

ORIGINAL ARTICLE

Immune-modulatory effect of probiotic *Bifidobacterium lactis* NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial

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BACKGROUND/OBJECTIVES: Probiotics are defined as 'living micro-organisms that when administered in adequate amounts confer a health benefit to the host'. Different probiotic strains have been investigated for beneficial effects on allergic disorders. The purpose of the current study was to evaluate the effect of orally administering the probiotic Nestlé culture collection (NCC)2818 *Bifidobacterium lactis* strain on immune parameters and nasal symptom scores in subjects suffering from seasonal allergic rhinitis (SAR).

SUBJECTS/METHODS: The study was a double-blinded, parallel, randomized placebo-controlled trial conducted during the peak of the pollen season. Adult subjects with clinical history of SAR and positive skin prick test to grass pollen were recruited. The subjects received *B. lactis* NCC2818 or placebo for 8 weeks and completed symptom questionnaires every week. Whole blood was collected at baseline (V1), 4 weeks (V2) and 8 weeks (V3) to measure immune parameters.

RESULTS: Concentrations of Th-2 cytokines, secreted by stimulated blood lymphocytes, were significantly lower in the probiotic group compared with the placebo group at V3 (interleukin (IL)-5, $P = 0.016$; IL-13, $P = 0.005$). Total nasal symptom scores were significantly lower in the second month of the study (weeks 5–8) in the probiotic group compared with the placebo group ($P = 0.03$). Also, percentages of activated CD63 expressing basophils were significantly lower in the probiotic group at V2 ($P = 0.02$).

CONCLUSIONS: Oral administration of the probiotic NCC2818 mitigates immune parameters and allergic symptoms during seasonal exposure. These promising results warrant that *B. lactis* NCC2818 be investigated further in large-scale trials for management of respiratory allergy.

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INTRODUCTION

Allergic rhinitis (AR) is of two types, seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR), in which symptoms last throughout the year.^{1,2} Management of AR requires avoidance of the causative allergen, treatment of the nasal symptoms with anti-histamines and corticosteroids, and in some cases allergen-specific immunotherapy. Intranasal corticosteroids are considered the gold standard approach to manage symptoms of AR. While they are effective in short term, many of the associated symptoms such as nasal congestion remain unaffected.^{3,4}

Recently, nutritional interventions with probiotics have been investigated for their beneficial effects on allergic disorders. Probiotics are defined by the WHO as 'living micro-organisms that when administered in adequate amounts confer a health benefit to the host'.⁵ The ability of certain probiotic strains to modulate the immune system is currently an area of intense research.^{6–8} The beneficial roles of probiotics, especially of the genera *Lactobacillus*

and *Bifidobacterium*, in allergic diseases have been investigated with increasing interest in animal models and human clinical trials^{9–11} where probiotics are sought to influence the gut microbiota composition and restore homeostasis of the host immune system.^{12,13}

Several probiotic strains have previously been evaluated for their effect in AR.^{14–20} *Lactobacillus casei* Shirota strain has been demonstrated to impact the immune system and allergic symptoms^{14,16} while *Lactobacillus plantarum* strains have been shown to inhibit allergic cytokines.¹⁵ In another study, *Bifidobacterium longum* BB536 strain alleviated symptoms of SAR.^{19–21} Studies with different *Lactobacillus paracasei* strains have shown potential for both SAR¹⁸ and PAR.¹⁷ In the current study, we have sought to examine the effect of oral administration of the *Bifidobacterium lactis* NCC2818 strain in reducing immune markers and alleviating allergic symptoms in subjects suffering from SAR to grass pollen (GP). Previous clinical studies on atopic

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eczema cohorts have shown a benefit for specific probiotic *B. lactis* strains.^{22–24} Our primary aim in conducting this study was to identify immune mechanisms via which the probiotic strain could mediate its anti-allergic effects. We have used *ex vivo* immune assays that are simple to implement in clinical trial settings.^{25–28} Here, we report our promising results with an 8-week oral administration of the probiotic *B. lactis* NCC2818.

PATIENTS AND METHODS

Subjects and design of study

The protocol was approved by the Ethics Commission of the Cantonal Hospital of Lausanne, Switzerland (Commission Cantonale d'Ethique pour la Recherche sur l'Etre Humain), under the reference 92/11. The study was a double-blinded, single-center, parallel, randomized placebo-controlled clinical trial performed at the Metabolic Unit, Nestlé Research Center, Lausanne, Switzerland (study no. 11.03.MET) during the GP season (between May and July 2011, peak of allergen load, confirmed by public health services). Twenty-seven adult subjects aged 20–65 years were invited to participate in the trial, of which twenty-four who gave written informed consent were screened for clinical history of SAR to GP > 2 years and a confirmatory skin prick test with a mix of GP (SOLUPRICK SQ, 6-grass mix, ALK-Abello AG, Horsholm, Denmark). Individuals with a mean wheal diameter ≥ 3 mm and a SAR clinical history were considered positive to GP-induced SAR. Exclusion criteria included history of anemia, congenital immunodeficiency, regularly taking anti-allergy or any other immunosuppressive medications, pregnancy, presence of common cold (flu-like) symptoms, regularly consuming probiotic-supplemented foods and subjects participating or having participated in another clinical trial in the month preceding the current study. Three subjects failed to meet the inclusion criteria and one declined to participate before the start of the study. Therefore, 20 subjects were randomized. Randomization was stratified on age, weight, height and body mass index. Subjects were assigned to either probiotic (10 subjects) or placebo (10 subjects) after a

two-week run-in period where probiotic products were not allowed for consumption. Total nasal symptom score (TNSS) questionnaires were filled at baseline. TNSS (scale of 0–12; individual symptoms scale 0–3, with 0 representing no symptoms and 3 for severe symptoms) was recorded weekly. At baseline visit (V1), subjects were given the products and the TNSS questionnaires. In addition, 10 ml whole blood was drawn in heparin-coated tubes. At V2 (4 weeks) and at V3 (8 weeks), TNSS questionnaires along with 10 ml of whole blood were collected. The study design is shown in Figure 1. All subjects completed the study.

Probiotic and placebo oral administration

Subjects included in the clinical trial took either probiotic *B. lactis* NCC2818 (NCC: Nestlé Culture Collection) (active) blended in maltodextrin or maltodextrin alone (placebo). Products were provided in 100-g cans with a calibrated spoon and were identical in packaging and appearance. Cans were labeled with two non-speaking codes per product by the manufacturing site (Nestlé PTC, Konolfingen, Switzerland), and each subject received two cans of the product, that is, one can for 4 weeks provided at V1 and V2. Each dose of the active product contained a minimum of 2×10^9 colony-forming units per gram of the probiotic mixed in maltodextrin. The products were to be taken orally (~ 2 g every day for a dose of 4×10^9 colony-forming units per day), diluted in cold milk or water, daily for 8 weeks. Probiotics, yoghurts and fermented milks were not permitted during the duration of the clinical trial.

Whole-blood assay

Heparinized, venous blood samples were collected at the three visits (V1: baseline, V2: 4 weeks and V3: 8 weeks). Whole-blood cells were cultured for 5 days in a 1:5 dilution (100 μ l of whole blood + 400 μ l of culture media) in triplicate with culture medium RPMI 1640 (Sigma, Buchs, Switzerland) that was complemented with 1% L-glutamine, 1% Penicillin/Streptomycin, 1% of non-essential amino acids (Invitrogen, Lucerne, Switzerland) and 0.1% Gentamycin (Sigma). Whole-blood cells were stimulated in 48-well plates (Milian, Meyrin, Switzerland) with anti-CD2 at a concentration of 2 μ g/ml

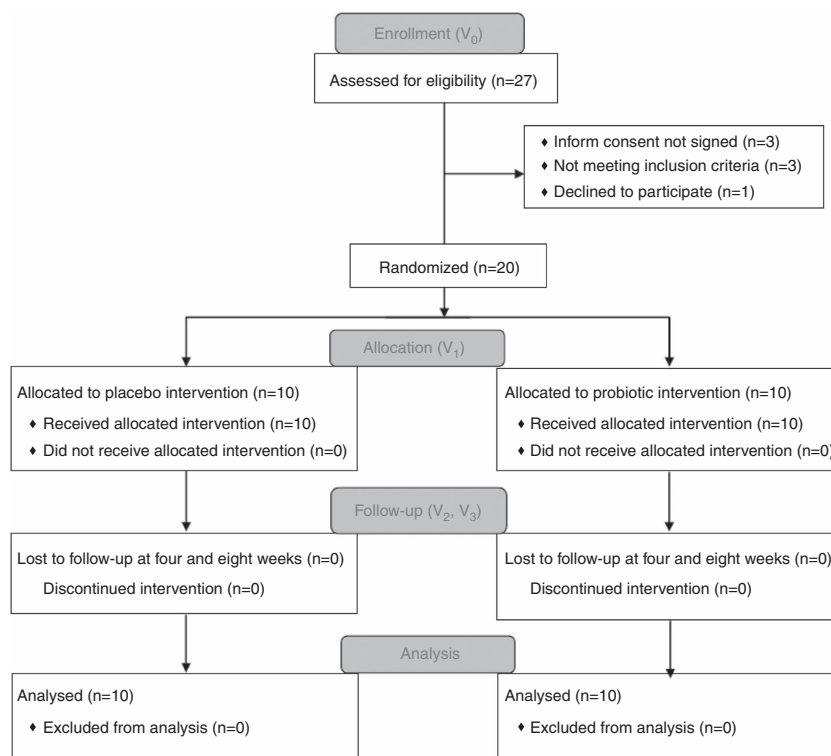


Figure 1. Protocol for the clinical trial comparing the efficacy of probiotic versus placebo treatment on seasonal allergic symptoms and immune parameters. The CONSORT flow diagram for the clinical trial is shown. Twenty subjects that met the inclusion criteria and signed informed consent forms were included. Subjects were randomly assigned to either placebo or *B. lactis* NCC 2818 probiotic treatment groups at the baseline visit (V1). Two follow-up visits after V1, at 4 weeks of treatment (V2) and at 8 weeks of treatment (V3), were scheduled for the subjects. All subjects completed the trial.

(Sanquin, Amsterdam, the Netherlands; clones: CLB-T11.1/1, CLB-T11.1/2) and anti-CD28 at a concentration of 4 µg/ml (Sanquin; clone: CLB-CD28/1). For allergen-specific stimulation, a 6-GP mix extract (ALK-Abello AG) was used at 100 µg/ml final concentration either alone or in combination with 20 ng/ml recombinant human interleukin (IL)-2 (BD Biosciences, San Jose, CA, USA). Cell supernatants were collected and stored at -20 °C. Cytokines in the supernatant (IL-5, IL-10, interferon-gamma (IFN-γ), IL-13, IL-1β and tumor necrosis factor (TNF)-α) were measured by a human multiplex kit (MesoScale Discovery, Gaithersburg, MD, USA).

Basophil activation test

Peripheral blood samples were collected at V1, V2 and V3. Fluorochrome-tagged monoclonal antibodies against CD3, CRTH2, CD203c and CD63 were added to 100 µl of whole blood in flow cytometry tubes (BD Biosciences) in the presence of allergen (GP) and positive controls (anti-IgE, from Beckman Coulter (Brea, CA, USA), A040174). Further processing of the samples was done using the Allergenicity Kit (# A17116, from Beckman Coulter) and instructions and reagents used as per the manufacturer's protocol. Erythrocytes were lysed before flow cytometry analysis. The processed samples were acquired via flow cytometry using a BD Fortessa machine (BD, San Jose, CA, USA). Raw data were analyzed by FlowJo software (Treestar, Ashland, OR, USA). Gating protocol is shown in Supplementary Figure 1.

Statistical analyses

A sample size of 10 subjects per group was judged sufficient to detect differences in immune parameters between the groups based on previous studies performed with probiotic strains in literature.^{14,15} The primary endpoints of this trial were the concentration of Th-2 cytokines (IL-5 and IL-13) under stimulated anti-CD2/CD28 conditions. As this was an exploratory study, no multiplicity correction for multiple testing was employed. Linear mixed models were fitted on log-transformed values of IL-5 and IL-13, adjusting for baseline values. Similar analysis was performed for the TNF-α and IL-1β cytokines. The TNSS data were analyzed as both monthly averages (at V2 and V3 compared with baseline V1) and weekly TNSS scores. Weekly TNSS data were modeled via linear mixed models on the log-transformed data, adjusting for baseline TNSS values, time, treatment and time*treatment interaction. Monthly TNSS scores were calculated as average over the 4 weeks preceding the visits and modeled via linear mixed models. The statistical analysis was performed in R 2.13.2.²⁹

RESULTS

Baseline demographics

Equal numbers of male and female adult subjects were assigned to placebo and probiotic groups (Figure 1). There were no statistical differences in age, weight, height and body mass index characteristics between the two groups. Baseline characteristics for the subjects that completed the study are given in Table 1. The values represent lower quartile (a), median (b) and upper quartile (c) for continuous variables. Numbers after percents are frequencies.

Safety and compliance

No minor or serious adverse events were reported for this trial. All subjects sent a filled TNSS questionnaire each week of the study. The remaining clinical trial product in the opened product cans was measured to monitor compliance and sent for microbiological analysis at the end of the study to the product manufacturing site (Nestlé PTC, Konolfingen, Switzerland). The measurements in the cans at the end of the study demonstrated that all subjects were compliant during the clinical trial. The results of the cell count analysis from the opened cans containing *B. lactis* NCC2818 showed that viable probiotic counts were 2.07×10^9 colony-forming units per gram of the clinical trial product, which is similar to the probiotic dose in the clinical trial samples at the time of production.

Oral administration of the probiotic *B. lactis* NCC2818 significantly decreases TNSS scores at V3

The changes in TNSS scores between V2 and V3 from baseline V1 are shown in Figure 2. The summary measures of monthly TNSS scores are shown in Table 2. The TNSS scores decreased significantly at V3 (after 8 weeks of treatment) in the probiotic group compared with the placebo (*P*-value = 0.03). No effect of the treatments was observed at V2 (4 weeks after the treatment;

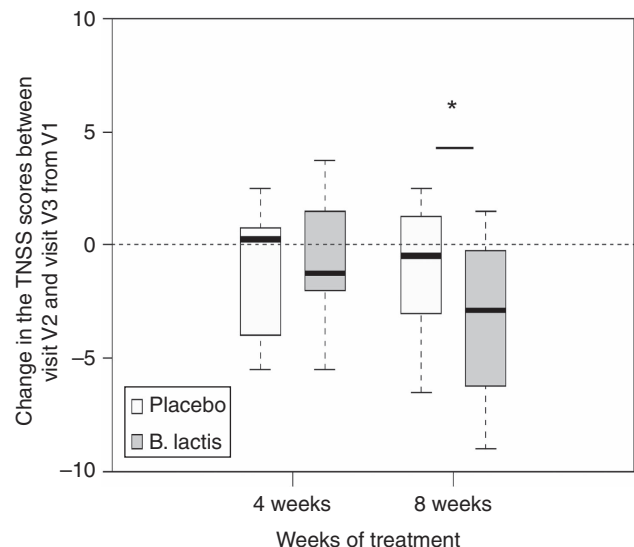


Figure 2. Oral administration of the probiotic *B. lactis* NCC2818 significantly decreases TNSS scores at V3. The changes in TNSS scores between visits V2 and V3 from baseline are shown. The TNSS scores decreased significantly at visit V3 (after 8 weeks of treatment) in the probiotic group compared with the placebo (*P*-value = 0.03). No effect of the treatments was observed at V2 (4 weeks after the treatment).

Table 1. Baseline characteristics of subjects

Characteristics	Placebo (N = 10)	<i>B. lactis</i> NCC2818 (N = 10)	Combined (N = 20)
Gender			
Female	60% (6)	60% (6)	60% (12)
Male	40% (4)	40% (4)	40% (8)
Age (years)	29.25 ^a , 34.00 ^b , 44.00 ^c	31.50 ^a , 35.50 ^b , 38.00 ^c	30.25 ^a , 35.00 ^b , 41.25 ^c
Weight (kg)	60.75 ^a , 68.25 ^b , 73.50 ^c	56.50 ^a , 64.75 ^b , 73.00 ^c	58.75 ^a , 66.25 ^b , 74.25 ^c
Height (cm)	168.50 ^a , 172.00 ^b , 179.50 ^c	164.00 ^a , 167.00 ^b , 176.75 ^c	164.75 ^a , 170.00 ^b , 178.50 ^c
BMI (kg/m ²)	21.40 ^a , 22.03 ^b , 23.43 ^c	20.38 ^a , 22.82 ^b , 24.49 ^c	20.99 ^a , 22.31 ^b , 23.77 ^c

Abbreviation: BMI, body mass index. ^aValues represent lower quartile. ^bMedian. ^cUpper quartile.

P -value = 0.32). Individual symptom scores were also lower after the fourth week of administration in the probiotic group (Supplementary Figure 2A–D) compared with placebo group.

Oral administration of the probiotic *B. lactis* NCC2818 significantly decreases the levels of Th-2 cytokines, IL-5 and IL-13, in *ex vivo*-stimulated whole-blood cultures at V3

Th-2 cytokines (IL-5 and IL-13) produced by whole-blood cells under *ex vivo*-stimulated conditions (anti-CD2/anti-CD28) were measured at V1, V2 and at V3. The box plots for differences between V2 and V1, and V3 and V1 in IL-5 (Figures 3a and b) and

Table 2. Summary measures of monthly TNSS

Visit	Placebo (N = 10)		<i>B. lactis</i> NCC2818 (N = 10)	
	Mean	s.d.	Mean	s.d.
V1	4.10	3.11	4.90	3.35
V2	3.05	1.86	3.92	1.89
V3	3.00	2.04	1.50	1.33 ^{*,**}

Abbreviation: TNSS, total nasal symptom score. ^{*} P = 0.03 (at V3 between the placebo and *B. lactis* NCC 2818 groups). ^{**} P = 0.008 (in *B. lactis* NCC 2818 group compared between V2 and V3).

IL-13 (Figures 3c and d) concentration under anti-CD2- and anti-CD28-stimulated conditions are presented. No differences were observed between the two groups at V2. IL-5 concentrations were, however, significantly lower in the probiotic *B. lactis* NCC2818-treated group compared with the placebo (P -value = 0.016) at V3. Similarly, the concentration of IL-13 decreased significantly at V3 in the probiotic group compared with the placebo (P -value = 0.005). Considerable variability between donor to donor responses was observed at V1 and V2; for IL-13 levels at V2, probiotic group: mean = 998.34.05 and s.d. = 1382.57, and placebo group: mean = 975.02 and s.d. = 1019.22. Interestingly, in the probiotic group at V3, this variability was minimal, that is, the probiotic group had lower allergic cytokine levels and lesser variation in the group; for IL-13 levels at V3, probiotic group: mean = 218.05 and s.d. = 151.13, and placebo group: mean = 967.53 and s.d. = 1205.63. Similar observations were observed for IL-5.

Pro-inflammatory cytokines TNF- α and IL-1 β are decreased at V3 with oral administration of the probiotic *B. lactis* NCC2818

The concentrations of pro-inflammatory cytokines TNF- α and IL-1 β in supernatants of *ex vivo*-stimulated (anti-CD2/anti-CD28) whole-blood cells were measured. The box plots for differences between V2 and V1, and V3 and V1 in TNF- α (Figures 4a and b) and IL-1 β (Figures 4c and d) concentration under anti-CD2- and anti-CD28-

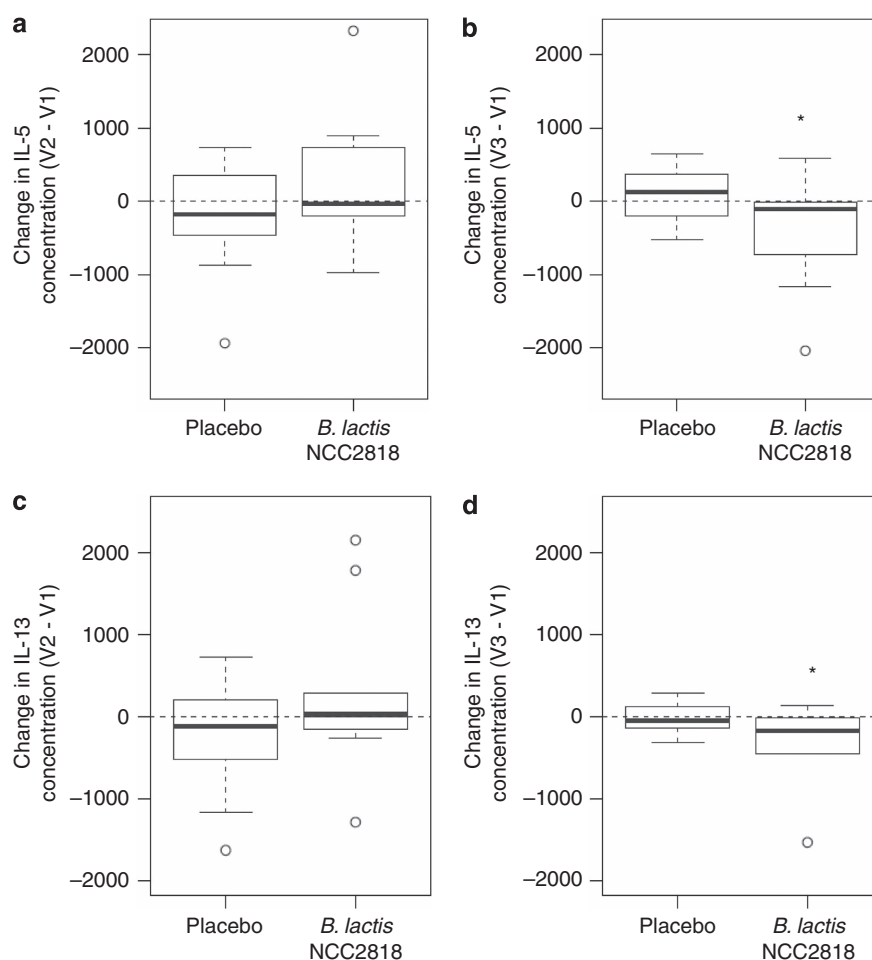


Figure 3. Oral administration of the probiotic *B. lactis* NCC2818 significantly decreases IL-5 and IL-13 levels in *ex vivo*-stimulated whole-blood cultures at V3. The box plots for differences between visit V2 and baseline V1, and visit V3 and baseline V1 in IL-5 (a, b) and IL-13 (c, d) concentration under anti-CD2 and anti-CD28-stimulated conditions are presented. IL-5 concentrations were significantly lower in the probiotic *B. lactis* NCC2818-treated group compared with the placebo (Figure 3b; P -value = 0.016) at V3. Similarly, the concentration of IL-13 also decreased significantly at V3 in the probiotic group compared with placebo (Figure 3d; P -value = 0.005).

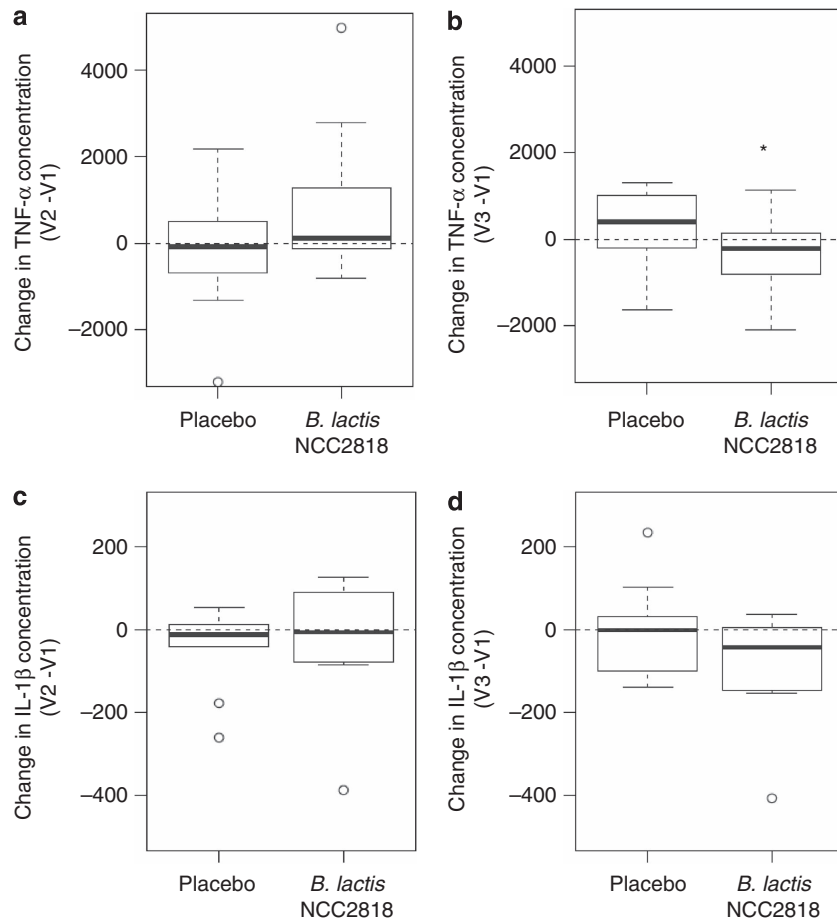


Figure 4. Reduced levels of TNF- α and IL-1 β in *ex vivo*-stimulated whole-blood cultures at V3 in probiotic *B. lactis* NCC2818-treated group. The box plots for differences between visit V2 and baseline V1, and visit V3 and baseline V1 in TNF- α (a, b) and IL-1 β (c, d) levels under anti-CD2- and anti-CD28-stimulated conditions are shown. TNF- α concentrations were significantly decreased in the probiotic *B. lactis* NCC2818-treated group compared with placebo (b; P -value = 0.04) at V3. Also, the concentration of IL-1 β was decreased at V3 in the probiotic group compared with the placebo (d; P -value = 0.06).

stimulated conditions are depicted. The results show that the levels of TNF- α cytokine at V3 were significantly decreased compared with V1 in the probiotic *B. lactis* NCC2818-treated group compared with the placebo (P -value = 0.04). There was also a trend in decreased IL-1 β cytokine levels at V3 in the probiotic-treated group (P -value = 0.06).

Probiotic *B. lactis* NCC2818 oral administration significantly decreases CD63 expression on activated basophils at V2

We compared the levels of CD63 and CD203c cell surface expression on *ex vivo*-activated basophils. Treatment effects on the percentages of activated CD63 and CD203 cells with two different stimulations, that is, nonspecific with anti-IgE and allergen (GP)-specific were analyzed. The box plots for differences between V2 and V1 (Figure 5a), and V3 and V1 (Figure 5b) indicate that subjects receiving *B. lactis* NCC2818 treatment have no significant differences in the expression of CD203c on activated basophil cells compared with placebo. Interestingly, CD63 expression on activated basophils in the probiotic-treated group was significantly decreased by allergen-specific (GP) stimulation (P -value = 0.02) at V2, compared with the placebo group (Figure 5d). For anti-IgE stimulation, there was also a decreased trend (Figure 5c; P -value = 0.08) in the probiotic-treated group compared with the placebo group at V2. This effect on CD63 expression was not observed at V3. Levels of GP-specific IgE were lower in the probiotic group at V2, although this difference was

not statistically significant. No differences were observed in total IgE levels between the groups (Supplementary Figure 3).

DISCUSSION

The primary aim of our current study was to elucidate immune mechanisms that are reflective of the beneficial effect of a probiotic strain on allergic symptoms. This was investigated in an exploratory clinical trial setting. An 8-week oral administration of the probiotic *B. lactis* NCC2818 to subjects with SAR to GP (May–July 2011) significantly decreased allergic and pro-inflammatory cytokines and reduced basophil cell activation compared with placebo treatment. The probiotic treatment also had a significant effect on AR symptoms in the second month of treatment at V3. After 4 weeks of treatment, at V2 in the trial, no statistically significant effects were observed on allergy-related (IL-5 and IL-13) and pro-inflammatory cytokines (TNF- α and IL-1 β) between the probiotic and placebo groups or on allergic symptoms.

These findings give two important pieces of information with respect to probiotics and their use for AR. First that for the beneficial effect of probiotics to manifest on the immune system and allergic symptoms, a continuous 8-week period of oral administration could be required to reach a possible beneficial outcome. In our study, *ex vivo* production of Th-2 cytokines and TNSS scores were improved in the probiotic-treated group at V3 (after 8 weeks), but not at V2 (after 4 weeks). One of the reasons could be that there is possibly an 'adjustment window' of the

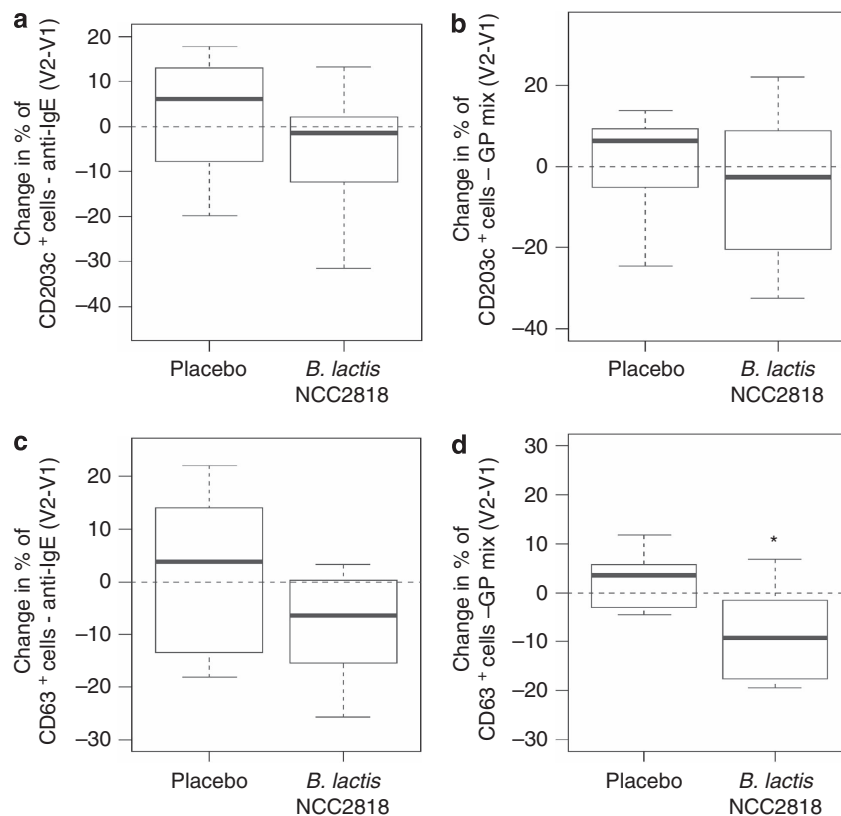


Figure 5. Probiotic *B. lactis* NCC2818 oral administration significantly decreases CD63 expression on activated basophils at V2. The box plots for differences in CD203c expression (a and b) and CD63 expression (c and d) between visit V2 and baseline V1 are shown. CD63 expression on activated basophils in the probiotic *B. lactis* NCC2818-treated group was significantly decreased for allergen-specific (GP) stimulation at V2 (d), compared with placebo group. For anti-IgE stimulation, there was a decreased trend (c; P -value = 0.08) in the probiotic-treated group compared with placebo group at V2. No differences in CD203c expression were observed between the two groups (a and b).

probiotic when administered to the host, so the initial response may mimic a 'low grade' inflammation. Following colonization of the probiotic in the gastrointestinal tract with continuous oral administration, the probiotic may then need time to impact the host immune system (in our case >4 weeks). It is also important to mention here that the study was done during the actual GP season, which makes the effect of the probiotic in our study even more impressive. A few published studies in the field of probiotics and AR are in line with our observations in this clinical trial and have showed a beneficial effect of the probiotic strains only after a >4-week administration.^{16,19,21,30,31}

We implemented the whole-blood assay and the basophil activation test in the current setting to correlate immune parameters to allergic symptoms and to be in alignment with recent recommendations suggested for probiotic-focused clinical trials.^{32,33} It is well known that in allergic individuals, significant cytokine levels are induced upon *ex vivo* stimulation^{28,34} and such increases in cytokine levels can be modulated by administration of mainstream treatments such as corticosteroids.³⁵ At V3, the probiotic-treated group demonstrated significantly reduced levels of both IL-5 and IL-13 in *ex vivo*-stimulated whole-blood cultures compared with the placebo group (Figure 3). Probiotic-treated group also had decreased levels of pro-inflammatory cytokines at V3 compared with the placebo group (Figure 4). TNF- α has also been implicated in the pathogenesis of AR and allergic effector cells, such as eosinophils, and mast cells are known to secrete TNF- α at sites of inflammation.^{36,37} No differences in the levels of the Th-1 cytokine IFN- γ and the regulatory cytokine IL-10 between the probiotic and placebo groups were observed (data not shown).

The basophil activation test is commonly used as a tool in the clinic to diagnose allergy,^{26,27} and the activation levels of basophils as measured by CD63 and CD203c expression are often reflective of the degree of sensitization or levels of allergen exposure. The reduction in CD63 expression, but not in CD203c surface expression, after 4 weeks of administration at V2 is reflective of the early impact the probiotic strain *B. lactis* NCC2818 has on the host immune system (Figure 5). These differences between the groups were not noticed at V3. In line with basophil activation were the lower levels of GP-specific IgE at V2 in probiotic group, although this difference was not statistically significant. No differences were seen in the levels of total IgE between the groups (Supplementary Figure 3).

Some investigators have utilized controlled settings, such as allergen exposure chambers²⁰ or nasal provocation settings,¹⁸ to determine the efficacy of probiotic strains. We wanted to conduct the trial during peak seasonal exposure to GP (between May and August) as reflective to the real life situation and investigate if the probiotic strain *B. lactis* NCC2818 could impact the immune system and have an effect on AR symptoms. The fact that in this challenging clinical trial setting, probiotic administration could impact on immunological parameters, mainly Th-2 cytokine levels and basophil activation, and alleviate allergic symptoms compared with placebo treatment suggests that the strain *B. lactis* NCC2818 should be investigated further in large-scale trials. The probiotic field has generally struggled in translating promising *in vitro* and *in vivo* findings obtained in preclinical models into health benefits in human intervention clinical trials. We feel that the best way forward is to examine the candidate probiotic strains in proof of concept human clinical trials where

the focus can be more on investigation of the different immune mechanisms via which a probiotic strain can impact on clinical symptoms, and then based on the findings engage in a subsequent large-scale trial with fewer outcomes and greater statistical power.

CONFLICT OF INTEREST

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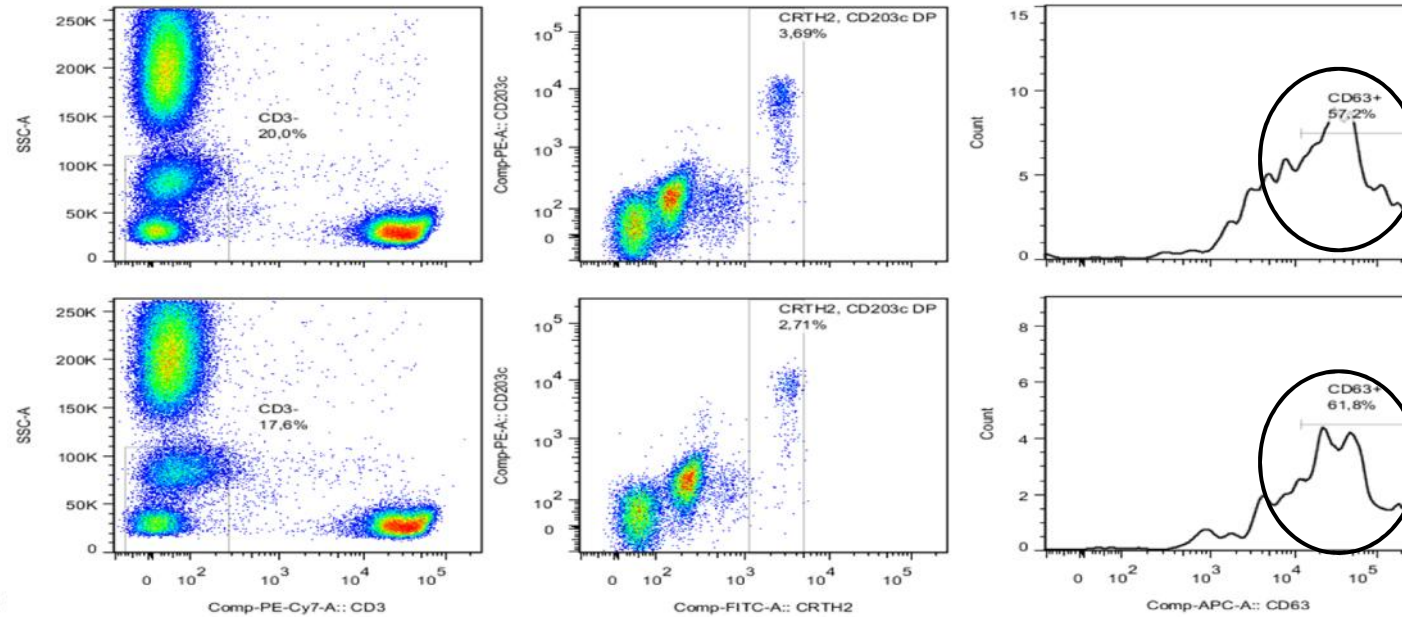
Supplementary Information accompanies the paper on European Journal of Clinical Nutrition website (<http://www.nature.com/ejcn>)

Supplementary Figure 1A

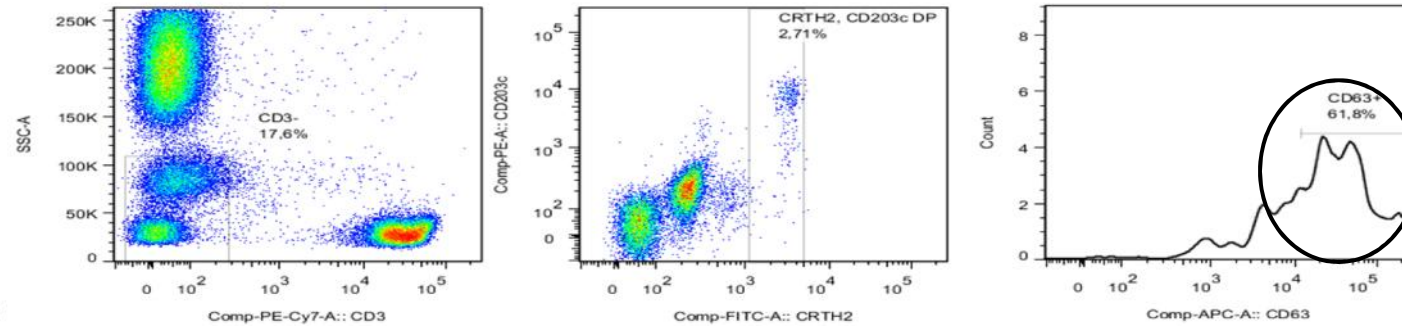
Anti-IgE

Placebo Treatment- Representative Donor

V1

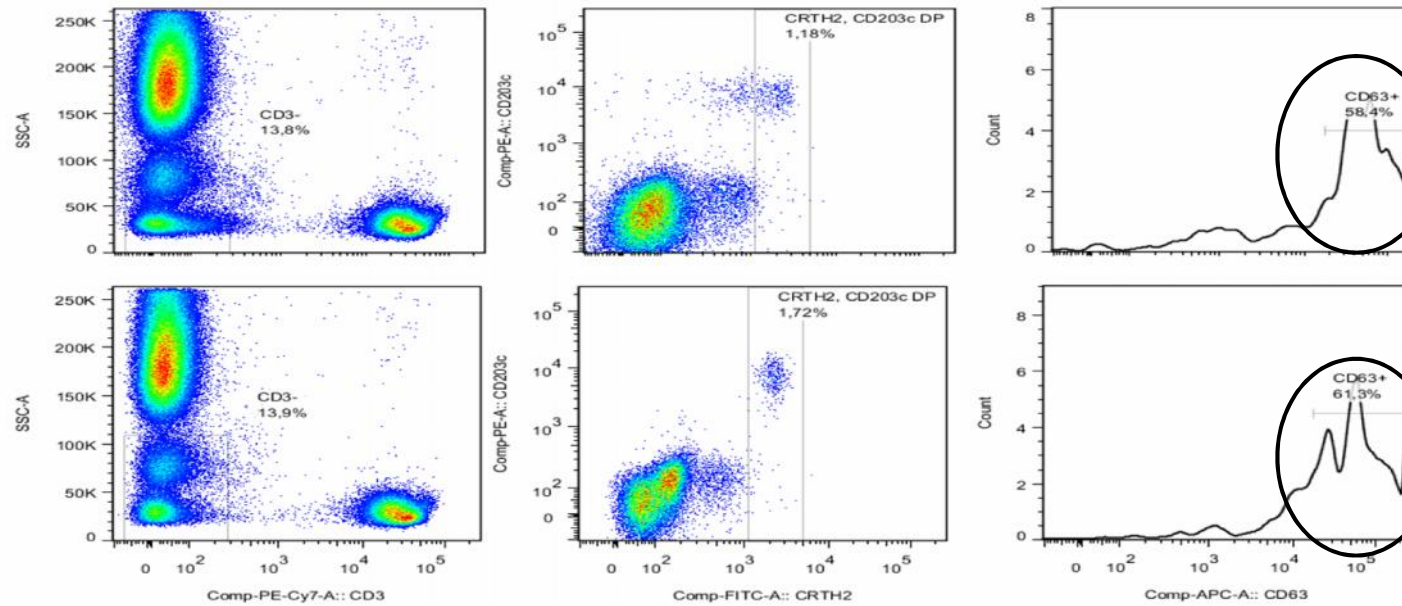


V2

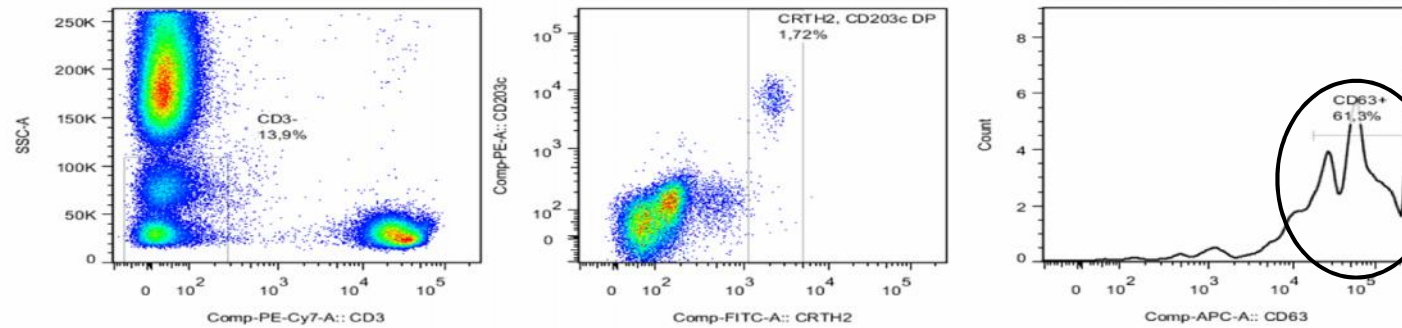


Grass Pollen (GP)

V1



V2

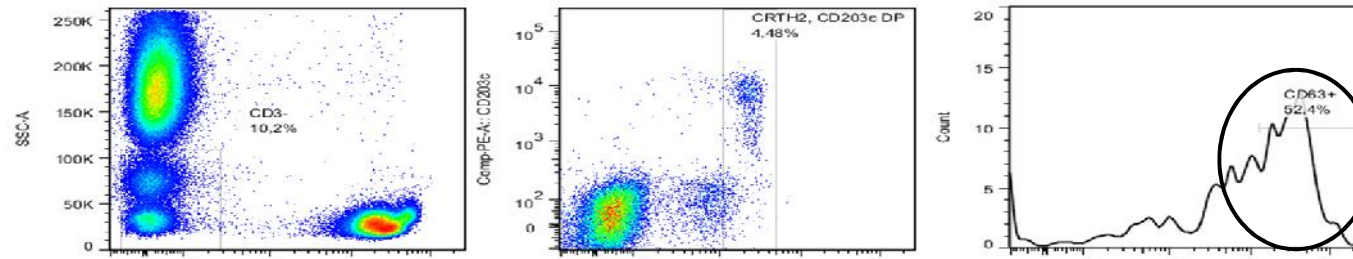


Supplementary Figure 1B

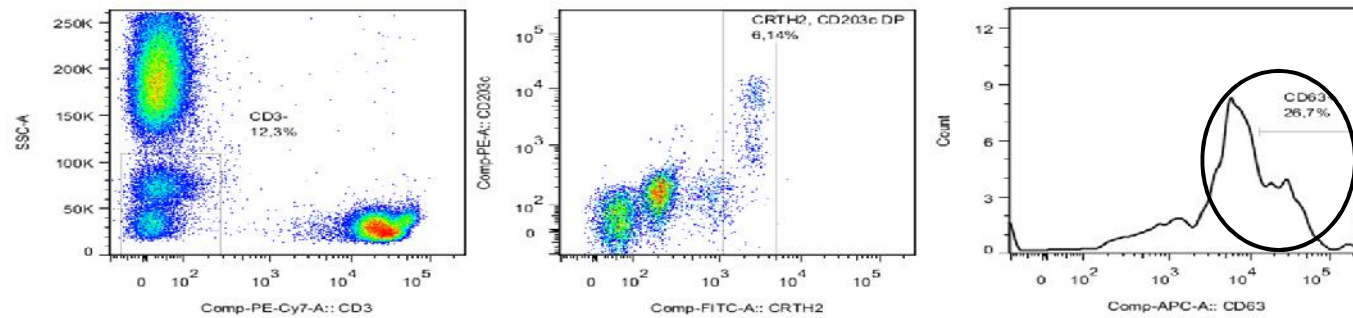
Anti-IgE

Probiotic Treatment- Representative Donor

V1

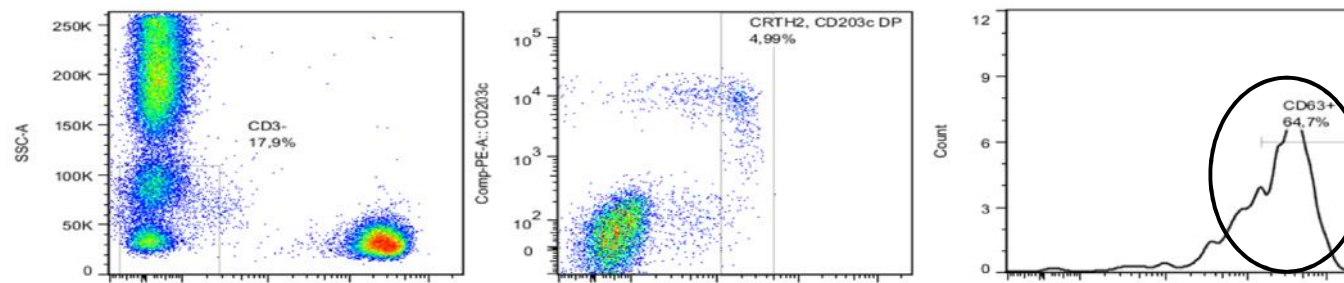


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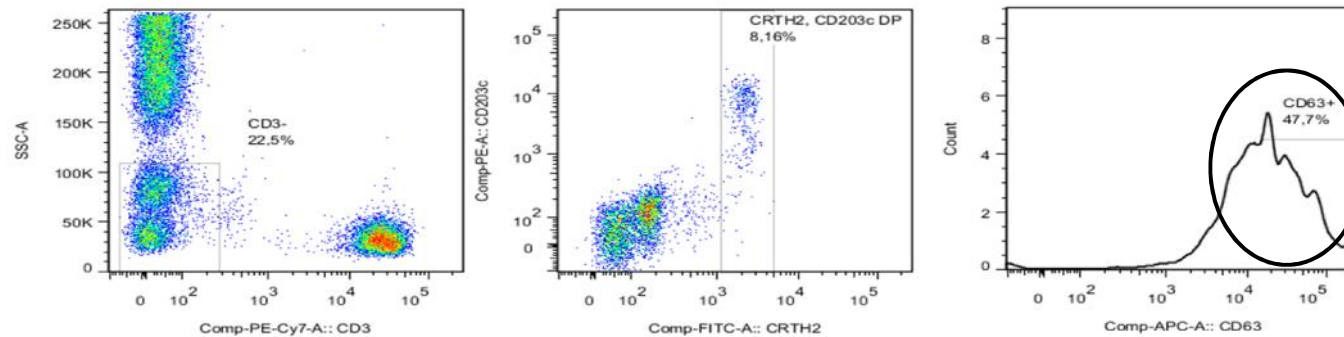


Grass Pollen (GP)

V1

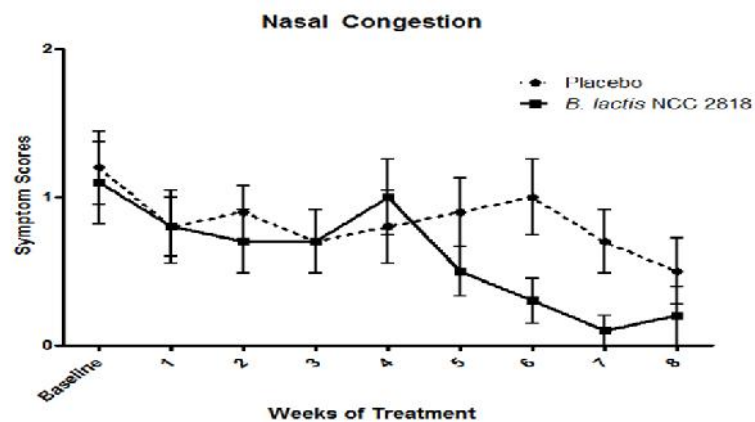


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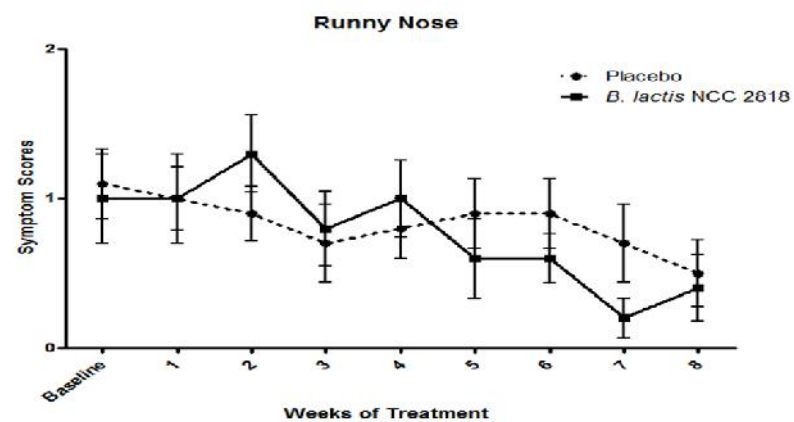


Supplementary Figure 2

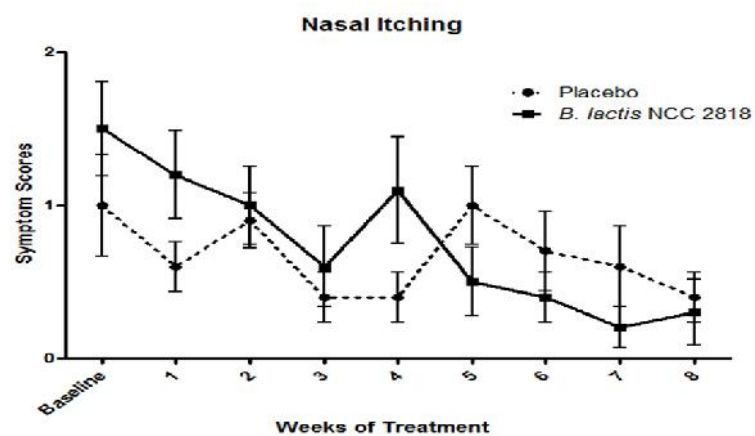
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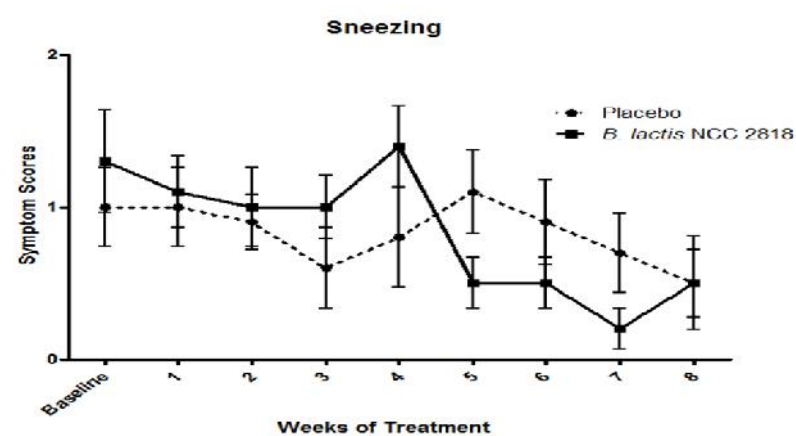
B



C

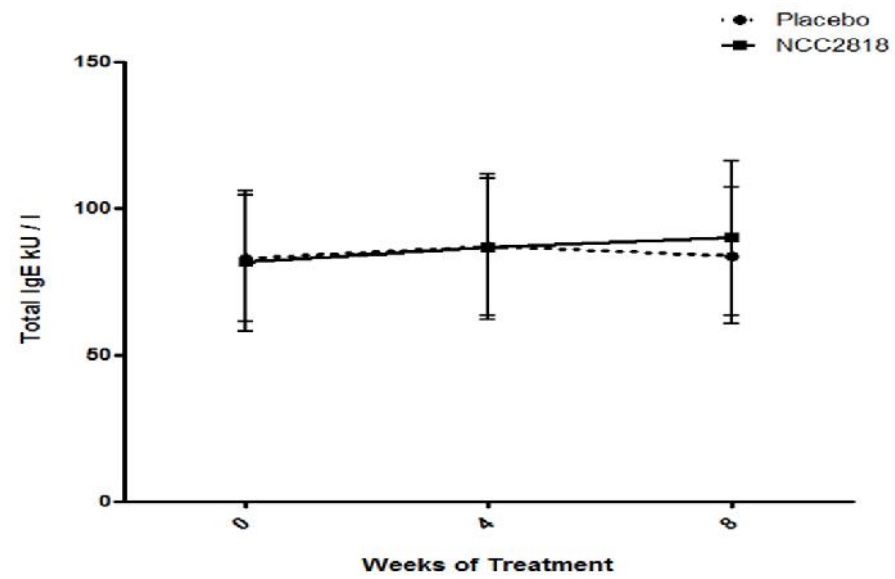


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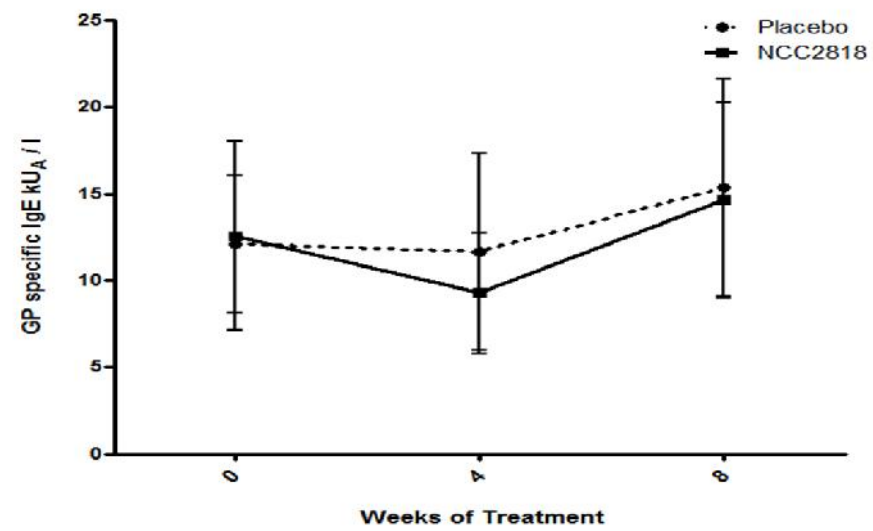


Supplementary Figure 3

A



B



Supplementary Figures

Supplementary Figure 1. Flow cytometry analysis of CD63 expression on activated basophil cells (identified as CD3^{neg} CRTH2^{pos} CD203c^{bright}) following *ex vivo* stimulation with anti-IgE and GP mix. Representative donors from placebo and probiotic NCC2818 treated groups at V1 and V2 visits in the clinical trial are shown in Supplementary Figure 1A (subject in placebo group) and 1B (subject in probiotic group). CD63 expression on activated basophils in the probiotic *B. lactis* NCC2818 treated group was decreased for both anti-IgE and GP stimulation at V2 in the donor in the probiotic group, compared to the placebo group (only highest GP mix stimulation shown).

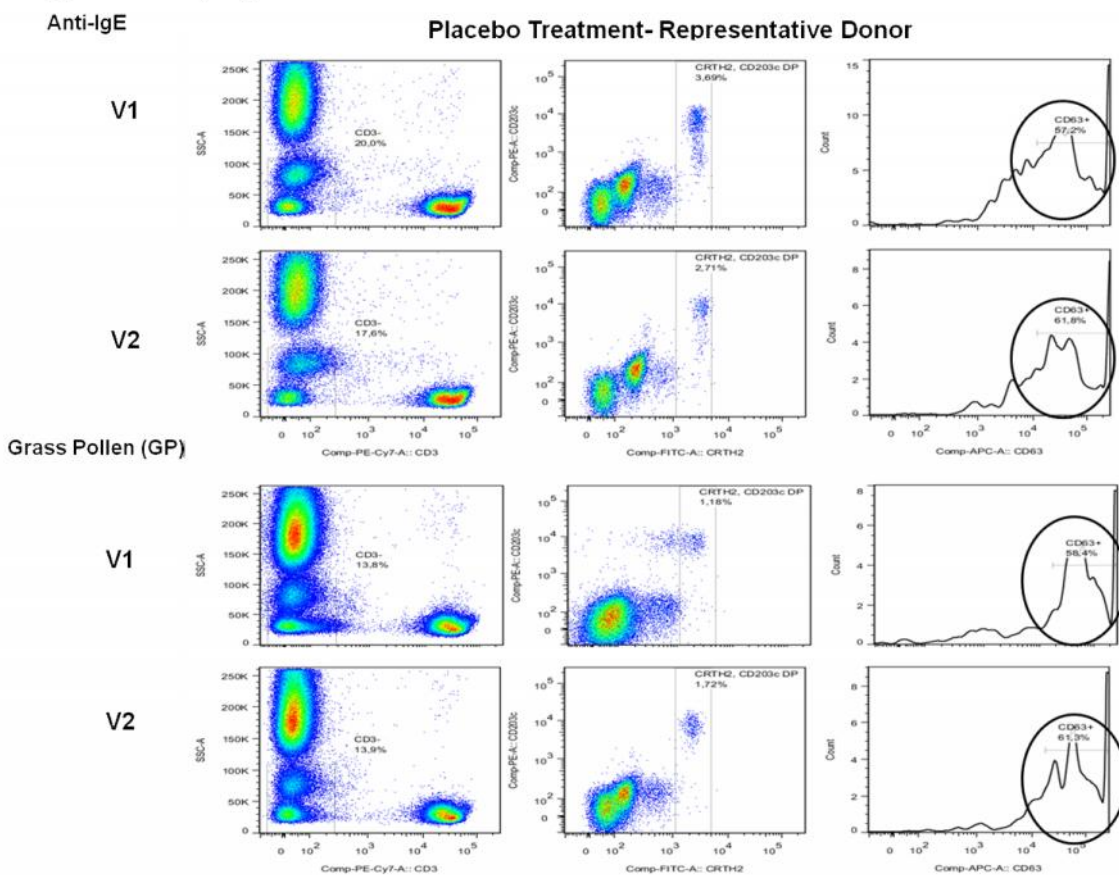
Supplementary Figure 2. Oral administration of the probiotic *B. lactis* NCC2818 decreases individual symptom scores

The changes in individual symptom scores from baseline to the end of the study are shown for the two treatment groups: placebo (circles, dotted lines) and probiotic (squares, solid line). The symptoms ~~mainly~~namely, nasal congestion (A), runny nose (B), nasal itching (C) and sneezing (D) are evaluated using a scale from 0-3 (0: no symptoms; 1: mild symptoms that are clearly present but easily tolerated; 2: moderate symptoms that are bothersome but tolerable, 3: severe symptoms that are hard to tolerate). Individual symptom scores were lower in the probiotic group compared with the placebo and this effect was visible after four weeks of treatment.

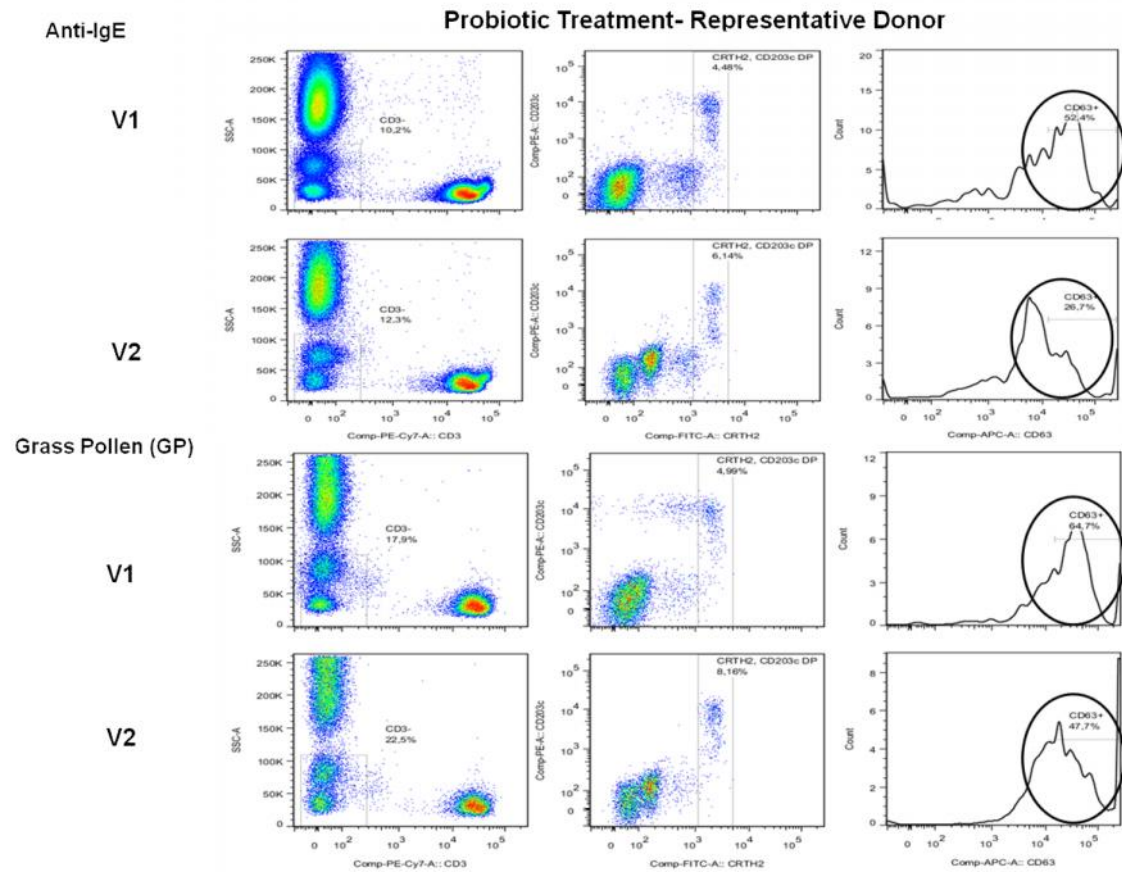
Supplementary Figure 3. Effect of treatment on total and GP specific IgE levels

Total and GP specific IgE levels are shown at the three visits (baseline V1, at four weeks V2 and at eight weeks V3) for the two treatment groups: placebo (circles, dotted lines) and probiotic (squares, solid line). No difference in total IgE levels (kU/l) were observed between the two groups (Figure A). GP specific IgE levels were detected in all subjects and were similar between the two groups at baseline. These levels were lower in probiotic NCC2818 treated groups at V2 (4 weeks) compared to placebo, but similar at V3 (8 weeks) between the treatment groups (Figure B). Analysis for total IgE and specific IgE to GP was performed using the ImmunoCAP 100 system (Phadia AB, Uppsala, Sweden)

Supplementary Figure 1A



Supplementary Figure 1B



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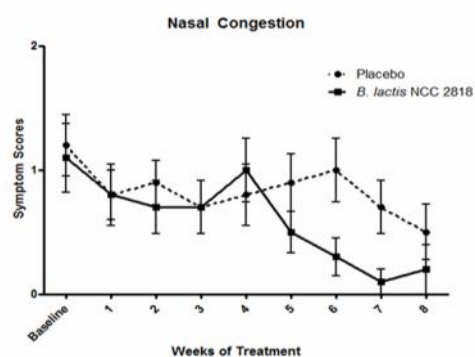
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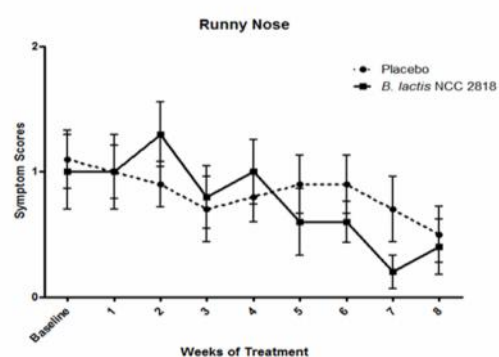
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Supplementary Figure 2

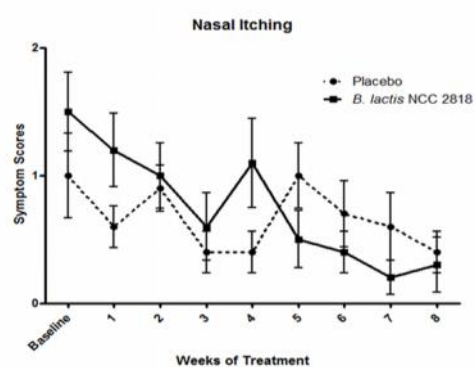
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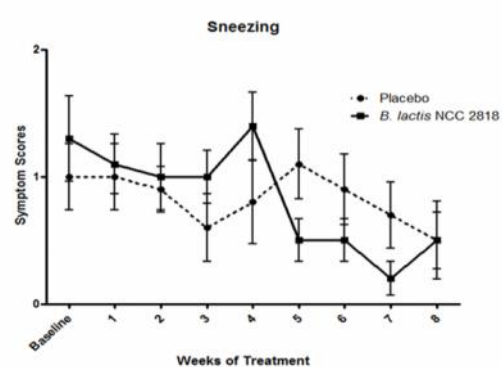
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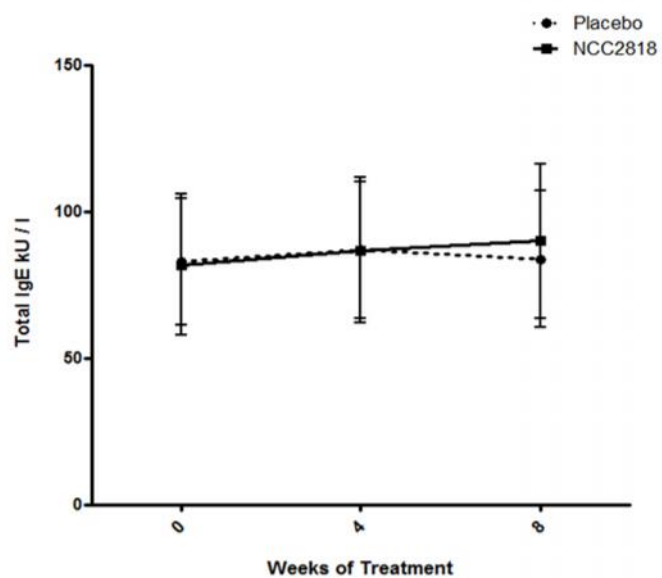
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Supplementary Figure 3

A**B**