

Treatment of Atopic Dermatitis Eczema With a High Concentration of *Lactobacillus salivarius* LS01 Associated With an Innovative Gelling Complex

A Pilot Study on Adults

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Goals: To evaluate the efficacy of a highly concentrated *Lactobacillus salivarius* preparation containing a gelling complex formed by *Streptococcus thermophilus* ST10 and tara gum in the treatment of atopic dermatitis (AD).

Background: Previous studies have demonstrated an improvement in AD symptoms after administration of the probiotic strain *L. salivarius* LS01. *S. thermophilus* ST10 and tara gum create a gelling complex that adheres to intestinal mucus and improves barrier function.

Study: A prospective, controlled pilot trial was carried out to evaluate how the association of *S. thermophilus* ST10 and tara gum could improve the activity of *L. salivarius* LS01 administered at high doses to adults with AD. Twenty-five patients were included into the study: 13 were treated for 1 month with the active formulation, whereas 12 represented the placebo group. Scoring Atopic Dermatitis index was determined before and at the end of probiotic administration. Fecal samples were also collected to evaluate changes in bacterial counts of *Staphylococcus aureus* and clostridia.

Results: A significant improvement in SCORAD index was observed in the probiotic group after 1 month of treatment, whereas no significant changes occurred in placebo patients. A slight decrease in fecal *S. aureus* count was observed in probiotic-treated patients.

Conclusions: Data obtained in this study suggest a potential role for *L. salivarius* LS01 in the treatment of AD. The addition of tara gum and *S. thermophilus* ST10 seems to improve the overall efficacy of the probiotic strain, in particular shortening the time required for the onset of the positive effects. Further studies to investigate the activity of this preparation are advisable.

Key Words: probiotics, dermatitis, *Lactobacillus salivarius*

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The authors declare that they have nothing to disclose.

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Atopic dermatitis (AD) is a chronic and relapsing inflammatory disease that primarily affects the skin, causing pruritic lesions, dryness, and staphylococcal infections.^{1–3} Given its symptoms, AD represents a substantial burden for patients, their families, and society as a whole.^{4,5} Onset of the disease usually occurs early in childhood and 15% to 30% of the pediatric population has been diagnosed with AD.⁶ Although AD may disappear over time, 40% to 60% of pediatric patients with AD continue to have symptoms, and the estimated prevalence in adults is 2% to 10%.^{6–8}

Different but partially overlapping clinical phenotypes have been recognized. Despite topical therapy and skin care effective in controlling AD, most patients present manifestations that do not respond to usual treatments.

AD is highly heritable, involving several genes related to skin barrier function and the immune system, but also environmental factors and lifestyle habits such as stress, infections, and allergens.^{9,10} Despite the fact that the detailed mechanisms underlying inflammation of AD skin are not fully understood, a defect in skin barrier function and an immune imbalance play a crucial role, leading to T-helper cell-type 2 (Th2)-biased immune responses.^{2,11,12} According to recent findings, these factors, combined with pruritus, are strictly related in a highly complex interplay that may lead to the development of AD.¹³ Mutations in filaggrin genes and abnormalities in tight junctions have been widely reported to occur frequently in AD patients.^{14,15} Alterations in skin barrier function are associated with transepidermal water loss, which is correlated with severity of AD. *Staphylococcus aureus* colonization, in turn, may favour the penetration of microbial antigens, which are responsible for subsequent sensitization.¹⁶ Both innate and adaptive immunity are involved in the development, maintenance, and flare-up of AD.¹⁴ A complex pattern of cytokines has been recognized as being involved in AD. In the acute phase of AD, the adaptive immune response is mediated by increased levels of Th2 and Th22 cytokines, which negatively affect epidermal differentiation, thus resulting in reduced filaggrin gene expression.¹⁴ The acute phase is characterized by the prevalence of a Th2 response and a reduction in Th17-cell expression.¹⁷ Moreover, after the formation of acute lesions, increased release of interferon- γ induces apoptosis of keratinocytes. As a consequence, Toll-like receptors, which are usually present on the surface of keratinocytes, are reduced along with keratinocyte-derived antimicrobial peptides.¹⁴ The chronic phase is dominated by a Th1 response associated with Th22

cells. Increased levels of IL-22, along with IL-31 and IL-9, are responsible for itching and pruritus.¹⁸ Pruritus, in turn, worsens skin barrier functions, thus closing a vicious circle initiated by a disruption of stratum corneum function.

Although the role of intestinal microbiota in the development of AD is still debated, differences in the composition of skin and intestinal microbiota between AD and healthy subjects have been reported by several studies.^{19–23} In particular, higher amounts of clostridia and qualitative differences in bifidobacteria population have been found in the gut of AD patients compared with healthy subjects.^{20,24} Because probiotics have important activity on the immune system, they may represent a valid option for treatment of AD. Numerous studies support the use of probiotics in AD treatment. Nonetheless, differences in outcome are related to composition and route of administration.^{25–29} As the mechanisms of action of probiotics are strictly strain dependent,³⁰ they should be evaluated for specific targets before any properties are claimed. Our previous studies^{31,32} performed with *Lactobacillus salivarius* LS01 in adult AD patients showed a favorable effect on AD symptoms by improving intestinal permeability, lowering *S. aureus* fecal counts, modulating cytokine release, and ameliorating SCORAD index. The observed improvement in intestinal barrier function led to the development of a new formulation containing a higher bacterial concentration of *L. salivarius* LS01 and an association with *Streptococcus thermophilus* ST10 and tara gum. *S. thermophilus* ST10 and tara gum form a gelling complex that is able to adhere to intestinal mucus, with mechanical activity that should enhance intestinal barrier function.

In this study, we present data obtained in a prospective, controlled pilot trial designed to evaluate the efficacy of this new formulation in an AD adult population.

METHODS

Study Population and Study Design

Patients aged 25 to 63 years treated by the Clinical Dermatology Unit of IRCCS Galeazzi Orthopaedic Institute in Milan, Italy, were included in the study. The inclusion criteria were: AD according to Hanifin and Rajka,³³ with predominant rough and fissured skin as well as pruritus for at least 2 months. Pregnant or lactating women were excluded.

The primary exclusion factors were the presence of chronic dermatosis such as seborrheic dermatitis, contact dermatitis, nummular eczema, psoriasis, ichthyosis, an immunodeficiency or any immunological disorder, scabies, cutaneous fungal infection, HIV-associated skin disorders, malignant diseases, T-cell lymphoma, Letterer-Siwe disease, progressive systemic diseases, serious internal diseases (eg, serious decompensated diseases of the heart, liver, and/or kidneys, or diabetes mellitus), or hypersensitivity toward one of the ingredients in the investigational product. Study participants who had been taking part in another study or had taken an investigational product during the last 4 weeks before the start of treatment were not allowed to take part in the study.

The study participants were allowed to continue to use any medication that they had been taking before the study at the same dose, unless the medication could be discontinued. Prohibited medications before the start of the study and during the study were the following: 30 days before the start of the study, physical ultraviolet therapy, anti-inflammatory medications used to treat AD,

and immunomodulating medications were not allowed. Fourteen days before the start of the study, nonsteroidal antirheumatic drugs, systemic glucocorticosteroids, tranquillizers, or antiemetic agents from the phenothiazine group were forbidden, and 7 days before the study, patients were not permitted to take antidepressants.

The study was designed as a prospective, controlled, pilot trial. Patients were randomized on a 1:1 ratio to receive the active treatment (group A) or a placebo (group B). A total of 35 patients were screened and 10 screening failures occurred; forbidden medications (pimecrolimus, antidepressants; $n = 2$), holidays ($n = 2$), refusal of fecal sampling ($n = 1$), no interest in participating ($n = 1$), and no reason specified ($n = 4$). Twenty-five patients were allocated into 2 groups: group A comprised 13 patients who were given the probiotic preparation, and group B included 12 patients receiving placebo, as control group.

The study was conducted according to ICH guidelines for Good Clinical Practice. All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000 and 2008. The study was carried out after obtaining informed consent from all the patients, and in line with the guidelines for experimental studies on humans applicable within our Institute. Informed verbal and written consent was obtained from all patients.

The objective SCORing Atopic Dermatitis (SCORAD) index and *S. aureus* and clostridial fecal counts were assessed before treatment (T0) and after 30 days of treatment (T1). As safety parameters, the occurrence of adverse events, hematological and clinical-chemical laboratory tests, and vital signs were assessed.

Probiotic Administration

The probiotic product (lot 001/13 Probiotal, Novara, Italy) used in the study contained a freeze-dried mixture of 5×10^9 CFU/sachet of *L. salivarius* LS01 (DSM 22775), 2×10^9 CFU/sachet of *S. thermophilus* ST10 (DSM 25246), and tara gum (125 mg). The placebo sachets contained gluten-free maltodextrins (lot 001/13, Probiotal). The products were maintained at 4°C in the dark until use, when 1 sachet was dissolved in water. Patients were given a box containing 30 sachets of either the probiotic mixture or the placebo, enough for 1 month of treatment.

Fecal Samples

Fecal samples were collected before treatment and at the end of 30 days treatment. Samples were stored at -80°C until analysis. One gram of feces was dissolved in 10 mL of a 0.5 g/L peptone saline solution and, after proper dilution in the same medium, aliquots of 10 and 100 μL were seeded onto Schaeffer blood agar (SCH), *Clostridium difficile* (CD) agar, and mannitol salt (MSA) agar. All media were purchased from BioMerieux (Marcy L'Etoile, France). After incubation in anaerobiosis for 72 hours at 37°C, colonies of spore-forming gram-positive bacilli grown on SCH and CD were identified by DNA sequencing. Colonies of gram-positive catalase-positive cocci grown on MSA plates after incubation for 48 hours in aerobiosis at 37°C were identified by means of biochemical tests (Vitek2 System, BioMerieux). The limit of detection was 2 log CFU/g of feces.

DNA Sequencing

Identification of clostridia was achieved by DNA sequencing of variable regions V1 and V3 of the 16S rRNA gene by pyrosequencing (PSQ96RA, Diatech, Jesi, Italy).

In summary, suspensions of about 10^{10} CFU/mL of bacteria from pure cultures were heated to 100°C for 10 minutes and centrifuged at $18,000g$ for 2 minutes. Supernatants were stored at -20°C until analysis. Primers and PCR settings were chosen as previously described by other authors.³⁴ The sequences obtained were inserted in BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for accurate identification.

Statistical Analysis

Differences in sex between the 2 groups were calculated using a χ^2 test. Differences in SCORAD and bacterial counts between treatment groups were evaluated by means of Student *t* test. Variations in SCORAD and bacterial counts at T0 and T1 in the same group were compared by *t* test for paired data.

RESULTS

Study Population

All of the 25 patients who agreed to commence the protocol completed the study. At the beginning of the study, no statistical differences were found between the placebo and treated groups in terms of age, male/female ratio, and SCORAD scores (Table 1).

Clinical Effects of Probiotic Administration

Patients treated with the probiotic (group A) generally experienced an improvement in their AD symptoms, especially in the first week of treatment, which was maintained during the treatment period. In particular, they reported an improvement in pruritus, which led to a decrease in the use of topical corticosteroids and emollient products. No differences in AD symptoms were noted by patients treated with placebo. No significant adverse events were registered during the study.

The SCORAD index significantly diminished in the active group from T0 to T1 ($P < 0.0001$, Fig. 1), whereas no variations were observed in the placebo group ($P = 0.274$, Fig. 1). After 1 month of treatment, the SCORAD index in group A was significantly lower than in group B ($P = 0.015$).

Changes in Fecal Microflora

No changes in fecal counts of clostridia were observed for the 2 groups (Fig. 2). In contrast, in 10 of the 13 patients in group A, a variable decrease in the amount of *S. aureus*, ranging from -1.04 to -0.18 log CFU/g, was observed ($P = 0.083$). No changes were observed in group B, with

TABLE 1. Clinical and Epidemiological Data for Patients Enrolled in the Study

Characteristics	Group A	Group B	<i>P</i>
No. patients	13	12	
Age (y)	43.6 ± 10.7	41.7 ± 11.8	0.679
Sex (male/female)	8/5	7/5	0.705
SCORAD index	40.62 ± 4.47	40.05 ± 3.71	0.757

Age and SCORing of Atopic Dermatitis (SCORAD index) are expressed as mean $\pm$ SD.

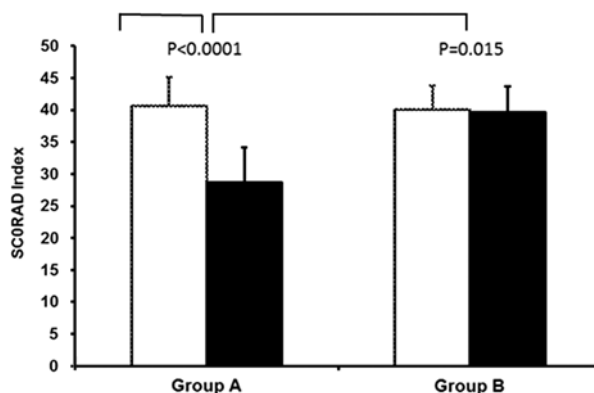


FIGURE 1. SCORing of Atopic Dermatitis index at baseline (white bars) and after 1 month of treatment (black bars). Group A: probiotic; group B: placebo.

only 1 patient showing a decrease in fecal *S. aureus*, as shown in Figure 2.

DISCUSSION

The rationale for the use of probiotics in allergic diseases including AD derives from their ability to rebalance the intestinal microbiota, and modulation of inflammatory mediators.

Strain-dependent effects of probiotics in AD have been described in several studies and have been summarized in recent reviews.^{29,35–37} Restoration of intestinal barrier function, stimulation of intestinal IgA production, and modulation of cytokine production have been observed in clinical studies, while probiotics have been shown in vitro to downregulate Th2 and regulatory cytokines.²⁹ However, despite the numerous studies performed, the efficacy of probiotic administration for the treatment or prevention of AD remains controversial. Moreover, as probiotic activity is strictly strain dependent, comparison of the results from different trials is complicated. Because of the strain-specific activity of probiotics, careful evaluation of their effects should be carried out before marketing of probiotics-based products.

In a previous study by our group, a 4-month administration of *L. salivarius* LS01 at a lower dose to adults with AD has been shown to be associated with an improvement in SCORAD index and a significant decrease in staphylococcal fecal content.³⁷ Therefore, we hypothesized that

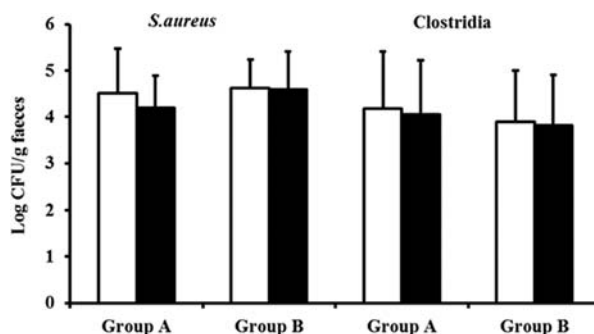


FIGURE 2. Fecal counts of *Staphylococcus aureus* and clostridia at baseline (white bars) and after 1 month of treatment (black bars). Group A: probiotic; group B: placebo.

the addition of *S. thermophilus* ST10 and tara gum to *L. salivarius* LS01 at higher concentrations could improve the effects of the *L. salivarius* strain alone. The major finding of this study is the improvement in SCORAD index observed after only 1 month of treatment by patients included in the active group. Improvement in SCORAD was reflected by the reporting of less intense pruritus suffered by treated subjects, particularly in the first week of treatment. Unfortunately, registration of consumption of topical corticosteroids or other remedies was not planned as part of the study design, so it is not possible to statistically evaluate these data. It has been reported that improvement in SCORAD index persists until probiotic supplementation is discontinued²⁷ and prolonged administration leads to a more persistent improvement in SCORAD index.³⁸ The previous observation that *L. salivarius* LS01 is able to persist in the gut after the end of administration³¹ suggests a longer lasting effect, which, however, needs to be confirmed by further studies. In the previous study,³¹ a significant decrease in fecal *S. aureus* was observed after 16 weeks of treatment. This trend was only partially confirmed by this study, in which the observed decrease was not so significant as previously reported. However, it should be noted that, in this study, probiotic treatment lasted only 30 days, which is much shorter compared with the previous study. It may be hypothesized that prolonged treatment should yield a more marked difference in fecal counts.

Colonization of skin and nares by *S. aureus* is a common feature in AD subjects and a correlation between skin *S. aureus* and barrier impairment has been reported.¹⁶ Conversely, fewer data are available on the role of *S. aureus* in the gut. Higher fecal counts of staphylococci have been found in AD patients compared with healthy subjects,^{39,40} leading to the hypothesis that intestinal *S. aureus* may influence skin symptoms in AD. Our study seems to confirm this hypothesis, although this is not supported by statistical analysis. It would be interesting in further studies to compare fecal *S. aureus* with biotypes isolated from skin, to better characterize the relationship between staphylococcal intestinal and skin colonization.

This study, like all pilot studies, presents some limitations: it has been conducted on a limited population and follow-up data after probiotic discontinuation are lacking. Nonetheless, it has shown the beneficial effects that administration of *L. salivarius* is able to induce in adult AD patients.

In conclusion, data obtained provide sufficient information for future studies aimed at providing a more in-depth evaluation of the effects of this new formulation in a larger population.

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