



## Original Article

# Is the effect of probiotics on allergic rhinitis confined to *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, or dust-sensitive children? A randomized prospective double-blind controlled trial

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## ABSTRACT

**Objective:** Probiotics have previously been shown to reduce the severity of atopic disease in infants and children. However, the immunological changes induced by this treatment that might account for the clinical improvement are still unclear. In this study, we examined the effect of *Lactobacillus rhamnosus* on the clinical symptoms and medication use among children with established allergic rhinitis. We also investigated the effect of probiotics on the white blood cell counts, red blood cell counts, platelet counts, eosinophil counts, and IgE antibody levels.

**Materials and Methods:** Atopic children with current rhinitis received  $4 \times 10^9$  colony forming units/g of *L rhamnosus* ( $n = 98$ ) or a placebo ( $n = 100$ ). Both were given this daily as a powder mixed with food or water. The SCORing Allergic rhinitis index (specific symptoms scores and symptom medication scores), which measures of the extent and severity of allergic rhinitis, was assessed for each patient at the five visits. These five scheduled visits were at 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 starting the treatment. The white blood cell, red blood cell, 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 *L rhamnosus* treatment, was no reduction in rhinitis symptoms or drug scores. No significant statistical differences were found between baseline and 3 months for the probiotic and placebo groups when any immunological and blood cell variable was examined.

**Conclusions:** Our data demonstrates that *L rhamnosus* treatment neither reduced rhinitis symptom scores nor altered immunological parameters in symptomatic children.

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## 1. Introduction

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 current symptoms of AR in Taiwanese children aged 6–8 years and

13–15 years are 29.8% and 18.3%, respectively [1]. The causes of AR are unknown but many cases, particularly in early childhood, are associated with sensitization to food proteins. 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 (e.g., dust mites, cats, and dogs) is strongly associated with AR.

Probiotics are defined as products or preparations containing viable numbers of microorganisms that are able to modify the host's microflora, thereby producing beneficial health effects [3].

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Two lines of argument have led to studies of the relationship between bowel flora and allergic disease. Firstly, lower counts of *Enterococci* and *Bifidobacteria* in infancy have been found in atopic versus nonatopic 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 nonbacterial antigens. Secondly, increased gut permeability may lead to increased exposure to food antigens and this has been associated with atopic dermatitis (AD) [6]. Probiotics may decrease this permeability and thus decrease systemic exposure to food antigens.

Isolauroi et al [7] have previously reported an improvement in the SCORing Atopic Dermatitis index of milk-allergic infants with mild AD following probiotic-supplemented hydrolyzed whey formula. Recently, Rosenfeldt et al [8] in a crossover study demonstrated an improvement in SCORing Atopic Dermatitis index in older children with AD who were treated with probiotics, but the improvement was only significant for atopic children. Indeed, *Lactobacillus paracasei* may improve the quality of life of adolescents with perennial AR [9,10]. Some studies have supported the effect of probiotics on rhinitis but the results remain controversial [11,12]. Double-blind controlled studies are needed to clarify the effects of probiotic bacteria on allergy-related disorders. We examined the effect of probiotic treatment on atopic children with rhinitis. Various specific clinical and immune parameters were assessed in a panel of allergen-sensitive patients before and after treatment and were compared with those from a matched group of untreated (UT) allergen-sensitive patients.

## 2. Materials and methods

### 2.1. Patients

A total of 240 (120 male and 120 female) age-matched Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), or dust-sensitive patients having perennial rhinitis and/or rhinitis plus mild asthma were recruited from February to March 2008. The criteria for inclusion in the study were as follows: age below 18 years; history of perennial allergic symptoms for at least 3 years; positive skin prick test for Dp, Df, or dust; and CAP positivity for Dp, Df, or dust (more than Class 1). This study was approved by the Research Ethics Committee of the Hualien Tzu-Chi General Hospital and informed consent was obtained from all patients.

### 2.2. Balancing of experimental groups

The selected patients were randomized into two groups (consisting of 120 UT patients and 120 patients treated with probiotics) taking into account age, sex, medication scores, and type and importance of symptoms.

### 2.3. Study design

The treatment group received  $4 \times 10^9$  colony forming units/g of *L rhamnosus* (American Type Culture Collection 53103). The control group received a placebo consisting of microcrystalline cellulose that looked and tasted the same as the probiotic. The powder was given once daily mixed in drink or food. A small number of the older children took the powder as an opaque capsule. The viability of the probiotic was tested monthly. Both patients and investigators were blind to the treatment groups. The study lasted for 12 weeks and patients were followed up for 7 months 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 starting the treatment. Parents received two phone calls during the treatment period to check on 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).

### 2.4. Questionnaire

All parents answered 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.

During the 12 weeks of the 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, which covered medication, other allergic diseases, and changes in life style or housing during the study, was completed at the end of treatment.

### 2.5. Clinical evaluation

Both the treated and UT patients maintained a weekly diary of allergic symptoms during the antigen exposure period. The SSS recorded were nasal blockage, nasal itching, sneezing, rhinorrhea, eye irritation and watering, wheezing, cough, and asthma. SMS were calculated from patient diaries, as described in a previous study [13]. Arbitrary scores were attributed to the 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 and 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 (Time 0, 1, 2, 3, and 4 visits) of the study, patient self-evaluation was carried out. Each one was asked for his/her overall evaluation of the treatment based on categories of symptoms gravity (from 0 to 9).

### 2.6. Immunological blood assessment

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

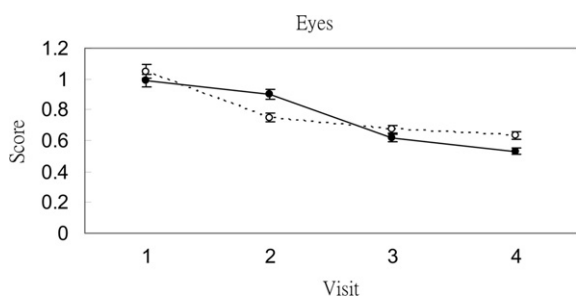
### 2.7. Statistical analysis

Statistical analysis was performed using paired and unpaired Student *t* tests, as appropriate. A *p* value of less than 0.05 was considered statistically significant.

**Table 1**  
Baseline characteristics of the study population by study group

Baseline characteristics	Group 1	Group 2
Number of patients	100	98
Mean age (yr) (SD)	8.0 (4.3)	8.1 (4.4)
Mean BW (kg) (SD)	27.6 (15.8)	28.2 (16)
Gender (M/F)	63/37	59/39
Family history of allergic disease	64	70
Rhinitis alone	51	57
Rhinitis + asthma	48	40
Follow-up (mo)	7	7
Duration of disease (yr) (SD)	3.8 (0.5)	3.8 (0.3)

BW = body weight; SD = standard deviation.  
Group 1 (placebo); Group 2 (LR).



**Fig. 1.** Specific eye symptom clinical scores were evaluated for the two groups. The study lasted for 12 weeks. 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. ○ = placebo; ● = *Lactobacillus rhamnosus*. \* $p < 0.05$  (vs. Visit 1 in each group).

### 3. Results

#### 3.1. Patients

The two groups did not differ in the terms of demographic variables, age, body weight, gender, family history, medication scores, and allergic symptoms. A total of 198 of the 240 enrolled patients (82.5%) completed the study in the year 2008. All patients were included in the safety analysis. The demographic information is shown in Table 1.

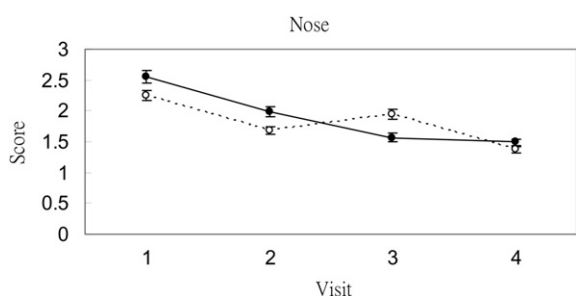
#### 3.2. Clinical efficacy of the probiotic therapy in terms of allergic symptoms

Clinical results for the 7 months follow-up were based on the patient self-evaluation scores obtained at the beginning and after 4, 8, and 12 weeks of treatment. The SSS of the *L rhamnosus* treated and UT patients at 4, 8, and 12 weeks were not significantly reduced in comparison with those at Time 1 (the start of probiotic treatment) in terms of eye, nose, and lung symptoms scores (Figs. 1–3).

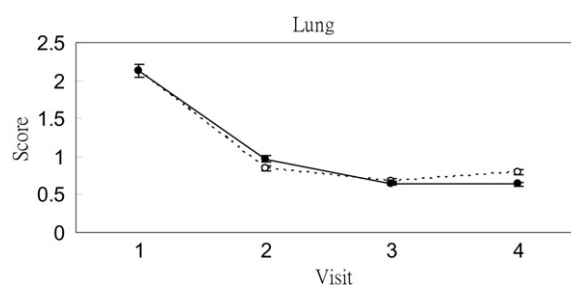
We further determined whether or not the probiotic was able to decrease drug use by analyzing the decrease in the drug score for allergic disease between Visit 1 (beginning of the probiotic treatment) and Visits 2, 3, or 4. There was no statistically significant change in medication scores for rhinitis for the two groups throughout the study as shown in Fig. 4.

#### 3.3. Blood and immunologic profile

Because of sampling difficulties, blood was only collected from 103 patients. Blood cell counts, total IgE, and blood eosinophil counts showed no differences between the placebo and probiotic groups (Table 2).



**Fig. 2.** Specific nasal symptom clinical score were evaluated for the two groups. The study lasted for 12 weeks. 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. ○ = placebo; ● = *Lactobacillus rhamnosus*. \* $p < 0.05$  (vs. Visit 1 in each group).



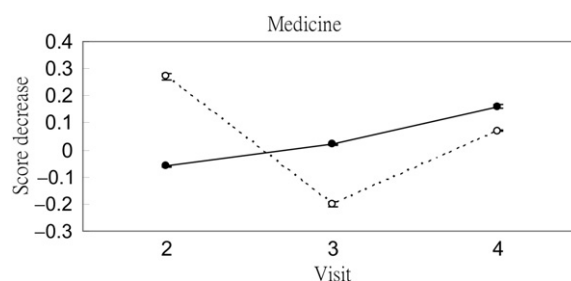
**Fig. 3.** Specific lung symptom clinical score were evaluated for the two groups. The study lasted for 12 weeks. There were four scheduled visits: at the beginning of the treatment (Visit 1), then 4 weeks (Visit 2), 8 weeks (Visit 3), and 12 weeks (Visit 4) after starting the treatment. ○ = placebo; ● = *Lactobacillus rhamnosus*. \* $p < 0.05$  (vs. Visit 1 in each group).

### 4. Discussion

This study's aim was to evaluate the effect of a probiotic on the clinical response to allergens in randomized Dp, Df, or dust-sensitive patients who had perennial rhinitis and/or asthma and conjunctivitis. Although a significant dropout rate (17.5%) was observed during the study, this did not alter the representativeness of the remaining members of the two groups as the baseline results for the mean values for SSS and SMS measures of remaining group members were similar to those of patients who dropped out. When the situation examined after 3 months of probiotic treatment, the *L rhamnosus*-treated group of patients did not show a clear-cut reduction in nasal, lung, and eye symptoms compared with the placebo group.

Bacteriotherapy with probiotic bacteria can affect the gut microbial flora in the host organism and thus have a beneficial effect on an individual's health and well being [14,15]. *L rhamnosus* (ATCC 53103), also called *Lactobacillus GG*, is an extensively studied intestinal probiotic strain that has been shown to influence specific and nonspecific immune responses in milk hypersensitive and healthy patients [16]. Over the last few decades, some reports have shown increasing enthusiasm for the potential health effects of probiotics. However, clinical studies of the efficacy of probiotics have as yet shown no therapeutic effect with respect to AR [17] and little or no benefit as a therapy for AD [8,18].

So far, there has been only one published double-blind, randomized, placebo-controlled trial and this showed no effect of *Lactobacillus GG* on AR in children [17]. The results of this earlier study needed to be interpreted with caution because the number of patients involved was small ( $n = 36$ ). Nonetheless, our findings agree with this earlier result and our study used a considerably larger group of patients ( $n = 240$ ). The results of the present double-blind and placebo-controlled study were also negative.



**Fig. 4.** Symptom medication score differences between the different visits. Symptom medication scores were evaluated for the two groups. The study lasted for 12 weeks. 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. ○ = placebo; ● = *Lactobacillus rhamnosus*. \* $p < 0.05$  (vs. placebo group at each visit).

**Table 2**  
Immunological and blood cell parameters throughout the study period

Group	n	Variables	Before-After		
			Mean (SD)	Median	p
1	51	WBCs	1.14 (2.60)	1.21	0.071
	51	Hb	-0.04 (0.79)	0	0.915
	51	Platelets	34.29 (48.68)	32	0.081
	51	Eosinophils	-0.93 (4.27)	-0.1	0.555
	51	IgE	-150.5 (632.76)	-35.85	0.375
2	52	WBCs	0.23 (1.90)	0.535	0.606
	52	Hb	0.16 (0.60)	0.1	0.286
	52	Platelets	15.13 (55.86)	12	0.296
	52	Eosinophils	-0.49 (3.51)	-0.8	0.528
	52	IgE	61.39 (284.82)	34	0.548

Hb = hemoglobin; SD = standard deviation; WBC = white blood cell.  
Group 1 (placebo); Group 2 (LR).

Allergy and AR symptoms in the treatment group were not reduced as compared with the control group.

On the other hand, there has been one study that suggests that *L. rhamnosus* may be useful and provide primary or secondary prevention of atopic sensitization in an animal model [19]. It seems probable that the probiotic therapy provided in the present study is unable to bring clinically important relief in young children because sensitization to Dp, Df, or dust has already occurred.

Various hypotheses have been proposed to explain the increase in asthma and atopic disease that is apparent in industrialized countries [20]. One hypothesis suggests that changes in the gut microbial flora, which have resulted from changes in diet and hygiene, might be the cause [21]. In line with the hypothesis, it has been shown that oral probiotic bacteriotherapy with *L. rhamnosus* does improve clinical symptoms by alleviating the intestinal inflammation associated with food allergy in small children [22,23]. The gut flora affects the Th1/Th2 lymphocyte balance [24]. We further explored the effect of *L. rhamnosus* in atopic children with rhinitis by examining some specific immune and blood parameters using this panel of allergen-sensitive patients before and after treatment. However, no difference was found in these parameters between the probiotic and placebo groups. This result agrees with some previous studies [25–27]. The exact mechanism by which the probiotic bacterial strain affects the regulatory mechanisms of the immune responses is not yet clearly defined. Further studies are needed to clarify this point.

In conclusion, supplementation of children with AR with *L. rhamnosus* neither reduced rhinitis symptom scores nor altered immunological parameters. These findings challenge the role of probiotics in the treatment of childhood atopic disease. Therefore, *L. rhamnosus* cannot be generally recommended for primary treatment of AR.

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