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Beneficial effect of *Lactococcus lactis* NCC 2287 in a murine model of eosinophilic esophagitis

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Abstract

Background: Eosinophilic esophagitis (EoE) is a severe inflammatory disease of the esophagus which is characterised histologically by an eosinophilic infiltration into the esophageal tissue. The efficacy of probiotics in the context of atopic diseases has been well-investigated but, to date, there has been no study which has evaluated probiotic effects on EoE inflammation. This study sought to identify a probiotic which improves esophageal inflammation in experimental EoE.

Methods: Two candidate probiotics, *Lactococcus lactis* NCC 2287 and *Bifidobacterium lactis* NCC 2818, were tested in a murine model of EoE elicited by epicutaneous sensitization with *Aspergillus fumigatus* protein extract. Administration of bacterial strains in drinking water were used respectively as a preventive or treatment measure, or continuously throughout the study. Inflammatory parameters were assessed in the esophagus, skin and lungs after allergen challenge.

Results: In this EoE model, supplementation with *L. lactis* NCC 2287 significantly decreased esophageal and bronchoalveolar eosinophilia but only when given as a therapeutic treatment. No significant effect on eosinophilia was observed when NCC 2287 was given as a preventive or a continuous intervention. NCC 2287 supplementation had no significant effect on immunoglobulin levels, skin symptom scores or on transepidermal water loss. Supplementation with another probiotic, *B. lactis* NCC 2818, had no significant effect on esophageal eosinophilia.

Conclusion: We identified a *Lactococcus lactis* strain, able to attenuate esophageal eosinophilic inflammation in a preclinical model of EoE. This effect is strain specific and

depends on the timing and duration of bacterial supplementation. Confirmation of these observations in human clinical trials is warranted.

Key words: Allergic inflammation, eosinophilic esophagitis, probiotics, supplementation, treatment.

Introduction

Eosinophilic esophagitis (EoE) is a severe and chronic, antigen/immune-mediated disease of the esophagus characterised clinically by symptoms related to esophageal dysfunction, and histologically by an eosinophil-predominant inflammation that is limited to the esophagus (1). This disease is a newly recognised condition that affects approximately 50 in 100'000 individuals in westernised countries (2;3). Studies showing that more than two thirds of EoE patients suffer, or have suffered from allergic conditions such as asthma or eczema, (4); and that the vast majority of EoE patients are sensitised to multiple food allergens (1;5;6) are testimony to the allergic background of the disease. Previously defined as an entity which was not responsive to proton pump inhibitors (PPI), a subset of patients have since been described as PPI-responsive (PPI-REE), thus positioning PPIs, together with topical steroids (1,7), as a first-line of pharmacological treatment for EoE patients (8). Other treatment options include avoidance of the food trigger, if known, and dilatation of the narrowed esophagus to decrease the risk of food impaction.

While several murine models of EoE exist (9), epicutaneous sensitisation models have recently gained attention in the scientific literature (10-12). Originally developed by Spergel *et al.* (13), the preclinical model used in this study is based on epicutaneous sensitisation to an allergen, followed by a single intranasal allergen challenge that induces esophageal eosinophilia (14;15). The model mimics three of the main allergic inflammatory manifestations frequently associated with human EoE namely eosinophilic esophagitis-like inflammation, atopic dermatitis-like symptoms, and allergic airway inflammation. While genetics play an important role in EoE inheritance (16;17), a recent twin cohort study has identified the environment as the main driver for disease onset (18). As probiotic microorganisms have been proposed as therapies for other allergic diseases with a strong environmental component we considered that exposure to specific microorganisms may be a promising strategy to prevent the onset of EoE or delay symptom relapse.

Probiotics are live microorganisms (19) that are widely available over the counter for a variety of health benefits ranging from gut comfort, to the prevention or reduction of allergy symptoms (20-23). Probiotics have been extensively studied and tested in various allergy models. However, most studies addressing the efficacy of probiotics in animal models, have limited their study to one probiotic strain and one type of inflammation, mainly allergic airway inflammation (24;25) or atopic dermatitis (26-29). Here, we hypothesised that probiotics may be able to dampen esophageal inflammation in a murine model of EoE. To our knowledge, there have been no human or preclinical studies which have examined the effect of probiotics on eosinophilic esophageal inflammation. The probiotic *Lactococcus lactis* NCC 2287 has previously been shown to decrease clinical scores in a food allergy model based on co-administration of ovalbumin and cholera toxin (30). In addition, NCC 2287 is a strong inducer of the immune-modulatory cytokine IL-10 and an inhibitor of the eosinophil survival cytokine IL-5 in Th2 skewed cultures of peripheral blood mononuclear cells (PBMC) (31). The probiotic *Bifidobacterium lactis* NCC 2818, is also known for its immune-modulatory properties in allergic diseases (31-32).

In the present study, we therefore tested *Lactococcus lactis* NCC 2287 and *Bifidobacterium lactis* NCC 2818, for their capacity to decrease esophageal inflammation in a murine model of EoE. Our study suggests that specific probiotics may be beneficial in reducing esophageal eosinophilic inflammation.

Methods:

Eosinophilic esophagitis model

The animal study protocol VD2382 was approved by the “Service Vétérinaire du Canton de Vaud”, Switzerland. Briefly, 5 week-old female BALB/c mice (BALB/cByJ JAX™ mice strain 001026 from Charles River; L'Arbresle, France) were anaesthetised under isoflurane (Baxter; Volketswil, Switzerland) on day 7. The backs of the mice were shaved with an electric razor and cleaned with 70% isopropanol solution (VWR; Nyon, Switzerland). Then, 100 µl of *Aspergillus fumigatus* (Af) protein extract (Greer Laboratories; Lenoir, NC, USA) at 2 mg/mL (Sensitized group (S.)) or 100 µl of a 0.9 % NaCl solution (non-sensitized group (N.S.); Merck; Zoug, Switzerland) was applied to a 1x1 cm patch of sterile gauze (Hartmann; Dermoplast, Chatenois, France) and secured to the skin with a bio-occlusive transparent dressing (Systagenix; Bioclusive, San Antonio, Texas, USA, Switzerland) and a Band-Aid (Mefix; Wasquehal, France). Patches were removed on day 14. After a 2-week resting period, a second, identical patch was applied on day 28 to the skin until day 35. The next day, mice were challenged intranasally

under isoflurane anaesthesia with 100 µg of *Af* diluted in 0.9% NaCl. On day 38, mice were anaesthetised using isoflurane and euthanised after collecting blood from the abdominal aorta. (Figure 1).

Bacterial biomass and intervention

Lactococcus lactis ssp. *lactis* NCC 2287 (CNCM I-4154) and *Bifidobacterium animalis* spp *lactis* NCC 2818 (CNCM I-3446) were sourced from the Nestlé Culture Collection (NCC) and added to the drinking water of sensitised (S.) mice at a final concentration of $5 \cdot 10^8$ CFU/mL. This corresponded to an intake of approximately $2 \cdot 10^9$ CFU per day. Bacterial strains were administered in either the preventive phase (S.-Preventive group; day 0 to day 28), the therapeutic treatment phase (S.-Treatment group day 28 to day 38) or given continuously throughout the study (S.-Continuous group; day 0 to day 38). Since the bacterial powders contained maltodextrin DE12 (Roquette; Lestrem, France), maltodextrin was used instead of probiotics in the drinking water of control groups (Figure 1).

Tissue processing and eosinophils staining

Esophageal tissues were fixed in 10% Formalin (Sigma; Buchs, Switzerland) immediately after harvest, washed twice in 70% ethanol (Merck Millipore; Darmstadt, Germany), stored in ethanol until embedding in paraffin (VWR, Tissue Tek VIP 5JR; Sysmex; Yverdon, Switzerland) and then cut into 3 µm sections (Zeiss Hyrax KS34; Histocom, Zug, Switzerland). Tissue sections were deparaffinised overnight at 60°C then in Histo-clear (Fisher Scientific, Reinach, Switzerland) and Xylol (VWR). After re-hydration, tissue sections were incubated in vital red solution (ABcam; Cambridge, UK) for one hour at room temperature and then washed twice in distilled water. Tissues were counterstained in 0.8% Methyl Green (Sigma) for 10 min at 60°C, washed in distilled water and then dehydrated before mounting with xylol mounting medium (VWR). Blinded eosinophil counts (maximum eosinophil count per high power field (x 400) were performed in duplicate by 2 individuals.

Bronchoalveolar lavage collection and staining

Bronchoalveolar lavage (BAL) was performed using 1 mL of phosphate buffered saline (PBS) (Sigma; Buchs, Switzerland) solution containing 0.2% bovine serum albumin (BSA) (Sigma).

BAL fluids were centrifuged for 10 minutes at 2000 rpm. Cell pellets were then suspended in 150 μ L PBS 0.2% BSA and transferred to slides using a cytospin (Thermo Fisher Scientific, Reinach, Switzerland) for 5 minutes at 500 rpm. Cells were then stained using DIFF-Quik staining kit (Medion Diagnostics; Miami, FL, USA) according to the manufacturer's instruction. Blinded differential cell counts were performed in duplicate by 2 individuals.

Scoring and clinical outcomes

Skin symptoms were assessed using a score from 0 to 6. The composite score was derived from values given to different symptoms, as follows: erythema = 1, dryness = 1, baldness = 1, excoriation = 1 or 2 (if more than one excoriation), lichenification = 1. Skin scores were assessed after removal of the second skin patch on day 37. Transepidermal water loss (TEWL) was measured in the patch area with a Tewameter (TM300; Courage + Khazaka electronic; Cologne, Germany) on day 37.

Specific IgG1 quantification

Ninety-six well plates (Nunc Maxisorp; VWR) were coated overnight at 4°C with *Af* protein extract (Greer Laboratories) at 50 μ g/ml in carbonate buffer. Plates were then washed with PBS-0.05% tween (Biorad; Reinach, Switzerland) and blocked with PBS-1% BSA (Sigma) for 1 h at 37°C. Diluted sera were incubated for 2 h at 37°C. After washing, plates were incubated 2 h at 37°C with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG1- (Southern Biotech; Bioconcept Allschwil, Switzerland), and then with the HRP substrate tetramethylbenzidine (TMB) (KPL; Socochim, Lausanne, Switzerland). The reaction was stopped using 1N hydrochloric acid (HCL; Merck). Absorbance was measured at 450 nm and the results expressed as optical density (OD) values.

Specific IgE quantification

Af (Greer Laboratories) was re-suspended in distilled water containing anti-proteases (Sigma) and Triton X100 (Sigma). This solution was sonicated for 5 min on ice, then centrifuged at 1000 rpm for 10 min. The upper phase was collected and its total protein content measured using a BCA protein assay kit (Thermo Scientific; Zug, Switzerland) according to the manufacturer's protocol. *Af* proteins were labelled with Digoxigenin-3-O-methylcarbonyl- ϵ -aminocaproic acid-N-hydroxysuccinimide ester (DIG; Roche, Basel, Switzerland) and purified according to the product protocol. Next, ninety six well plates were coated with 100 μ L of 2 μ g/ml rat anti-mouse IgE (BD-Bioscience) in carbonate buffer and incubated overnight at 4°C. Plates were then

washed in PBS 0.05% tween and blocked with 200 μ L PBS containing 10% foetal calf serum (FCS; Bioconcept), for 1 h at room temperature. Diluted sera were incubated 2 h at room temperature. After washing, DIG- labelled antigen was added and the plates incubated for a further 90 min at room temperature. Plates were then washed and incubated with HRP-labelled anti-DIG antibody (Roche, Switzerland) for 1 h at room temperature. Colour was developed using TMB and the reaction stopped using 1N HCl (Merck). Plates were read at 450 nm and the results expressed as OD values.

Statistical analyses

The exact Wilcoxon non-parametric statistical test was used to compare groups. Statistical analyses were performed using the software R 2.14.1. Results with a p -value ≤ 0.05 were considered as significant. Data are expressed as median $\pm$ standard error (SE) of the median. For each outcome, the significantly different responses of sensitised (S.) and non-sensitised (N.S.) mice confirmed the validity of the model. All figures are representative of one experiment out of two independent experiments. For the two probiotics, two separated experiments were performed: one exploratory (n=8 mice per group) and one confirmatory experiment (n=8 to 10 mice per group). For the confirmatory experiment, the sample size for each group was calculated according to the outcomes obtained in the exploratory experiment.

Results

Oral supplementation with *Lactococcus lactis* NCC 2287 in the treatment phase decreases esophageal eosinophilia

In order to test whether probiotics could decrease esophageal eosinophilia (EoE) in a mouse model of EoE, the strain NCC 2287 was added to the drinking water either as a preventive measure (day 0 to day 28; n=8), as a treatment (day 28 to day 38; n=10) or as continuous exposure (day 0 to day 38; n=10) (Figure 1). Maximum eosinophil count was significantly greater in the esophagus of sensitised mice (S., n=10) challenged with *Af* extract than in the esophagus of non-sensitised mice (N.S.; n=8) challenged with *Af* extract (13.00 ± 2.84 vs 2.50 ± 1.06 , $p=0.025$; respectively) (Figure 2A and 2B). Interestingly, sensitised (S.) mice receiving NCC 2287 from day 28 to 38 (S.-Treatment) had significantly less esophageal eosinophilia than the non-supplemented sensitized (S.) group (5.50 ± 1.89 vs. 13.00 ± 2.84 , $p=0.03$; respectively) (Figure 2A and 2B). However, there was no significant effect on esophageal eosinophilia when the NCC 2287 strain was given as a preventive measure (from day 0 to day 28) or when it was given continuously, throughout the study (day 0 to day 38).

Oral supplementation with *Lactococcus lactis* NCC 2287 in the treatment phase decreases bronchoalveolar eosinophilia

Prior studies have emphasised the strong association between esophageal and lung eosinophilia (12;28). In our model, a single intranasal injection of *Af* extract significantly increased the level of total bronchoalveolar lavage (BAL) eosinophilia in the S. group ($n=10$, $p=0.001$, Figure 3). We thus investigated whether a supplementation with NCC 2287 from day 28 to 38 (S.-Treatment, $n=10$) could also decrease BAL eosinophilia. A significant decrease in the total eosinophil numbers in the BAL was observed in the S.-Treatment group (1896.8 ± 512.0) compared to the non-supplemented S. group (2797.8 ± 1016.5 , $p=0.038$). These results suggest that NCC 2287 given as a therapeutic treatment in our experimental model, can decrease both bronchoalveolar and esophageal eosinophilic infiltration.

Specific IgE and IgG1 levels are not affected by oral administration of *Lactococcus lactis* NCC 2287 in the treatment phase

In order to demonstrate that NCC 2287 supplementation from day 28 to day 38 had no effect on sensitisation, we measured anti-*Af* IgE and anti-*Af* IgG1 levels in the serum (Figure 4A and 4B). Both anti-*Af* IgE and anti-*Af* IgG1 levels were increased in the S. group compared to the N.S. group (0.57 ± 0.19 vs 0.06 ± 0.00 , $p<0.001$; for anti-*Af* IgE and 0.48 ± 0.26 vs 0.10 ± 0.01 , $p=0.006$ for anti-*Af* IgG1). As expected, NCC 2287 supplementation after sensitisation (S.-Treatment) has no effect on the anti-*Af* IgE levels (0.54 ± 0.21 vs 0.57 ± 0.19 , $p=0.912$; Figure 4A) or on the anti-*Af* IgG1 levels (0.84 ± 0.32 vs 0.48 ± 0.26 , $p=0.280$; Figure 4B). These results suggest that the supplementation with NCC 2287, in the treatment phase, does not modulate the sensitisation under these specific conditions.

***Lactococcus lactis* NCC 2287 supplementation in the treatment phase does not affect skin symptom scores or transepidermal water loss**

To evaluate whether NCC 2287 also modulates atopic dermatitis-like symptoms in these mice, skin symptom scores and transepidermal water loss (TEWL) were assessed. Significant increases in skin symptom scores and TEWL were observed in the S. group when compared to the N.S. group (2.00 ± 0.47 $n=10$ vs. 0.00 ± 0.00 $n=8$; $p=0.007$ and 36.68 ± 7.60 $n=10$ vs. 11.88 ± 1.00 $n=8$, $p=0.002$; respectively). Supplementation with NCC 2287 from day 28 to 38 had no

significant effect on the skin symptom scores (2.00 ± 0.00 n=10 vs. 2.00 ± 0.47 n=10, $p=0.209$; Figure 4C) or on the TEWL (41.31 ± 5.35 n=10 vs. 36.68 ± 7.60 n=8, $p=0.353$; Figure 4D) when compared to the non-supplemented S. group. These results suggest that therapeutic treatment with NCC 2287 in this experimental model does not confer any beneficial effect on allergic skin inflammation.

The decrease in esophageal eosinophilia induced by *Lactococcus lactis* NCC 2287 oral supplementation is strain specific

We tested whether the reduction in esophageal eosinophilia could also be observed with another bacterial species. In contrast to *Lactococcus lactis* NCC 2287, administration of *B. lactis* NCC 2818, a probiotic known for its immune-modulatory properties in allergic diseases (31-32), at a similar dose during the therapeutic treatment phase, had no significant effect on esophageal eosinophilia of sensitised (S.) mice (9.5 ± 2.75 , vs. 13 ± 4.76 , $p=0.331$, respectively, n=8 in each group) (Figure 5). These results suggest that not all probiotics are able to attenuate eosinophilia in esophageal tissue when administered as a therapeutic treatment.

Discussion

In this study, we show that *L. lactis* NCC 2287 specifically decreases esophageal eosinophilia in a murine model of EoE. Our study also demonstrates that the time frame of supplementation is important since the beneficial effect of *L. lactis* NCC 2287 was only observed when the microorganism was given as a therapeutic treatment. Altogether, our data suggest that *L. lactis* NCC 2287 may be an interesting candidate strain for reducing eosinophilic esophagitis inflammation in humans.

To the best of our knowledge, there is no report to date, showing a beneficial effect of probiotics in a mouse model for eosinophilic esophagitis. Thus, our study is the first to report that a specific probiotic strain, *L. lactis* NCC 2287, may influence esophageal eosinophilia. In contrast, no beneficial effect was observed with *B. lactis* NCC 2818 supplemented at the same dose and at the same time period. In *in vitro* models, such as those using stimulated PMBCs or epithelial cells co-cultured with dendritic cells (33-35), probiotics have been shown to induce different pro-inflammatory cytokine, and immune-modulatory cytokine responses (31). It has been shown that *B. lactis* NCC 2818 and *L. lactis* NCC 2287 both induce high amounts of IL-10 in a Th2 skewed PBMC model, suggesting a possible immuno-regulatory effect of these strains (31). However, in

the same model, *L. lactis* NCC 2287 is a stronger inducer of IFN- γ , and is more potent at reducing IL-5 levels than *B. lactis* NCC 2818 (31). The predictive value of these assays in human disease is debatable, nevertheless, they convincingly highlight the critical fact that probiotics are not equal when confronted with the host immune environment. The specific strain of *L. lactis* NCC 2287 used in our study, has already been shown to confer some benefits in the food allergy model described by Zuercher *et al.*, (30). When *L. lactis* NCC 2287 was given in the treatment phase of their study, a reduction in clinical and inflammatory parameters was observed while specific IgE and IgG1 levels were not modified (30). The specific mechanism of action of NCC 2287 during allergy treatment is not known. In general, probiotic effects on allergic inflammation are mainly attributed to their capacity to increase the CD4⁺/CD25⁺ Foxp3⁺ regulatory T cell pool in peripheral lymph nodes (24-26;36), to decrease sensitisation efficacy (24-25) and/or to modulate cytokine expression (37). While, T regulatory cells have been associated with decreased esophageal eosinophilia in another EoE model (38), it has recently been shown that other regulatory mechanisms may be involved. Probiotics have been shown to dysregulate microRNAs and to stabilize *IL10* mRNA expression in human monocyte *ex vivo* (39). These results suggest that other epigenetic mechanisms could explain the effect observed in the esophagus and that the understanding of probiotic effect is at its infancy. In the same model as the one used in this paper, Akei *et al.* have shown that esophageal eosinophilia is IL-5-dependent. The counteracting effect of NCC 2287 on IL-5 expression seen in Th2 skewed PBMCs (31) and in an ovalbumin / cholera toxin-induced food allergy model (30) may thus partially explain the decrease in esophageal eosinophilia seen with NCC 2287 treatment in the current study. Taken together, these results suggest that *L. lactis* NCC 2287 may lead to a strong reduction in gastrointestinal Th2 inflammation independently of the level or route of antigen sensitisation in experimental allergy models.

Previous studies have highlighted the strong link between lung and esophageal inflammation (40). Furthermore, 14-70% of EoE patients also present with asthma (1;41). It is thus possible that the effect of *L. lactis* NCC 2287 on lung eosinophilia and esophageal eosinophilia is due, at least in part, to a common effect of the probiotic on eosinophil recruitment. However, oral supplementation with *B. lactis* NCC 2818 had no significant effect on EoE, although it significantly reduced bronchoalveolar eosinophilia (Supplementary Figure 1D). Our data suggest that a decrease in eosinophils in the lung is not necessarily concomitant with a significant decrease in esophageal eosinophilia. These results are not surprising as numerous preclinical studies have shown that lung eosinophilia can be reduced with probiotic

supplementation (24;25), or can be reduced secondary to changes in the gut microbiota (42;43).

Depending on the study, 4-60% of EoE patients have been reported to have a clinical history of atopic dermatitis (1;41). A probiotic with a dual effect on atopic dermatitis and EoE would thus be valuable. Numerous human clinical trials testing probiotic effects on atopic dermatitis have been conducted but meta-analyses have given controversial results (44-49). Recent literature shows that nutritional intervention with probiotics can reduce the risk of developing the disease or decrease the severity of the symptoms (45). However there are other studies reporting only mild effects of probiotics in the prevention of atopic dermatitis (49) or showing mild or no effect on symptom improvement. In our model, we showed that *L. lactis* NCC 2287 effectively decreases the atopic dermatitis skin score and TEWL but only when given as a preventive measure from day 0 to 28 (Supplementary Figure 1A). Similar to the results in the esophagus, this beneficial effect of the probiotic on the skin was lost if the supplementation was prolonged until the end of the experiment (day 0 to 38, Supplementary Figure 1A). It appears therefore that *L. lactis* NCC 2287 is able to modulate both atopic dermatitis and EoE, but the intervention times needed to achieve both effects in our model do not coincide. Taken together, our observations suggest that the time-windows of intervention may determine the tissue specificity of the benefit. However, the relevance of the timing of the supplementation is not well-addressed in the current literature.

Only a few studies have clearly demonstrated the differential effect of probiotics on the treatment and/or prevention of allergic inflammation. In our study, we identified the puzzling but reproducible fact that a shorter intervention, from day 28 to 38, was more efficacious on esophageal eosinophilia than a continuous intervention during the whole study period. This finding, while surprising, indicates that the host may somehow adapt to the probiotics or that the cytokine milieu induced by the probiotic supplementation may not always be beneficial for the inflamed tissues. Our results highlight the dichotomy between immediate but transient immunomodulation and a longer term imprinting for a sustained benefit. As such, careful design of human clinical trials testing the effect of probiotics on EoE is necessary as our results suggest that a very early supplementation may preclude a beneficial effect of the strain on EoE. This concept of adaptation of the host to a new microbial strain is not new but evidence in the probiotic literature is scarce.

In conclusion, this study demonstrates the beneficial effect of a specific probiotic strain on eosinophilic esophageal inflammation. This effect is not only dependent on the intervention

schemes but also on the nature of the probiotics strain used. Our results suggest that probiotics differ in their capacity to prevent or manage allergic response, and as well as in the allergic manifestations they target. Finally, our findings showing that a specific probiotic may be beneficial in the management of EoE opens a new area of research that may lead to an improved health outcome in EoE patients. Probiotic supplementation will not have a similar efficacy to pharmacological compounds (such as glucocorticoids). Rather, probiotics allow a more holistic approach which may re-establish and/or maintain homeostasis. Their administration may permit lower doses of corticosteroid use and, thereby, reducing the likelihood of side-effects. Furthermore, probiotic use may attenuate symptoms when the culprit food is consumed, or delay dilatation. Probiotics may thus be regarded as a complement to the current treatments, whether this be glucocorticoid treatment, food avoidance or dilatation. More research and human clinical trials confirming the effect of probiotics in EoE patients are warranted.

Figure legends

Figure 1: Eosinophilic esophagitis model and nutritional intervention

Two patches containing 200 µg of *Af* protein extract (sensitised group (S.)) or saline (non-sensitised group (N.S.)) were applied to the shaved back of the mice on day 7 to 14 and on day 28 to 35. Mice were all challenged intranasally with 100 µg of *Af* under isoflurane anaesthesia on day 36. Sacrifice was performed on day 38. *L. lactis* NCC 2287 or *B. lactis* NCC 2818 were given *ad libitum* in the drinking water of sensitised mice at a dose $5 \cdot 10^8$ CFU / mL as a preventive intervention (from day 0 to 28 (S.-Preventive)), a therapeutic treatment (from day 28 to 38 (S.-Treatment)) or given continuously throughout the model (from day 0 to 38 (S.-Continuous)).

Figure 2: Effect of *L. lactis* NCC 2287 on esophageal eosinophilia

L. lactis NCC 2287 was supplemented during the prevention phase (day 0 to 28), the treatment phase (day 28 to 38), or continuously (day 0 to 38). Results are expressed as eosinophil count per high power field. Histograms are expressed as median $\pm$ SE median (A). Eosinophil stained with vital red in esophageal tissue sections and counterstained with methyl green are shown (B). N=8 for non-sensitised (N.S.) and prevention (S.-Prevention) groups and n=10 for sensitised (S.), treatment (S.-Treatment) and continuous intervention (S.-Continuous).

Figure 3: Effect of *L. lactis* NCC 2287 on bronchoalveolar lavage (BAL) eosinophilia

L. lactis was given during the treatment phase (day 28 to 38). Eosinophil counts in BAL are

expressed as median $\pm$ SE median. N=8 for non-sensitised (N.S.) n=10 for sensitised (S.) and treatment (S.-Treatment) groups.

Figure 4: Effect of *L. lactis* NCC 2287 on specific immunoglobulins, skin symptoms and transepidermal water loss (TEWL)

Anti-Af specific IgE (A) and Anti-Af IgG1 (B) are expressed as optical density (OD) values. *L. lactis* was given in treatment phase (day 28 to 38). Diamonds represent individual mice with median $\pm$ interquartile ranges. Skin score (C) and TEWL (D) were assessed on day 37. Data are expressed as median $\pm$ SE median with n=8 for non-sensitised (N.S.) and n=10 for sensitised (S.) and treatment (S.-Treatment) groups.

Figure 5: Effect of *B. lactis* NCC 2818 on esophageal eosinophilia

Maximum eosinophil counts per high power field are shown as median $\pm$ SE median, n=8 for sensitised (S.) group and n=8 for treatment (S.-Treatment) group.

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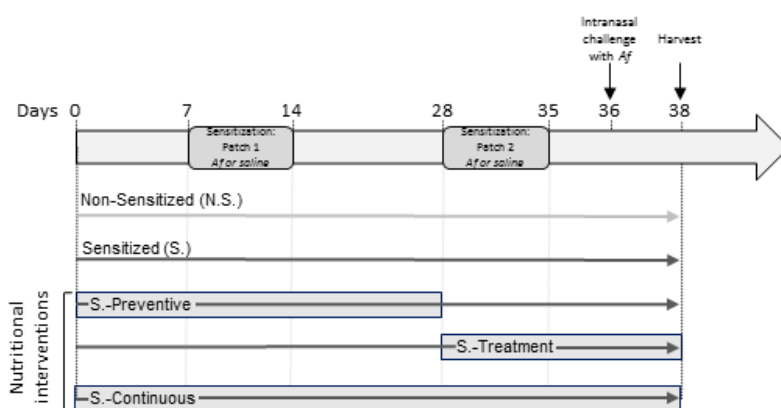


Figure 1

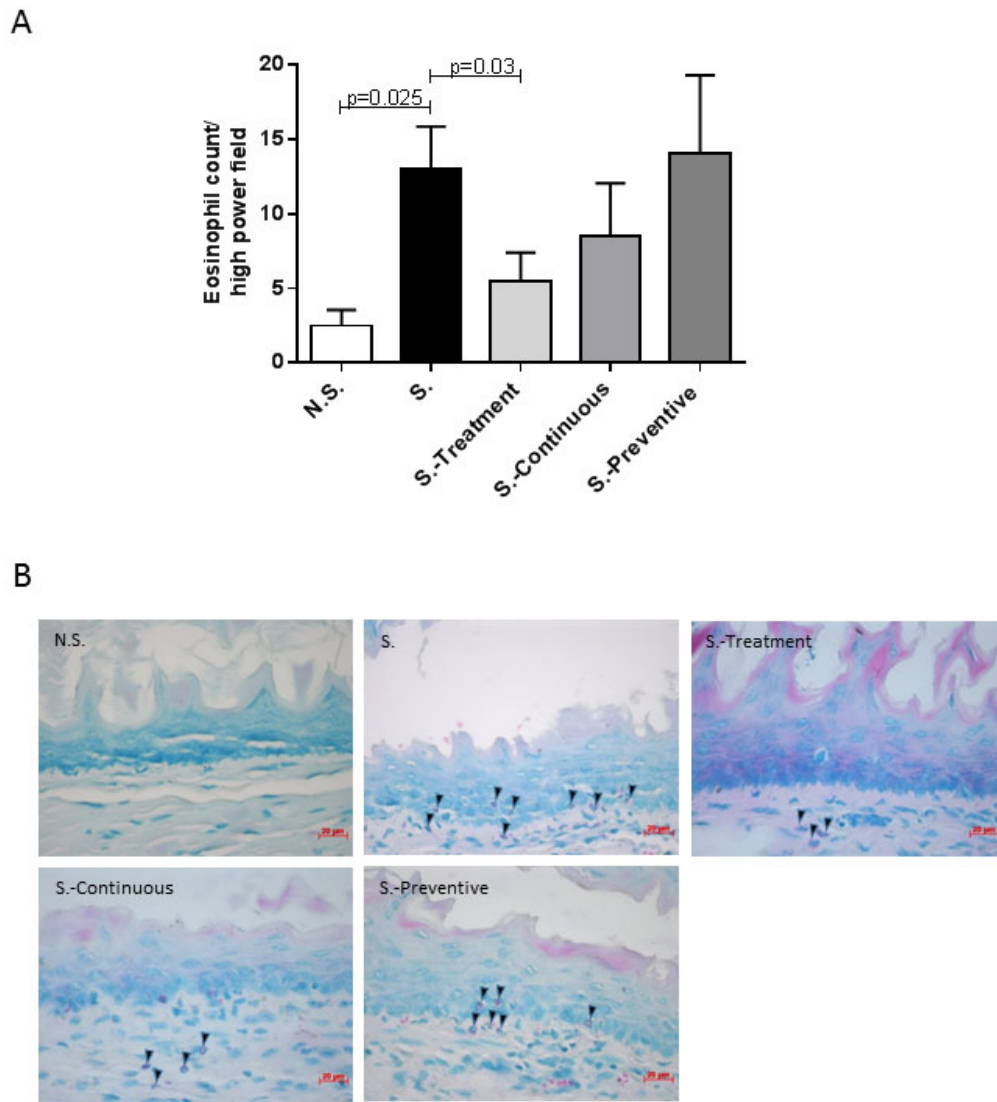


Figure 2

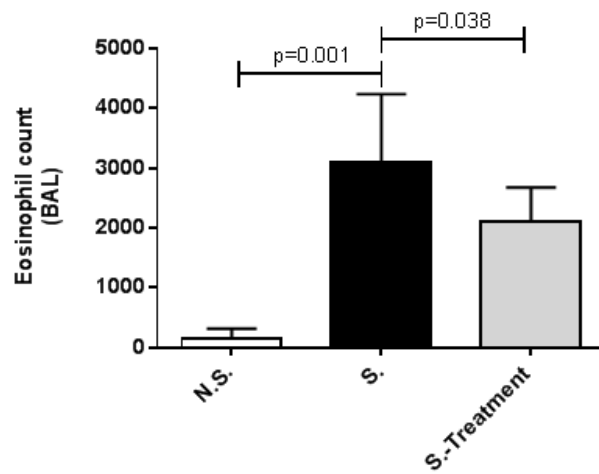


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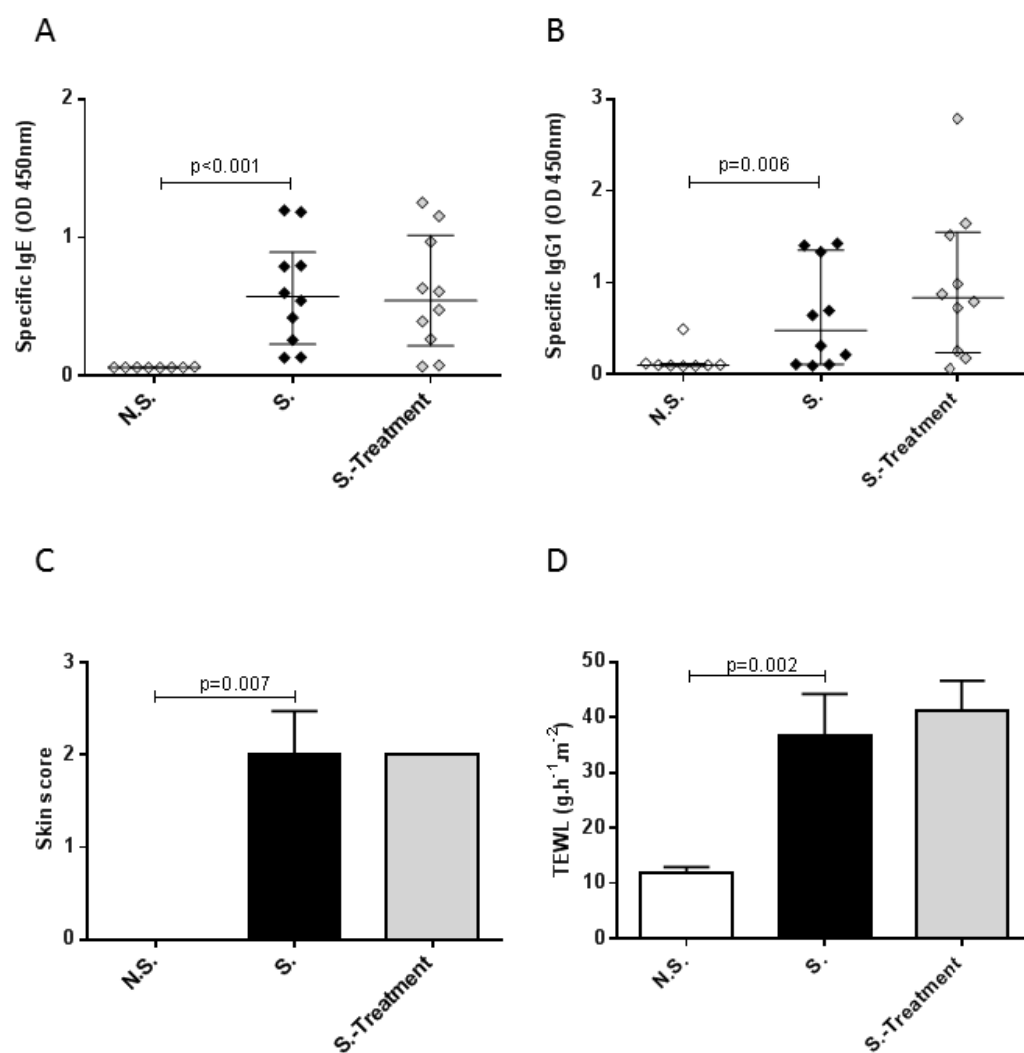


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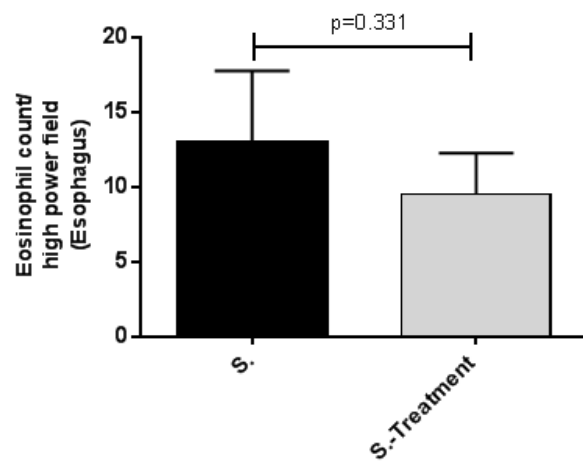
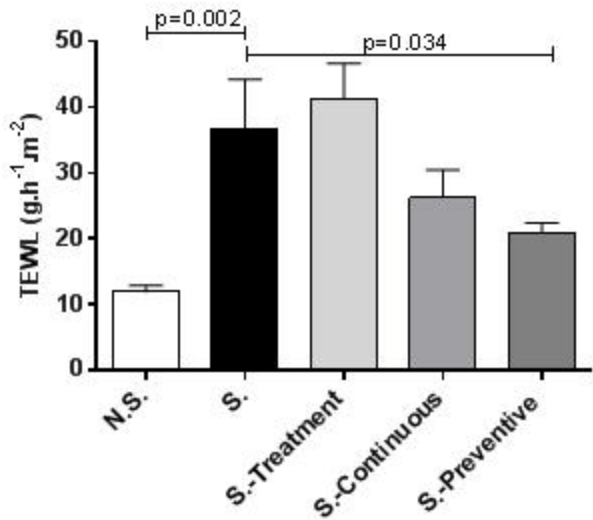


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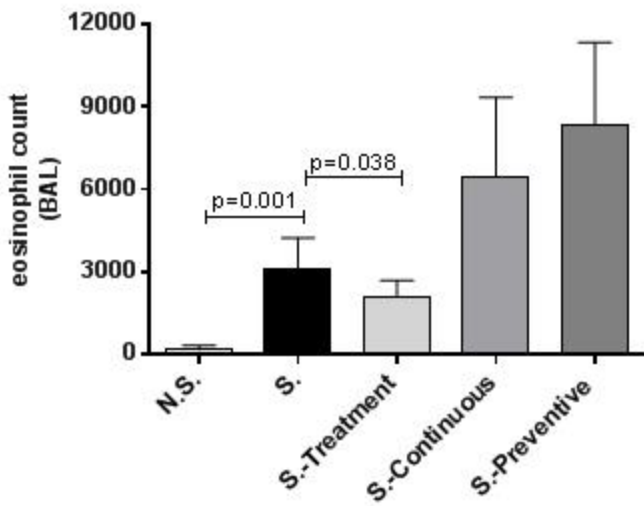
Supplementary Figure 1

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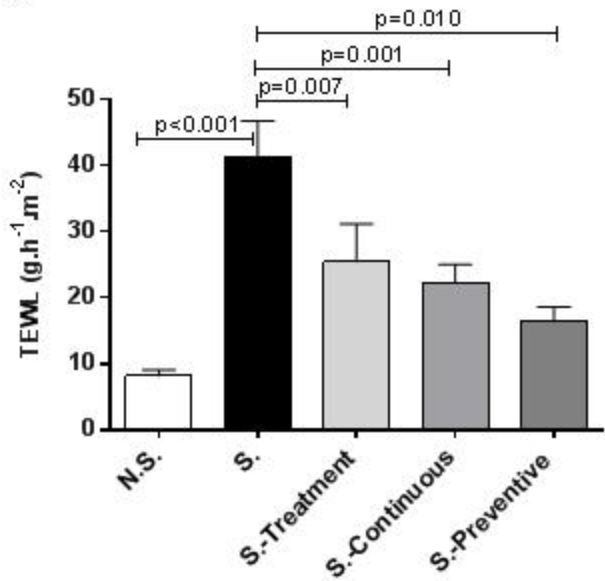


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