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Effect of *Lactobacillus paracasei* ST11 on a nasal provocation test with grass pollen in allergic rhinitisJ. Wassenberg¹, S. Nutten², R. Audran¹, N. Barbier¹, V. Aubert¹, J. Moulin², A. Mercenier² and F. Spertini¹¹Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland and ²Nestle Research Centre, Lausanne, Switzerland

Clinical & Experimental Allergy

Summary

Background Probiotics have been associated with prevention and improvement of symptoms in atopic diseases such as atopic dermatitis. However, few studies exist that document their efficacy for upper airways allergies such as allergic rhinitis.

Objective To investigate the effect of short-term oral administration of *Lactobacillus paracasei* ST11 on a nasal provocation test (NPT) with grass pollen.

Methods Thirty-one adult volunteers with allergic rhinitis were enrolled in a randomized, double-blind, placebo-controlled study, based on two 4-week cross-over periods of product consumption (ST11-fermented milk vs. placebo), separated by a wash-out period of 6–8 weeks. Objective and subjective clinical parameters of NPT as well as systemic and nasal immunological parameters were compared between the two treatment periods (registration number: NCT 011 50 253).

Results Subjects that received ST11-fermented milk had lower nasal congestion than subjects under placebo (visual analogical scale; $P < 0.05$). Nasal pruritus followed the same trend. However, no significant change in combined nasal reaction threshold was observed between the two periods. IL-5 secretion by peripheral blood mononuclear cells and serum allergen-specific IgG4 were significantly lower in ST11-fermented milk group compared to placebo group. IL-8 and IL-10 secretion followed the same trend.

Conclusion and Clinical Relevance Short-term treatment with ST11-fermented milk before NPT significantly improved a clinical marker of NPT (subjective nasal congestion) and down-regulated systemic immune markers (IL-5 from peripheral blood mononuclear cells and serum IgG4). These data strongly suggest that probiotics may down modulate key parameters of allergic rhinitis and warrant future evaluation in seasonal trials.

Keywords allergic rhinitis, IL-5, immunomodulation, nasal provocation test, probiotics

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Introduction

Allergic rhinitis is a highly prevalent disease occurring in over 500 million people around the world [1]. The current therapeutic recommendations for allergic rhinitis include aeroallergen avoidance when feasible, pharmacotherapy and allergen-specific immunotherapy [1]. However, the latter ones can cause significant side effects. Under these circumstances, nutritional interventions that are easy to implement in daily life represent an attractive strategy to reduce allergic symptoms.

There is growing interest in orally administered probiotics for fighting allergic diseases. The rationale for developing probiotic interventions stems from the so-called

'hygiene hypothesis' [2], which proposes that, as a consequence of altered microbial stimulus, the shaping of the immune system during early infancy might be biased towards allergy and autoimmunity [3]. A number of studies have highlighted differences in the composition of intestinal microbiota of allergic vs. non-allergic subjects [4–6]. More than 25 randomized, double-blind, placebo-controlled clinical trials have been conducted with different strains of probiotics. They mostly aimed at preventing or treating atopic dermatitis [7–11] and led to divergent results depending on the clinical design, the target population and the probiotic strains [12–15]. Few studies only have considered the prevention or treatment of allergic rhinitis. Four main probiotic strains (*Bifidobacterium longum* BB536, *Lactobacillus casei*

Shirota, *Lactobacillus paracasei* Lp33, *Lactobacillus acidophilus* L92) have been shown to alleviate symptoms in patients suffering from pollen or dust mite allergy and to improve their quality of life [16–20]. In addition, two studies suggested that probiotics are able to improve symptoms also in children with allergic rhinitis [21, 22].

The goal of this study was to evaluate the protective effect of a fermented milk containing *L. paracasei* ST11 strain in a well-reproducible experimental design, i.e. a nasal provocation test (NPT), in volunteers with grass pollen allergic rhinitis. The strain ST11 was selected on the basis of *in vitro* and *in vivo* preclinical studies. Cultured with naïve murine splenocytes, ST11 induced high levels of IL-10 and IL-12 [23]. In germ-free mice mono-associated with ST11, this strain induced a TH1-like pattern of serum immunoglobulin (Ig) isotypes, characterized by increased amounts of total and probiotic-specific IgG2a without influencing IgG1 levels [24]. Finally, a beneficial effect of orally administered ST11 has been established in a mouse model of respiratory allergy (C. Pellaton et al., manuscript in preparation). To verify these properties in the human situation, fermented milk containing ST11 or a placebo was administered out of the pollen season to adults with allergic rhinitis and challenged with grass pollen extracts in a cross-over setting during a period of 4 weeks.

Patients and methods

Population

Thirty-one volunteers with a history of grass pollen allergic rhinitis were enrolled in a single centre study with the following inclusion criteria: (1) age 18–35 years; (2) history of allergic rhinitis to grass pollen confirmed by either a positive skin prick test (weal diameter > 3 mm) or specific IgE for grass pollen (> 0.35 kU/L) as titrated by UniCAP 100 (Pharmacia Diagnostics, Uppsala, Sweden) and no history of perennial rhinitis, and (3) a nasal reaction threshold of 10 000 standardized quality units (SQs)/mL grass pollen or less at the screening phase. Volunteers were excluded from the study when presenting any medical condition that could influence the study (pregnancy, viral or bacterial airway

infection, active allergic rhinitis), or uncontrolled asthma (peak expiratory flow < 20% of volunteer's best personal value), when treated with antihistamine or antibiotic treatment < 2 weeks before enrolment or during the trial. Seven subjects were smokers declaring < 10 cigarettes/day except two of them (10 cigarettes/day). The determination of the sample size (minimum of 30 volunteers in a cross-over design) was based on a reduction of one allergen dose level in 30% of the subjects as a discriminating threshold (power of 90% and type I error of 5%).

The clinical research protocol was approved by the Ethical Review Board of the Faculty of Biology and Medicine, Lausanne. Written informed consent was obtained from each volunteer before the start of the study.

Study product

ST11-fermented milk contained a lactic acid bacterial starter strain (*Lactobacillus helveticus* NCC1643) $\geq 5 \times 10^7$ colony-forming unit (CFU)/mL and the probiotic candidate strain *L. paracasei* ST11 (CNCM I-2116; NCC2461) $\geq 1 \times 10^7$ CFU/mL. Acidified milk was used as placebo. Both ST11-fermented milk and placebo were provided blind by the producer (Nestlé, PTC Lisleux, France) in 80 mL containers (80 kcal/100 g), leading to a daily intake of $4\text{--}9 \times 10^{10}$ CFU/day/subject. Packages and contents were undistinguishable for colour and taste. Each batch of ST11-fermented milk or placebo was consumed within 6 weeks of production and stored between 4 and 10 °C. Other fermented products (including any kind of yoghurts) and any product containing probiotics were proscribed during and for 2 weeks before treatment periods.

Study design

The study was designed as a randomized, double blind, placebo-controlled, cross-over trial, performed outside of grass pollen season (October 2006–March 2007; registration number: NCT 011 50 253). Enrolled volunteers were randomly assigned to receive either ST11-fermented milk or placebo during a first period of 4 weeks (period A), then during a second period of 4 weeks (period B) separated by a wash-out period of 6–8 weeks (Fig. 1). The choice of the

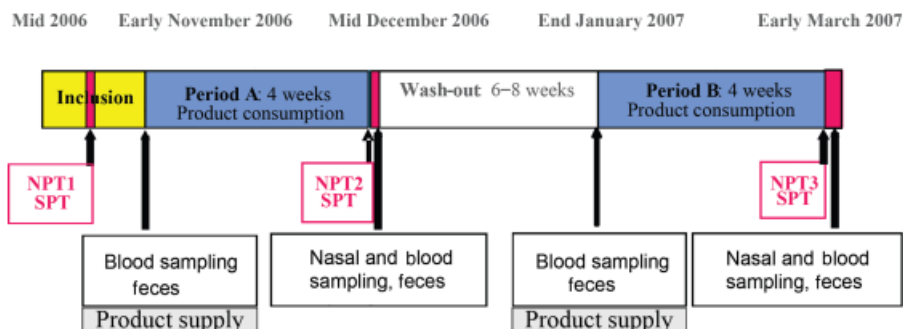


Fig. 1. Schematic representation of the cross-over, placebo-controlled study. NPT, nasal provocation test; SPT, skin prick test.

duration (4 weeks) of the treatment period was mainly based on the time constraints linked to the fact that the study had to be performed in between two pollen seasons, i.e. August to February in Switzerland, while ensuring an adequate wash-out period between two product consumption periods. Safety and tolerability were evaluated on the basis of the number and type of reported adverse events. Volunteers' compliance was followed through diary cards and presence of the ST11 strain (as measured by RT-PCR with strain-specific primers) in stools samples collected at the beginning and the end of each treatment period.

A NPT was performed as described previously [25] at the inclusion visit and at the end of each treatment period. Briefly, 0.2 mL of diluent (ALK-Abello, Horsholm, Denmark) was nebulized into each nostril using a DeVilbiss atomizer. During the first 10 min after the challenge, the number of sneezes and the amount of secretion (by weighing handkerchiefs) were recorded. At the end of the 10 min observation, nasal itchiness and subjective nasal congestion were evaluated by means of a visual analogical scale (VAS; score range: 0 = absent to 10 = severe). When indicated, nasal samples (NS) were collected (Fig. 1) followed by the determination of peak nasal inspiratory flow (PNIF) (Youtlen Flow meter, Airmed; Clement Clarke International Ltd., London, UK) and minimal cross-sectional area (MCA) as measured by acoustic rhinometry (AR) (Rhinocheck RK100, Stimotron Medizinische Geräte, Wendelstein, Germany) [26, 27], with the same devices during the whole study. The same sequence was repeated at each allergen concentration every twenty minutes. The same batch of standardized allergens (mixture of six grass pollens; ALK wässrig SQ; ALK-Abello) was used throughout the study. Serial dilutions of the allergen extract (100, 330, 1000, 3300, 10 000, 33 000 SQ/mL) were prepared immediately before the NPT in phosphate-buffered saline (PBS). Nasal reaction threshold (combined threshold) was reached when at least two of the three following clinical criteria were fulfilled: (1) ≥ 5 sneezes, (2) ≥ 0.5 g nasal secretion in the first 10 min after challenge; (3) a decrease in PNIF $\geq 40\%$ or MCA $\geq 30\%$ of baseline whatever came first [27, 28]. The primary end-point of the study was defined as the difference before and after treatment in allergen dose levels necessary to reach the combined threshold. Each clinical criterion and their respective thresholds were also examined separately as secondary end-points. Other secondary end-points included nasal itchiness and subjective nasal congestion by VAS as well as immune markers from blood and NS as described below.

Nasal sampling

Nasal cells and nasal mucus were collected prior to NPT, after the 33 000 SQ/mL dose level and 24 h later by brushing three times the head of the inferior turbinate and the nasal septum of the same nostril with a PBS-

humidified brush (Cytobrosse[®], Charles Berdat, Courroux, Switzerland); the brush was then gently washed during 1 min in 1 mL PBS [29]. After centrifugation at 450 g for 10 min at 4 °C, cells were resuspended in PBS at 10^6 cells/mL, and fixed on glass slides by centrifugation at 500 r.p.m. for 5 min (Cytospin Shandon, Astmoor, UK). After staining (Diff-Quick, Dade-Behring, Düringen, Switzerland), at least 200 cells were counted and identified.

Nasal mucus samples were collected by absorption on two strips (6 × 30 mm/strip) of filter paper (Whatman No. 903, Whatman Paper Ltd., Maidstone, UK), placed on the nasal septum and on the inferior turbinate, for 10 min [30]. After removal, strips were incubated in 2 mL silicone-coated tubes with 800 µL of a protease inhibitor cocktail (Complete, Roche, Mannheim, Germany) in distilled water at 4 °C and agitated for 2 h to elute proteins from filters. Supernatants were finally buffered with PBS and stored at -80 °C until analysis.

Blood samples

Blood samples were collected on sodium citrate before the treatment periods and 24 h after the NPTs at the end of the treatment periods (Fig. 1). Sera were stored at -80 °C until use. Peripheral blood mononuclear cell (PBMC) purification and culture conditions were previously described [31]. PBMC were stimulated with 90 µg/mL of grass pollen extract (ALK Wässrig SQ; ALK-Abello), with a cocktail of vaccine antigens (5 µL/mL Candida mannan (NIBSC, London, UK), 10 µg/mL tetanus toxoid, 5 µg/mL Tuberculin Purified Protein derivative (PPD, SSI, Copenhagen, Denmark) or medium alone. Culture supernatants were collected after 6 days of culture and stored at -20 °C.

Biological and immune markers

Albumin and total IgA were measured in nasal fluids by nephelometry (detection limits: 17 and 0.24 mg/L, respectively; BN II, Dade Behring). IgG, IgG4 and IgE specific for a mix of grass pollen in sera, specific IgA, tryptase and eosinophil cationic protein (ECP) in nasal fluids were measured by the CAP System (detection limits 2 mg/L, 0.02 mg/L, 0.35 kU/L, 0.01 mg/L, 1 µg/L and 2 µg/L, respectively; UniCAP 100, Pharmacia Diagnostics, Uppsala, Sweden). Cytokines (IL-4, IL-5, IL-8, IL-10 and IFN- γ) were measured in nasal fluid or PBMC culture supernatants by multiplexed bead-based flow cytometry (detection limits: 0.6, 0.2, 0.55, 1.19 and 0.87 pg/L, respectively; Fluorokine multiplex kit, R&D Systems Inc., Minneapolis, MN, USA) using a Luminex 100 analyser.

Statistical analysis

Statistical analyses were performed using the software SAS 8.02 (SAS Institute, Cary, NC, USA). For the primary

outcome and individual threshold parameters, differences were analysed by a Cochran–Mantel's test. For clinical scores, a mixed model was performed with subject as random effect and treatment, allergen dose ($\log_{10}$ -transformed), visit and the interaction between treatment and allergen dose as fixed effects. For cellularity data, they were transformed using a power transformation. Mixed models were applied on the transformed data. Cytokines and Igs data were analysed using Wilcoxon's signed-rank test. Number of sneezes was calculated using a Poisson regression. The treatment effect (treatment *minus* placebo) on PBMC cytokines and Igs was calculated on the evolution from baseline (post-treatment *minus* pre-treatment values) for each type of stimulation.

Results

Demographic and baseline data

Thirty-one volunteers were enrolled into the study. One subject was excluded because of antibiotic intake during the period B of the study. Thirty volunteers (12 men and 18 women) completed the study and were included in the analysis. Most of the subjects (28/30; 93.3%) were Caucasian and 23.3% (7/30) were smokers. Detailed demographic data are described in Table 1.

Compliance and safety

According to daily cards, the mean days of consumption of ST11-fermented milk and placebo acidified milk were 29.6 ± 0.7 and 29.7 ± 0.9 days, respectively. *L. paracasei* ST11 was also detected in the stools of each subject having consumed ST11-fermented milk and in none of those under placebo (Table S1). Importantly, the strain was

undetectable at the end of the wash-out period. No adverse events related to ST11-fermented milk consumption were reported during the study.

Nasal provocation test

No significant difference in allergen dose level at reaction threshold (primary outcome) was observed for the combined NPT index. However, subjective congestion score based on VAS was significantly improved in the ST11-fermented milk group ($P=0.04$, as tested using a mixed statistical model, see 'Statistical analysis') (Fig. 2a). This effect was logically more apparent in the lower range of allergen dose levels of NPT. Pruritus score using VAS showed the same trend, although not reaching statistical significance (Fig. 2b). In contrast, combined threshold (Fig. 3) and individual NPT parameters (sneezing, obstruction measured by PNIF and MCA and nasal secretions) (data not shown) did not differ between the two arms of treatment (ST11-fermented milk vs. placebo).

Cell-mediated immunity

PBMC stimulated *in vitro* with grass pollen proliferated and released IFN- γ , IL-4, IL-5, IL-8 and IL-10. After NPT, less IL-5 was secreted by cells collected from volunteers having consumed ST11 than from the placebo group

Table 1. Subject characteristics

	N	Mean	SD	Minimum	Maximum
Patient age (derived, in years)	30	26.8	4.5	20.0	35.0
Height adults (cm)	30	172.3	8.6	154.5	190.0
Weight adult (kg)	30	66.0	13.0	47.0	90.0
Body mass index (BMI; kg/m ²)	30	22.1	2.9	17.4	28.2

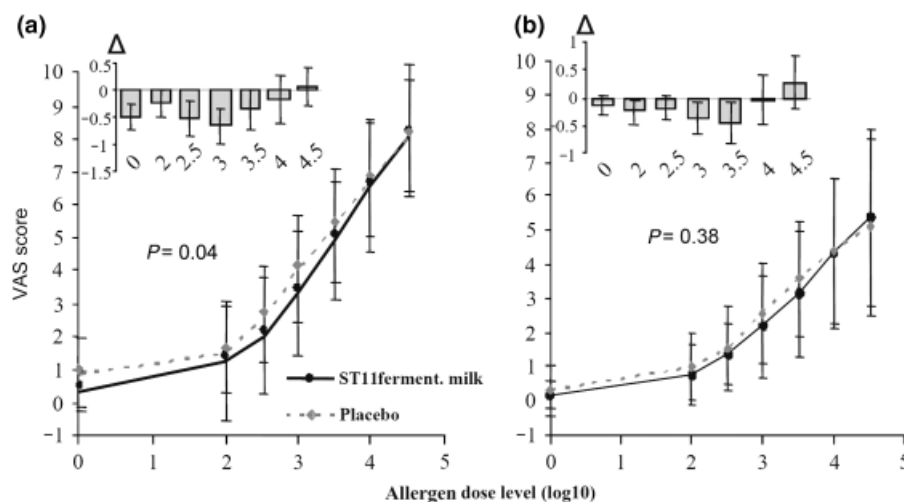


Fig. 2. Mean visual analogical scale (VAS) congestion (a) and pruritus (b) scores according to the allergen dose level (mean $\pm$ SEM). Inset, treatment effect by difference of VAS congestion (a) and pruritus (b) scores at the different allergen dose levels between the ST11-fermented milk group and the placebo group (ST11-fermented milk group *minus* placebo group).

($P=0.03$) (Figs 4a and b). This effect was not observed after non-specific stimulation of PBMC with a cocktail of vaccine antigen or in unstimulated PBMC. A similar non-

statistically significant trend was observed for IL-8 and IL-10 secretion. There was no difference between the two groups for IL-4 and IFN- γ .

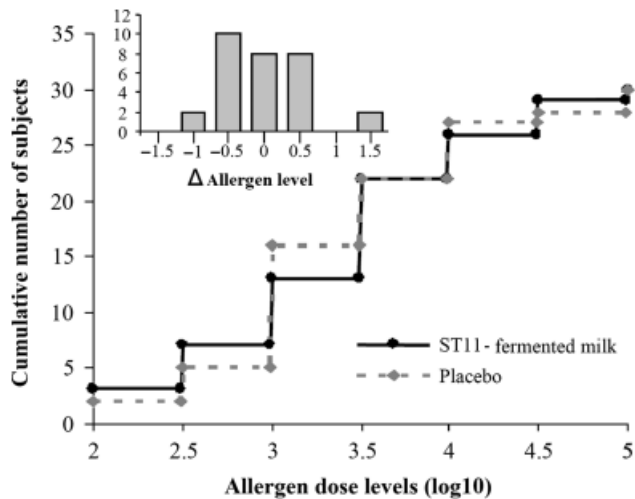


Fig. 3. Cumulative number of patients reaching the combined reaction threshold according to dose level of grass pollen allergen. *Inset*, difference (δ) in allergen dose level necessary to reach the threshold between ST11-fermented milk group and placebo group (>0 , in favour of ST11-fermented milk; <0 , in favour of placebo).

Antibody response

Grass pollen-specific IgG4 levels were significantly lower in the ST11-fermented milk group than in the placebo group ($P=0.049$) (Figs 5a and b). No difference in the levels of grass pollen-specific IgE and IgG was observed between the two groups.

Nasal cytology

Measurement of total leucocytes, neutrophils and eosinophils performed before NPT, after the 33 000 SQ/mL dose level and 24 h later showed a significant rise in all three cell populations. The number of eosinophils tended to be lower after the 33 000 SQ/mL dose level in the ST11-treated group as compared with the placebo group (median 520.8 ± 149.8 vs. 3909 ± 1085 eosinophils/sample in ST11-treated and placebo groups, respectively, $P=0.37$).

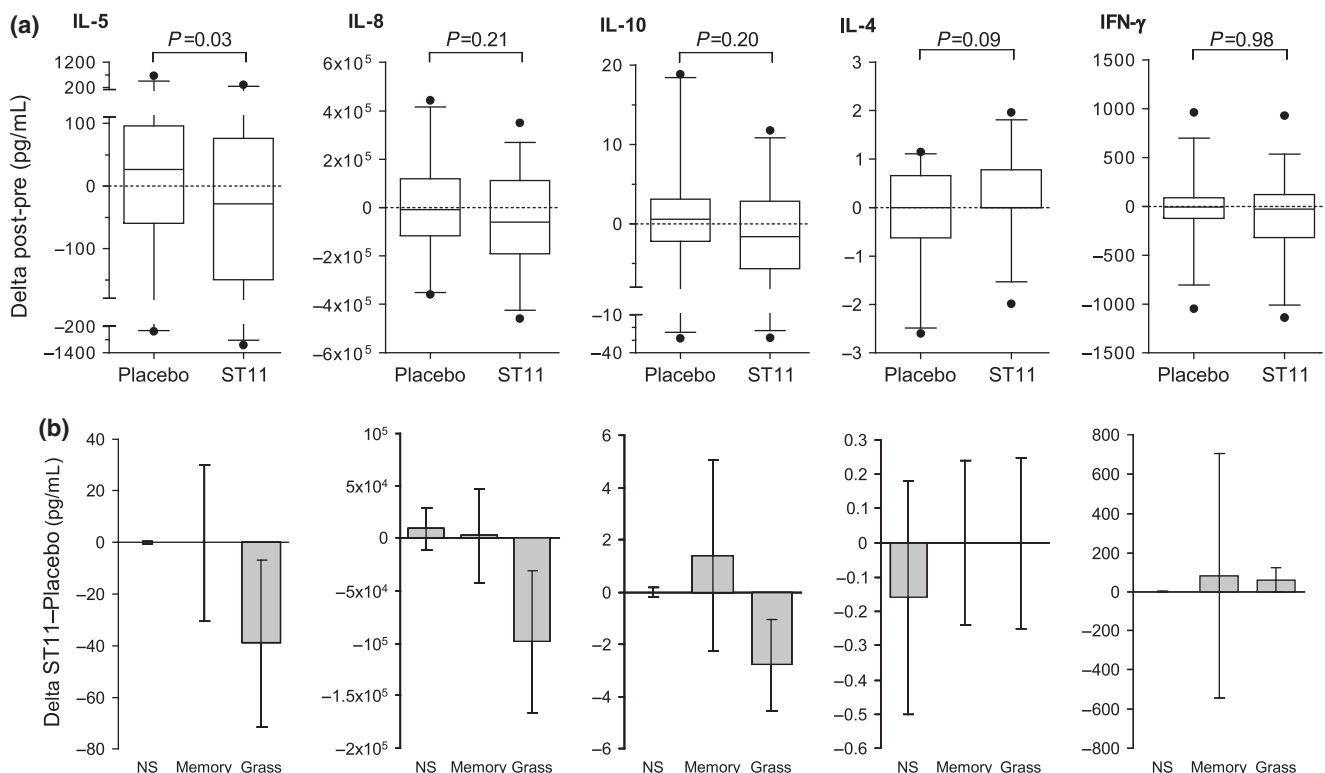


Fig. 4. Cytokine secretion by peripheral blood mononuclear cell (PBMC). (a) Post-NPT PBMC responses to grass pollen extract [Delta: post-treatment minus pre-treatment (baseline) value] after ST11 or placebo treatment. Box and whiskers show interquartile range of individual delta cytokine concentrations. (b) Treatment effect [treatment (ST11) minus placebo] on specific and non-specific PBMC cytokine responses (NS, non-stimulated; Memory, vaccine antigen cocktail; Grass, grass pollen) calculated on the evolution from baseline (post-treatment minus pre-treatment values). Data are expressed as medians $\pm$ SEs to the medians.

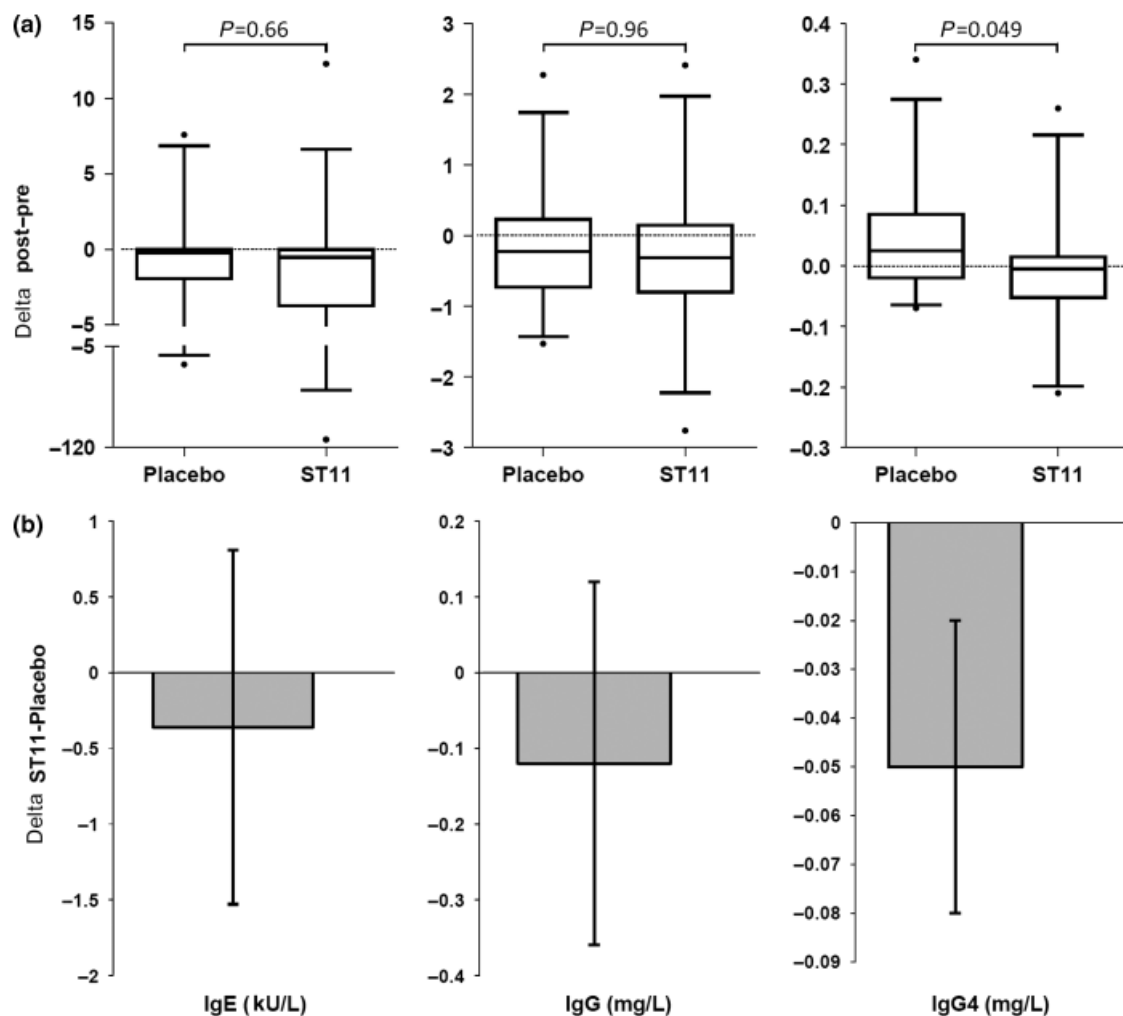


Fig. 5. Grass pollen-specific IgEs. (a) Grass pollen-specific IgE, IgG and IgG4 in sera drawn 24 h after nasal provocation test (NPT) following treatment with ST11 or placebo. Box and whiskers show medians and quartiles of individual post-treatment *minus* pre-treatment (baseline) Ig concentrations. (b) Treatment effect [treatment (ST11) *minus* placebo] on grass pollen-specific IgE, IgG and IgG4 calculated on the evolution from baseline (post-treatment *minus* pre-treatment values). Data are expressed as medians $\pm$ SEs to the medians.

Nasal fluid markers

Ex vivo levels of IL-4, IL-5, IL-8, IL-10 and IFN- γ , *ex vivo* levels of albumin, ECP, tryptase, total IgA and grass pollen-specific IgA were all analysed at the different time-points of NPT. None of these parameters significantly differed between the two treatment groups (data not shown).

Discussion

The beneficial effect of probiotic administration during early infancy in preventing the development of atopic diseases remains controversial and today only a few strains have demonstrated benefits [8, 32, 33]. These conflicting results reflect the complexity of the allergic syndrome, the different characteristics and potentials of the various probiotic strains as well as the diversity in

clinical settings [15]. Nonetheless, this approach remains attractive and needs to be further investigated. The majority of published studies have evaluated the effect of probiotics on atopic eczema. Fewer studies have examined the efficacy of probiotics in the field of respiratory allergy, yet successful mitigation of symptoms has been reported in different clinical designs [16]. The trial described in this report aimed to modulate established allergic symptoms or allergic markers in adults in the well-reproducible and -standardized NPT. To the best of our knowledge, this is the first report on the effect of a short-term probiotic treatment on an NPT with grass pollen in young adults suffering from seasonal allergic rhinitis. The administration of the probiotic *L. paracasei* ST11 demonstrated a significant beneficial effect during NPT on the subjective measurement of nasal congestion, accompanied by a similar trend on the sensation of nasal pruritus in volunteers from the active arms. However, the trial did not reach

its primary end-point, i.e. the demonstration of a significant difference in allergen dose level at reaction threshold for the combined NPT index. The choice of the *L. paracasei* ST11 strain tested in this clinical study was driven by positive pre-clinical results (*in vitro* and *in vivo* studies: C. Pellaton et al., in preparation) which we further wished to confirm in a pilot human trial. Taking into account the limited duration (4 weeks) of the treatment, the improvement of two classical subjective markers of allergic rhinitis during the trial is highly encouraging.

Among the possible mechanisms that could underlie the beneficial effect of probiotic strains, the decrease in IL-5 secretion by grass pollen-specific T cells in the probiotic-treated group is particularly relevant. IL-5 plays a crucial role in the allergic reaction by supporting eosinophil differentiation, maturation and life span [34]. Although the differences between the two groups were relatively small, the decrease in IL-5 production by PBMC post-active treatment showed that despite the short-term administration of probiotics, this approach might have significant influence on key cytokines of the allergic reaction. Furthermore, IL-8 and IL-10 secretion by grass pollen stimulated PBMC also tended to decrease. Globally, these data were suggestive of the correction of an imbalance excessively in favour of Th2 cytokines.

We did not reasonably expect in this clinical setting a decrease in allergen-specific IgE, since receptor-bound IgE half-life is about 3 weeks [35]. Yet, the significant decrease in serum allergen-specific IgG4 was unexpected. As IL-10 is an important regulator of IgG4 production [36, 37] in association with Th2 cytokines [38, 39], the decrease in IL-5 together with the trend in decreased IL-10 secretion by PBMC may be logically associated with the decrease in allergen-specific IgG4. Of note, the difference between groups was rather small. We also considered that pro-inflammatory cytokine secretion may possibly decrease at the inflammation induction site (nasal mucosa) upon probiotic treatment and thus analysed NS at different time-points. No difference in the *ex vivo* production of cytokines, in total cell counts as well as in eosinophil infiltration was observed in NS between the two arms. Upon NPT, there was however a clear stimulation of cytokine secretion as well as of eosinophil influx into the nasal fluid, validating the NPT methodology (data not shown). The absence of difference between groups in the NS parameters may be related to a lower sensitivity of the *ex vivo* measurements as compared with the *in vitro* allergen-specific T cell stimulation. No significant anti-inflammatory effects on the nasal mucosa itself were observed as suggested by unaffected levels of albumin in nasal secretions, a marker of fluid extravasation upon allergen exposure (data not shown) [34]. Similarly, the treatment had no impact on the production of typical

eosinophil by-products such as ECP, or mast cell by-products such as tryptase (data not shown). Although we cannot rule out that the timing for the nasal sampling was not optimal in our protocol, a longer period of treatment could have changed our observations.

In conclusion, a single clinical marker (subjective nasal congestion, a parameter of high impact on quality of life of patients [40]) and two systemic immune markers (IL-5 and IgG4) were significantly reduced after *L. paracasei* ST11 consumption, a finding further supported by the non-significant trend for a decrease in a few key cytokines or clinical makers including IL-10 and IL-8 secreted by grass pollen-stimulated PBMC and nasal pruritus, respectively.

Clinical relevance

A profound modification of an established immune response was not expected from probiotics administered for a limited period of time (4 weeks) in adults. Nonetheless, the clinical and biological effects noted in this study can be rationally linked, and in this sense acquire all their validity, although the full clinical significance of the observed clinical and immune alterations remains to be examined further. Remarkably the improvement in nasal congestion (as measured by VAS) observed in a recent study from our group after desloratadine as compared to placebo was very comparable to our current data with probiotics [27]. This strongly suggests that in seasonal conditions probiotics may also be active on allergen induced congestion and pruritus. However, these data need to be appreciated within the context of a NPT and cannot readily be transposed to seasonal exposure. Our data were furthermore supported by a recent seasonal study on volunteers with allergic rhinitis treated for 5 months with *L. casei* Shirota [18]. In the active group, antigen-specific IL-5, IL-6 and IFN- γ responses were significantly reduced.

The mechanisms underlying the modulation of key cytokines production post-probiotic treatment will need to be further investigated with emphasis on the potential role of innate immunity and inducible T regulatory cells. Altogether, these data support the notion that selected probiotics may down modulate key parameters of allergic rhinitis and might be used for the design of functional foods targeting management of respiratory allergy symptoms.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Detection of *L. paracasei* ST11 strain in feces of subjects during trial phases.

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Supplementary Table *detection of L. paracasei ST11 strain in feces of subjects during trial phases.*

Subject study number	After period A	After wash-out	After period B
1	-	-	3.00<ST11<7.21
2	-	-	3.00<ST11<7.70
3	3.00<ST11<8.18	-	-
4	3.00<ST11<7.39	-	-
5	-	-	3.00<ST11<5.00
6	3.00<ST11<3.30	-	-
7	3.00<ST11<6.91	-	-
8	-	-	3.00<ST11<7.48
9	3.00<ST11<6.76	-	-
10	3.00<ST11<6.94	-	-
11	-	-	3.00<ST11<8.28
12	-	-	3.00<ST11<7.85
13	3.00<ST11<5.00	-	-
14	-	-	3.00<ST11<4.94
15	3.00<ST11<7.48	-	-
16	-	-	3.00<ST11<6.30
17	-	-	3.00<ST11<6.92
18	3.00<ST11<6.30	-	-
19	3.00<ST11<7.07	-	-
20	3.00<ST11<7.30	-	-
21	-	-	3.00<ST11<6.97
22	3.00<ST11<3.70	-	-
24	-	-	3.00<ST11<6.11
25	-	-	3.00<ST11<7.25
27	3.00<ST11<7.12	-	-
28	-	-	3.00<ST11<7.85
29	-	-	3.00<ST11<8.23
30	3.00<ST11<8.81	-	-
31	-	-	3.00<ST11<7.60
32	-	-	3.00<ST11<7.95
33	-	-	3.00<ST11<6.11

*Stool samples were collected after the first period of consumption of the products (After period A), after the wash-out period (After wash-out) and after the second period of consumption of the products (After period B). Briefly, feces samples (1g/ml) were plated at various dilutions on MRS-Agar (Difco) supplemented with phosphomycin, trimethoprim and sulfamethoxazole (Sigma-Aldrich, Buchs, Switzerland) and incubated anaerobically for 48h at 37°C. All colonies were picked and used to run RT-PCR using ST11-specific primers. Results are expressed in log colony forming unit/g feces (- : not detected: no ST11 detected in the samples of the placebo treated group).). Of note : numbers 23 and 26 were not attributed to any subject. Subject 19 was excluded because of antibiotic intake during the period B of the study.