

Tolerability and Clinical Outcome of Coseasonal Treatment with *Escherichia coli* Strain Nissle 1917 in Grass Pollen-Allergic Subjects

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Key Words

Escherichia coli strain Nissle 1917 · Grass pollen allergy · Immunoglobulin A · Rhinoconjunctivitis · Symptom-medication score

Abstract

Background: Nonpathogenic *Escherichia coli* strain Nissle 1917 (EcN) has immunomodulatory properties and can act on different cells which are important for the allergic immune response. Herein, we investigated the efficacy and tolerability of EcN in subjects with grass pollen-dependent allergic rhinoconjunctivitis. **Methods:** Grass pollen-allergic subjects were randomly allocated to receive EcN in a double-blind, placebo-controlled manner. The treatment was performed from 2 months before onset until the end of one grass pollen season (in total: 6 months). The clinical symptom score and the intake of symptomatic medications were assessed. A skin prick test and grass pollen-specific immunoglobulin (Ig) E and IgA were evaluated before and after treatment. **Results:** Our results show that coseasonal treatment with EcN in grass pollen-allergic subjects was not superior to placebo as assessed using the symptom-medication score ($p = 0.257$). Interestingly, an increase [median (range)] in grass pollen-specific IgA was detectable in the EcN group [20,556 LU/ml (1,812–60,800)] versus placebo [5,246 LU/ml

(944–50,467)] ($p = 0.048$). **Conclusions:** The results indicate that 6 months of coseasonal nonspecific immunomodulation by EcN is not sufficient to achieve clinical efficacy in grass pollen-allergic subjects. Future approaches in which such immunomodulators are combined with an allergen-specific protocol might enhance the clinical efficacy of the allergen-specific treatment.

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Introduction

The increase in allergic diseases has been linked to changed hygiene practices which entail reduced childhood infections by different microbes [1]. It has been shown that microorganisms such as *Escherichia coli* strain Nissle 1917 (EcN) modulate the immune response towards TH₁ and regulatory T cells (Treg) and display a preventive or therapeutic potential in allergic diseases [2, 3].

EcN, serotype O6:K5:H1, is a Gram-negative bacterial strain [4]. It adheres to the intestinal mucus and antagonizes the growth of pathogenic bacteria [4, 5]. The immunomodulatory capacity of EcN is delivered by its cell surface antigen, a specific truncated O6 lipopolysaccharide [6]. An important feature of EcN-mediated immuno-

modulation is the regulation of pro- and anti-inflammatory cytokine production by direct interactions with immune cells [7]. It has been previously shown that EcN has immunomodulatory activity in vitro on T cell and B cell subsets by promoting a shift towards TH₁ cells, a reduction of the low immunoglobulin (Ig) E receptor (CD23) which facilitates IgE-mediated allergen presentation, and upregulation of the costimulatory molecule CD86 [8]. Previous in vivo experiments have demonstrated an immunomodulatory impact of orally given EcN, including the observation of upregulation of FoxP3+ cells and increased production of regulatory acting cytokines like TGF- β and IL-10 in the skin of EcN-treated mice [9]. Furthermore, it has been shown by other groups that EcN may increase the secretion of IgA [10] or can inhibit the degranulation of mast cells [11]. All of these findings point to the possibility that orally given EcN might be of therapeutic interest in allergic rhinoconjunctivitis.

Therefore, we hypothesized that EcN administration may be clinically effective in subjects suffering from grass pollen-allergic rhinoconjunctivitis and initiated a placebo-controlled clinical trial.

Material and Methods

Subjects

This clinical pilot study was set up in February 2009 and carried out as a randomized, double-blind, placebo-controlled clinical trial. The trial protocol was approved by the ethics committee of Berlin (Germany) and the responsible authority (BfArM, Bonn, Germany). It is registered in ClinicalTrials.gov (identifier: NCT01013259) and was conducted in accordance with the principles of the Declaration of Helsinki. All subjects were recruited in the Allergy-Center-Charité, Berlin, Germany. Written informed consent was given before inclusion. The main inclusion criteria were: age 18–65 years, a history of clinically relevant grass pollen-dependent rhinoconjunctivitis, a positive skin prick test (SPT, ≥ 3 mm), and detection of specific IgE (≥ 0.7 kU/l) to grass pollen extract. A relevant retrospective symptom score (rSS, >12 points) for the previous grass pollen season (2008) was required as well.

The main exclusion criteria were: a previous specific immunotherapy to grass pollen or an ongoing specific immunotherapy, use of EcN (12 weeks before), use of antibiotics or sulfonamides towards Gram-negative bacteria (4 weeks before), any immunosuppressive therapy, lactation, and pregnancy.

Subjects were randomized and treated in a double-blinded manner either with EcN or placebo (fig. 1a). Block randomization without stratification was done by an independent person. Thirty-four subjects were enrolled into this study and 30 participants completed the entire study course (EcN group, $n = 16$; control group, $n = 14$; fig. 1b). The subjects in the EcN and control groups were well matched and did not differ significantly regarding baseline characteristics (table 1).

Table 1. Baseline characteristics of the study subjects

Variable	Placebo (n = 17)	EcN (n = 17)
Female/male	8/9	12/5
Age, years	36 (19–49)	35 (20–54)
Height, cm	174 (156–190)	170 (162–183)
Weight, kg	75 (53–93)	66 (51–89)
Ethnic origin	Caucasian	Caucasian
Total IgE, kU/l	252 (28–1,417)	96 (16–383)
sIgE, kU/l	23 (8–100) ^a	14 (1–100) ^a
SPT, mm	8 (5–12)	7 (5–12)
rSS, points	17 (12–21)	17 (12–21)

Values are presented as medians (range) unless otherwise stated. No significant differences were found.

^a One subject with grass-sIgE >100 kU/l.

Treatment

The study medication was kindly provided by Ardeypharm (Herdecke, Germany) and used according to the instructions in the summary of product characteristics. Thus, EcN or placebo administration started with 1 capsule per day for 4 days and was continued with 2 capsules per day according to the permitted dosage. The EcN capsules contained $2.5\text{--}25 \times 10^9$ colony-forming units (cfu) of EcN per capsule. The placebo capsules were identical to the EcN capsules apart from the EcN, including capsule size, color, and taste. Treatment compliance was controlled via the record of daily intake in the subjects' diary cards. Additionally, the consumed capsules were monitored.

Clinical Assessment

The symptom-medication score (SMS) defined according the recommendations of Canonica et al. [12] served as the primary efficacy parameter. The SMS was assessed daily by the subjects and documented in the subjects' diary cards. The compliance of documentation was over 96% for all subjects. The difference between EcN and placebo treatment was assessed by calculation of the area under the curve (AUC) of the SMS during the grass pollen season. As secondary clinical efficacy parameters, SPT, the conjunctival provocation test, and the rhinoconjunctivitis quality of life questionnaire [13] were assessed before and after treatment. Adverse events (AEs) were documented at each visit by history and inspection of the subjects' diary cards.

Ig Measurements

The total and grass pollen-specific IgE (grass-sIgE) were measured using the Thermo Fisher Scientific ImmunoCAP System (Uppsala, Sweden) according to the manufacturer's instructions.

Specific IgA was detected by ELISA as previously described [14] using grass pollen extract (Sigma-Aldrich, St. Louis, Mo., USA). Briefly, grass pollen extract coated on 96-microwell plates (NUNC; Thermo Fisher Scientific Inc., Waltham, Mass., USA) were used and incubated with subjects' sera. The captured grass pollen-specific IgA (grass-sIgA) was detected with biotinylated anti-human-IgA1 (Affinity Bioreagents; Thermo Fisher Scientific) and measured photometrically at 405 nm.

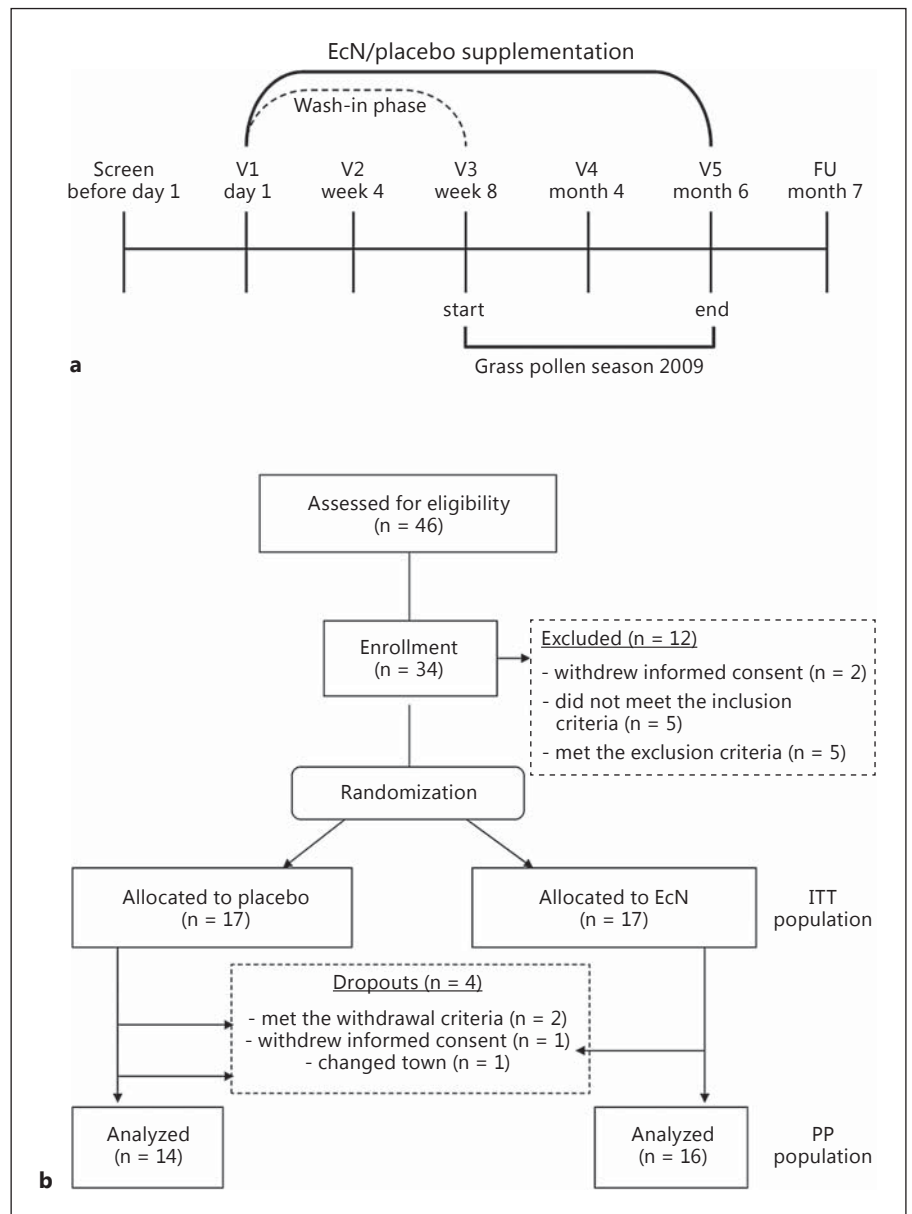


Fig. 1. Study schedule (**a**) and consort flow chart (**b**). PP = Per-protocol; ITT = intent-to-treat.

Pollen Count

The local pollen counts were kindly provided by the Deutscher Polleninformationsdienst. The period for assessment was specified as 14 days before and 31 days after the grass pollen peak (45 days).

Statistical Analysis

Primary and secondary analyses were performed in the per-protocol population, while safety analyses were performed in the intent-to-treat population. Due to the exploratory character of this study, a sample size of 30 subjects plus a dropout rate of 10% was predicted.

Data were subjected to nonparametric analysis by applying either the Mann-Whitney test (unpaired data) or the Wilcoxon test (paired data) using the SPSS Statistics 18.0 software package (Chicago, Ill., USA). All data are presented as medians (range).

Results

SMS upon EcN Administration Was Not Superior to Placebo

The SMS was chosen as the primary efficacy parameter (fig. 2a). From the first day of intervention, the SMS was consistently higher in the EcN group compared to placebo. No statistical significance was detectable at any time point. The SMS was comparable between EcN and placebo throughout the observational period. The AUC values of the defined grass pollen season (45 days) showed

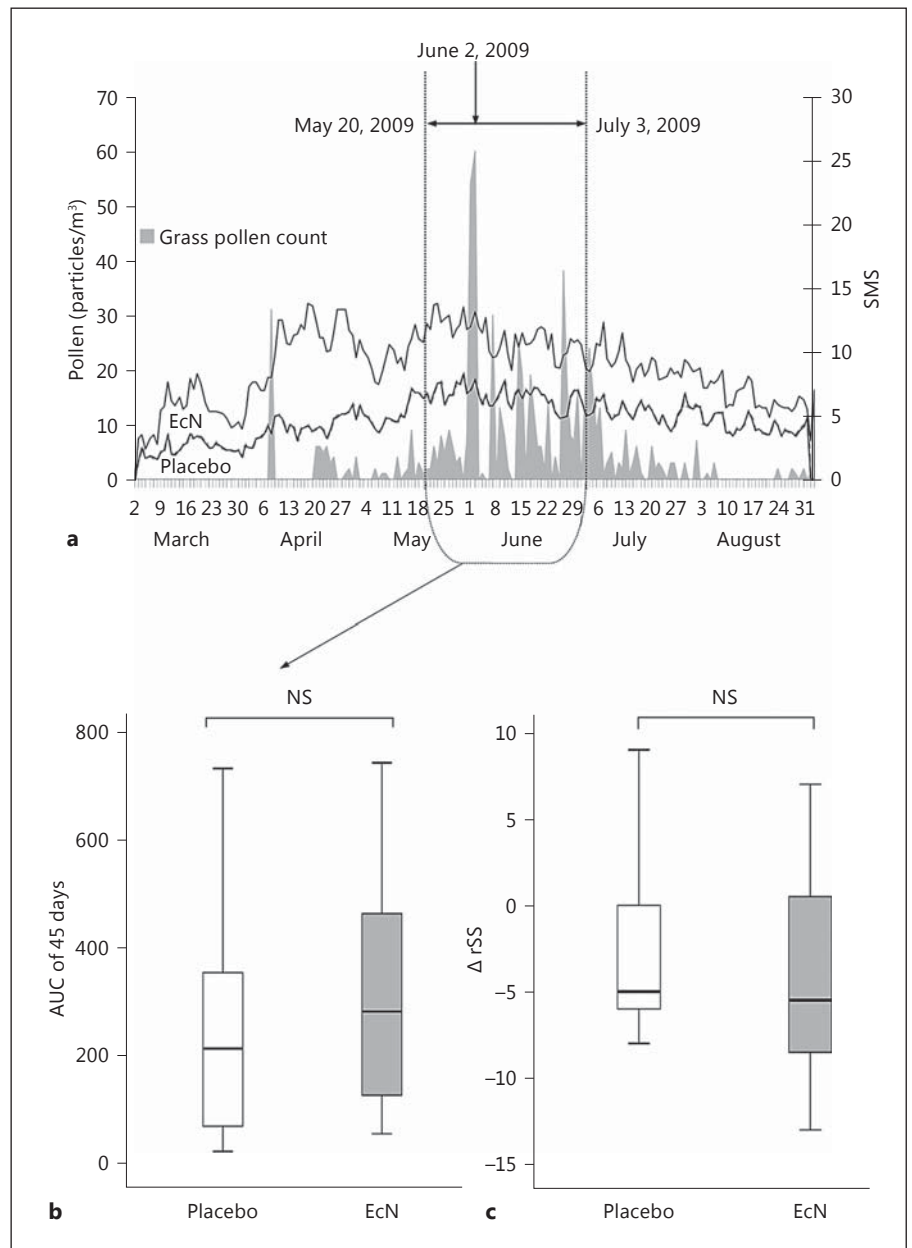


Fig. 2. Clinical symptoms in the treatment groups. **a** The SMS is shown for the complete supplementation period with EcN (n = 16) or placebo (n = 14). **b** The corresponding AUC was calculated for the defined grass pollen season (45 days). No significant differences in AUC values were detected between EcN and placebo (p = 0.257). **c** The rSS was lower in the grass pollen season in 2009 than in 2008 in both groups. The rSS is depicted as delta (rSS 2009 to rSS 2008).

no significant differences between EcN and placebo (fig. 2b, p = 0.257). The rSS of 2009 was lower than that of 2008 in both groups (fig. 2c). The additional clinical assessments (SPT, conjunctival provocation test, and rhinoconjunctivitis quality of life questionnaire) were comparable between placebo and EcN before, during, and after the treatment (data not shown).

The overall compliance was very good. No subject of the per-protocol population discontinued the treatment for more than 10 days. Documentation of the daily SMS was over 96% for all subjects analyzed.

Increased Grass-sIgA Response in EcN-Treated Subjects

To investigate whether oral EcN administration alters the humoral immune response as previously described in mice [9], total IgE, grass-sIgE, and grass-sIgA were measured before and after treatment (fig. 3). Total IgE and grass-sIgE remained stable upon EcN and placebo treatment. In contrast, grass-sIgA was significantly elevated in EcN-treated subjects [20,556 LU/ml (1,812–60,800)] compared to placebo [5,246 LU/ml (944–50,467)] (p = 0.048).

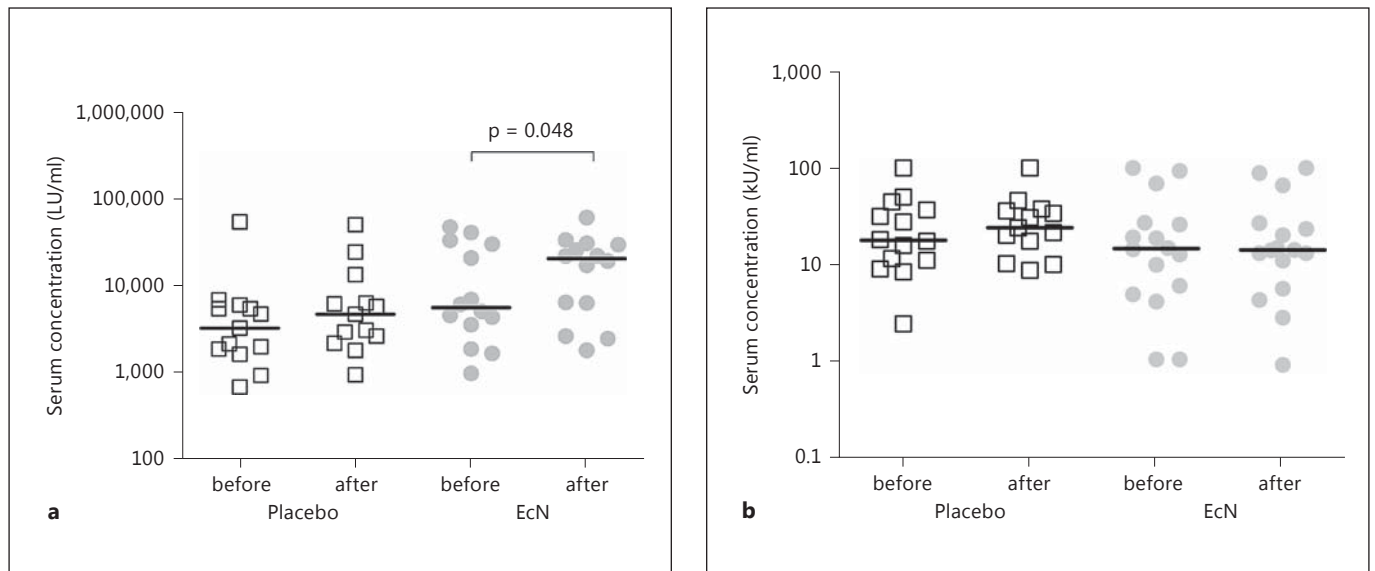


Fig. 3. Impact of EcN supplementation on grass-sIgA and grass-sIgE. The serum concentration of grass-sIgA was increased after EcN supplementation compared to placebo ($p = 0.048$; placebo, $n = 13$; EcN, $n = 14$) (a), while grass-sIgE remained unchanged in both groups (NS; placebo, $n = 14$; EcN, $n = 16$) (b).

Table 2. Safety data of EcN administration

Category	Placebo (n = 17)	EcN (n = 17)	Total (n = 36)
AEs	91 (56)	71 (44)	162 (100)
Intensity			
Mild	59 (65)	37 (52)	96 (59.3)
Moderate	32 (35)	33 (47)	65 (40.1)
Severe	0 (0)	1 (1)	1 (0.6)
Relation to EcN/placebo			
None	77 (85)	54 (76)	131 (80.9)
Possible	14 (15)	16 (23)	30 (18.5)
Sure	0 (0)	1 (1)	1 (0.6)

Values are presented as numbers (%). In total, 162 AEs were observed, i.e. 91 in the placebo group and 71 in the EcN group.

Safety and Tolerability of EcN Administration

In total, 162 AEs occurred during the study (91 in the placebo group and 71 in the EcN group) (table 2). The AE intensity was mild (59%, 96 AEs) to moderate (40%, 65 AEs). No serious AEs were observed. Possible relations to the study drug (EcN or placebo), i.e. adverse drug reactions (ADRs), were documented in 19% (31 ADRs). These included diarrhea, abdominal pain, and flatulence. Subjects with these ADRs recovered within a few days. In one

subject, flatulence was almost certainly related to EcN and this subject ceased participation in the clinical study. No unblinding was necessary.

The tolerability of the study medication judged by the subjects was 'very good' in 10 and 'good' in 4 subjects treated with EcN, compared to 7 and 6 subjects in the placebo group, respectively. Furthermore, 1 subject in each group assessed the tolerability as 'moderate' and 2 subjects taking EcN-containing capsules assessed the tolerability as 'poor'.

Discussion

This is the first clinical pilot study to assess whether EcN is clinically effective in subjects suffering from allergic rhinoconjunctivitis. In this randomized, double-blind, placebo-controlled clinical trial we demonstrated that coseasonal treatment of grass pollen-allergic subjects with EcN was not superior to placebo with regard to the SMS during one grass pollen season, while the safety and tolerability were excellent. However, significant changes in the grass pollen-specific humoral immune response were detectable, indicating the potential of oral EcN supplementation to achieve immunomodulation in humans as such. This finding supports and extends our previous data showing that EcN is immunologically active on T cell and B cell function [8, 9].

We chose the SMS as the primary outcome parameter to evaluate clinical efficacy. The SMS seemed to be higher in the EcN group at the beginning of the study and seemed to never be below the SMS of the placebo group during the grass pollen season. However, this might be due to variances in scoring among the subjects. Besides that, the SMS is highly related to the pollen count. The grass pollen count in 2009 was low. On most days of the grass pollen season, the pollen count was <20 pollen grains/ m^3 . Only 4 peaks with a maximum of 40 pollen grains/ m^3 were observed. The highest count was 60 pollen grains/ m^3 ; this occurred for only 2 days and was still low compared to earlier seasons. In 2008, the highest grass pollen count was 140 pollen grains/ m^3 (information from the Deutscher Polleninformationsdienst). To date, no defined threshold for pollen counts and their clinical effects exists. However, pollen counts <20 pollen grains/ m^3 are considered not to elicit relevant clinical symptoms.

Additionally, the global retrospective assessment of symptoms (rSS) was assessed and compared between 2008 and 2009. Interestingly, a reduced rSS was noticed in both groups in 2009. This result indicates that the grass pollen season in 2009 was generally weak. The lack of adequate antigen stimulation is furthermore suggested by the failure of an increase in grass-sIgE.

A recent study showed that allergen release from pollen may vary between different seasons [15]. Thus, it was concluded that the strength of symptoms may not directly correlate with pollen counts. This knowledge is of great clinical importance and should be considered in further clinical studies. It also points to the fact that more accurate markers to determine the true allergen exposure are urgently needed.

In addition, extended treatment (e.g. perennial or coseasonal for 3 grass pollen seasons) may have increased the clinical efficacy. On the other hand, one should also consider the fact that isolated nonspecific immunomodulation is probably not sufficient to achieve clinical efficacy in grass pollen-allergic subjects. Therefore, such immunomodulation with EcN should be combined with allergen-specific protocols as it has been successfully initiated with monophosphoryl lipid A (MPL) given subcutaneously [16]. MPL is a bacterial component (lipopolysaccharide) derived from the cell walls of *Salmonella minnesota*. It promotes a TH_1 -driven immune response and is successfully used as an effective adjuvant for specific immunotherapy [16, 17]. An optimized allergen/adjuvant ratio may improve the treatment protocols of specific immunotherapy by lowering the allergen dose, increasing

the safety/efficacy profile, and shortening the treatment period [18].

Measurement of the humoral immune response showed that EcN supplementation had no effect on grass-sIgE. Nevertheless, we observed a significant increase in grass-sIgA. This finding supports our previous results from ovalbumin-sensitized mice, where we also observed increased sIgA serum concentrations after oral EcN supplementation [9]. Also other bacteria and bacterial components have been previously described to promote systemic IgA production [19]. As the regulatory cytokine TGF- β plays an important role in Ig synthesis, this might be relevant for the observed increase in IgA [20]. Indeed, our previous data suggested that EcN may promote the onset of FoxP3⁺ Tregs [9]. Tregs are known to play an important role in regulation of the immune response upon allergen exposure through production of their functional cytokines IL-10 and TGF- β [2]. Therefore, this mechanism might be relevant in this clinical context as well. However, more data is needed to prove whether this increase in sIgA is likewise relevant for clinical efficacy in grass pollen-allergic subjects.

The impact of EcN on humoral immunity may be partially determined by the colonization conditions in the host's gut [19]. As some subjects had no increase in grass-sIgA after EcN treatment, we assume that inappropriate colonization of EcN in the intestine of those subjects might be responsible for the ineffective immunomodulation. To prove this hypothesis, parallel analysis of stool samples will be required in future studies.

Finally, the safety and tolerability of EcN supplementation were good. In the present study, the majority of the AEs were not related to the supplementation of EcN. These observations are in line with previous studies in humans indicating that EcN is a safe nonpathogenic strain [21, 22].

In conclusion, our results show that isolated nonspecific immunomodulation by EcN has an impact on in vitro surrogate markers but remains without clinical relevance.

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