitate the clinical application of probiotics.

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