Probiotics Reduce Gut Microbial Translocation and Improve Adult Atopic Dermatitis

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Background: It has been suggested that probiotics modulate atopic dermatitis (AD) progression, but no data are actually available on their mechanisms of action and on their ability to act as immunomodulators in this pathology.

Objective: The aim of this randomized double-blinded active treatment versus placebo study was to evaluate clinical efficacy of an intake of a combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) for the treatment of adult AD patients.

Methods: Forty-eight patients were enrolled in the study (randomization ratio 2:1) and treated with a combination (LS01 and BR03) or placebo (maltodextrin) for 12 weeks. Clinical efficacy was assessed from baseline by changes in the SCORAD index and DLQ index improvement. Analysis on the gut permeability barrier, immunologic parameters, and changes in fecal microbiota and recovery of probiotics were performed at baseline, at the end of therapy, and 2 months later.

Results: Patients receiving probiotics showed a significant improvement in clinical parameters (SCORAD, $P < 0.0001$ and DLQ index, $P = 0.021$) from baseline. The probiotics reduced microbial translocation ($P = 0.050$), immune activation ($P < 0.001$), improved T-helper cell (Th)17/regulatory T cell (Treg) ($P = 0.029$) and Th1/Th2 ($P = 0.028$) ratios. None of these changes were observed in the placebo group.

Conclusions: Our results suggest that this specific mixture of probiotics (LS01 and BR03 strains) may induce beneficial effects for clinical and immunologic alterations in adult AD. This combination could be considered as adjuvant therapy for the treatment of AD in adult patients.

Key Words: atopic dermatitis, probiotics, microbiota, T-helper cells, regulatory T cells

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Atopic dermatitis (AD) is an inflammatory and pruritic chronic relapsing skin disorder. There is direct and indirect evidence that its prevalence has increased 2- to 3-fold over the last 30 years.[1] The barrier defects in AD seem to go beyond the skin and involve the intestinal mucosa[2] where indigenous intestinal microflora contributes to the mucosal barrier function and stabilizes intestinal permeability.[3]

Probiotics were used in irritable bowel syndrome[4] and seemed to be able to modulate mucosal immune responses and reduce gastrointestinal inflammation in infants with food allergies.[5] These observations are likely to be secondary to the fact that the normal gut microflora plays a critical role in developing and maintaining normal immune functions and tolerance.[6,7]

Probiotics have additional immunomodulatory action[8] by exerting stimulatory effects on the intestinal innate and adaptive immune system,[9,10] enhancing mucosal barrier functions,[11] inducing the production of anti-inflammatory cytokines,[12] and facilitating the maintenance of immune tolerance.[13,14]

Some studies suggest that probiotics could prevent the development of atopic diseases[15,16] and might have beneficial effects in the treatment of these disorders.[17–19] These effects were not observed in all studies and, to date, probiotics cannot be recommended for treating atopic eczema.[20] The different probiotics strains used, the study designs, the characteristics of populations enrolled, and the duration of treatments may justify these contrasting results. Moreover, few studies are actually available for the adult population, despite demonstrating the positive effects of probiotics.[21–26] Thus, clinical trials are needed to evaluate the effects of probiotics on AD in adults and to explain their biological and immunologic mechanisms of action.

We chose the *Lactobacillus salivarius* (patented *L. salivarius* LS01 DSM 22775) strain that, in a preclinical evaluation, reduced the release of type 2 cytokines and induced an improvement in the T-helper cell (Th)1/Th2 ratio.[27] Furthermore, type 2 cytokines [interleukin (IL)-4 and IL-13] play an important role in the pathogenesis of AD and dominate inflammatory milieu in atopic skin lesions by reducing filaggrin production.[26]

Bifidobacteria strains were selected, because they were present in a lesser proportion in atopic eczema infant feces[28–31] and, in particular, a supply of *Bifidobacterium breve* was found to be effective.[32] Clinical, immunologic, and microbiological parameters were evaluated before treatment, at the end of treatment and 2 months later. This aimed to assess the effectiveness of a new combination of these probiotics (LS01 and BR03).

MATERIALS AND METHODS

Patients and Study Design

Sixty adult patients suffering from AD were enrolled at the Allergy Unit of Ospedale Sacco in Milan. Forty-eight

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The authors declare that they have nothing to disclose.

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were assessed for eligibility. The study was conducted from April to September 2010. Patients were randomized with a 2:1 ratio in a double-blinded placebo-controlled study to receive active treatment (probiotic, n = 32, group A: 14 males and 18 females; aged, 32.44 ± 1.47 y or placebo, n = 16, group B: 6 males and 10 females; aged, 30.91 ± 2.79 y; Fig. 1).

Thirty-two patients (23 in group A and 9 in group B) had respiratory allergy and 6 (3 in both groups) had food allergies. Contact allergy dermatitis was reported by 5 patients (3 in group A and 2 in group B). Seven patients had no history of allergies (5 in group A and 2 in group B).

Probiotic and placebo sachets were matched for size, shape, and volume of contents. Group A received a mixture of *Lactobacillus* LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604 at a dose of 1×10^9 colony-forming units (CFU)/g each in maltodextrin and group B received maltodextrin alone, twice daily for 12 weeks. Supplements were stored as stable freeze-dried powder (in sachet packets provided by Probiotical Spa, Italy). Compliance was monitored through a dose count (returned sachet packets). During the study, patients were allowed to use oral antihistamines or emollient cream and were asked to limit their exposure to the sun as much as possible, at least in the first 3 months. None of the patients changed their diet, but they had to avoid any fermented food products containing live microorganisms and antibiotics administration. The data on allergic sensitization were obtained from clinical symptoms together with the results of skin prick tests for respiratory or food allergens and through the CAP system test (IgE > 0.35 kU/L were considered positive).

Patients who were receiving probiotics, antibiotics, or immune-modulating agents 6 months before the study period or oral steroid treatment 1 month before enrollment were excluded from the study.

The study was approved by the Local Ethics Committee, and all patients gave their written informed consent when assessed for eligibility.

Symptom Scores and Quality of Life Questionnaire

The diagnostic criteria of AD were confirmed following published clinical guidelines in 2004.[33] The clinical severity was evaluated using the SCORAD index, as it is one of the most validated scoring systems.[34] This system considers both objective signs (severity and extension) and subjective signs (pruritus and loss of sleep). The range of the SCORAD score is 0 to 80, and the eczema was graded as mild (SCORAD score 0 to 15), moderate (SCORAD score 16 to 40), or severe (SCORAD score > 40).

To measure how much patients evaluated the improvement of AD disability symptoms during the study, they had to answer the DLQ index (DLQI) questionnaire.[35] The questionnaire was structured with each question having 4 alternative responses: "not at all," "a little," "a lot," or "very much" with corresponding scores of 0, 1, 2, and 3, respectively. The DLQI was calculated by summing the score of each question, resulting in a maximum of 30 and a minimum of 0. The highest score corresponded to the greatest impairment of quality of life. A single investigator, blinded to the treatment arm, performed all SCORAD assessments, whereas the DLQI was performed by the patients at weeks 0, 12, and 20.

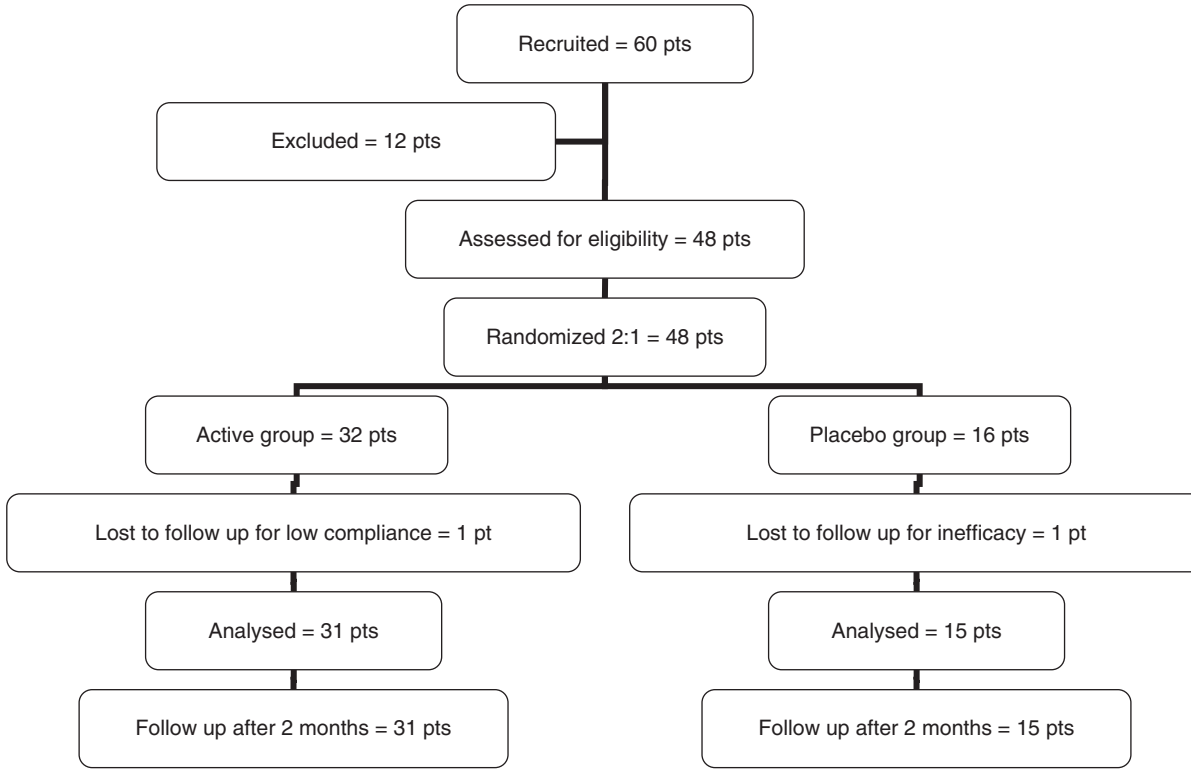

FIGURE 1. CONSORT flow diagram: Enrollment and follow-up diagram of patients included in the study.

Stimulation of Peripheral Blood Mononuclear Cell (PBMC)

PBMC were isolated from whole-blood samples using a lymphocyte separation medium (Cedarlane Laboratories Limited, Hornby, ON, Canada). The PBMC were incubated for 18 hours in the presence/absence of the LPS antigen (Sigma-Aldrich, St. Louis, MO). For the cytokine analysis, 10 μg/mL Brefeldin A (Sigma-Aldrich) was added to cell cultures during the last 6 hours to block protein secretion.

Immunophenotypic Analysis

Activated $CD8^+$ T lymphocytes were evaluated using 50 μL of EDTA-treated peripheral blood incubated for 10 minutes at room treatment with flourochrome-labeled monoclonal antibodies (mAbs): CD8 PC5, CD38PE, and CD45RO FITC. After incubation, erythrocyte lysis and fixation of stained cells were performed using the Immuno-Prep EPICS Kit and Q-prep Work Station (Coulter Electronics, Miami Lakes, FL). Samples were then analyzed in flow cytometry as previously described.[36]

Identification of Th1, Th2, Th17, and Regulatory T Cells (Treg) Lymphocyte Subpopulations

Th1 lymphocytes were identified as interferon (IFN)-γ-secreting, Tbet-expressing $CD4^+$ T cells using the following mAbs: CD4 PC7, Tbet PC5, and IFNγ FITC and Th2 subsets were identified as IL-4-secreting, GATA3-expressing $CD4^+$ T cells using the following mAbs: CD4 PC7, GATA3 PC5, IL-4 PE FITC mAbs. mAbs specific for CD4 FITC, RORγT PE, and IL-17 PC5 were used for Th17 identification (IL-17-secreting, RORγT-expressing $CD4^+$ T cells) and Treg were identified with CD4 PC7, CD25 ECD, FoxP3 PC5, TGF-β PE, and IL-10 FITC mAbs (IL-10-secreting and TGF-β-secreting, Foxp3-expressing $CD25^+$ $CD4^+$ T cells). Unstimulated and stimulated PBMC were washed in phosphate-buffered saline and stained with CD4 and CD25 mAbs for 15 minutes in the dark. The intracellular staining was performed following the manufacturer's protocol (eBioscience Inc., San Diego, CA).

Plasma LPS Concentration

Plasma LPS concentration was measured with the LAL Chromogenic Endopoint Assay (Hycult biotechnology, Uden, the Netherlands) according to manufacturer's instructions. LPS concentration was expressed in pg/mL and calculated based on a standard curve.

Quantification of Cultivable Bacteria in Fecal Samples

Fecal samples were collected at baseline, after 3 months treatment, and 2 months after suspension of treatment, and stored at −80°C until analysis. Quantification was performed for Enterobacteriaceae, Staphylococci, Lactobacilli (in particular *Lactobacillus* LS01 DSM 2275) and Bifidobacteria (in particular *Bifidobacterium* BR03 DSM 16604). The total aerobes were also quantified.

The weighed fecal samples were serially diluted in a sterile physiological solution (NaCl 9 g/L). The appropriate dilutions were plated onto all media and incubated at 37°C in accordance with the following schedule: total aerobes: AS, 10% CO_2 for 24 hours; enterobacteriaceae: MC, aerobiosis for 24 hours; staphylococci: MSA, aerobiosis for 48 hours; lactobacilli: MRS, 10% CO_2 for 48 hours; bifidobacteria: BSM, anaerobiosis for 72 hours.

All colonies of differing morphology were identified according to: growth on selective medium, Gram staining, colony and cell morphology, and the catalase and oxidase tests.

The gut microbiota composition was expressed as counts (mean ± SD of $\log_{10}$ CFU per gram of wet feces) of each bacterial group.

Changes in the colony counts of the different groups of microorganisms were calculated as follows: [($\log_{10}$ CFU/g at T12 or T16) − ($\log_{10}$ CFU/g at T0)].

Molecular Identification of *Lactobacillus* LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604

Lactobacillus LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604 were previously identified according to their particular morphology: *L. salivarius* LS01 forms round, sharp-shaped, cream-white colonies of a 2 to 4 mm diameter on MRS agar; *B. breve* BR03 forms round, sharp-shaped, fuchsia colonies of a 1 to 2 mm diameter on BSM agar.

On each plate containing supposed colonies of *L. salivarius* and *B. breve*, 10 of these colonies were randomly selected and identified by polymerase chain reaction (PCR) using specific primers and conditions for *L. salivarius* and *Bifidobacterium* spp. according to the Italian Superior Institute of Health standards.[37] All the colonies identified as *L. salivarius* or *B. breve* by PCR were further analyzed using PFGE to ensure they belonged to the LS01 or BR03 strains. Pulsed-field gel electrophoresis (PFGE) was performed as previously described.[38–40] PFGE profiles were compared with those obtained with the reference strains *L. salivarius* LS01 and *B. breve* BR03.

Statistical Analysis

Clinical data were analyzed according to the intention-to-treat approach. If there were no data for the last visit, the tests were repeated twice, assigning the variable: (1) the last value observed that is hypothesizing no further improvement and (2) the mean value for the whole sample. To account for the potential confounding effect, the analysis of variance was performed including the clinical variable initial value (SCORAD and DQLI) in the model.

Variations in bacterial counts were analyzed by means of the Wilcoxon-Mann-Whitney test and immune responses in the 2 groups of patients were evaluated using a *t* test.

Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL).

RESULTS

Clinical Results

Forty-six patients completed the trial; 2 patients (1 in each group) were lost at follow-up and were excluded by final analysis (Fig. 1).

No significant adverse event was recorded during the study in the 2 groups and compliance was similar. Antihistamine treatment was similarly used by individuals in the 2 groups.

Patients receiving probiotics showed a significant reduction of SCORAD at the end of treatment ($P = 0.001$). This persisted after suspension ($P = 0.006$) (Table 1) and an improvement in DLQI (baseline vs. 3 mo after treatment: $P = 0.024$; baseline vs. 2 mo after suspension: $P = 0.001$).

As compared with baseline, DLQI decreased significantly in the active treatment arm, whereas we did not find any difference in the placebo arm.

TABLE 1. SCORAD score and DLQ Index†

	Probiotic Pts (n = 31)			Placebo Pts (n = 15)			
	Baseline	After 3-mo Treatment	2 mo After Suspension	Baseline	After 3-mo Treatment	2 mo After Suspension	P*
SCORAD score	46.25 ± 3.70	29.45 ± 2.01	22.63 ± 2.81	45.00 ± 2.60	40.21 ± 1.53	38.43 ± 1.38	Baseline vs after 3-mo treatment: $P < 0.001$ baseline vs. 2 mo after suspension: $P = 0.006$
DLQ index	9.16 ± 0.80	6.58 ± 1.25	4.73 ± 0.75	6.52 ± 0.40	8.50 ± 1.27	5.29 ± 0.97	Baseline vs. after 3-mo treatment: $P = 0.024$ baseline vs. 2 mo after suspension: $P = 0.001$

*Significant differences in probiotic patients.
†SCORAD score and DLQ index in probiotic and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension. Mean values and SE are shown.
DLQ indicates Dermatology Life Quality; pts, patients.

No significant differences were reported in placebo individuals either for SCORAD or DLQI (Table 1).

Plasma LPS

Plasmatic LPS concentration is a marker of altered gut barrier permeability, and it was reported to be associated with Toll-like receptor–dependent immune activation. Patients receiving probiotics showed a reduction in LPS plasma concentration that was maintained after suspension of treatment (baseline vs. 3-mo treatment: $P = 0.050$; baseline vs. 2 mo after suspension: $P < 0.001$; 3-mo treatment vs. 2 ms after suspension: $P < 0.001$) (Fig. 2A). In placebo patients, plasma LPS levels increased during the study (baseline vs. 2 mo after suspension: $P = 0.004$; 3-mo treatment vs. 2 mo after suspension: $P = 0.016$) (Fig. 2A). Plasma LPS concentrations were significantly higher in the placebo group compared with the probiotic group at the end of study period ($P < 0.001$).

Activated T Lymphocytes

The percentage of CD8/CD38/CD45RO T cells indicates systemic immune activation. A progressive reduction of activated lymphocytes was observed during the study period in probiotic-treated individuals alone (baseline vs. 2 mo after suspension: $P < 0.001$; 3-mo treatment vs. 2 mo after suspension: $P < 0.001$) (Fig. 2B).

Tregs

Tregs play a crucial role in tolerance and in immune regulation. Tregs were evaluated both in unstimulated conditions and after stimulation with LPS. Unstimulated Tregs were significantly increased by probiotics; this effect persisted 2 months after treatment suspension (baseline vs. 2 mo after suspension: $P = 0.002$; 3 mo treatment vs. 2 mo after suspension: $P = 0.034$) (Figs. 3A, B). A similar trend was observed for LPS-stimulated Tregs. These results suggest that probiotic supplementation is effective in reducing gut alterations, improving its tolerogenic functions.

Th1, Th2, and Th17 Subpopulations

Probiotics induced a significant increase in Th1 and reduction of Th2 and Th17 T-cell subpopulations. In particular, IFN-γ-secreting Tbet-expressing $CD4^+$ T cells (Th1) significantly increased in individuals receiving probiotics both in basal and in stimulated conditions (unstimulated: baseline vs. 3-mo treatment: $P = 0.003$; LPS-stimulated: baseline vs. 3-mo treatment: $P = 0.025$; baseline vs. 2 mo after suspension: $P = 0.019$) (Figs. 4A, B). Two months after suspension, a significant rereduction in Th1 cells was observed in probiotic patients ($P < 0.001$).

Th2 cells (IL-4-secreting, GATA3-expressing $CD4^+$ T cells) were significantly reduced by probiotics but reincreased after treatment suspension (unstimulated: baseline

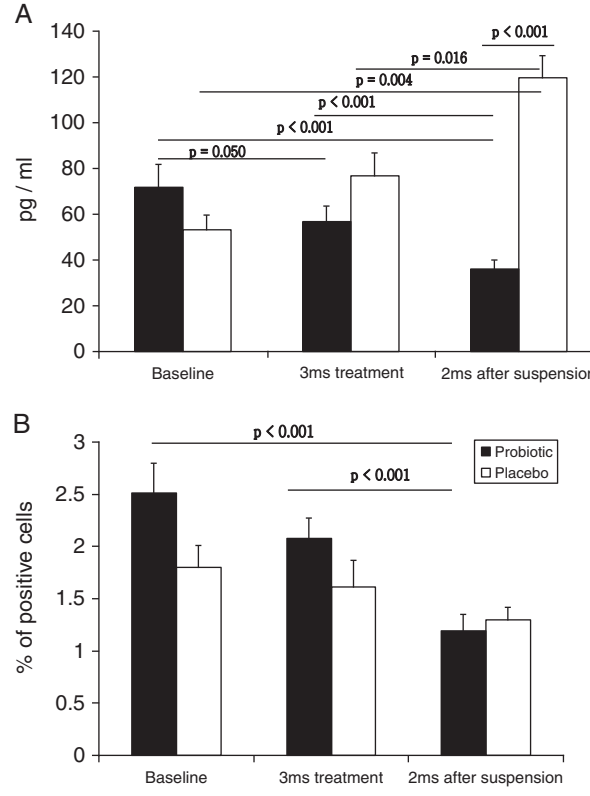

FIGURE 2. LPS and activated T cells: Plasma LPS concentrations (A) and percentage of CD38-CD45RO-CD8-expressing T cells (B) in probiotics and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension of treatment. Mean values, SE, and *P*-values are shown.

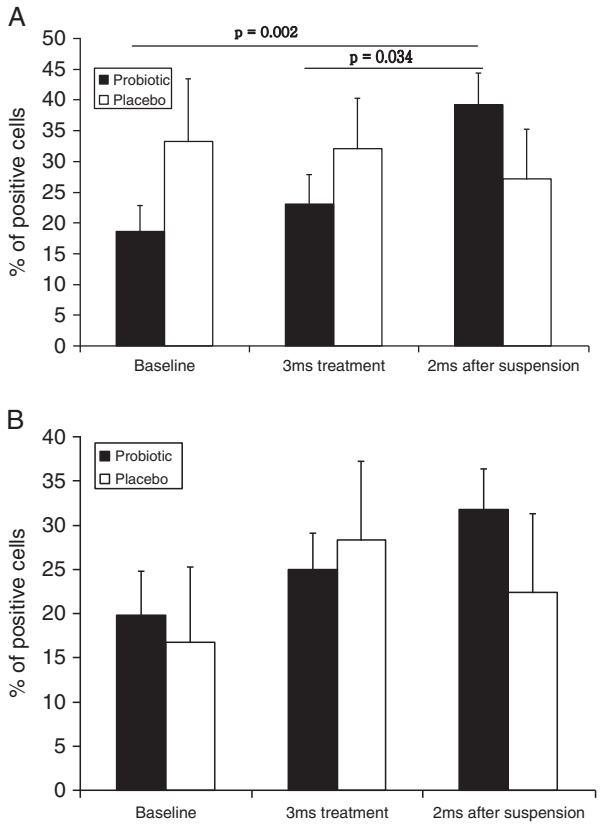

FIGURE 3. Regulatory T cells: TGF-β-secreting and IL-10-secreting regulatory T cells in unstimulated (A) and LPS-stimulated conditions (B) in probiotics and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension of treatment. Mean values, SE, and *P*-values are shown. IL indicates interleukin; TGF, transforming growth factor.

vs. 3-mo treatment: $P = 0.016$; 3-mo treatment vs. 2 mo after suspension: $P = 0.005$; LPS-stimulated: 3-mo treatment vs. 2 mo after suspension: $P = 0.045$) (Figs. 4C, D).

In individuals receiving probiotics IL-17-secreting, RORγT-expressing $CD4^+$ T cells (Th17) reduced after 3 months of treatment (unstimulated: baseline vs. 3-mo treatment: $P = 0.037$; LPS-stimulated: baseline vs. 3-mo treatment: $P = 0.046$) (Figs. 4E, F).

Two months after treatment suspension, significant differences were observed between probiotic and placebo patients in Th2 and in Th17 cells ($P = 0.008$ and 0.031, respectively). No significant differences were reported in Th1, Th2, and Th17 subpopulations in the placebo group.

In analyzing the Th subpopulations ratios, we observed that a significant increase in the Th1/Th2 ratio was detected after 3 months of probiotics ($P = 0.028$) (Fig. 5A). This effect was lost after treatment suspension ($P = 0.002$). On the contrary, the Th17/Treg ratio progressively decreased in the probiotic group, and this reduction was maintained even 2 months after the suspension of probiotic treatment ($P = 0.029$). Neither Th1/Th2 nor Th17/Treg ratio changed in the placebo group during the study period, whereas the Th17/Treg ratio was significantly higher in the placebo group compared with the probiotic group after 3 months of treatment ($P = 0.037$) (Fig. 5B).

These data suggest that probiotics are able to restore a favorable balance between the different Th subtypes involved in the AD pathogenesis.

Correlations

A negative correlation was only observed in probiotic patients at the end of treatment between SCORAD and LPS-stimulated Treg ($P = 0.020$) and between the percentage of CD8/CD38/CD45RO T cells and LPS-stimulated Th1 cells ($P = 0.046$). Again, in the same group of patients, a significant positive correlation was detected between plasma LPS concentrations and unstimulated Th2 cells ($P = 0.016$) and LPS-stimulated Th17 ($P = 0.004$) and between the percentage of CD8/CD38/CD45RO T cells and LPS-stimulated Th17 ($P = 0.037$) (data not shown).

These correlations suggest that both microbial translocation and immune activation are associated with different Th subtypes in AD and can worsen the disease (Th2 and Th17). However, clinical improvement induced by probiotic treatment is related to a recovery of the immune system's tolerogenic functions (Treg).

Changes in Fecal Microbiota and Recovery of *Lactobacillus* LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604

In placebo patients, there was no significant change in fecal flora. A significant decrease in the staphylococcal load was observed in treated patients after 3 months of treatment ($P = 0.037$). This decrease is significant when compared with placebo patients ($P = 0.047$). This reduction remained statistically relevant 2 months after probiotic suspension. Significant changes were not detected in other bacterial groups.

The recovery of probiotic strains in feces from treated patients is shown in Table 2. All colonies with the typical morphology, identified by PCR and analyzed by PFGE, corresponded to the previously identified *Lactobacillus* LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604 strains.

L. salivarius LS01 was recovered in all subjects after 3 months of treatment, in quantities of 10^2 to 10^5 CFU/g. *B. breve* BR03 was found in 90% of treated subjects after 3 months of treatment, in quantities of 10^6 to 10^8 CFU/g.

Both strains were also isolated 2 months after probiotic suspension in some patients, but in a lower quantity than the quantity observed immediately after the end of the treatment.

DISCUSSION

The combination of probiotics (*Lactobacillus* LS01 DSM 2275 and *Bifidobacterium* BR03 DSM 16604) studied here improved clinical and immunologic parameters in adults suffering from moderate to severe AD. At the end of probiotic treatment, we particularly observed: (1) a significant improvement in SCORAD and DLQI; (2) a reduction in microbial translocation (plasma LPS levels) and immune activation (CD8/CD38/CD45RO cells); (3) an increase in Tregs; and (4) an improvement in Th1/Th2 and Th17/Treg ratios. Notably, this mixture was able to colonize the gastrointestinal tract of patients and reduce staphylococcal load in the feces. No significant differences were reported by dividing patients in terms of presence or absence of respiratory or food allergies and contact dermatitis.

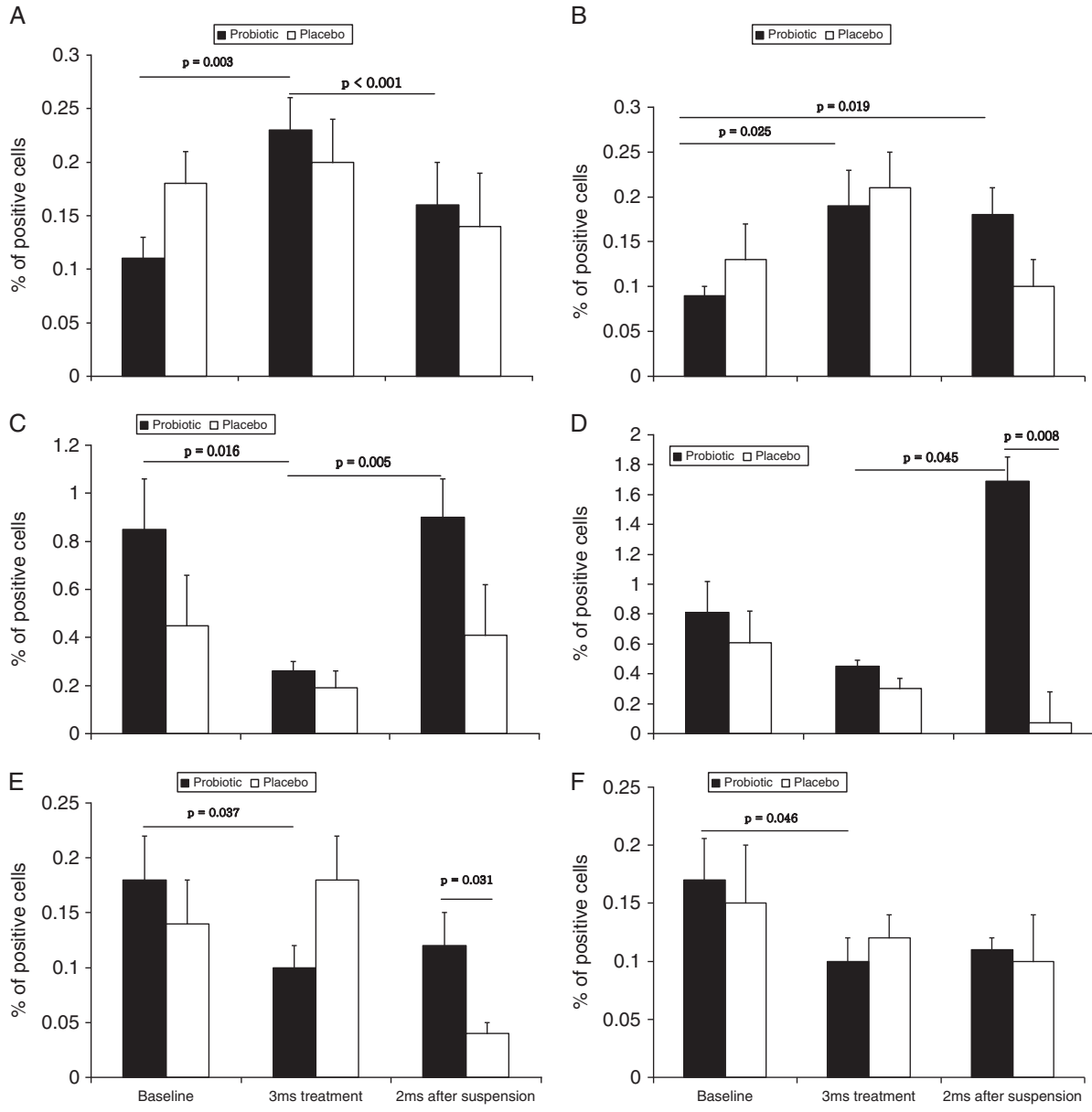

FIGURE 4. Th1, Th2, and Th17 cells: IFN-γ-secreting Tbet-expressing Th1 cells in unstimulated (A) and LPS-stimulated conditions (B); IL-4-secreting GATA3-expressing Th2 cells in unstimulated (C) and LPS-stimulated conditions (D); IL-17-secreting RORγt-expressing Th17 cells in unstimulated (E) and LPS-stimulated conditions (F) in probiotic and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension of treatment. Mean values, SE, and *P*-values are shown. IFN indicates interferon; IL, interleukin; Th, T-helper cell.

Clinical improvement was evident after 3 months of probiotic treatment and continued for 2 months after treatment suspension. We also observed the continuance of the 2 bacterial strains in the gastrointestinal tract even after the suspension of treatment, suggesting its ability to colonize the intestine for long time.

Alteration in gut permeability plays a key role in the AD pathogenesis and in other inflammatory diseases.[41] We observed a significant reduction of microbial translocation during and after probiotic treatment. These data indicate that microbial translocation is effectively involved in the AD pathogenesis and that recovery of gut barrier functions could improve the clinical outcome of AD.

Studies on the human immunodeficiency virus infection have shown an association between microbial translocation and immune activation with an excessive turnover of T cells. The percentage of CD8/CD38/CD45RO T cells, considered to be an important prognostic parameter in human immunodeficiency virus, is one of largely accepted markers of LPS-mediated immune activation.[42] As expected, in AD patients enrolled in our study, we observed that plasma LPS reduction is associated with a decrease in CD8/CD38/CD45RO cells. These data demonstrate that our probiotics combination has strong immunomodulatory activity as a consequence of their ability to improve gut barrier functions.

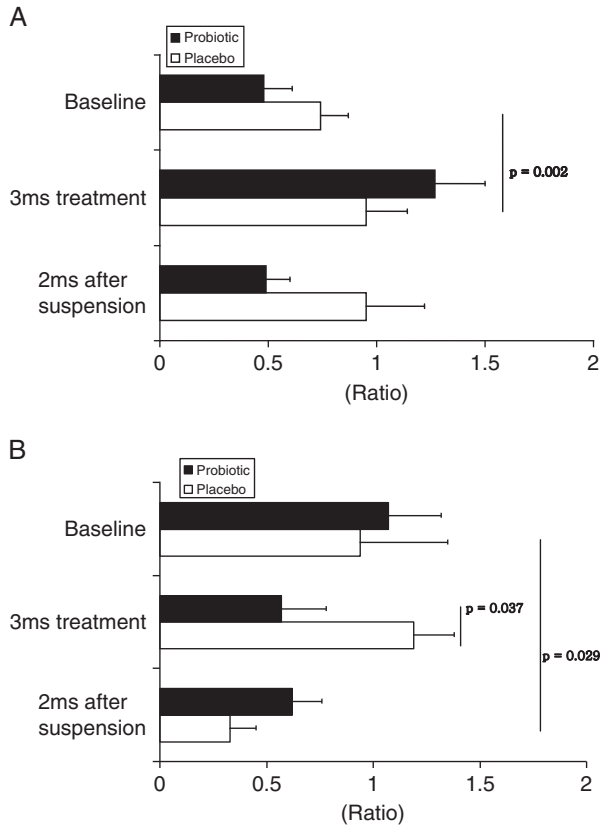

FIGURE 5. Th1/Th2 and Th17/Treg ratios: Th1/Th2 (A) and Th17/Treg (B) ratios in probiotic and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension of treatment. Mean values, SE, and *P*-values are shown. Th indicates T-helper cell; Treg, regulatory T cell.

A decrease in Tregs has been reported in patients with AD and asthma, and the number of Treg is inversely correlated with IgE, eosinophilia, and IFNγ levels.[43,44] Our probiotics combination induced a significant increase in Treg percentage. The beneficial effects of probiotics could be due to a recovery of Treg-mediated immune tolerance. Moreover, a negative correlation was detected between Treg and SCORAD score, suggesting a protective role of these cells in AD evolution.

Th2 cells play a critical role in the pathogenesis of allergic reactions and are responsible for the acute phase of AD, whereas Th1 cells have a protective function associated with the chronic phase of disease. Th17 cells are increased in acute, but not in chronic, lesions of AD skin[45] and positively correlate with disease severity.[46] These data show that this probiotic formulation upregulates Th1 functions and downregulates Th2 and Th17 activity, improving Th1/Th2 and Th17/Treg ratios.

Th2 and Th17 were positively correlated with plasma LPS levels, suggesting an interaction between microbial translocation and the activation of unfavorable Th subtypes in the pathogenesis of this disease. In our study, we did not observe a significant change in microbial composition between the different groups, with the exception of staphylococci that was found to be correlated with disease severity that could be due to IgE hypersensitivity[47,48] or the production of exotoxins with superantigen properties.[49,50] We speculate that this genus could play a crucial role in the skin and in the intestine of patients with AD and that administration of our probiotics combination can rebalance it.

The main limitations of this study include the low patient number and the absence of data on filaggrin, actually considered the most recent pathognomonic marker of AD. Nevertheless, immunologicy and microbiology data support the clinical results and encourages the use of probiotics in adult AD.

Further studies are required to investigate the use of probiotics in adult AD, to explore whether a single strain works better than an association, the duration of treatment, and overall safety.

The higher number of patients with respiratory allergy observed in the probiotic group is a further limitation of our study and could be responsible for the increased LPS concentration and immune activation reported in this group at baseline.

In conclusion, this novel probiotics mixture was well tolerated and resulted in the colonization of gut microbiota and beneficial clinical effects in AD that are associated with a beneficial immune modulation. Overall data suggest that this treatment could be a beneficial adjunct therapy for treating AD in adults.

TABLE 2. Fecal Flora Composition†

	Baseline	After 3-mo Treatment	2 mo After Suspension
Probiotic patients			
Total aerobes	7.7 ± 1.0	7.9 ± 0.9	8.2 ± 0.7
Enterobacteriaceae	6.8 ± 1.3	7.3 ± 1.2	6.7 ± 2.6
Staphylococci	4.0 ± 1.6	2.8 ± 1.5*,**	2.3 ± 1.6*,**
Lactobacilli	5.4 ± 2.0	5.1 ± 1.8	5.5 ± 0.4
Bifidobacteria	9.1 ± 0.6	8.8 ± 0.5	8.4 ± 0.6
Placebo patients			
Total aerobes	7.1 ± 1.5	7.5 ± 1.2	7.7 ± 1.3
Enterobacteriaceae	5.8 ± 1.7	6.7 ± 1.6	6.2 ± 1.5
Staphylococci	4.1 ± 0.9	3.9 ± 1.1	4.0 ± 1.1
Lactobacilli	5.4 ± 1.9	5.2 ± 1.6	5.4 ± 1.8
Bifidobacteria	8.5 ± 1.1	8.6 ± 0.6	8.6 ± 0.9

*Significant decrease ($P < 0.05$) compared with baseline.

**Significant difference ($P < 0.05$) compared with placebo patients at the same time.

†Effect of probiotic administration on fecal flora. Bacterial counts are expressed as mean ± SD of $\log_{10}$ per gram of wet feces.

REFERENCES

1. Diepgen T. Is the prevalence of atopic dermatitis increasing? In: Williams HC, ed. *Atopic Dermatitis*. Cambridge: Cambridge University Press; 2000:96–112.
2. Rosenfeldt V, Benfeldt E, Valerius NH, et al. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr*. 2004;145:612–616.
3. Simon G, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology*. 1994;86:74–93.
4. Ringel Y, Ringel-Kulka T. The rationale and clinical effectiveness of probiotics in irritable bowel syndrome. *J Clin Gastroenterol*. 2011;45(suppl):S145–S148.
5. Del Giudice MM, Leonardi S, Maiello N, et al. Food allergy and probiotics in childhood. *J Clin Gastroenterol*. 2010; 44(suppl 1):S22–S25.
6. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy*. 2000;55:688–697.

7. Heller F, Duchmann R. Intestinal flora and mucosal immune responses. *J Allergy Clin Immunol*. 2003;38:165–172.
8. Lammers KM, Brigidi P, Vitali B, et al. Immunomodulatory effects of prebiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol*. 2003;38:165–172.
9. Mueller C, Macpherson AJ. Layers of mutualism with commensal bacteria protect us from intestinal inflammation. *Gut*. 2006;55:276–284.
10. Rakoff-Nahhoum S, Medzhitov R. Role of the innate immune system and host-commensal mutualism. *Curr Top Microbiol Immunol*. 2006;308:1–18.
11. Ramakrishna BS. Probiotic-induced changes in the intestinal epithelium: implication in the gastrointestinal disease. *Trop Gastroenterol*. 2009;30:76–85.
12. Pessi T, Sütas U, Hume M, et al. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy*. 2000;30:180–188.
13. Huurre A, Laitinen K, Rautava S, et al. Impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization: a double-blind placebo controlled study. *Clin Exp Allergy*. 2008;38:1342–1348.
14. Kilpi T, Kero J, Jokinen J, et al. Common respiratory infections early in life may reduce the risk of atopic dermatitis. *Clin Infect Dis*. 2002;34:620–626.
15. Björkstén B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Clin Immunol*. 2001;108:516–520.
16. Taylor AL, Dunstan JA, Prescott AS. Probiotics supplementation for the first 6 month of life fails to reduce the risk of atopic dermatitis and increase the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol*. 2007;119:184–191.
17. Kamat A, Ancuta P, Blumberg RS, et al. Serological markers for inflammatory bowel disease in AIDS patients with evidence of microbial translocation. *PLoS One*. 2010;5:e15533.
18. Vanderhoof JA. Probiotics in allergy management. *J Pediatr Gastroenterol Nutr*. 2008;47(suppl 2):S38–S40.
19. Betsa GI, Papadavid E, Falagas ME. Probiotics for the treatment or prevention of atopic dermatitis: a review of the evidence from randomized controlled trials. *Am J Clin Dermatol*. 2008;9:93–103.
20. Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J, et al. Probiotics for the treatment of eczema: a systematic review. *Clin Exp Allergy*. 2009;39:1117–1127.
21. Yim JH, Duk K, Ja KK, et al. Therapeutic effects of probiotics in patients with atopic dermatitis. *J Microbiol Biotechnol*. 2006;16:1699–1705.
22. Yoschida Y. Clinical effects of probiotic *Bifidobacterium breve* supplementation in adults patients with atopic dermatitis. *Yonago Acta Medica*. 2010;53:37–45.
23. Roessler A, Friedrich U, Vogelsang H, et al. The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention. *Clin Exp Allergy*. 2008;38:93–102.
24. Drago L, Iemoli E, Rodighiero V, et al. Effects of *Lactobacillus salivarius* LS01 (DSM 22775) treatment on adult atopic dermatitis: a randomized placebo-controlled study. *Int J Immunopathol Pharmacol*. 2011;24:1037–1048.
25. Kaur S, Kullisaar T, Mikelsaar M. Successful management of mild atopic dermatitis in adults with probiotics and emollients. *Eur J Med*. 2008;3:215–220.
26. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol*. 2007;120:150–155.
27. Drago L, Nicola L, Iemoli E, et al. Strain-dependent release of cytokines modulated by *Lactobacillus salivarius* human isolates in an in vitro model. *BMC Res Notes*. 2010;3:44–47.
28. Bjorkesten B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol*. 2001;108:516–520.
29. Kalliomaki M, Kijavainen P, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol*. 2001;107:129–134.
30. Murray CS, Tannock GW, Simon MA, et al. Fecal microbiota in sensitized wheezy and non sentitived non wheezy children: a nested case control study. *Clin Exp Allergy*. 2005;35:741–745.
31. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol*. 2012;129:434–440.
32. Hattori K, Yamamoto A, Sasai M, et al. Effects of administration of bifidobacteria on fecal microflora and clinical symptoms in infants with atopic dermatitis. *Arerugi*. 2003;52:20–30.
33. Einchenfield LF. Consensus guidelines in diagnosis and treatment of atopic dermatitis. *Allergy*. 2004;59:86–92.
34. Schmitt J, Langan S, Williams HC, European Dermato-Epidemiology Network. What are the best outcome measurements for atopic eczema? A systematic review. *J Allergy Clin Immunol*. 2007;120:1389–1398.
35. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI) a simple practical measure for routine clinical use. *Clin Exp Dermatol*. 1994;19:210–216.
36. Piconi S, Parisotto S, Rizzardini G, et al. Hydrxycloroquine drastically reduces immune activation in HIV-infected, ART-treated, immunological non responders. *Blood*. 2011;118:3263–3272.
37. Aureli P, Fiore A, Scalfaro C, et al. Traditional microbiological methods and molecular methods for the analysis of probiotic food supplements for human use: a report ISTISAN 08/36 of Italian Institute of Health. 2008:1–58.
38. Coudeyras S, Marchandin H, Fajon C, et al. Taxonomic and strain-specific identification of the probiotic strain *Lactobacillus rhamnosus* 35 within the *Lactobacillus casei* group. *Appl Environ Microb*. 2008;74:2679–2689.
39. Wall R, Fitzgerald G, Hussey S, et al. Genomic diversity of cultivable Lactobacillus populations residing in the neonatal and adult gastrointestinal tract. *FEMS Microbiol Ecol*. 2007;59:127–137.
40. Briczinski EP, Roberts RF. Technical note: a rapid pulsed-field gel electrophoresis method for analysis of Bifidobacteria. *J Dairy Sci*. 2006;89:2424–2427.
41. Del giudice MM, Rocco A, Capristo C. Probiotics in the atopic march: highlights and new insights. *Dig Liver Dis*. 2006; 38(suppl 2):S288–S290.
42. Douek D. HIV disease progression: immune activation, microbes, and a leaky gut. *Top HIV Med*. 2007;15:114–117.
43. Orihara K, Narita M, Tobe T, et al. Circulating Foxp3 + CD4 + cell numbers in atopic patients and healthy control subjects. *J Allergy Clin Immunol*. 2007;120:960–962.
44. Ito Y, Adachi Y, Makino T, et al. Expansion of FOXP3-positive CD4 + CD25 + T cells associated with disease activity in atopic dermatitis. *Ann Allergy Asthma Immunol*. 2009;103: 160–165.
45. Toda M, Leung DY, Molet S, et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol*. 2003;111:875–881.
46. Koga C, Kabashima K, Shiraishi N, et al. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol*. 2008;128:2625–2630.
47. Breuer K, Wittmann M, Bösche B, et al. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). *Allergy*. 2000;55:551–555.
48. Ong PY, Patel M, Ferdman RM, et al. Association of staphylococcal superantigen-specific immunoglobulin e with mild and moderate atopic dermatitis. *J Pediatr*. 2008;153: 803–806.
49. McFadden JP, Noble WC, Camp RD. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol*. 1993;128:631–632.
50. Skov L, Olsen JV, Giorno R, et al. Application of Staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol*. 2000;105:820–826.