RESEARCH PAPER

Effect of Probiotics on Allergic Rhinitis in Df, Dp or Dust-Sensitive Children: *A Randomized Double Blind Controlled Trial*

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Objective: To study, we examined the effect of *Lactobacillus salivarius* on the clinical symptoms and medication use among children with established allergic rhinitis (AR).

Design: Double blind, randomized, controlled trial.

Setting: Hualien Tzu-Chi General Hospital.

Methods: Atopic children with current allergic rhinitis received 4 × 10^9 colony forming units/g of *Lactobacillus salivarius* (*n*=99) or placebo (*n*=100) daily as a powder mixed with food or water for 12 weeks. The SCORing Allergic rhinitis index (specific symptoms scores [SSS] and symptom medication scores [SMS]), which measures the extent and severity of AR, was assessed in each subject at each of the visits - 2 weeks prior to treatment initiation (visit 0), at the beginning of the treatment (visit 1), then at 4 (visit 2), 8 (visit 3) and 12 weeks (visit 4) after starting treatment. The WBC, RBC, platelet and, eosinophil counts as well as the IgE antibody levels of the individuals were evaluated before and after 3 months of treatment.

Results: The major outcome, indicating the efficacy of *Lactobacillus salivarius* treatment, was the reduction in rhinitis symptoms and drug scores. No significant statistical differences were found between baseline or 12 weeks in the probiotic and placebo groups for any immunological or blood cell variables.

Conclusions: Our study demonstrates that *Lactobacillus salivarius* treatment reduces rhinitis symptoms and drug usage in children with allergic rhinitis.

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Allergic rhinitis (AR) is a common childhood disease that often persists into adulthood. The prevalence of childhood allergic disease has increased dramatically in recent decades in many parts of the world, including Taiwan [1]. The prevalence of reported symptoms of AR in Taiwanese children aged 6-8 and 13-15 years has been reported to be 29.8% and 18.3%, respectively [1]. The causes of AR are not well understood, but sensitization to food proteins may play a role. Children who are atopic and develop dermatitis are at a significantly increased risk of developing atopic asthma and rhinitis in later childhood [2]. This immune response includes both IgE antibodies and helper T cells type 2 (Th2), which are thought to contribute to inflammation in the respiratory tract. Moreover, sensitization to indoor allergens (eg, dust mites, cats, and dogs) is strongly associated with allergic rhinitis.

Probiotics are products or preparations containing viable numbers of microorganisms that are able to modify the host's microflora, thereby producing beneficial health effects [3]. Lactobacilli are considered to induce reactions involving Th1 cells and to improve allergic diseases. Two lines of argument have provided the framework for studies on the relationship between bowel flora and allergic disease. First, lower counts of *Enterococci* and *Bifidobacteria* in infancy have been found in atopic *vs.* non-atopic children and these differences precede sensitization [4, 5]. The early colonization of the bowel with probiotic bacteria such as *Enterococci* and *Bifidobacteria* are hypothesized to more effectively mature the gut mucosal immune system and promote tolerance to non-bacterial antigens. Secondly, increased gut permeability may lead to increased exposure to food antigens, which has been associated with atopic dermatitis (AD) [6]. Probiotics may decrease gut permeability thereby decreasing systemic exposure to food antigens.

Isolauri, *et al*. [7] have previously reported an improvement in the SCORing Atopic Dermatitis (SCORAD) index in milk-allergic infants with mild AD following probiotic-supplemented hydrolyzed whey formula [8]. Recently, Rosenfeldt, *et al*. [8], using a cross-over study design, demonstrated an improvement in the SCORAD index in older children with AD who were treated with probiotics; however, the improvement was only significant is allergic patients. *Lactobacillus*

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paracasei may improve the quality of life of adolescents with perennial allergic rhinitis [9, 10]. The effect of probiotics on rhinitis, remain controversial [11, 12]. Studies have demonstrated that oral administration with *L. salivarius* prior to and during allergen sensitization and airway challenges leads to a suppression of various features of the asthmatic phenotypes, including specific IgE production, airway inflammation, and development of airway hyperresponsiveness in an animal model [13]. Therefore, it is worth while examining whether *L. salivarius* could improve allergic symptoms in humans. We examined the effect of probiotic treatment on atopic children with rhinitis. Various specific clinical and immune parameters were assessed in allergen-sensitive patients before and after treatment, and were compared with those of untreated (UT) allergen-sensitive patients.

METHODS

The study was conducted in the pediatric clinic of Hualien Tzu Chi General Hospital between February and December 2009. Children aged between 6 to 12 years; history of perennial allergic symptoms for at least 3 years; positive skin prick test (SPT) for Dp, Df or dust, and Unicap system (Pharmacia Diagnostics, Uppsala, Sweden) positivity for Dp, Df, or dust (more than class 1) were recruited in the study. Patients who had previously been treated with immunotherapy, and those with recurrent respiratory tract and infectious diseases, were excluded. This study was approved by the Research Ethics Committee of the Hualien Tzu-Chi General Hospital and informed consent was obtained from all subjects.

Randomization was performed by doctors, who were not involved in this study design. All of the enrolled patients were randomly assigned to the *L. salivarius* group or the placebo group according to computer-generated permuted-block randomization. The selected patients were randomized into two groups (consisting of 120 UT patients and 120 patients treated with probiotic) taking into account age, sex, medication scores, type and importance of ocular symptoms (itching, redness, or weeping), nasal symptoms (sneezing, rhinorrhea, itching or nasal blockage), and lung symptoms (cough, sputum, dyspnea, or wheezing). The treatment group received 4×10^9 colony forming units/g of *Lactobacillus salivarius* PM-A0006 supplied by ProMD Biotech Co., Ltd. The control group received a placebo consisting of microcrystalline cellulose that looked and tasted the same as the probiotics. All patients who met the eligibility criteria were randomized into either the probiotic-treated group or the control group. The powder (500 mg) was given once daily mixed in drink or food. A small number of older children (>10 years) took the powder as an opaque capsule. The viability of the probiotic was tested monthly. Both subjects and investigators were blind to the treatment groups. Study duration was 12 weeks, followed by a 7 month observational phase to observe disease manifestations. There were five scheduled visits: 2 weeks before starting the treatment (visit 0), at the beginning of the treatment (visit 1), then 4 weeks (visit 2), 8 weeks (visit 3), and 12 weeks (visit 4) after treatment was initiated. Parents received two phone calls during the treatment period to check on patient progress and compliance (6 and 9 weeks after the beginning of the treatment). At each visit the severity of the child's AR was evaluated using the specific symptoms scores (SSS) and symptom medication scores (SMS) [14,15].

All parents completed a questionnaire (visit 0) about AR and the allergic disease history of their child, the family's history of allergic diseases, and any current oral or topical medication currently in use. During the 12 week study parents were asked to complete a weekly diary of medication use, health problems, and the presence and severity of AR in the child to aid recall for the questionnaire at the study visits. A final questionnaire was completed at the last office visit, which included previous medication usage, other allergic diseases, and changes in life style or housing during the study.

Both the treated and UT patients maintained a weekly diary of allergic symptoms during the antigen exposure period. Specific symptoms scores (SSS) were recorded for nasal blockage, nasal itching, sneezing, rhinorrhea, eye irritation and watering, wheezing, cough, and asthma. Symptom medication scores (SMS) were calculated from patient diaries, as described previously [14,15]. During the study, all patients received same medications in the whole period according to individual allergic status and allowed to take the following medications if required. Scores were calculated based on drugs used (0.5 points for each dose of nasal corticosteroids and 2 points for each dose of antihistamine). Patients were instructed to use local steroids plus antihistamines only if their symptoms did not improve. Patients were also asked to report each administration or variation of the initial drug therapy in the diary. Patients were also instructed to stop their medication at least 7 days before blood sampling. At each time point (visit 0, 1, 2, 3, and 4) of the study, a patient self-evaluation was completed. Each patient was also asked for his/her overall evaluation of the treatment based on categories of symptoms gravity.

Blood samples were taken before and after treatment, to examine total IgE, peripheral blood cell counts, and blood eosinophil counts.

Of the 240 randomized patients, 21 from the probiotic group and 20 from the placebo group did not complete the study and another 106 patients (44 probiotic and 49

placebo) dropped out of the blood trial due to sampling difficulties and withdrawal of consent. ***Fig.* 1** shows the relevant patient flow chart.

Statistical analysis: Statistical analysis was performed using paired and unpaired Student's t-tests, as appropriate. A p-value of less than 0.05 was considered statistically significant. All analyses utilized SPSS 13.0 Statistical Software.

RESULTS

A total of 240 (120 boys) age-matched *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df), or dust-sensitive patients with perennial rhinitis and/or rhinitis plus mild asthma were recruited from February to March 2009. The two groups did not differ in terms of demographic variables, age, body weight, gender, family history, medication scores, and allergic symptoms. A total of 199 out of the 240 enrolled patients (82.9%) completed the study in the year 2009. All patients were included in the safety analysis. The demographic information is shown in ***Table* I**.

Results for the three-month follow-up were based on the patient self-evaluation scores obtained at treatment onset and after 4, 8, and 12 weeks of treatment. Study duration was 12 weeks, followed by a 7 month observational phase to observe disease manifestations. The

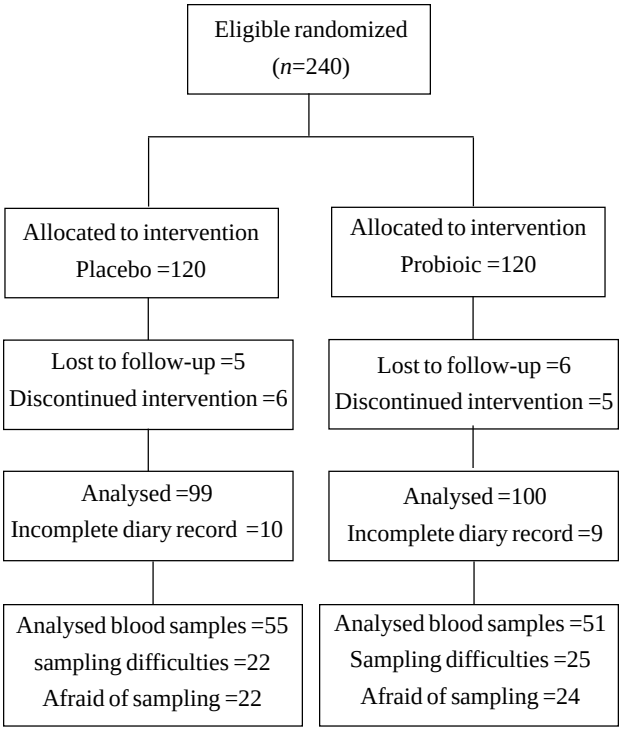

FIG.1 *CONSORT diagram of the study.*

TABLE I BASELINE CHARACTERISTICS OF THE STUDY POPULATION

	Placebo N=100	*Probiotics groups N=99*
Age (y)	8.0 (2.1)	8.0 (1.9)
Body weight (kg)	27.6 (4.3)	27.0 (4.9)
Gender (M/F)	63/37	59/40
*Family history *n*	64	70
Rhinitis alone, *n*	51	52
Rhinitis + asthma, *n*	48	46
Duration of disease (y)	3.8 (0.5)	3.8 (0.3)
Mean Specific eye scores	4.1 (0.9)	4.3 (0.8)
Mean Specific nasal scores	8.8 (1.1)	8.7 (1.2)
Mean Specific lung scores	8.3 (1.2)	8.2 (1.1)
Drug scores	2.8 (1.5)	2.9 (1.6)
WBC (10^3/mm^3)	8.64 (2.8)	8.49 (2.5)
Eosinophils (%)	5.36 (4.1)	4.87 (3.8)
IgE (IU/mL)	543.38 (154.2)	572.07 (153.2)

*Values are mean (SD) unless indicated; *of allergic disease.*

SSS of *Lactobacillus salivarius*-treated at 8 and 12 weeks were significantly reduced in comparison with those UT patients, specifically for eye and nose symptom scores (eye scores: 1.0 ± –0.5 vs. 2.4 ± –0.9 at 8 weeks and 0.6 ± –0.3 vs. 2.1 ± –0.7 at 12 weeks, P=0.001 and 0.000; nasal scores: 5.1 ± –0.9 *vs* 6.5 ± –1.2 at 8 weeks and 3.1 ± –0.8 *vs* 5.1 ± –1.5 at 12 weeks, P=0.001 and 0.000) (***Table* II *a, b***). In *Lactobacillus salivarius*-treated patients a significant reduction in lung SSS was not observed (***Table* II c**). The effect of probiotics on drug use was determined by analyzing the drug score for allergic disease at visit 1, 2, 3, and 4. There was a statistically significant change in medication scores for rhinitis at visit 4 between the *Lactobacillus salivarius*-treated group and UT group (2.4±_0.9 vs. 2.8 ±_1.1 , *P=0.006*) (***Table* II d**).

Due to sampling difficulties, blood was only collected from 106 patients (44.2%). There was no difference between the groups in blood and immunologic profile level before the study. Blood cell counts, total IgE, and blood eosinophil counts were not statistically different between visit 4 and 1 in *Lactobacillus salivarius*-treated or UT groups (***Table* III**).

DISCUSSION

The aim of this study was to evaluate the effects of a probiotic on the clinical response to allergens in Dp, Df or dust-sensitive patients. To the best of our knowledge, there have been no published double-blind, randomized,

TABLE II SPECIFIC SYMPTOM- AND DRUG-SCORES

Visit	*Placebo*	*Probiotics group*	*P value*
Specific eye symptom scores			
1	4.1 (0.9)	4.0 (0.8)	0.262
2	3.0 (0.7)	3.2 (0.9)	0.125
3	2.4 (0.90)	1.0 (0.5)	0.001*
4	2.1 (0.70)	0.6 (0.3)	0.000*
Specific Nasal Symptom Scores			
1	8.8 (1.1)	8.7 (1.2)	0.583
2	5.8 (2.2)	5.1 (1.5)	0.052
3	6.5 (1.2)	5.1 (0.9)	0.001*
4	5.1 (1.5)	3.1 (0.)	0.000*
Specific Lung Symptom Scores			
1	8.3 (1.2)	8.2 (1.1)	0.363
2	3.5 (0.9)	3.5 (1.0)	0.885
3	2.3 (0.7)	2.2 (0.5)	0.236
4	2.4 (1.4)	2.1 (0.5)	0.06
Specific Medication Scores			
1	2.8 (1.5)	2.9 (1.6)	0.769
2	2.7 (0.6)	2.6 (0.5)	0.737
3	2.9 (1.0)	2.6 (0.8)	0.083
4	2.8 (1.1)	2.4 (0.9)	0.006*

*There were four scheduled visits: at the beginning of the treatment (visit 1), then 4 weeks (visit 2), 8 weeks (visit 3) and 12 weeks (visit 4) after starting the treatment. All values in mean (SD). * p<0.05 (Probiotics vs placebo group at each visit).*

placebo-controlled trials examining the effect of *Lactobacillus salivarius* on atopic disease in patients with rhinitis. The major results, indicating the efficacy of *Lactobacillus salivarius* treatment, was the reduction in rhinitis symptoms and drug scores. We had also performed a study to determine the clinical significance. Most healthy children did not experience any medicine problem related to allergic symptoms, and nasal and ocular SSS of the healthy participants were similar to the *Lactobacillus salivarius*-treated patients' at 8 or 12 weeks (data not shown). Taken together, these observations shows that subjective symptoms are good parameters to assess the condition of allergic rhinitis. Even though a significant dropout rate (17.1%) was observed in the current study, the mean values for SSS and SMS in the remaining group members were similar to those of patients who dropped out. When examined after 3 months of probiotic treatment, the *Lactobacillus salivarius*-treated group reported reduced nasal and eye symptoms compared with the UT group.

The currently reported findings are compatible with previously published *in vitro* results [16-18]. The consumption of *Lactobacillus salivarius* strains induces a significant increase in IL-10 production. IL-10 cytokine can downregulate the production of Th1 cytokines and induce the development of regulatory T cells [19, 20]. Therefore, the *Lactobacillus salivarius* strain acts as an immuno-modulator with anti-inflammatory effects in the regulation of the response to antigen challenge in allergic disease.

TABLE III IMMUNOLOGIC AND BLOOD CELL PARAMETERS

Group	*Variable*	*visit 1 to visit 4* *Mean difference (SD)*
Placebo (*n*=51)	WBC	1.14 (2.60)
	Eosinophil	-0.93 (4.27)
	IgE	-150.5 (632.76)
Probiotics (*n*=55)	WBC	1.05 (2.11)
	Eosinophil	0.72 (4.54)
	IgE	40.34 (392.55)

P value >0.05 for all comparisions (visit 1 vs visit 4)

No difference was found in specific immune and blood parameters between the probiotic and placebo group. This result is in line with previous studies [21-23]. Several studies have indicated that T-regulatory cells play an important role in regulating allergen-specific inflammatory responses. $CD4^+$ $CD25^+$ $Foxp3^+$ cells are recruited into both lungs and draining lymph nodes and can suppress allergen-induced mucous hypersecretion, airway eosinophilia and hyperresponsiveness [24-27]. In addition, the natural resolution of an allergic airway response to Der p1 in mice was shown to be dependent on $CD4^+$ $CD25^+$ $Foxp3^+$cells that appear in the lungs and drain mediastinal lymph nodes following an airway challenge. Recently, studies revealed that *L. salivarius* AH102 did not alter T-regulatory cell number in animal model tested [28]. The exact mechanism for the effects of this strain of probiotics on the regulatory mechanisms of the immune responses in humans is not as yet clearly defined. Further studies are needed to clarify this point.

In conclusion, we found that a marked reduction of the symptom scores was observed during treatment with *Lactobacillus salivarius*, with differences in anti-allergic drug intake in patients. The magnitude of the reduced symptom scores is a clinically important issue to investigate the application of probiotics.

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