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The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention

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Summary

Background Probiotic bacteria are proposed to alleviate atopic dermatitis (AD) in infants. There are few indications about the effect of probiotics on AD in adults.

Objective The purpose of this study was to elucidate the influence of a probiotic drink containing a combination of the probiotics *Lactobacillus paracasei* Lpc-37, *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 (*B. lactis* 420) in healthy volunteers and in patients with AD on clinical and immunological parameters and their detection in feces.

Methods A double-blind, placebo-controlled, randomized cross-over study was conducted in 15 healthy adults and 15 patients with AD. The probiotic product or placebo was given over 8 weeks. A 2-week washout period was interconnected before the intervention was crossed. At the end of each period, blood and stool samples were collected. In patients, the severity of AD was evaluated using the Scoring of Atopic Dermatitis (SCORAD).

Results *L. paracasei* and *B. lactis* were recovered in high numbers in feces after supplementation, whereas *L. acidophilus* marginally increased. In patients, the SCORAD tended to decrease by 15.5% ($P = 0.081$). Major lymphocyte subsets were not affected by the probiotic intervention. However, $CD57^+$ increased significantly ($P = 0.034$) in healthy subjects after probiotic intake and was not changed in patients, whereas $CD4^+CD54^+$ decreased significantly ($P = 0.031$) in patients with AD and remained uninfluenced in healthy subjects. The expression of $CD4^+CD25^+$ T cells was similar in healthy subjects and AD patients. The phagocytic activity of monocytes and granulocytes was significantly increased in healthy subjects after probiotic intervention ($P = 0.014$).

Conclusion *L. paracasei* Lpc-37 and *B. lactis* 420 are able to colonize the intestine transiently. This study reveals that the probiotics differently modulate peripheral immune parameters in healthy subjects and patients with AD.

Keywords atopic dermatitis, *Bifidobacterium*, $CD4^+CD25^+$, $CD57^+$, human, immune system, inflammation, *Lactobacillus*, probiotic, real-time PCR

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Introduction

The incidence of atopic dermatitis (AD) in children as well as in adults has increased over the past decades [1]. Among the genetic disposition, the so-called 'hygiene hypothesis', which postulates that the environmental changes in the industrialized world have led to a reduced microbial contact in the early childhood, is discussed as a potential cause for the increased prevalence [2, 3]. In this context, the researchers' interest is focused on the role of

probiotics as 'beneficial' microorganisms to counteract the lessened stimulation of the immune system. Oral bacteriotherapy with probiotics may alleviate allergic inflammation, by reducing local and systemic inflammatory markers and allaying the severity and the extent of AD in children [4–6, 8]. However, the immune-stimulating capacity of probiotics varies among strains and is dependent on the immune status of the subjects [7].

Most studies that investigated the effects of probiotic bacteria on AD were conducted in infants with a mean age

from 6 to 21 months [5, 6, 9]. Recently, Kalliomäki et al. [10] and Rautava et al. [11] determined the effect of the probiotic *Lactobacillus rhamnosus* GG given pre- and post-natally to mothers with a family history of atopic disease and their newborns, respectively. They showed a preventive effect of the probiotic strain against the development of atopic eczema in the high-risk children in later life (≤ 4 years of age). However, there is little evidence concerning the influence of probiotics on the immune system in adult patients with AD. The role of probiotics in young adults has been studied previously with respect to respiratory allergies but these regimens have been unsuccessful in improving the clinical symptoms and allergy-associated blood parameters, respectively [12, 13]. In addition, differences in the composition of the microbiota have been reported between infants with atopic eczema and healthy subjects [14]. Three previous studies referred to a decreased proportion of Lactobacilli and Bifidobacteria for the benefit of Staphylococci and Clostridia [15–17]. The microbiota of the gut is the most important source of bacterial stimulation. Mainly, their composition affects the types of signals that transfer immune response via the gut-associated lymphoid tissue. Some strains are responsible for facilitating the induction of specific immunity, while others increase intestinal inflammatory response [18]. As a consequence, the concept of shifting the microbiota by oral administration of health-promoting microorganisms seems to be an effective way to balance the microbiota and influence the immune system [19].

In the current study, healthy subjects and patients with AD were chosen because of their different immune status to investigate the probiotic effects. The primary aim of this trial was to evaluate the probiotic potential of a combination of *Lactobacillus paracasei* Lpc-37, *Lactobacillus acidophilus* 74-2 and *Bifidobacterium lactis* 420 to affect the immune system in healthy adults and patients with mild or moderate AD. Moreover, the administered probiotic species were detected in stool samples after each study period to evaluate their colonization and to assess whether this ability is involved in the regulation of immune response.

Methods

Subjects

For the study, 15 healthy subjects and 19 patients with AD were recruited. Three patients with AD withdrew before starting the trial. From these 31 volunteers, 15 healthy subjects (five males and 10 females) and 15 patients with moderate AD (three males and 12 females) completed the study (Table 1). One patient with AD dropped out because of disease that necessitated a medication with corticosteroids, which was an exclusion criterion. Further exclusion criteria were pregnant or breastfeeding women, immune

Table 1. Baseline data of healthy subjects and patients with AD enrolled in the study

	Healthy subjects (n = 15)	Patients with AD (n = 15)	P-value
Age (years)	24 $\pm$ 3	23 $\pm$ 3	0.317*
BMI (kg/m ²)	22 $\pm$ 3	22 $\pm$ 3	0.514*
IgE (kU/L)	31 $\pm$ 26	1062 $\pm$ 2106	0.002†
ECP (μ g/L)	10.4 $\pm$ 7.3	11.4 $\pm$ 9.8	0.754*
MDC (pg/mL)	614 $\pm$ 250	938 $\pm$ 328	0.005*
IL-16 (pg/mL)	134 $\pm$ 47	230 $\pm$ 126	0.013*

Values are presented as mean $\pm$ standard deviation.

*Student's *t*-test.

†Mann-Whitney *U*-test.

AD, atopic dermatitis; BMI, body mass index; ECP, eosinophilic cationic protein; MDC, macrophage-derived chemokine.

suppressive or cytostatic therapy or systemic steroid and phototherapy or systemic therapy of AD within the last 4 weeks before the start of the study, active infections of the skin, relevant asthma disease, which must be treated with corticoids, intake of antihistamines, antibiotic treatment, subjects with an indigestibility or allergy against the components of milk or lactose intolerance, long-term treatment with systemic steroids, depot steroids, long-acting antihistamines, tranquilizers and psycho-pharmaceuticals, respectively. The topical glucocorticoid Advantan[®] (Scheming, Berlin, Germany) (1 mg/g) was administered in AD patients as the only medical treatment in the case of need. There were no differences in the usage of Advantan[®] between the probiotic and placebo period.

As a precondition for participating in this study, the subjects were provided information in writing and verbally about the details of the study. Informed consent was obtained from all volunteers. Before the beginning of the study, all participants were subject to a medical examination in the Clinic for Dermatology and Dermatologic Allergology of the Friedrich Schiller University, Jena. The AD was diagnosed according to the atopic score of Diepgen [20]. The severity of eczema was evaluated using the Scoring of Atopic Dermatitis (SCORAD) score [21]. A skin prick test was performed for the exclusion of type 1 sensitivity to cow's milk. The study was approved by the Ethical Committee of the Medical Faculty of the Friedrich Schiller University of Jena (administration number 1452–11/04).

Study design

The study was designed as a placebo-controlled and cross-over study that allowed each volunteer to serve as his or her own control. In the 3-week pre-period and throughout the 20-week study duration, all volunteers had to eliminate any pro- and pre-biotic products other than the intervention yoghurt drink from their diet. At the

beginning of the study, all subjects were randomly assigned to two groups. One group (eight patients, seven healthy subjects) received 2×100 mL/day of the probiotic drink and the other group (seven patients, eight healthy subjects) received 2×100 mL/day of the placebo drink over 8 weeks. After a 2-week washout period, the intervention was crossed between the groups and the respective products were consumed for another 8 weeks. To acquire the individual nutritional habits, the participants had to keep a nutrition diary for 5 days sticking to their usual nutrition at the beginning of the study and at the end of each intervention period. At the same time, fresh stool samples were collected and venous blood samples were taken after overnight fasting. Additionally, after the washout period, fresh stool samples and venous blood samples were taken in order to control the continuance of the effect. In patients, the severity of AD (SCORAD) was assessed by the same physician every 4 and 2 weeks after the washout period, respectively. For checking the constitution of the body, a bioelectrical impedance analysis (BIA) was carried out at the beginning and at the end of the study. BIA is an easily applicable and non-invasive method to assess body composition concerning body cell mass, fat-free mass, total body water, etc. [22].

Yoghurt

The probiotic yoghurt drink (Zott GmbH & Co KG, Mertingen, Germany) contained *Streptococcus thermophilus* and was enriched with the probiotic cultures *L. paracasei* Lpc-37 (3.9×10^8 CFU/g), *L. acidophilus* 74-2 (2.9×10^4 CFU/g) and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 (*B. lactis* 420, 5.9×10^4 CFU/g) (Danisco Deutschland GmbH, Niebüll, Germany). These strains were selected because of their ability to survive gastrointestinal transition and to adhere to human intestinal cells (confirmed by *in vitro* assays, Danisco Innovation, Kantvik, Finland and Ouwehand et al. [23]). At the expiration date, each charge of the test preparations was controlled for viability of the strains by plate counting. Cultivation of *L. acidophilus* 74-2 and *L. paracasei* Lpc-37 was performed on Difco™ Lactobacilli MRS Agar (Difco Laboratories GmbH, Augsburg, Germany) for 3 days at 37 °C. *B. lactis* 420 was cultivated on RCM 5.9 Agar (Merck KGaA, Darmstadt, Germany) for 5 days at 37 °C. The placebo drink had an identical composition except for the probiotic cultures and had the same appearance, taste and smell as the probiotic drink. The contents of carbohydrate, fat and protein were comparable in all batches of yoghurt drinks.

Quantitative real-time polymerase chain reaction

DNA extraction. For the extraction of bacterial DNA, 1 g of fresh feces was suspended in 6 mL Ringer's solution

(0.9% NaCl, 0.042% KCl, 0.024% NaHCO₃). The samples were agitated for 30 min and centrifuged for 5 min at 300 g, in order to separate the coarse components. Then, 2 mL of the supernatant was centrifuged at 10 000 g. The cell pellets obtained were subjected to DNA isolation with the Invitek DNA Kit for stool samples (Invitek, Berlin, Germany) according to the manufacturer's instructions. The DNA was eluted in 100 µL elution buffer and stored at -20 °C until analyses.

Quantitative real-time polymerase chain reaction

The PCR mastermix for the quantification of *L. paracasei* and *L. acidophilus* contained $1 \times$ SYBR® Premium Ex Taq™ (Takara Bio Inc., Otsu, Japan) and 0.2 µM of each of the primers Lp1 and Lc2 for *L. paracasei* and 0.3 µM of each of the primers La1 and La2 for *L. acidophilus* following the PCR profile as described previously [24]. The PCR mastermix for the quantification of *B. lactis* contained $1 \times$ PCR buffer with 1.5 mM MgCl₂ (Qiagen, Hilden, Germany), additional 2.5 mM MgCl₂, 2.5 U Hot Star Taq-Polymerase (Qiagen), 0.2 µM of the FAM (6-carboxyfluorescein)-labelled TaqMan probe PBlact174F, 0.9 µM of each of the primers Blact144F and Blact226R and 0.8 µM dNTPs. The mastermix for the amplification of the 16S rRNA-encoding genes contained $1 \times$ PCR buffer with 1.5 mM MgCl₂ (Qiagen), additional 3.5 mM MgCl₂, 2.5 U Hot Star Taq-Polymerase (Qiagen), 0.2 µM of the JOE (carboxy-4', -5'-dichloro-2', 7'-dimethoxyfluorescein)-labelled TaqMan probe PBac1060F, 0.9 µM each of the primers Bac944F and Bac1108R and 0.8 µM dNTPs following the PCR profile as described previously [25]. The quantitative RT-PCR amplifications obtained with this primer pair were subsequently used to calculate the relative concentrations (%) of the DNA of the target taxa of interest vs. total bacterial 16S rDNA.

PCR Mastermix aliquots of 16.0 µL per reaction were dispensed using a model CAS-1200 liquid handling system (Corbett Life Science, Sydney, Australia) and 4.0 µL of a DNA sample was added to the reaction tubes. The PCR was carried out on a Rotor-Gene model 3000 quantitative PCR thermocycler (Corbett Life Science). The PCR profile for the quantification of *B. lactis* DNA consisted of a first denaturation at 95 °C for 15 min, followed by 40 cycles at 94 °C for 20 s and 60 °C for 45 s. The fluorescence data were acquired at the end of each elongation step in the respective channel FAM or JOE. All samples were analysed in duplicate. The data were analysed using the Rotor-Gene Software version 6.0 (Corbett Life Science). The absolute quantification was carried out using a standard curve obtained from amplifications of known DNA quantities. For this purpose, pure cultures of the strains were cultivated, a series of dilutions were prepared and DNA was extracted from each dilution step as described above. In parallel, cell counts of the strains were

determined by conventional plate counting. From each dilution step, 100 µL of the cultures were plated onto selective agar plates and incubated under anaerobic conditions as described above. Total microbial counts are reflected by 16S rRNA contents. For the relative quantification, the results obtained for the probiotic cultures were normalized to the results obtained for total 16S rRNA.

Clinical evaluation

Scoring of Atopic Dermatitis. The clinical severity of the AD was evaluated according to the standardized scoring system developed by the European task force for AD [21].

Immunological evaluation

Cellular immunological parameters. Major leucocyte subset phenotypes were counted in ammonium-heparinized blood samples within 6 h after blood withdrawal. The following fluorochrome-conjugated monoclonal antibodies were applied to determine lymphocyte populations and subsets: CD3⁺, CD19⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, CD3⁺CD16⁺ and CD56⁺, CD3⁺HLA-DR⁺, CD25⁺, CD4⁺CD25⁺, CD57⁺, CD8⁺CD57⁺ (Becton Dickinson, Heidelberg, Germany), CD54 [inter-cellular adhesion molecule-1 (ICAM-1)] and CD4⁺CD54⁺ (BD Biosciences PharmingenTM, Heidelberg, Germany). Samples were analysed on a flowcytometer FACScanTM using the blue-green excitation light (488 nm argon-ion laser) with SimulSET software (Becton Dickinson). The results are expressed as the percentage of mononuclear cells that stained positively for each cell surface marker.

Phagocytic and oxidative burst activity

In vitro phagocytic and oxidative burst activity of granulocytes and monocytes was determined using the commercial test kits Phagotest[®] and Phagoburst[®] (Orpegen, Heidelberg, Germany) according to the manufacturer's instructions.

Humoral immunological parameters

Serum samples of peripheral blood were obtained after a blood clotting time of 30 min and centrifugation of coagulated blood samples. Serum eosinophilic cationic protein (ECP) was analysed by means of RIA (Pharmacia Diagnostics, Freiburg i.Br., Germany). The detection limit was 2 µg/L. The concentrations of macrophage-derived chemokine (MDC), sE-selectin and IL-16 in serum were determined with a commercial sandwich ELISA (R&D Systems, Wiesbaden, Germany). The concentration of IgE in serum was determined on the basis of a fluorescent

enzyme immunoassay using the CAP[®]-system (Pharmacia Diagnostics). The measuring range was 2–5000 kU/L. All assays were run in duplicate.

Statistical analyses

A power analysis was performed using PASS 6.0 (NCSS Statistical Software, Kaysville, UT, USA) based on data from the literature and our study group to evaluate sample size. All data were checked for a normal distribution applying the Kolmogorov–Smirnov test. Statistical analyses were conducted by SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). To evaluate data in terms of statistical significance, repeat-measures ANOVA with the general linear model was applied to identify changes in parameters over time, unless otherwise noted. This model considers both individual and inter-individual changes over time. Differences between the healthy volunteers and the patients with AD were analysed by inserting the study group (healthy vs. patients) as inter-subjects. Furthermore, the intervention sequence as a consequence of the cross-over design was considered by inserting the sequence as a covariate. No influence of sequence was determined for any of the analysed parameters.

Data that were not normally distributed were analysed using Wilcoxon's signed-rank test to evaluate changes over time and the Mann–Whitney *U*-test to evaluate differences between the groups, respectively. For each comparison, a *P*-value of <0.05 was considered to be significant. Significant differences over time within the study groups are indicated by unequal arabic superscripts. Significant differences between the study groups are marked by Greek letters. Results are expressed as mean ± standard deviation (SD).

Results

Subjects characteristics

All volunteers completed the study in good health, and no adverse effects of the regular intake of the probiotic drink were reported by the study participants. The two study groups (healthy subjects and patients with AD) did not differ concerning age and body constitution. However, the baseline concentrations of IgE, MDC and IL-16 were significantly elevated in patients with AD compared with the healthy subjects (Table 1). Nutrient and dietary fibre uptake remained stable during the study and was in accordance with the recommendations of the German Nutrition Society (data not shown).

Quantification of probiotic bacteria in fecal samples

Fecal concentrations of *L. paracasei*, *L. acidophilus* and *B. lactis* were determined by real-time PCR. The numbers

Table 2. Mean counts (log₁₀ CFU/g) of administered probiotic bacteria in feces of healthy subjects and patients with AD

	Healthy subjects (n = 15)			Patients with AD (n = 15)		
	Probiotic	Washout	Placebo	Probiotic	Washout	Placebo
<i>Lactobacillus paracasei</i>	8.4 ± 0.6 ^a	7.3 ± 0.8 ^b	6.8 ± 0.4 ^b	8.1 ± 0.7 ^a	6.9 ± 0.6 ^b	7.0 ± 0.7 ^b
<i>Lactobacillus acidophilus</i>	6.3 ± 3.2	7.9 ± 1.2	6.4 ± 2.5	6.9 ± 3.1	5.9 ± 3.3	5.8 ± 2.4
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	7.5 ± 2.2 ^a	3.3 ± 2.6 ^b	3.2 ± 2.5 ^b	7.2 ± 2.7 ^a	3.6 ± 2.8 ^b	4.0 ± 2.9 ^b

Cell counts are presented as mean ± standard deviation.

Detection limit for the probiotic bacteria in fecal samples was log₁₀ 3 CFU/g.

Significant differences between means of unequal superscript letters for each species within the study groups, $P < 0.05$.

AD, atopic dermatitis.

of *L. paracasei* and *B. lactis* in feces increased significantly during the probiotic intervention period compared with the placebo period. Two weeks after cessation of the probiotic drink, a significant decrease in the fecal counts of *L. paracasei* and *B. lactis* was observed (Table 2).

Regarding the fecal concentrations of *L. acidophilus*, no significant influence of the regular probiotic intake was determined ($P < 0.05$). The numbers of detected bacteria in feces were not significantly different between healthy subjects and patients with AD.

Scoring Atopic Dermatitis

Before the beginning of the probiotic intervention, the mean clinical SCORAD in the 15 patients with AD was 24.0 [95% confidence interval (CI), 20.6–26.7]. After 8 weeks of probiotics, the SCORAD decreased from 15.5% ($P = 0.081$) to 20.3 (95% CI, 17.0–23.6). During the placebo period, the SCORAD score changed slightly by 8.0% ($P = 0.380$) from 24.9 (95% CI, 20.7–28.9) to 22.9 (95% CI, 20.0–25.8). Two weeks after the cessation of the intervention, similar SCORAD values were detected, with 21.9 (95% CI, 19.3–24.4) after probiotics and 21.7 (95% CI, 18.1–25.3) after placebo, respectively. Comparing the two end-points of intervention with a clinical SCORAD of 20.3 vs. 22.9, it can be shown that the decrease in SCORAD was more obvious after the probiotic period ($P = 0.063$, Fig. 1). Overall, in 10 out of 15 patients with AD an improvement of the skin conditions was observed as a result of the 8-week probiotic intake. In the residual five patients, the SCORAD score remained largely unaffected in the probiotic period.

Cellular immune response

Few differences were observed for the expression of lymphocyte subsets caused by the intervention with the probiotic cultures. The expression of CD25⁺ and the proportion of CD4⁺CD25⁺ T cells remained unaffected in patients with AD, whereas a significant difference between the placebo and washout period, but not between the probiotic and placebo period, was observed in healthy

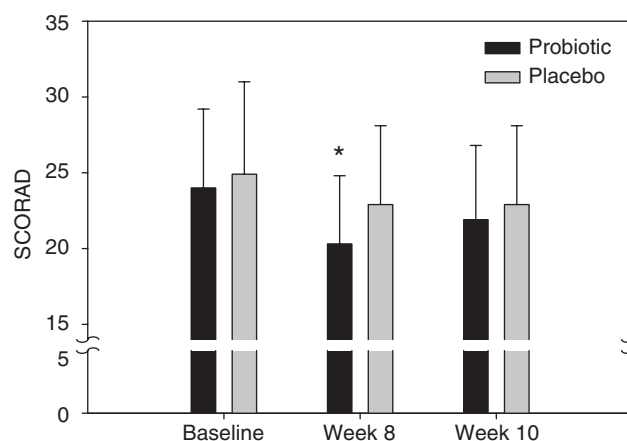


Fig. 1. Changes in the Scoring of Atopic Dermatitis (SCORAD) score after supplementation with probiotics and placebo (week 8) and after the 2-week washout period (week 10) in patients with atopic dermatitis (n = 15). *Trend compared with the placebo value ($P < 0.08$), general linear model procedure.

participants. CD57⁺, mainly expressed on the natural killer cells, was significantly increased during the probiotic period in healthy subjects ($P = 0.034$). After the 2-week washout period without probiotic supplementation, the value declined and remained at that level in the placebo period. In patients with AD, this parameter was not affected. In contrast, in patients the expression of CD4⁺CD54⁺ decreased significantly ($P = 0.031$) in the probiotic period. However, the effect did not persist in the following washout period. For CD3⁺CD4⁺, a significant reduction after the 2-week washout period was observed in healthy subjects ($P = 0.003$). However, no difference existed between the probiotic and the placebo values (Table 3). The expression of CD3⁺ T cells, CD19⁺ B cells, CD3⁺CD8⁺ T cells, natural killer cells (CD3⁺CD16⁺ and CD56⁺), CD3⁺HLA-DR⁺ T cells, CD8⁺CD57⁺ and CD54 (ICAM-1) remained unaffected throughout the study.

The phagocytic activity of monocytes and granulocytes was significantly increased in healthy volunteers, with $99.0 \pm 0.8\%$ after probiotic supplementation compared

Table 3. Mean percentage values of various lymphocyte subsets in the peripheral blood in healthy subjects and patients with AD (% of total lymphocytes)

	Healthy subjects (<i>n</i> = 15)			Patients with AD (<i>n</i> = 15)		
	Probiotic	Washout	Placebo	Probiotic	Washout	Placebo
CD3 ⁺	71.4 ± 7.9	68.9 ± 5.3	71.2 ± 6.2	69.1 ± 9.3	69.7 ± 7.5	69.7 ± 6.2
CD19 ⁺	9.9 ± 2.7	10.2 ± 3.2	10.0 ± 2.8	11.9 ± 3.1	12.5 ± 3.8	12.3 ± 2.9
CD3 ⁺ CD4 ⁺	46.1 ± 5.7 ^a	42.4 ± 4.2 ^b	45.3 ± 4.3 ^a	43.4 ± 8.3	45.6 ± 5.7	45.3 ± 3.3
CD3 ⁺ CD8 ⁺	26.9 ± 11.0	26.9 ± 8.0	28.3 ± 9.6	22.4 ± 4.7	22.8 ± 4.1	22.9 ± 4.6
CD3 ⁺ CD16 ⁺ and CD56 ⁺	15.6 ± 7.8	16.5 ± 5.8	15.1 ± 5.4	15.0 ± 9.1	13.4 ± 7.4	12.7 ± 5.9
CD3 ⁺ HLA-DR ⁺	6.9 ± 3.1	7.1 ± 3.4	5.8 ± 3.1	6.4 ± 2.1	5.5 ± 2.3	6.1 ± 2.1
CD25 ⁺	11.3 ± 4.3 ^{ab}	10.5 ± 3.5 ^b	13.1 ± 4.8 ^a	11.7 ± 3.2	11.0 ± 3.4	12.9 ± 3.1
CD4 ⁺ CD25 ⁺	9.5 ± 3.2 ^{ab}	8.6 ± 2.7 ^b	11.3 ± 3.5 ^a	9.7 ± 2.8	8.9 ± 2.3	10.5 ± 2.1
CD57 ⁺	8.7 ± 5.2 ^a	5.7 ± 3.1 ^b	5.3 ± 3.7 ^b	6.3 ± 4.4	6.9 ± 5.0	6.7 ± 3.5
CD8 ⁺ CD57 ⁺	7.0 ± 4.1	7.7 ± 3.5	7.4 ± 2.3	5.9 ± 2.8	5.9 ± 2.5	6.0 ± 2.4
CD54 ⁺	69.1 ± 11.5	68.9 ± 9.9	68.1 ± 10.3	62.1 ± 10.1	63.0 ± 8.0	63.2 ± 8.3
CD4 ⁺ CD54 ⁺	21.1 ± 8.7	22.5 ± 6.9	21.0 ± 8.0	15.1 ± 5.0 ^b	17.1 ± 3.7 ^a	18.3 ± 4.9 ^a

Values are presented as mean ± standard deviation.

Significant differences between means of unequal superscript letters within the study groups, $P < 0.05$.

AD, atopic dermatitis.

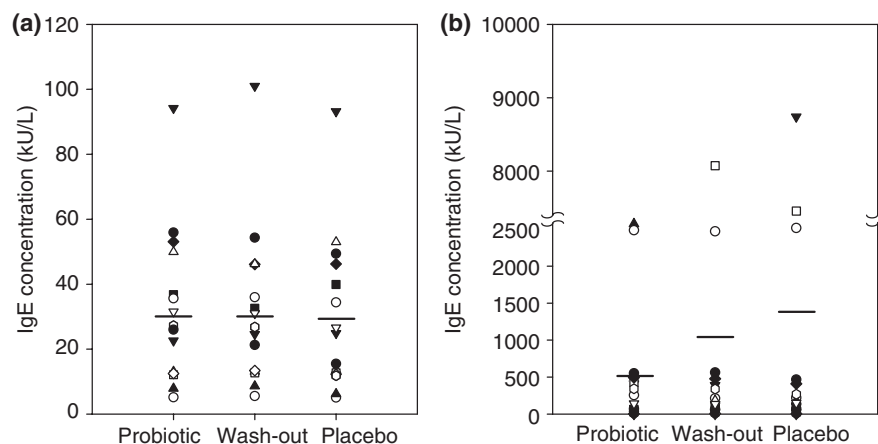


Fig. 2. Serum concentrations of IgE in (a) healthy subjects and (b) patients with atopic dermatitis after an 8-week probiotic intake, after a 2-week washout and after an 8-week placebo intake, respectively. Each symbol represents individual concentrations and each line characterizes the mean value of each group ($n = 15$). There were no significant differences over time within the study groups ($P < 0.05$), Wilcoxon's signed-rank test.

with the placebo with an activity of $96.7 \pm 3.3\%$ ($P = 0.014$). The cessation of probiotics showed a return similar to the placebo values after 2 weeks ($95.7 \pm 3.9\%$, $P = 0.005$). In patients with AD, no influence on the phagocytic activity of monocytes and granulocytes by the probiotic intervention was observed. Oxidative burst activity also remained unaffected in this group (probiotic: $96.4 \pm 6.5\%$, placebo: $97.5 \pm 2.3\%$), whereas in healthy subjects the burst activity increased to $98.6 \pm 1.1\%$ in the probiotic period and declined in the washout period to $94.1 \pm 7.5\%$ ($P = 0.029$). In the placebo period, lower burst activity was shown than in the probiotic period, but not significant statistically ($95.7 \pm 7.7\%$, $P = 0.156$). The levels

of phagocytic and burst activity did not differ between healthy subjects and patients with AD.

Humoral immune response

The concentrations of IgE remained unaffected during the intervention study in healthy subjects. However, they showed significantly lower IgE levels than the patients with AD ($P < 0.01$, Mann-Whitney U -test). After an 8-week consumption of probiotics, IgE concentrations decreased by 62% in patients with AD compared with the placebo, but this difference was not significant statistically ($P = 0.649$, Wilcoxon's signed-ranked test, Fig. 2).

Although the severity of AD is often associated with increased IgE levels, no significant relationship between IgE levels and the SCORAD could be found in this study ($r = 0.099$, $P = 0.510$).

No effect of the probiotics was observed in healthy subjects for the concentrations of ECP (11.6 ± 8.0 vs. 8.7 ± 10.3 $\mu\text{g/L}$), MDC (636 ± 286 vs. 646 ± 275 pg/mL), IL-16 (141 ± 37 vs. 126 ± 55 pg/mL) and sE-selectin (25.7 ± 11.2 vs. 26.5 ± 11.0 ng/mL ; probiotic vs. placebo) and in AD patients (ECP: 11.1 ± 10.2 vs. 13.7 ± 14.0 $\mu\text{g/L}$; MDC: 950 ± 288 vs. 947 ± 313 pg/mL ; IL-16: 217 ± 93 vs. 240 ± 121 pg/mL ; sE-selectin: 25.8 ± 15.4 vs. 25.6 ± 16.6 ng/mL ; probiotic vs. placebo). The differences in the baseline values for MDC and IL-16 between healthy volunteers and patients with AD (Table 1) were maintained over the study.

Discussion

In the present study, healthy subjects and patients with AD consumed a triple-strain probiotic drink containing *L. paracasei* Lpc-37, *L. acidophilus* 74-2 and *B. lactis* 420 to investigate its influence on the clinical, immunological and fecal parameters. Up to now, few studies exist that have studied the effect of multiple probiotic strains administered in food products [26, 27].

The survival of gastrointestinal transition by the probiotic bacteria and their adhesion to intestinal mucosa provide the basis for colonization and mediation of immune system-stimulating signals [28, 29]. In the present study, *L. paracasei* and *B. lactis* increased significantly in feces after probiotic administration, indicating good colonization of these probiotics. In contrast, the levels of *L. acidophilus* remained unaffected (Table 2). These findings may be explained by the different concentrations of the three supplemented strains in the probiotic product. It is worth mentioning that after daily ingestion of about 10^7 CFU of *B. lactis* 420, nearly 10^7 CFU/g of *B. lactis* were detected in feces. Ouwehand et al. [23] showed very good adhesion properties to the intestinal mucus isolated from newborns as well as to mucus from adults for *B. lactis* 420. This observation argues for successful colonization and persistence of this strain in the human intestine. However, the significant decrease 2 weeks after cessation suggests the transience of this effect for *B. lactis* and also for *L. paracasei*. This observation agrees with the results of previous human studies that documented a washout of the ingested probiotic strains shortly after the intake was discontinued [30–32].

Although in the present study an improvement of the clinical SCORAD as a result of the probiotic intervention in the 15 patients with AD was observed, it did not reach statistical significance ($P = 0.081$). An unequivocal effect of probiotics on the skin conditions as demonstrated in studies with newborns and infants could not be deter-

mined in this study with adult patients. Consequently, probiotics can support the therapy of mild and moderate AD and may be suitable for the prevention of acute flare-up, but there hardly exists an effect in the treatment of acute exacerbated AD.

The immune-stimulating properties of lactobacilli and bifidobacteria are based on the mucosal-bacterial cross-talk via the gut-associated lymphoid tissue. In this study, little effects were observed for systemic parameters by dietary intervention with a triple-strain probiotic product. No major differences were determined for the main lymphocyte subsets like CD3^+ T cells, CD19^+ B cells, $\text{CD3}^+\text{CD8}^+$ T cells, natural killer cells ($\text{CD3}^-\text{CD16}^+$ and CD56^+), $\text{CD3}^+\text{HLA-DR}^+$ T cells, $\text{CD8}^+\text{CD57}^+$ and CD54^+ . Moreover, the proportions of these parameters were within the normal range and comparable to the results of recent studies [33–36]. In healthy subjects, the helper/inducer T lymphocytes ($\text{CD3}^+\text{CD4}^+$) decreased significantly 2 weeks after the probiotic intake was discontinued. The same trend was observed by De Vrese et al. [37], who documented a reduction in the severity of common cold episodes in healthy adults as a result of a probiotic intervention. Although the natural killer cells ($\text{CD3}^-\text{CD16}^+$ and CD56^+) and the suppressor/cytotoxic T cells bearing CD57^+ ($\text{CD8}^+\text{CD57}^+$) remained unaffected in healthy subjects, the expression of CD57^+ , as a further natural killer cell marker, was significantly increased after 8 weeks of probiotics compared with the placebo period. This can be explained by the fact that the CD57^+ antigen is expressed by only 50–60% of peripheral blood natural killer cells and furthermore, only about 50% of the peripheral blood lymphocytes that express CD57^+ are actually natural killer cells [38–40]. The CD57^+ -marked T cells play an important role in recognizing and killing both tumour cells and virus-infected cells [41]. They produce larger amounts of IFN- γ than normal T cells and increase proportionally with age possibly to compensate for the immunosenescence in the elderly [42]. A decrease in the proportion of natural killer cell markers was observed in people with suboptimal immune status resulting from smoking, malnourishment, and exhaustive exercise, mental or physical stress [33, 42–44]. Thus, the finding of elevated CD57^+ expression in the healthy subjects indicates immune system stimulation and may lead to a lower incidence or duration of infections. These results are consistent with those obtained from recent studies conducted with other probiotic strains [33, 35, 45].

$\text{CD4}^+\text{CD25}^+$ T-regulatory cells are involved in the modulation of allergic and autoimmune reactions by suppressing immune responses [46]. In NC/Nga mice, an increase in the proportion of splenic $\text{CD4}^+\text{CD25}^+$ T cells was positively correlated with the severity of dermatitis, but no effect of probiotic *L. rhamnosus* GG could be shown on this parameter [47]. In this human study, the expression of peripheral $\text{CD4}^+\text{CD25}^+$ T cells was also not

affected by the probiotics in patients with AD and no relationship between the SCORAD and peripheral CD4⁺CD25⁺ T cells could be demonstrated ($r=0.188$, $P=0.215$). However, the proportion of CD4⁺CD25⁺ T cells was similar in healthy subjects and AD patients as described previously [48].

ICAM-1 (CD54⁺) is a cell adhesion molecule that is expressed inter-alia on CD4⁺ T lymphocytes and is up-regulated in response to inflammation, cellular stresses and virus infections [49, 50]. Increased levels of ICAM-1 were shown in atopic patients, indicating inflammatory processes [51, 52]. Interestingly, in this study, the exclusive expression of CD54⁺ and of CD4⁺ T cells did not change as a result of the probiotic intervention, but the expression of CD54⁺ on the T helper (T_H) cells decreased significantly in patients with AD. This suggests that the expression of this cell surface marker was shifted within the lymphocytes.

In the present study, the administration of *L. paracasei* Lpc-37, *L. acidophilus* 74-2 and *B. lactis* 420 stimulated the cellular immune function in healthy subjects, indicated by increased phagocytic and oxidative burst activity. The levels of activity were relatively high at all reading points. Nevertheless, phagocytic and oxidative burst activities of monocytes and granulocytes were significantly elevated in the probiotic period. This result is in agreement with those of previous reports [53–56]. Interestingly, no change in these parameters could be shown in patients with AD. Peltó et al. [57] examined the effect of probiotic *Lactobacillus* GG on the expression of phagocytosis receptors in healthy subjects and milk-hypersensitive subjects. In the healthy subjects, an increased expression of phagocytosis receptors was observed, whereas in milk-hypersensitive subjects the milk-induced increased expression of these receptors was suppressed by the probiotic intervention. Thus, the results of this previous study are similar to the findings of our study demonstrating that the immune response may be differently modulated in healthy subjects and in patients with AD. Probably, the underlying mechanism is associated with the existing differences in the composition of the microbiota. In healthy subjects, a stable intestinal microbiota is developed, whereas in AD patients an imbalance of the intestinal environment exists. Hence, the probiotics find different environmental conditions influencing the host–bacterial crosstalk by competing for epithelial attachment sites. Possibly, different pathways of bacterial–enterocyte crosstalk may induce different immune responses in both, healthy subjects and AD patients. Previous studies have demonstrated that the interaction of lactobacilli with M cells induced mainly the specific immune response, while the interaction with the follicle-associated epithelium cells from Peyer's patches generally induced the non-specific immune response [58]. Additionally, the mediation of immunoregulatory signals

through dendritic cells, which are the principal stimulators of naïve T_H cells, may be considered [59].

To the best of our knowledge, this is the first study evaluating the effect of a triple-strain probiotic product containing *L. paracasei* Lpc-37, *L. acidophilus* 74-2 and *B. lactis* 420 on the immune system of healthy subjects and patients suffering from AD. The probiotics *L. paracasei* and *B. lactis* administered are able to colonize transiently the intestine in both healthy and AD subjects and to affect the immune system. The results for the peripheral immune parameters in healthy volunteers and patients with AD indicate that the immune response is influenced by the immunological status of the host. No significant improvement of the skin conditions could be shown. Nevertheless, symptoms tended to be allayed. In that, a larger clinical trial over a longer period is warranted to confirm the findings of this study.

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