

Nasal administration of a probiotic assemblage in allergic rhinitis: A randomised placebo-controlled crossover trial

Anders Mårtensson^{1,2,*} | Franziska U. Nordström^{2,3,*}  | Charlotte Cervin-Hoberg^{2,3} | Malin Lindstedt⁴ | Christina Sakellariou⁴  | Anders Cervin⁵ | Lennart Greiff^{2,3}

¹Department of ORL, Head and Neck Surgery, Helsingborg Hospital, Helsingborg, Sweden

²Department of Clinical Sciences, Lund University, Lund, Sweden

³Department of ORL, Head and Neck Surgery, Skåne University Hospital, Lund, Sweden

⁴Department of Immunotechnology, Lund University, Lund, Sweden

⁵Department of ORL, Royal Brisbane & Women's Hospital, University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia

Correspondence

Franziska U. Nordström, Department of ORL, Head and Neck Surgery, Skåne University Hospital, SE-221 85, Lund, Sweden.

Email: franziska_ulrike.nordstrom@med.lu.se

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Abstract

Background: Topical probiotics have been suggested as a treatment option for allergic rhinitis, as they may skew the immune response towards a beneficial type-1 non-allergic profile. So far observations in man have exclusively involved oral intake. The aim of this study was to examine whether a topical/nasal administration of a probiotic assemblage (PA) affects quality of life, symptoms and signs of allergic rhinitis in a nasal allergen challenge (NAC) model.

Methods: In a placebo-controlled and crossover design, 24 patients with seasonal allergic rhinitis were randomised to topical/nasal administration with a PA of *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A or placebo for 3 weeks. Participants and investigators were blind to treatment allocation. The last week of each treatment period was combined with a NAC series. Efficacy variables were "Mini-Rhinoconjunctivitis Quality of Life Questionnaire" (Mini-RQLQ), "Total Nasal Symptom Score" (TNSS), "Peak Nasal Inspiratory Flow" (PNIF) and "Fractional Exhaled Nitric Oxide" (FeNO). In addition, to assess whether or not the PA produced any pro-inflammatory effect *per se*, soluble analytes were monitored in nasal lavage fluids. Finally, bacterial cultures, sampled using swabs from the middle nasal meatus, were assessed for the presence of the PA by MALDI-TOF analysis.

Results: Administration of the PA did not produce any nasal symptoms (cf. placebo). An innate immune response was discerned within the PA run (cf. baseline), but no change in nasal lavage fluid levels of cytokines/mediators was observed cf. placebo except for IL-17/IL-17A (a minor increase in the PA run). Administration of the PA did neither affect Mini-RQLQ, TNSS, PNIF nor FeNO. No evidence of persistent colonization was observed.

Conclusions: Topical/nasal administration of a PA comprising *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A, while likely evoking a minor innate immune response yet being safe, does not affect quality of life, symptoms or signs of allergic rhinitis. Trial registration: not registered.

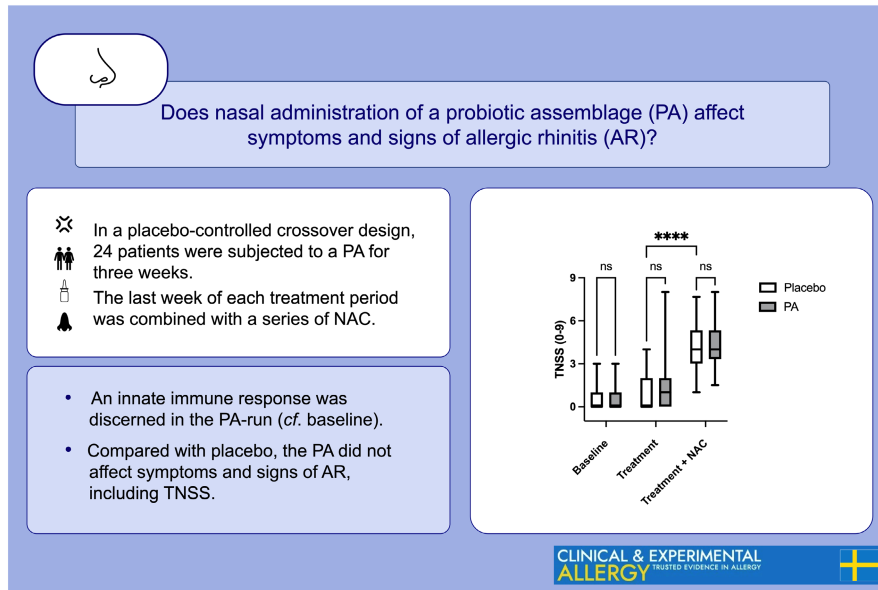
Anders Mårtensson and Franziska U. Nordström have contributed equally to this work.

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KEYWORDS

Allergy, cytokines, *Lactobacillus*, *Lactococcus*, mini-RQLQ, nasal allergen challenge, topical/nasal probiotics



GRAPHICAL ABSTRACT

Probiotics have been suggested as a treatment option for allergic rhinitis (AR). So far observations in humans have exclusively involved oral intake. In a placebo-controlled crossover design, biological effects and clinical efficacy of a topical/nasal probiotic assemblage (PA) were examined in a nasal allergen challenge model in patients with seasonal AR. Topical/nasal administration of the PA was safe. Compared with placebo, the PA did not affect quality of life, symptoms and signs of AR.

1 | INTRODUCTION

The “hygiene hypothesis,” introduced by Strachan et al. in 1989,¹ states that less microbes in the homes of developed countries (i.e. clean domestic milieus) are associated with a rise of allergic/inflammatory disorders. Subsequently, it was expanded into a “biodiversity hypothesis” by von Hertzen et al.² A global decline in biodiversity (comprising pathogens, commensals and other organisms) contributes to the cause of these conditions. In parallel, experimental observations provided insights into T cell populations and their functions, indicating key immunological mechanisms for the interaction between inflammatory processes produced by allergen and infectious agents. For example, that Th1 cell actions, including release IFN γ , might down-regulate Th2 cell, allergy-associated features.³

A possibility that has emerged from the above-mentioned context is that measures mimicking infections may reduce symptoms of allergic disorders. In agreement, in allergic rhinitis (AR), we have demonstrated that topical/nasal treatment with a Toll-like receptor-7 (TLR-7) agonist, a receptor for single-stranded viral RNA, produces a type-1 response (local production of IL-6, TNF α , IFN γ and IP-10) and a parallel reduction in responsiveness to allergen (i.e. reduced symptoms).⁴ A similar mechanism has been suggested for probiotics acting on “pattern recognition receptors” (e.g. TLRs).⁵⁻⁷

Key Messages

- Effects of a probiotic assemblage (PA) were examined in a nasal allergen challenge model.
- Nasal administration of the PA was well tolerated.
- In allergic rhinitis, the PA did not affect symptoms and signs of the disease.

However, even if various probiotics and probiotic assemblages (PA) may produce different effects, the single study in man so far on topical/nasal administration of lactic acid bacteria did not produce any discernable type-1 response: that is, unaffected lavage fluid levels of IL-6, IL-8, MIG, IL-15, EGF, IP-10 and IL-1RA.⁸

A further mechanism suggested to be involved in disease-modifying effects of probiotics is a stabilizing effect of the epithelial lining, proposed to result in reduced release of pro-inflammatory factors.⁹⁻¹¹ However, supporting data are restricted to animal observations and to bronchial airway/lung assessment after nasal administration of probiotics.^{12,13} In a mouse model of AR, *L. rhamnosus* was suggested to attenuate allergen-induced production of type-2 cytokines (IL-5 and IL-13) and reduce allergen responsiveness.¹² Similarly, Hisbergues et al.¹⁴ reported that *L. plantarum* prevented Th2 immune

responses in mice sensitized to house dust mite. Previous observations in man exclusively involve oral intake of probiotics, and the results, although showing some degree of efficacy, do not convincingly demonstrate a clinically relevant effect.¹⁵⁻¹⁷

To the best of our knowledge, although suggested as a possibility,^{6,12} no studies have focussed on administration of topical/nasal probiotics in patients with AR. In this study involving individuals with seasonal AR, we examined effects of a topical/nasal PA comprising *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A in a nasal allergen challenge (NAC) model.^{18,19} Accordingly, out of the pollen season, the PA was administered by a nasal spray device for 3 weeks in a placebo-controlled and crossover design. Two weeks into each treatment period, once daily NAC commenced and continued for 7 days. A range of parameters were monitored including the "Mini-Rhinoconjunctivitis Quality of Life Questionnaire" (Mini-RQLQ) with its nasal subdomain, "Total Nasal Symptom Scores" (TNSS), "Peak Nasal Inspiratory Flow" (PNIF) and "Fractional Exhaled Nitric Oxide" (FeNO). In addition, cytokines/mediators in nasal lavage fluids were monitored to assess any pro-inflammatory effect of the PA. Finally, aspects of colonisation of the PA were examined.

2 | MATERIAL AND METHODS

2.1 | Study design

The study was of a randomized, double-blinded, placebo-controlled and crossover design and involved administration of a topical/nasal PA to patients with seasonal AR in a NAC model. It was conducted in Sweden outside the pollen season of 2019 and covered 14 weeks (Table 1). Rescue medications were allowed on request (desloratadine, 5 mg, once daily). The study was approved

by the Swedish Ethical Review Authority (2019-04204), conducted according to good clinical practice, and after written informed consent. Outcome measures are described below. Adverse events and side effects were monitored in case record forms.

2.2 | Patients

Twenty-five patients with strictly seasonal AR to birch or grass (timothy) pollen allergen were recruited. One individual withdrew his/her consent before starting administration of the study drug/placebo (Table 2). The diagnosis was verified by a skin prick test (SPT; Soluprick, ALK), which also included house dust mite, cat and dog allergen.

Inclusion criteria: Positive SPT for birch or grass pollen allergen (weal diameter ≥ 3 mm), no history of AR outside the pollen season and age ≥ 18 years. **Exclusion criteria:** Respiratory tract infection within 2 weeks, chronic rhinosinusitis, treatment with antibiotics (within 4 weeks), structural abnormalities of the nasal airway, asthma, immune deficiency, sensitization to house dust mite and previous immune therapy. **Medication washout:** No medication, except for occasional paracetamol, was allowed for 4 weeks prior to the study.

2.3 | Visits

The study comprised an inclusion visit (Visit 1), visits at the start of each treatment period (Visits 2 for the 1st period and Visits 10 for the 2nd), visits during the NAC series (Visits 3-9 for the 1st period and Visits 11-17 for the 2nd), and a follow-up visit (Visit 18; Table 1). A washout period of 4 weeks was instituted between the 1st and the 2nd run. Patients randomized to the PA in the 1st run were subjected

TABLE 1 Study scheme of treatment periods and nasal allergen challenge (NAC) series in relation to monitoring/sampling in a crossover design

	Inclusion	Treatment week 1							Treatment week 2							Treatment week 3							4 week	Treatment week 1							Treatment week 2							Treatment week 3							2 week														
Visit	1	2																					3	4	5	6	7	8	9	10	11																					12	13	14	15	16	17	18	
Treatment		[Grey]																					[Green]								[Grey]																					[Green]							
Placebo		[Grey]																					[Green]								[Grey]																					[Green]							
NAC		[Grey]																					[Green]								[Grey]																					[Green]							
TNSS		[Grey]																					[Green]								[Grey]																					[Green]							
PNIF		[Grey]																					[Green]								[Grey]																					[Green]							
Mini-RQLQ		[Grey]																					[Green]								[Grey]																					[Green]							
FeNO		[Grey]																					[Green]								[Grey]																					[Green]							
Nasal lavage		[Grey]																					[Green]								[Grey]																					[Green]							
Blood		[Grey]																					[Green]								[Grey]																					[Green]							
Examination	[Grey]	[Grey]																					[Green]								[Grey]																					[Green]							[Grey]
ESwab		[Grey]																					[Green]								[Grey]																					[Green]							
Titration		[Grey]																					[Green]								[Grey]																					[Green]							
SPT		[Grey]																					[Green]								[Grey]																					[Green]							

Abbreviations: FeNO, fractional exhaled nitric oxide; Mini-RQLQ, rhinoconjunctivitis quality of life questionnaire; NAC, nasal allergen challenge; PNIF, peak nasal inspiratory flow; SPT, skin prick test; TNSS, total nasal symptom score.

TABLE 2 Clinical characteristics of the patients

Category	Data (n = 24)
Sex male, n (%)	9 (37.5)
Age median (min-max)	27 (19-49)
Total IgE, kU/L median (min-max)	89.9 (16.5-562)
Birch-specific IgE, kU/L median (min-max)	0.6 (0-100)
Timothy-specific IgE, kU/L median (min-max)	9.5 (0-86.4)
Sensitized to birch, n (%)	12 (50)
Sensitized to timothy, n (%)	23 (95.8)
Nasal allergen challenge, birch/timothy n (%)	6/18 (25/75)

to placebo in the 2nd and *vice versa*. Timing of recordings of outcome measures in relation to NAC is indicated below.

At Visit 1, inclusion and exclusion criteria were considered and written informed consent was obtained. An ENT examination was performed, including a rhino-endoscopy, and ESwab samples (Copan) were collected from the middle meatus bilaterally for bacterial culturing and analysis. Finally, an allergen titration procedure was performed in order to establish the daily dose to be used in the NAC model (see below).

Visits 2 and 10 included a baseline collection: Mini-RQLQ, TNSS, PNIF and FeNO. Moreover, at Visit 2, the patients received instructions regarding storage of study drug (refrigerator), treatment and recording of symptoms. Furthermore, at Visits 2/10 and 9/17, material was obtained for laboratory tests: that is, blood sampling for total and specific IgE and nasal lavage fluids for analysis of cytokines/mediators.

Visits 3 and 11, after 2 weeks treatment and prior to the NAC series, included the same measurements as at Visits 2 and 10. Visits 3-9 in the 1st run and 11-17 in the 2nd run included daily NACs with recording of TNSS and PNIF 10 min after each allergen challenge. Finally, at Visit 18, ENT examinations with rhino-endoscopies and ESwab samplings were performed.

2.4 | Probiotics, randomization and blinding

PA and placebo were provided by Essum Pharma. The PA was *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A in a ratio of 1:1:1. The placebo was a composition of rice starch, maltodextrin and sucrose. Randomization numbers were allocated to the patients according to a list generated by Essum Pharma. Patients, physicians and medical staff were blinded to the treatment order.

Two-hundred microlitre per nostril was administered twice daily for 3 weeks using a nasal spray device (Pump 100 APF SNAP 20, Aptar pharma). For the PA, this translated to 1.9×10^{10} CFU/per dose. Compliance was assessed by comparing the weight of the nasal spray bottles at baseline (Visits 2 and 10) with the weight after 2 and 3 weeks of treatment (Visits 3 and 11 and Visits 9 and 17), respectively. In addition, viability of the PA was randomly assessed by Essum Pharma after the return of the spray bottles.

2.5 | Allergen titration and nasal allergen challenge

In order to establish repeatable, symptom-producing yet individually tolerable allergen challenge doses, allowing for daily challenges for 7 days, a titration procedure was performed.¹⁸ Diluent followed by increasing doses of allergens was administered at 10-min intervals using a spray device delivering 100 µl per spray, resulting in effective doses of 100, 300, 1000 and 3000 SQ (standardized quantity) units per nasal cavity (ALK). This scheme was followed until the subject responded with five sneezes or a symptom score of 2 or more on a scale of 0-3 for either nasal secretion or blockage.²⁰ The dose that produced this effect was used in the NAC series, that is, once daily for seven consecutive days in each treatment run (Table 1).

2.6 | Outcome measures

Mini-RQLQ comprises 14 items divided into 5 domains (activity limitations, practical problems, nose symptoms, eye symptoms and other symptoms). All questions were scored on a seven-point scale (ranging from 0 = no impairment to 6 = severe impairment), with lower scores indicating a better QoL.²¹ Mini-RQLQ was recorded at baseline (Visit 2 and 10), after 2 weeks' treatment (Visit 3 and 11) and at Visit 9/17 prior the NAC.

Sneezes, blockage, rhinorrhoea and either itchy nose or sneezes (the most predominant) were scored by the subject at baseline (Visit 2 and 10) and after 2 weeks treatment (before the first NAC; Visit 3 and 11) on a 4-point scale: 0: no symptoms, 1: mild, 2: moderate and 3: severe symptoms, added to a TNSS.²² Similarly, nasal symptoms were scored 10 min after each allergen challenge (Visits 3-9 in the 1st run, Visits 11-17 in the 2nd run).

After scoring of TNSS, at the visits described above, PNIF was assessed as a measure of nasal blockage. A nasal inspiratory flow meter equipped with a facial mask was used (Clement-Clarke). The highest of three measurements was registered.

Airway allergy/inflammation is associated with high levels of nitric oxide (NO), which can be measured as FeNO in exhaled air.^{23,24} In this study, the Niox Vero system was used (Circassia). Airway allergy/inflammation is also associated with *blood eosinophilia* and the occurrence of *allergen-specific* and *total IgE*: Venous blood samples and clinical protocols were used. FeNO, blood eosinophilia and IgE were assessed at the same time-points as Mini-RQLQ.

Nasal lavages were performed for analysis of cytokines/mediators in order to assess any pro-inflammatory effect of the PA. Fifteen millilitre of a sodium chloride solution (0.9%) was instilled into each nasal cavity using the Nasaline system (Squip). After 2 min, the fluids were collected and centrifugated (4°C, 10 min). Supernatants were stored at -20°C until analysis. Nasal lavages on Visit 9/17 were performed 24 h after the second last NAC and prior to the last NAC.

Markers were analysed by Luminex profiling using a human pre-mixed discovery assay (Bio-Techne) on the Bio-PlexR 200 system (Bio-Rad Lab). The analytes comprised: TNFα, IFNγ, IP-10, MIP-1α, MIP-1β, MCP-1, IL-6, IL-8, IL-10, IL-17/IL-17A, IL-33, ST2, IL-4, IL-5,

IL-12p70, IL-13 and eotaxin. To maintain high quality, coefficient of variance (CV) filtration was performed and values exceeding a 20% CV cut-off were omitted. Extrapolated values were used in case readouts were out of the detection range of the assay.

Microbiology: At Visits 1 and 18, ESswabs were inserted bilaterally in the middle nasal meatus for sampling of liquids followed by bacterial culturing. This was followed by MALDI-TOF analysis (matrix-assisted laser desorption/ionization time of flight) for detection of the probiotic strains used in this study, in order to explore whether or not the patients were colonized after the study treatment.

2.7 | Statistics

Quantitative data were described as box plots with medians, interquartile ranges (IQR) and range and categorical data (qualitative variables) as frequencies. Paired data were compared using the Wilcoxon signed-rank test. p -values $<.05$ were considered statistically significant. The focus was on comparisons between the PA and placebo, but also within each treatment group in order to explore effects of the PA. Calculations were done with Prism version 9 (GraphPad).

3 | RESULTS

One individual opted not to participate after Visit 1. Accordingly, 24 patients were randomized. Their characteristics are presented in Table 2. In the 1st run, 13 patients received the PA and 11 placebo, and *vice versa* in the 2nd run. During the course of the study, no response to the placebo was observed. In the NAC series, six patients received birch pollen allergen (1 at the 100 SQ-U dose level, 1 at 300 SQ-U and 3000 SQ-U, respectively, and 3 at 1000 SQ-U). Eighteen patients received timothy pollen allergen (3 at the 100 SQ-U dose level, 7 at 300 SQ-U and 1000SQ-U, respectively, and 1 at 3000 SQ-U). Two patients in the placebo group received a single-dose desloratadine (5 mg) each during the NAC series. One patient in the PA group received one dose desloratadine each day throughout the NAC series, and an additional patient in the PA group received

a single-dose desloratadine during the NAC series. One patient was excluded because of non-compliance.

There were no differences in total *Mini-RQLQ* scores between the PA and placebo at baseline ($p = .26$, Visit 2/10), after 2 weeks treatment ($p = .83$, Visit 3/11) or at the end of the NAC series (while the treatment continued; $p = .84$, Visit 9/17; Figure 1). Minor yet statistically significant increases in total *Mini-RQLQ* scores occurred between observations at baseline (Visit 2/10) and after 2 weeks treatment (Visit 3/11) in the PA run ($p = .04$) as well as the placebo run ($p = .04$; Figure 1). Compared with recordings prior to each NAC series (Visits 3/11), total *Mini-RQLQ* scores increased at the end of the NAC series (Visit 9/17), but this trend failed to reach statistical significance in the PA run ($p = .27$) as well as the placebo run ($p = .11$; Figure 1).

There were no differences in *Mini-RQLQ nasal domain* scores between the PA and placebo at baseline ($p = .21$, Visit 2/10), after 2 weeks treatment ($p = .23$, Visit 3/11) or at the end of the NAC series (while the treatment continued; $p = .94$, Visit 9/17; Figure 1). No statistically significant changes in scores occurred between observations at baseline (Visit 2/10) and after 2 weeks treatment (Visit 3/11) in the PA run ($p = .09$) or the placebo run ($p = .08$; Figure 1). Compared with recordings prior to each NAC series (Visits 3/11), *Mini-RQLQ nasal domain* scores increased at the end of the NAC series (Visit 9/17), and this trend reached statistical significance in the placebo run ($p = .04$), but not in the PA run ($p = .14$; Figure 1).

There were no differences in *TNSS* between the PA and placebo at baseline ($p > .99$, Visit 2/10), after 2 weeks treatment ($p = .46$, Visit 3/11) or at the end of the NAC series (while the treatment continued; $p = .98$, Visit 7–9/15–17; Figure 2). A minor increase in *TNSS* occurred between observations at baseline (Visit 2/10) and after 2 weeks treatment (Visit 3/11). This reached statistical significance in the PA run ($p = .03$), but not in the placebo run ($p = .22$; Figure 2). Compared with recordings prior to each NAC series (Visits 3/11), *TNSS (10-min post-challenge)* increased at the end of the NAC series (Visit 7–9/15–17), and this reached statistical significance in the PA run ($p < .0001$) as well as the placebo run ($p < .0001$; Figure 2).

There were no differences in *PNIF* between the PA and placebo at baseline ($p = .96$, Visit 2/10), after 2 weeks treatment ($p = .13$,

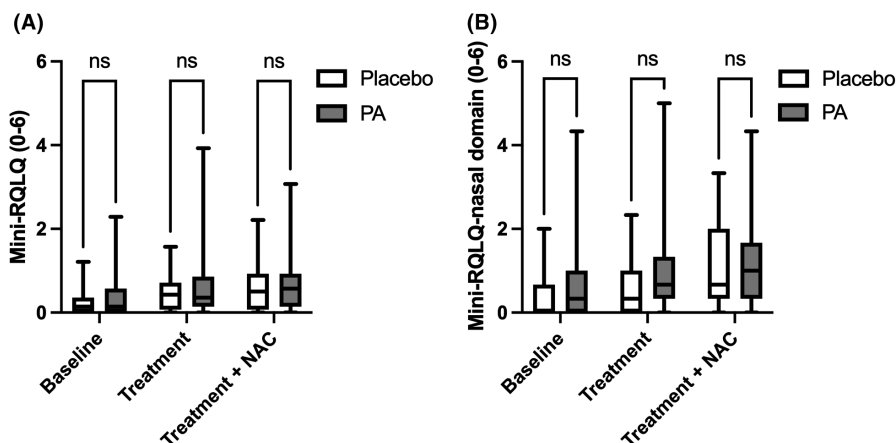
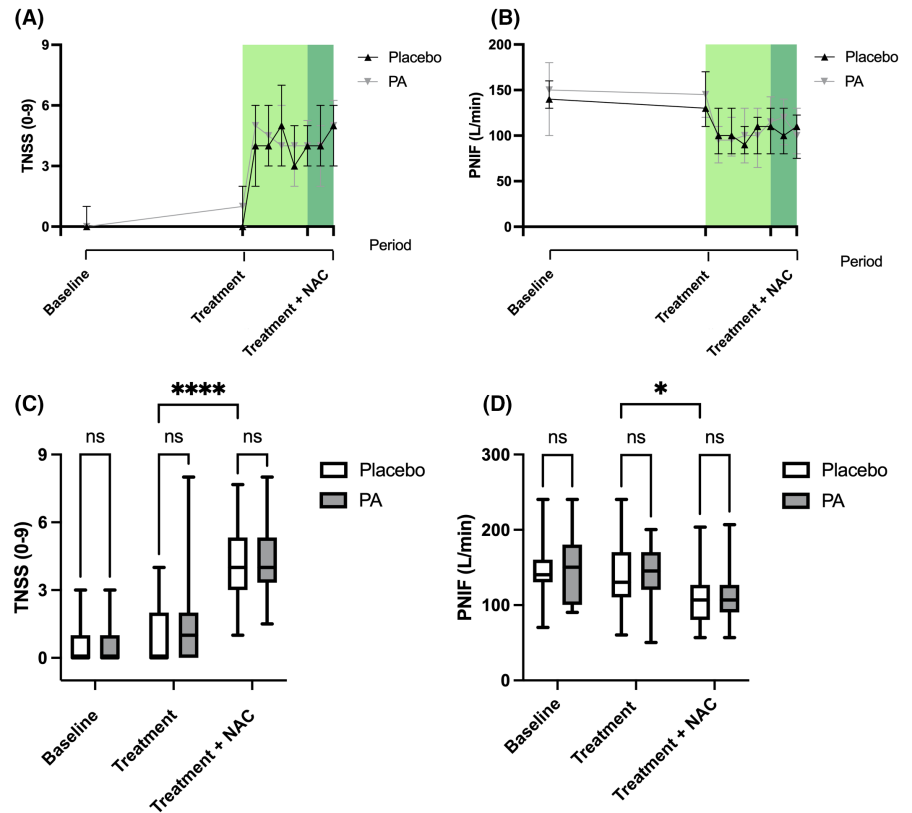


FIGURE 1 Total *Mini-RQLQ* scores (A) and *Mini-RQLQ nasal domain* scores (B) at baseline (Visit 2/10), after 2 weeks treatment (Visit 3/11) and after 1 week NAC series with continued treatment (Visit 9/17). There were no differences in either of the scores at any time-point between the PA run and the placebo run. Data are presented as box plots: medians with IQRs and ranges

FIGURE 2 Total Nasal Symptom Score and PNIF at baseline (Visit 2/10), after 2 weeks treatment (Visit 3/11 prior to NAC) and after 1 week NAC series (Visits 3–9/11–17, marked green) with continued treatment (A, B). Mean scores of the last 3 days of the NAC series were used in the analysis (marked dark green; C, D). The PA produced mild symptoms, but did not produce any change in PNIF rates. During the NAC series, TNSS scores increased and PNIF rates decreased. There were no differences in either of the scores at any time-point between the PA run and the placebo run. Data are presented as medians with IQRs (A, B) or as box plots: medians with IQRs and ranges (C, D). * indicates $p < .05$; **** indicates $p < .0001$



Visit 3/11) or at the end of the NAC series (while the treatment continued; $p = .86$, Visit 7–9/15–17; Figure 2). No statistically significant changes in scores occurred between observations at baseline (Visit 2/10) and after 2 weeks treatment (Visit 3/11) in the PA run ($p = .14$) or the placebo run ($p = .40$; Figure 2). Compared with recordings prior to each NAC series (Visits 3/11), PNIF decreased at the end of the NAC series (Visit 7–9/15–17), and this difference reached statistical significance in the PA run ($p = .003$) as well as the placebo run ($p = .01$; Figure 2).

Data for FeNO, specific IgE and total IgE were obtained at Visits 2/10, 3/11 and 9/17 (Table 1). For all parameters, no statistically significant differences were observed between the PA and placebo at baseline (Visit 2/10), after 2 weeks treatment (Visit 3/11) or at the end of the NAC series (while the treatment continued; Visit 7–9/15–17; data not shown). No statistically significant changes occurred for either of the markers between observations at baseline (Visit 2/10) and after 2 weeks treatment (Visit 3/11) in the PA run or the placebo run. Compared with recordings prior to each NAC series (Visits 3/11), no changes were observed for either of the markers at the end of the NAC series (Visit 7–9/15–17).

There were no differences in either of the nasal lavage fluid cytokines/mediators between the PA and placebo at baseline (Visit 2/10) or after 2 weeks treatment (Visit 3/11), except for IL-17/IL-17A (a minor increase in the PA run; Figure 3). Statistically significant increase in TNF α , MIP-1 α , MIP-1 β and MCP-1, IL-6, IL-8, IL-10 and ST2 was observed after 2 weeks topical treatment with the PA (Visit 3/11) cf. baseline (Visit 2/10; Figure 3). Statistically significant increase in MCP-1 and IL-6 was observed after 2 weeks topical

treatment with placebo (Visit 3/11) cf. baseline (Visit 2/10; Figure 3). At the end of the NAC series, nasal lavages obtained 24 h after the last allergen challenge did not reveal any specific cytokine profiles for either the PA run or the placebo run. Thus, the data were not investigated further.

Compliance with regard to topical/nasal probiotic administration was good (data not shown), and viability of the bacteria of the PA was $5 \times 10^9 - 1 \times 10^{10}$ CFU/ml at return of the nasal spray devices. At the end of the study, after a treatment-free interval of 2 weeks, no patient showed the presence of the bacteria of the PA (data not shown). No severe adverse event related to the PA was reported during the study. The spray was generally well tolerated, but two patients recorded a burning sensation, one itching and one pain in association at administration of the PA.

4 | DISCUSSION

In this study, involving patients with seasonal AR examined in a NAC model, we demonstrate that topical/nasal administration of a PA (i.e. *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A) is without effect on symptoms and signs of the condition. The observation, which is the first of its kind, suggests that topical/nasal probiotics, at least the PA evaluated in this study, is not a viable option as treatment for AR.

The PA in this study was chosen based on demonstrated effects in vitro and on tolerance and safety in man.^{8,13,25,26} It was administered using a nasal spray device as 200 μ l of a 9.5×10^{10} CFU/

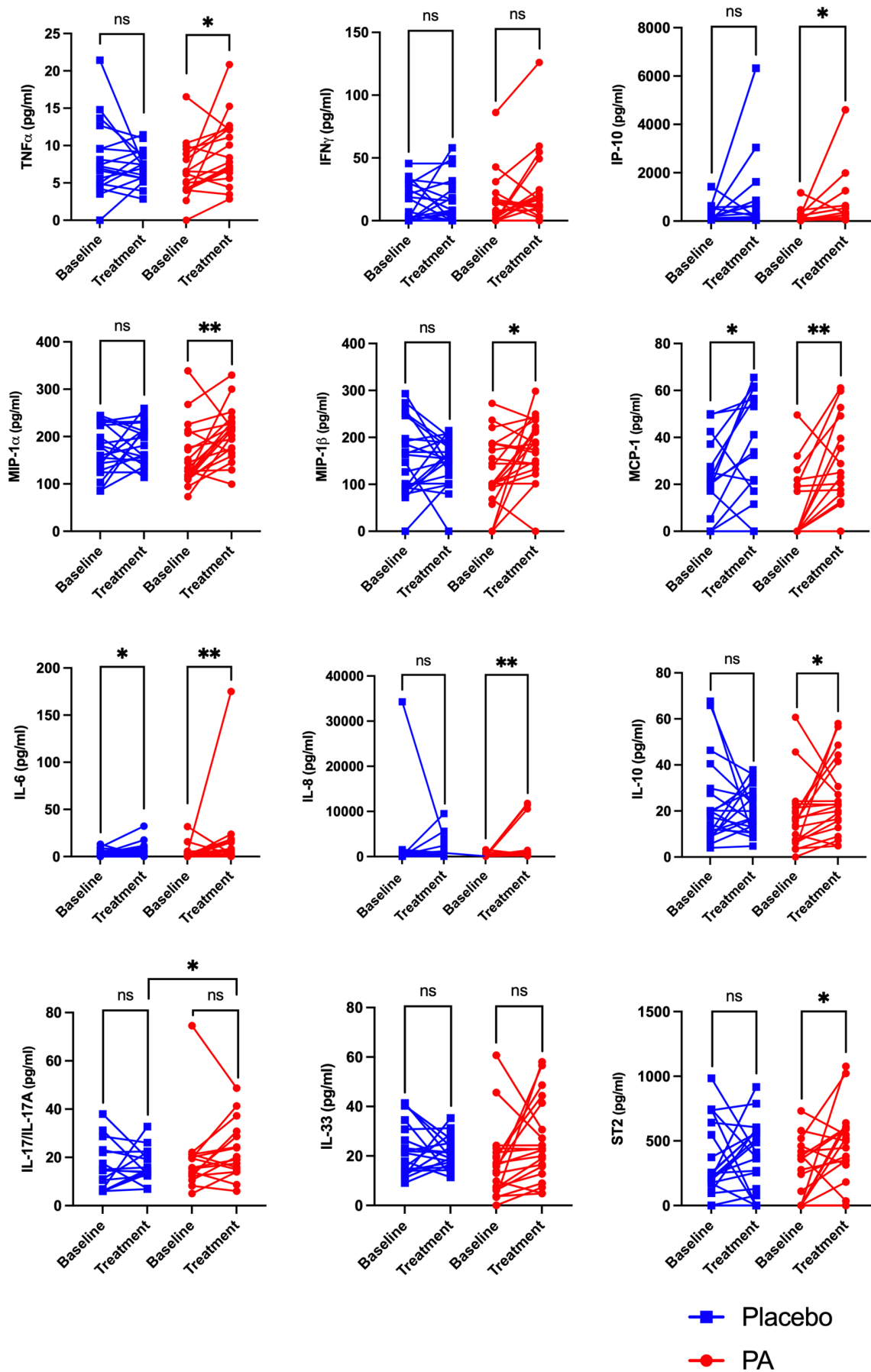


FIGURE 3 Nasal lavage fluid levels of cytokines/mediators at baseline (*Visit 2/10*) and after 2 weeks treatment (*Visit 3/11*) prior to the NAC series. There were no differences in either of the analytes at any time-point between the PA run and the placebo run, except for IL-17/IL-17A (a minor increase in the PA run) at *Visit 3/11*. Statistically significant increases in TNF α , MIP-1 α , MIP-1 β and MCP-1, IL-6, IL-8, IL-10 and ST2 were observed after 2 weeks topical treatment with the PA (*Visit 3/11*) cf. baseline (*Visit 2/10*). Statistically significant increases in MCP-1 and IL-6 were observed after 2 weeks topical treatment with placebo (*Visit 3/11*) cf. baseline (*Visit 2/10*). * indicates $p < .05$; ** indicates $p < .01$

ml solution per nostril twice daily for 3 weeks. The dose was selected as “moderate,” for example, as compared to that of an earlier study focussing on chronic rhinosinusitis (with another PA): that is, 200 μ l of a 1×10^{11} CFU/ml solution per nostril twice daily for 2 weeks.²⁷ In agreement with previous observations,^{8,27} the PA was well tolerated. For example, no discernible change in Mini-RQLQ scores was observed and no nasal symptoms were produced (cf. placebo). Taken together, our observations confirm the notion that probiotics administered to the nasal cavity does not cause any adverse events. Furthermore, as evidenced by negative cultures 2 weeks after the final treatment run, no colonization with the PA occurred.

The effect of the topical/nasal PA was assessed in a NAC model involving 7 days repeated allergen exposure. Benefits of the model are that interindividual differences in allergen sensitivity are levelled and that fluctuations in allergen exposure, which characterize natural allergen exposure and make studies of crossover design difficult, are avoided. Accordingly, in the model, a treatment can be evaluated against placebo and against other treatments.^{18,19} It is well established that AR impairs QoL.²⁸ However, in this study no such effects could be discerned through the brief 7 days NAC series either for the total Mini-RQLQ or its nasal subdomain. Similarly, no change in FeNO was observed. In contrast, symptoms of AR were produced as expected as well as corresponding reductions in PNIF (reflecting nasal blockage). The PA failed to affect nasal symptoms and PNIF compared with placebo, indicating that it (i.e. *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A) was without effect on AR.

In this study, we hypothesized that the PA might skew the immune system into a type-1 response, which could affect symptoms of AR. Compared with placebo, no marked such effect was observed. However, it was evident that many cytokines/mediators were increased after 2 weeks in the PA run compared with baseline, whereas this was rarely seen in the placebo run. Taken together, we therefore might conclude that a mild innate immune response was indeed evoked, differing from a clearer type-1 response by an absence of IFN γ production. The response was thus similar, but not identical, to our previous observation of a type 1-response-producing effect of topical/nasal administration of a TLR-7 agonist, which attenuated symptoms of AR at seasonal allergen exposure.⁴ In contrast to that observation, the PA did neither affect symptoms of AR nor PNIF. Whether or not other doses of the present PA, or other PAs, may produce a clearer type-1 response and affect symptoms of AR remains to be elucidated.

Our findings disagree with the notion emerging from studies in mouse models of allergic airway inflammation involving topical *L. rhamnosus* GG/GR-1, *L. paracasei* NCC2461 and *L. plantarum*

NCC1107/NCIMB8826¹²⁻¹⁴ resulting in reduced allergen-induced type-2 cytokine production and eosinophil recruitment and, consequentially, attenuation of airway symptoms. Indeed, our observations suggest that such findings may not immediately be transferable to humans. Furthermore, the experimental observations focused on inflammation of the bronchial airways/lung and not the upper/nasal airway. In the present study, aspects of inflammation, assessed by levels of cytokines/mediators in nasal lavages, were not intelligible, likely because of the concomitant administration of allergen and the PA. Furthermore, the nasal lavages might not have been timed accurately in relation the allergen exposure to detect transient increases in type 2-cytokines.

A comparison between the present study and previous observations on effects of oral intake of probiotics in AR may warrant consideration. To the best of our knowledge, all previous studies in man focussing on effects of probiotics in AR involve oral administrations.²⁹⁻³¹ Some of these report no effects on QoL,³²⁻³⁴ while a series of others (meta-analysed) suggests improvements.¹⁵ The mechanisms involved may comprise a systemic effect on the immune system and not any effect initiated locally, such as stabilizing effects on the nasal epithelium and reduced release of pro-inflammatory factors. However, in comparison with key pharmaceuticals for AR, that is, corticosteroids and antihistamines, the effect of oral probiotics appears to be minor and likely of limited clinical relevance. The PA used in this study has not previously been used orally in allergic rhinitis.

Study limitation considerations: The objective of this study was to examine effects of the PA on symptoms and signs of allergic rhinitis. Accordingly, no control group (healthy individuals) was included, and no comparison was made between healthy individuals and patients with allergic with regard to effects of the PA administration. The level of symptoms reached during the NAC series was in agreement with previous observations in the model.^{18,19} Notably, it was titrated to be on the lower side, because it had to be tolerable when the challenge was repeated throughout the NAC series. The probiotic assemblage was administered concomitant with the nasal allergen challenge; thus, the cytokine signalling obtained could not be attributed to either intervention.

In conclusion, the PA produced a mild innate immune response. Nevertheless, 3 weeks of topical/nasal treatment with the PA consisting of *Lactobacillus rhamnosus* SP1, *Lactobacillus paracasei* 101/37 and *Lactococcus lactis* L1A did not affect symptoms of AR compared with placebo.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

AUTHOR CONTRIBUTIONS

AM, FN, CC-H, AC and LG contributed to the conception and design of the study. AM, FN and CC-H coordinated patient selection and clinical sampling. CS and ML designed and performed the cytokine analysis. CC-H, AM and FN performed data input. AM, FN and LG analysed the data and wrote the manuscript. CC-H, CS, ML and AC contributed with revision and editing of the manuscript. All authors read and approved the manuscript.

ETHICAL APPROVAL

The study was approved by Swedish Ethical Review Authority and complies with the Declaration of Helsinki. All patients provided written consent.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Franziska U. Nordström  <https://orcid.org/0000-0003-3730-094X>

Christina Sakellariou  <https://orcid.org/0000-0002-2376-7808>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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