

INVESTIGATIVE REPORT

Differential Immunomodulating Effects of Inactivated Probiotic Bacteria on the Allergic Immune Response

Claudia RASCHE¹, Christin WOLFRAM¹, Michael WAHLS² and Margitta WORM¹

¹Department of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, and ²Department of Otorhinolaryngology, Bundeswehrkrankenhaus Berlin, Germany

Bacterial stimulation plays an important role in modulating the allergic immune response. The aim of this study was to investigate the effects of inactivated probiotic *Lactobacillus acidophilus* and non-pathogenic *Escherichia coli* strain Nissle on the phenotype and function of T- and B-cells. Peripheral blood mononuclear cells from patients with grass-pollen allergy ($n=10$) and non-allergic patients ($n=19$) were co-stimulated with inactivated bacteria and grass-pollen allergen. Expression of CD23, CD80, CD86 and CD69 were analysed, and the intracellular production of interleukin-4 and interferon- γ was measured by direct *ex vivo* flow cytometry after stimulation. Both bacteria induced a significant up-regulation of CD69 expression on T lymphocytes ($p=0.001$). CD23 expression was significantly increased following stimulation with allergen ($p=0.008$), but reduced after stimulation with *Lactobacillus* and significantly reduced with *E. coli* plus allergen ($p=0.029$). CD80 expression was reduced after stimulation with *Lactobacillus* in the allergic group only ($p=0.021$). By contrast, CD86 expression was significantly increased after stimulation with *Lactobacillus* ($p=0.049$) and distinctly increased with *E. coli* in both groups ($p=0.001$). The cytokine patterns of CD69-positive T lymphocytes from allergic patients showed a T-helper2-dominated response after allergen stimulation (interferon- γ /interleukin-4-ratio 0.2), directed into a T-helper1-like response by stimulation with both types of bacteria (interferon- γ /interleukin-4-ratios 1.5–2.0 in both groups). These data show that both types of bacteria modulate the allergic immune response by the alteration of CD23 and co-stimulatory molecule expression. Regarding cytokine production, the data suggest a differential response to both bacteria depending on the atopic state, but a clear promotion of T-helper1-dominated response in allergic donors. **Key words: allergy; T-cells; *Escherichia coli* Nissle 1917; *Lactobacillus acidophilus*.**

(Accepted October 16, 2006.)

Acta Derm Venereol 2007; 87: 305–311.

Claudia Rasche, Charité, Universitätsmedizin Berlin, Department of Dermatology and Allergy, Schumannstr. 20/21, DE-10117 Berlin, Germany. E-mail: margitta.worm@charite.de

New perspectives concerning the prevention of atopic disorders and their treatment are of current interest as these diseases are continuously increasing. Estimates of worldwide prevalence indicate that 20% of the population have asthma and rhinitis, 5% have atopic dermatitis and 3% have food allergy (1).

There are several hypotheses about the causes of this increase, one of the most controversial of these is the so-called “hygiene hypothesis” (2). This associates the increase in allergic diseases with changes in the interactions between people and the microbes in their ecosystem consequent to a “western lifestyle” (3). Extensions of this hypothesis focus on the reduced diversity and magnitude of the “microbial burden” in early life, which is thought to lead to less bacteria-derived maturation signals being encountered during early immune development (4).

Considering this hypothesis, microbial products functioning as immunological stimulators have been assumed to play an important role in the prevention and treatment of allergic diseases (5, 6), but their clinical efficacy has shown diverse results and their usage is preliminary (5). Among these microbial products are the so-called “probiotics”, a group of living or inactivated micro-organisms of potential benefit when ingested (7). The most commonly used probiotics are *Lactobacilli* and *bifidobacteria*, but enterococci and *Escherichia coli* have also been proposed as probiotics (5, 8). Previous studies have shown beneficial effects of probiotic micro-organisms, even in adults, caused by promoting the gut barrier function to prevent unfavourable intestinal micro-ecological alterations (9). Research into extensions of the hygiene hypothesis to adults appeared to show that allergic recurrences were improved or prevented in rhinitis patients by eating yoghurt (10), but no effects were seen on birch pollen allergy after oral treatment with *Lactobacillus rhamnosus* (11).

The present study analysed the influence of chill-inactivated probiotic *L. acidophilus* and non-pathogenic *E. coli* Nissle 1917 on the phenotype and function of T- and B-lymphocytes from adult allergic and non-allergic individuals. In order to elucidate the immunomodulatory effects of these bacterial components in humans an *in vitro* assay was used, followed by direct *ex vivo* analysis

of peripheral lymphocytes, representing systemic bacterial administration and their impact on gut-associated lymphoid tissue lymphocytes.

Previous studies have shown that a T-helper (TH)₂-dominated response via the increased production of interleukin (IL)-4 can be arrested by cross-regulatory cytokines such as interferon gamma (IFN γ) (12). Focusing on the allergen-specific immune response, the ability of allergen (A) and of the above-mentioned bacterial components (LC-lactobacilli, EC-*E. coli* Nissle) to modulate the peripheral TH₁/TH₂ balance was investigated in adult allergic (a) and non-allergic (na) individuals. In addition, the state of activation and the expression of important co-stimulatory molecules in the presence of the bacteria was analysed by direct *ex vivo* flow cytometry.

MATERIALS AND METHODS

Patients

The study included 29 age- and sex-matched adult patients from the Department of Otorhinolaryngology (Bundeswehrkrankenhaus, Berlin, Germany), who gave written consent. The study was approved by the local ethics committee. Ten allergic patients suffering from seasonal allergic rhinitis were diagnosed as being sensitized to grass pollen according to skin prick testing (ALK, Hamburg, Germany) and detection of specific IgE (Pharmacia, Freiburg, Germany) to grass pollen (CAP-class \geq 2). Nineteen non-allergic, non-grass-pollen sensitized healthy individuals were included as controls. Exclusion criteria were: administration of systemic and local steroids within 14 days prior to the study; the use of antihistamines during the 3 days prior to the study; or current specific immunotherapy or other immunomodulating medication. None of the patients had allergic symptoms at the time of investigation, as the study was performed during the winter season, and none had any other severe diseases.

Cell separation and culture

Heparinized blood samples were taken once from patients and control individuals. Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized whole blood by Ficoll density gradient centrifugation and washed repeatedly in phosphate buffered saline (PBS) before being resuspended and cultured in RPMI 1640 medium (Sigma, Munich, Germany) supplemented with 10% foetal calf serum, glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μ g/ml). PBMCs were incubated in flat-bottomed 24-well plates at 37°C in a water-saturated atmosphere containing 5% CO₂.

Stimulants

PBMCs were stimulated with grass pollen extract (500 SQ/ml, provided by ALK Scherax, Hamburg, Germany), -80°C chill-inactivated *E. coli* Nissle (Mutaflor, Ardeypharm, Herdecke, Germany) at a 1:1 ratio, or *L. acidophilus* (Paidoflor, Ardeypharm) at a 2:1 ratio (13). The grass pollen extract additionally contained anti-CD28 (0.5 μ g/ml, BD PharMingen, Heidelberg, Germany) for co-stimulation. As a positive control, PBMCs were incubated with staphylococcal enterotoxin B (SEB) (1 μ g/ml, Sigma). The negative control contained unstimulated cells. PBMCs were collected after 18 h for surface staining and after 16 h for intracellular staining. Grass pollen extract was analysed with regard to endotoxin content, measured by the

chromogenic Limulus amoebocyte lysate assay (Bio Whittaker) and showed 222 pg/ml in the allergen dilution of 100,000 SQ/ml. Accordingly, 500 SQ/ml of allergen extract contains a negligible amount of endotoxin, which has no modulating effect.

Flow cytometry

The expression of surface CD20, CD23, CD80, CD86, CD4 and CD69, and of intracellular IL-4 and IFN γ were assayed by flow cytometry (all antibodies were "mouse-anti-human" from BD/PharMingen). PBMCs (1×10^6) were incubated with the specific antibodies and the appropriate isotype controls (BD/PharMingen) for 30 min at 4°C in 100 μ l staining buffer (2% bovine serum albumin in PBS, 0.002% sodium azide) following a 10-min-blocking with 5 μ l Beriglobin[®] (Aventis-Behring, Marburg, Germany). Cells for intracellular staining were additionally incubated with Brefeldin A (1 μ l/ml, Sigma) for 3 h after 13 h of incubation, and permeabilized with 500 μ l FACS-permeabilizing solution (BD Bioscience, Heidelberg, Germany) before staining. Finally, the cells were washed and fixed in PBS with 2% paraformaldehyde.

Flow cytometry of 4-fold labelled cells was performed with a FACScalibur (Becton Dickinson, Heidelberg, Germany) flow cytometer and evaluated using Cell Quest software (Becton Dickinson). Lymphocytes were gated by anti-CD20 or anti-CD4 fluorescence, the percentage of the double-positive cells after deduction of the isotype control values was determined.

Preceding analysis

Dose-response experiments were initially carried out using increasing bacterial concentrations (i.e. 0.25×10^6 , 0.5×10^6 , 1×10^6 and 2×10^6 *E. coli* / *L. acidophilus* per 1×10^6 cells) for stimulation. Subsequently, a time course analysis covering 6, 18, 24 and 48 h of stimulation was performed, using the determined bacterial concentration. PBMCs were stained with CD4 and CD69 Allophycocyanin, respectively, CD20 and CD23, CD54 or CD86 to analyse their level of activation (data not shown). Mitochondrial activity in response to all the stimulants used was analysed using 10 μ l WST-1 reagent (4-(3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulphonate, Boehringer Mannheim) per 100 μ l cell-stimulant preparation (as described above) and measured half-hourly for 4 h by spectrophotometric quantification (OD 450 nm) after 18 h of stimulation.

Statistical analysis

Analysis of the statistical significance of within-group changes was carried out using Brunner's analysis and the Wilcoxon test for non-parametric data. Descriptive statistical analysis, table calculations and diagrams were produced using Excel 2000 and SPSS 11.0. The values in Table I represent the mean \pm standard error of the mean or ratio of means.

RESULTS

Mitochondrial activity assay

With respect to cell viability, the addition of bacteria caused a reduced mitochondrial activity strongest after stimulation with *L. acidophilus* (OD_{LC} 0.18; OD_{EC} 0.19) in comparison with SEB (OD 0.4) and allergen-stimulated (OD_A 0.29) as well as unstimulated cells (OD_U 0.28). Incubation of bacteria alone did not show any effect on the mitochondrial activity measured.

Table I. Intracellular measurement of cytokine production by CD4-positive T lymphocytes in allergic (a) and non-allergic (na) individuals. Expression of interleukin (IL)-4 and interferon (IFN) γ on CD4+ T-lymphocytes, determined by flow cytometry in unstimulated cells (U) and following incubation with grass pollen allergen (A), *E. coli* Nissle (EC) and *L. acidophilus* (LC). Each value represents the mean \pm SEM as a percentage of CD4+ T-lymphocytes

| | CD4+ | | | |
|------|-------------------|-------------------|--------------------|-------------------|
| | IL-4 | | IFN γ | |
| | a | na | a | na |
| U | 0.27 \pm 0.14 | 0.55 \pm 0.09 | 0.10 \pm 0.08 | 0.31 \pm 0.05 |
| A | 0.34 \pm 0.18 | 0.66 \pm 0.08 | 0.16 \pm 0.08 | 0.43 \pm 0.07 |
| LC | 0.65 \pm 0.28* | 0.97 \pm 0.23* | 0.48 \pm 0.18* | 0.87 \pm 0.15* |
| LC+A | 0.91 \pm 0.32** | 1.08 \pm 0.22** | 0.49 \pm 0.18** | 0.74 \pm 0.19* |
| EC | 0.17 \pm 0.14 | 0.88 \pm 0.19* | 0.14 \pm 0.12 | 0.70 \pm 0.20* |
| EC+A | 0.37 \pm 0.19 | 0.91 \pm 0.30** | 0.57 \pm 0.21*** | 0.80 \pm 0.25** |

* $p < 0.05$ compared with U; ** $p < 0.05$ compared with U, A; *** $p < 0.05$ compared with U, A, EC.

Increased T-cell activation by microbacterial stimulation

First, the T-lymphocytes were investigated with regard to the state of activation via surface CD69 expression (14). A significant up-regulation of CD69 expression was detected following allergenic (median: A_a 3.9%, $p_a = 0.004$; A_{na} 2.9%, $p_{na} = 0.001$) and bacterial stimulation with *L. acidophilus* in both groups (median: LC_a 5.0%; LC+A_a 5.7%, $p_a = 0.001$; LC_{na} 3.7%; LC+A_{na} 4.4%, $p_{na} = 0.001$) in comparison with basal CD69 expression (median: U_a 2.1%; U_{na} 2.0%). In the presence of *E. coli*, CD69 expression showed a distinct up-regulation, which was highly significant with or without the addition of allergen (median: EC_a 29.2%; EC+A_a 36.0%, $p_a = 0.001$; EC_{na} 23.8%; EC+A_{na} 29.5%, $p_{na} = 0.001$).

Triggering of TH1-dominated T-cell responses by *L. acidophilus*, lactobacilli and *E. coli*

As the balance of TH1/TH2 response plays a central role for the development of allergic disease (15), intracellular IL-4 and IFN γ production was measured on

CD4-positive T-lymphocytes by intracellular staining (Fig. 1). Stimulation with grass pollen extract led to an increase in IL-4 production (median: U_a 0.27%; A_a 0.34%) and, to a lesser extent in IFN γ production in the allergic group (median: U_a 0.1%; A_a 0.16%).

Analysis of the IFN γ /IL-4-ratio of allergen specific T-lymphocytes is presented in Fig. 2 and shows a TH2-dominated response after allergen exposure, especially in the allergic group (CD69+ IFN γ /IL-4-ratio_A 0.2; 0.75_{na}). In contrast to these findings, TH1-dominated responses were observed following the incubation with *L. acidophilus* in both groups (CD69+ IFN γ /IL-4-ratio_{LC} 2.0; 1.5_{na}). However, stimulation with lactobacilli plus allergen resulted in a TH2-like response in allergic and non-allergic individuals (CD69+ IFN γ /IL-4-ratio_{LC+A} 0.67; 0.84_{na}). As seen in Table I, this may be due to a significant increase in IL-4 production (median: LC+A_a 0.91%; LC+A_{na} 1.08%, $p = 0.041$), whereas IFN γ production remained basically unchanged in the allergic group (median: LC_a 0.48%; LC+A_a 0.49%; LC_{na} 0.87%; LC+A_{na} 0.74%).

A significant increase in IFN γ production was present after stimulation with *E. coli* and allergen (median: EC+A_a 0.57%, $p_a = 0.007$; EC+A_{na} 0.80%, $p_{na} = 0.001$), whereas single *E. coli*-stimulation caused a strong up-regulation of IFN γ in non-allergic individuals only (median: EC_a 0.14%; EC_{na} 0.70%; $p_{na} = 0.001$, see Table I). Finally, *E. coli* also had little effect on the production of IL-4 in the allergic group. Non-allergic subjects showed a significant increase (median: EC_a 0.17%; EC_{na} 0.88%; $p_{na} = 0.001$). As shown in Fig. 2, the IFN γ /IL-4-ratio of allergen-specific T-lymphocytes reflects these findings of a TH1-dominated response following exposure to *E. coli* (CD69+ IFN γ /IL-4-ratio_{EC} 2.0; 1.75_{na}) as well as *E. coli* and allergen (CD69+ IFN γ /IL-4-ratio_{EC+A} 4.0; 2.66_{na}).

E. coli stimulation enhanced CD80 and CD86 expression

The importance of co-stimulatory molecules, especially the up-regulation of CD86 but not CD80 following

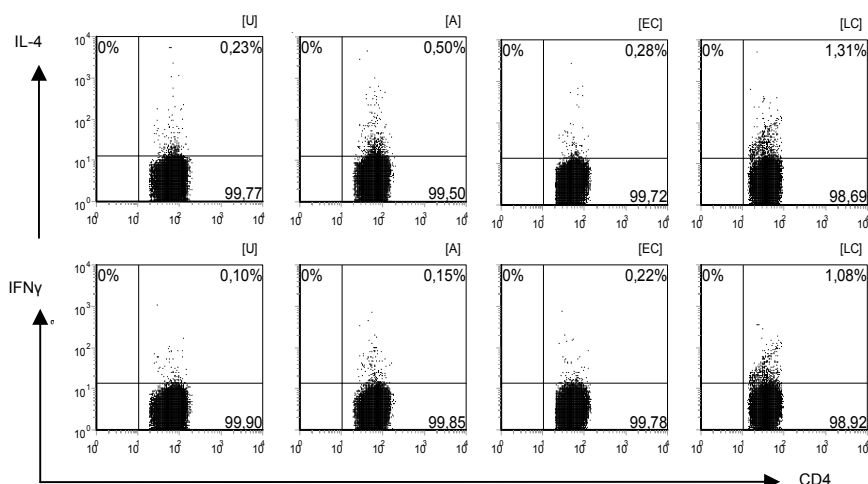


Fig. 1. Representative example of the intracellular production of IL-4 and IFN γ in the presence of *E. coli* Nissle 1917 (EC) and *L. acidophilus* (LC) and combined stimulation with allergen (EC+A, LC+A) compared with unstimulated T-cells (U) and allergen (A). Scatter plots, showing intracellular flow cytometry of cytokines in peripheral blood CD4-positive T lymphocytes in allergic individuals following 16 h stimulation (see Materials and Methods).

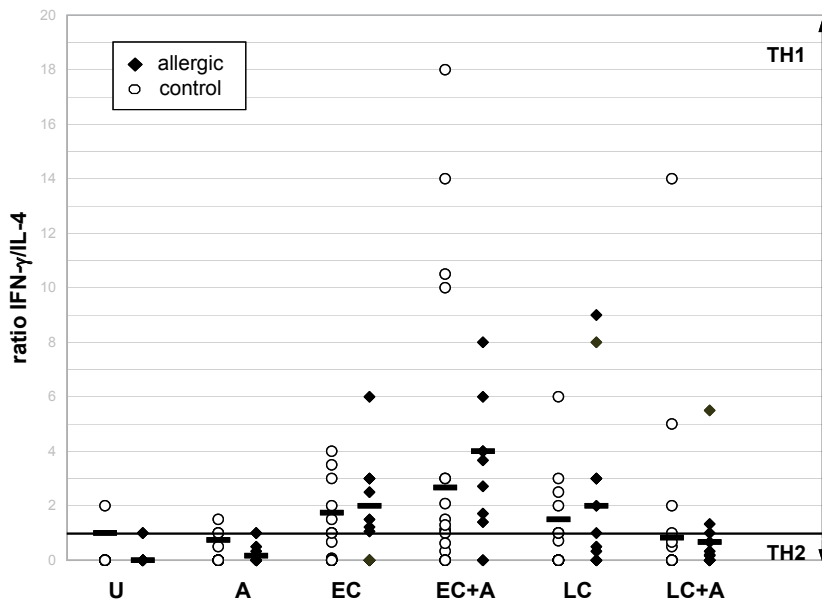


Fig. 2. Induction of TH1-dominated immune response in activated CD4-positive T-lymphocytes following stimulation with non-pathogenic *E. coli* Nissle (EC) and *L. acidophilus* (LC) in comparison with unstimulated cells (U) and stimulation with allergen (A). Ratios calculated from the percentages of CD69-IL-4 and CD69-IFN γ double-labelled CD4-positive T-lymphocytes, $n_a=7$, $n_{na}=13$. A ratio less than 1 means a predominance of IL4 compared with IFN γ , representing a TH2 response, and vice versa.

allergenic or IL-4 stimulation in the development of TH2-cells in patients with atopic diseases has been described repeatedly (16, 17). Our results show that grass pollen extract induced a small, but significant, increase in CD86 expression only in the allergic subjects (median: U_a 4.26%; A_a 5.6%; $p=0.025$) and had no impact on the expression of CD80 on B-lymphocytes (median: U_a 10.6%; A_a 10.4%).

We then investigated the influence of the above-mentioned bacteria on the expression of co-stimulatory molecules. As shown in Fig. 3, stimulation with *L. acidophilus* reduced the percentage of B-cells positive for CD80 in the allergic group (median: LC_a 7.4%, $p=0.021$). *E. coli* caused an up-regulation of CD80 in both groups, which was statistically significant only in the non-allergic subjects (median: EC_a 13%; EC_{na} 16%; $p_{na}=0.001$) and similarly after stimulation with *E. coli* and allergen (median: EC+A_a 13%; EC+A_{na} 16%, $p=0.001$).

As shown in Fig. 4, CD86-expression on B lymphocytes following bacterial stimulation showed a significant increase in both groups (median: EC_a 21%; EC+A_a 21%; EC_{na} 20.7%; EC+A_{na} 24.8%, $p_a=0.007$, $p_{na}=0.001$; LC_a 12.6%; LC_{na} 9.9%, $p_a=0.049$, $p_{na}=0.001$), except for the increase following *L. acidophilus* plus allergen,

which did not reach statistical significance (median: LC+A_a 15%; LC+A_{na} 6.5%).

Modulation of CD23 expression by inactivated bacteria

CD23, the IL-4-induced and IFN γ -modulated low-affinity receptor for IgE, plays a crucial role in regulating T- and B-cell interactions within the allergic response (18). Therefore we analysed the effect of bacterial and allergenic stimuli on the expression of CD23 on peripheral B-lymphocytes in allergic and non-allergic subjects. We observed higher basal expression and significantly higher up-regulation of CD23 after exposure to allergen within the allergic group (median: U_a 11.9%; A_a 15.0%, $p=0.012$) than the non-allergic control (median: U_{na} 5.1%; A_{na} 5.4%). *E. coli* reduced the expression of CD23 compared with allergenic stimulation (median: EC_a 7.1%; EC_{na} 6.0%), which appeared to be significant after co-stimulation with *E. coli* and allergen in the allergic group only (median: EC+A_a 8.2%; EC+A_{na} 5.7%, $p_a=0.029$).

Incubation with *L. acidophilus* led to decreased expression of surface CD23 in the allergic group in

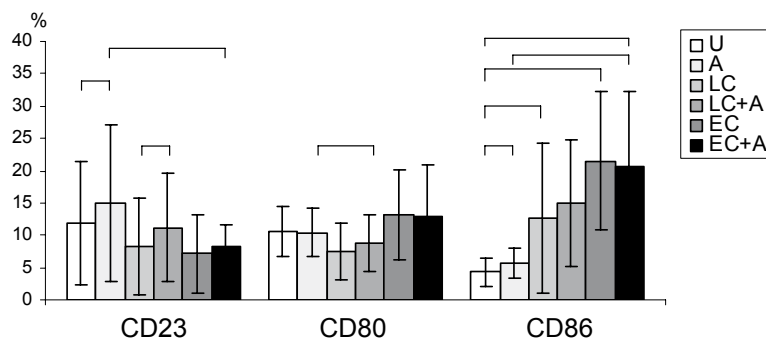


Fig. 3. CD23 and co-stimulatory molecule expression upon stimulation with bacteria. Percentage of CD23-, CD80- and CD86-positive B-cells in allergic individuals. Flow cytometry was performed following 18 h of stimulation. Mean values \pm SEM (brackets indicate $p < 0.05$). U: unstimulated ($n=8$); A: allergen ($n=8$); LC: lactobacilli ($n=7$); EC: *E. coli* Nissle ($n=6$); LC+A ($n=7$); EC+A ($n=6$).

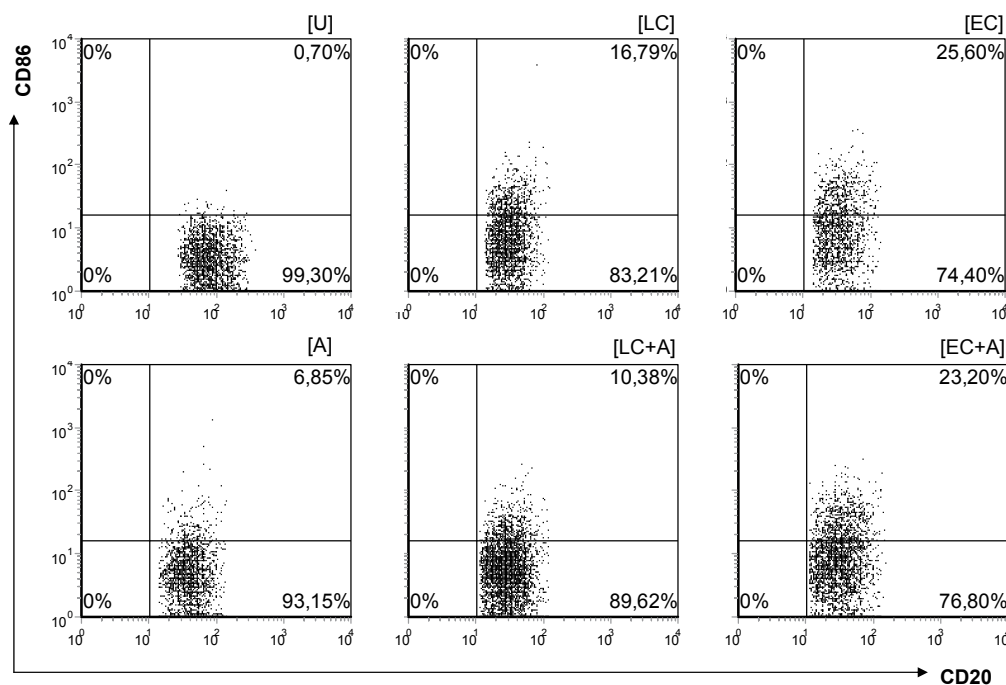


Fig. 4. Increase in CD86 expression following stimulation with grass pollen allergen (A), *E. coli* Nissle 1917 (EC) and *L. acidophilus* (LC) in comparison with unstimulated cells (U). Scatter plots of flow cytometry of peripheral blood CD20-positive B-lymphocytes in allergic individuals following 18 h stimulation (see Materials and Methods).

particular, but this did not reach statistical significance (median: LC_a 8.3%). The addition of allergen increased the percentage of CD23-positive B-cells significantly compared with *L. acidophilus* in both groups (median: LC+A_a 11.2%; LC+A_{na} 9.3%; $p_a = 0.001$, $p_{na} = 0.03$).

DISCUSSION

This study investigated the immunomodulatory effects of inactivated probiotic *L. acidophilus* and non-pathogenic *E. coli* in an *ex vivo* model on peripheral lymphocytes from sensitized allergic individuals and healthy controls.

The results show that stimulation with allergen caused a TH2-like response with enhanced IL-4 production in both groups and up-regulation of CD86 and CD23 on B-lymphocytes in allergic individuals. This immune response was profoundly modulated by the bacterial components *L. acidophilus* and *E. coli*. Single bacterial stimulation resulted in a TH1-dominated response in both groups, as reported in previous clinical studies (5). Furthermore, the expression of CD23, but also the co-stimulatory molecules CD80 and CD86 were modulated by both *L. acidophilus* and *E. coli*.

The shift in the TH1/TH2-balance measured via IL-4 and IFN γ following stimulation with non-pathogenic bacteria demonstrates the possible therapeutic potency especially of non-pathogenic *E. coli* Nissle. Although some studies indicate that human cytokine activities fail to exhibit an exclusively pro-TH1 or -TH2 pattern (19), our results show rather a reduction in one pathway.

Moreover, allergic patients showed decreased CD23 expression in the presence of bacteria, especially *E. coli*.

These observations are closely related to the modulating effects of IL-4 and IFN γ on the expression of CD23. As previous studies describe increased CD23 expression caused by IL-4 and a decreased CD23 expression caused by IFN γ , the above-mentioned cytokine patterns following bacterial stimulation explain our findings (18). The *in vivo* relevance has been shown, but could not be confirmed clinically (20, 21). Increased CD23 expression in patients suffering from atopic dermatitis and allergic rhinitis has been shown previously by our group (22). Furthermore, former studies show that IFN γ inhibits CD23 mRNA and sCD23 production on human B-cells (18). sCD23, which was not measured in this study, has cytokine properties alone, but is also able to elicit strong inflammatory action by stimulating the production of TNF α and IL-1 β and co-stimulates IL-2-induced INF γ production (23). Decreased shedding of sCD23 results in decreased IgE production (24).

In addition, the co-stimulatory molecules CD80 and CD86 were modulated by both types of bacteria. Non-pathogenic *E. coli* resulted in up-regulation of CD80 and CD86, indicating a non-specific stimulation of the B-cell system. This was consistent with with an increased production of specific antibodies and proliferative activity, as described previously by Cukrowska et al. (25). By contrast, incubation with probiotic *L. acidophilus* led to inhibition of CD80 but up-regulation of CD86 especially in allergic individuals. Thus, our findings may support the hypothesis of CD80 functioning as a more neutral differentiation signal instead of a TH1-maintaining one and – also explaining the *E. coli*-derived results – of both molecules directing the development of TH1 and TH2 in a less contrasting pattern (17, 26).

As it is shown that T-cell activation does not necessarily need antigen presenting cell signalling for activation (27), differences between the bacteria may be based on toll-like receptor (TLR)-mediated regulation of pathogen-related responses, since there are different cascades of activation mechanisms of Gram-positive and Gram-negative bacteria (28, 29). Without focusing on TLR-mediated cascades, the basic activity of lymphocytes plays a crucial role in the distinct TH1-like responses on bacterial stimulation in the presence of allergen, especially the allergic group. If one assumes preventive or even maturing effects of *L. acidophilus* and even non-pathogenic *E. coli* beyond childhood, these may be also explained via the TLR model. As the hygiene hypothesis focuses only on the maturation of the immune system towards TH1 phenotype in childhood (30), TLR pathways may also be involved in the maturation of the normal adult immune system. It remains unclear whether the above-mentioned TH1-responses following stimulation with probiotic and apathogenic bacteria as seen in our study show prolonged effects on driving the maturation of the adult immune system. However, the current study does not lead to precise conclusions about the comparative potency of both types of bacteria.

The strong TH1-like responses following bacterial stimulation also seen in the non-allergic controls suggest that priming signals may exist and consequently prevent over-expression of TH2-dominated responses. These effects might play a role not only in childhood (31). Previous studies using murine models indicate that such immunomodulatory effects are derived from long-term administration of mutated bacterial agents. Such effects may also result in long-term down-regulation of hypersensitivity reactions secondary to antigen-specific TH1/TH2 imbalances (32). As it is even hypothesized that TH1- and TH2-mediated conditions might frequently coexist and that the contrasting TH1/TH2-model might be an oversimplification (33), we assume, that long-term immunomodulating is possible beyond priming in infancy.

Another interesting observation is the shift of the allergen-specific TH2 response towards a TH1-like response following stimulation with *E. coli* Nissle plus allergen. This effect was not seen with *L. acidophilus*. Whether these observations reflect the clinical findings of a poor reduction in symptoms after oral *L. acidophilus* treatment requires further investigation (34). On the other hand, oral *E. coli* application has been reported to reduce the incidence of IgE-dependent allergies, namely respiratory and skin manifestations, in children when administered after birth (6, 34).

In summary, our observations indicate that inactivated non-pathogenic *E. coli* and probiotic *L. acidophilus* have immunomodulating capacities, as we determined profound changes in the TH balance. Clinical studies also indicate the applicability of probiotic agents for preventive use (35, 36).

The promotion of a TH1-dominated immune response by long-term application of bacteria, and especially *E. coli*, may be critical, as autoimmune diseases are characterized by a TH1-dominated immune response with high levels of TH1-type cytokines, e.g. IFN γ . On the other hand, it has recently been shown in a clinical trial that oral administration of *E. coli* Nissle maintains the remission of ulcerative colitis, an autoimmune disease of the gut (37).

E. coli Nissle, but not *lactobacilli*, were shown to induce high levels of IL-10 and IL-12, which might be due to the content of lipopolysaccharides in Gram-negative bacteria only. Therefore IL-12-promoted induction of IFN γ may be the cause of the differences in modulation of TH1/TH2 balance seen when comparing the types of bacteria. Finally, changes in the expression of surface molecules, such as CD86 and CD23, might also originate from this pro-inflammatory immune response (38).

We conclude that probiotic *L. acidophilus* and non-pathogenic *E. coli* Nissle exhibit immunomodulatory effects on lymphocytes by influencing the cytokine pattern, indicating a potential for preventive and therapeutic efficacy in the treatment of TH2-dominated allergic diseases. *E. coli* in particular increases the production of IFN γ under allergen challenge, which might also result in reduced symptoms in allergic individuals. However, in order to elucidate the role of non-pathogenic *E. coli* Nissle in modulating allergic symptoms and its mechanism of action, further investigation are needed.

REFERENCES

1. Baena-Cagnani CE, Serra H, Teijeiro A, Croce JS. Prevention of allergy and asthma. *Clin Exp All Rev* 2003; 3: 51–57.
2. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299: 1259–1260.
3. Matricardi PM, Bonini S. High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the Hygiene hypothesis? *Clin Exp Allergy* 2000; 30: 1506–1510.
4. Prescott SL. Allergy: the price we pay for cleaner living? *Ann Allergy Asthma Immunol* 2003; 90: 64–70.
5. Matricardi PM, Bjorksten B, Bonini S, Bousquet J, Djukanovic R, Dreborg S, et al. Microbial products in allergy prevention and therapy. *Allergy* 2003; 58: 461–471.
6. Lodinova-Zadnikova R, Cukrowska B, Tlaskalova-Hogenova H. Oral administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). *Int Arch Allergy Immunol* 2003; 131: 209–211.
7. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics – approaching a definition. *Am J Clin Nutr* 2001; 73: S361–S364.
8. Jansen GJ, Wildeboer-Veloo AC, van der Waaij D, Degener JE. *Escherichia coli* as a probiotic? *Infection* 1998; 26: 232–233.
9. Bongaerts GP, Severijnen RS. Preventive and curative effects of probiotics in atopic patients. *Med Hypotheses*.

- 2005; 64: 1089–1092.
10. Aldinucci C, Bellussi L, Monciatti G, Passali GC, Salerni L, Passali D, et al. Effects of dietary yoghurt on immunological and clinical parameters of rhinopathic patients. *Eur J Clin Nutr* 2002; 56: 1155–1161.
 11. Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *Allergy* 2002; 57: 243–246.
 12. Cross ML, Stevenson LM, Gill HS. Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria? *Int Immunopharmacol* 2001; 1: 891–901.
 13. Baati L, Fabre-Gea C, Auriol D, Blanc PJ. Study of the cryotolerance of *Lactobacillus acidophilus*: effect of culture and freezing conditions on the viability and cellular protein levels. *Int J Food Microbiol* 2000; 59: 241–247.
 14. Testi R, Phillips JH, Lanier LL. T cell activation via Leu-23 (CD69). *J Immunol* 1989; 143: 1123–1128.
 15. Martinez FD. The coming-of-age of the hygiene hypothesis. *Respir Res* 2001; 2: 129–132.
 16. Nakada M, Nishizaki K, Yoshino T, Okano M, Masuda Y, Ohta N, et al. CD86 (B7-2) antigen on B cells from atopic patients shows selective, antigen-specific upregulation. *Allergy* 1998; 53: 527–531.
 17. Hofer MF, Jirapongsananuruk O, Trumble AE, Leung DY. Upregulation of B7.2, but not B7.1, on B cells from patients with allergic asthma. *J Allergy Clin Immunol* 1998; 101: 96–102.
 18. Corominas M, Mestre M, Bas J, Buendia E. Distinct modulation by interferon-gamma (IFN-gamma) of CD23 expression on B and T lymphocytes of atopic subjects. *Clin Exp Immunol* 1998; 112: 276–280.
 19. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003; 8: 223–246.
 20. Hanifin JM, Schneider LC, Leung DY, Ellis CN, Jaffe HS, Izu AE, et al. Recombinant interferon gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993; 28: 189–197.
 21. Reinhold U, Kukel S, Brzoska J, Kreysel HW. Systemic interferon gamma treatment in severe atopic dermatitis. *J Am Acad Dermatol* 1993; 29: 58–63.
 22. Frotscher B, Anton K, Worm M. Inhibition of IgE production by the imidazoquinoline resiquimod in nonallergic and allergic donors. *J Invest Dermatol* 2002; 119: 1059–1064.
 23. Daniels BB, Askew SL, van de Venter M, Oosthuizen V. Production of biologically active recombinant human soluble CD23 and its effect on PBMCs isolated from hyper-IgE blood. *Cell Immunol* 2005; 234: 146–153.
 24. Wheeler DJ, Parveen S, Pollock K, Williams RJ. Inhibition of sCD23 and immunoglobulin E release from human B cells by a metalloproteinase inhibitor, GI 129471. *Immunology* 1998; 95: 105–110.
 25. Cukrowska B, LodInova-ZadnIkova R, Enders C, Sonnenborn U, Schulze J, Tlaskalova-Hogenova H. Specific proliferative and antibody responses of premature infants to intestinal colonization with nonpathogenic probiotic *E. coli* strain Nissle 1917. *Scand J Immunol* 2002; 55: 204–209.
 26. Thompson CB. Distinct roles for the costimulatory ligands B7-1 and B7-2 in T helper cell differentiation? *Cell* 1995; 81: 979–982.
 27. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR. Heat shock protein 60 enhances CD4 CD25 regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 2006; 116: 2022–2032. Epub 2006 Jun 2008.
 28. Muzio M, Mantovani A. The Toll receptor family. *Allergy* 2001; 56: 103–108.
 29. Sabroe I, Parker LC, Wilson AG, Whyte MK, Dower SK. Toll-like receptors: their role in allergy and non-allergic inflammatory disease. *Clin Exp Allergy* 2002; 32: 984–989.
 30. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30: 1230–1234.
 31. Cross ML, Gill HS. Can immunoregulatory lactic acid bacteria be used as dietary supplements to limit allergies? *Int Arch Allergy Immunol* 2001; 125: 112–119.
 32. Li XM, Srivastava K, Grishin A, Huang CK, Schofield B, Burks W, et al. Persistent protective effect of heat-killed *Escherichia coli* producing “engineered,” recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003; 112: 159–167.
 33. Sheikh A, Smeeth L, Hubbard R. There is no evidence of an inverse relationship between TH2-mediated atopy and TH1-mediated autoimmune disorders: Lack of support for the hygiene hypothesis. *J Allergy Clin Immunol* 2003; 111: 131–135.
 34. Wheeler JG, Shema SJ, Bogle ML, Shirrell MA, Burks AW, Pittler A, et al. Immune and clinical impact of *Lactobacillus acidophilus* on asthma. *Ann Allergy Asthma Immunol* 1997; 79: 229–233.
 35. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000; 30: 1604–1610.
 36. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357: 1076–1079.
 37. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, et al. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; 53: 1617–1623.
 38. Cross ML, Ganner A, Teilab D, Fray LM. Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. *FEMS Immunol Med Microbiol* 2004; 42: 173–180.