Lactobacillus rhamnosus induces peripheral hyporesponsiveness in stimulated CD4⁺ T cells via modulation of dendritic cell function¹⁻³

Henri Braat, Jan van den Brande, Erik van Tol, Daan Hommes, Maikel Peppelenbosch, and Sander van Deventer

ABSTRACT

Background: Although it is widely recognized that the intake of so-called probiotic microorganisms is beneficial in chronic mucosal inflammation and topical allergic disease, the immunologic details explaining how such bacteria can exert these effects remain obscure. Objective: We determined whether Lactobacillus rhamnosus can modulate T cell responses in vitro and in vivo.

Design: In vitro, human monocyte-derived dendritic cells (DCs) matured in the presence of L. rhamnosus were used to instruct naive CD4⁺ T cells; subsequently, the T cell response was assessed with the use of CD3/CD28 and interleukin (IL) 2. Cytokine production by ex vivo–stimulated naive cells and memory T cells was measured before and after oral supplementation with L. rhamnosus in 6 healthy volunteers and 6 patients with Crohn disease.

Results: A decreased T cell proliferation and cytokine production, especially of IL-2, IL-4, and IL-10, was observed in CD3/CD28-stimulated T cells derived from L. rhamnosus–matured DCs. This T cell hyporesponsiveness was associated with enhanced DC–T cell interaction and normal responsiveness of T cells for IL-2. In vivo oral supplementation of L. rhamnosus for 2 wk induced a similar T cell hyporesponsiveness, including impaired ex vivo T helper subsets 1 and 2 responses without up-regulation of immunoregulatory cytokines in cohorts of both healthy volunteers and patients with Crohn disease.

Conclusions: We propose that L. rhamnosus modulates DC function to induce a novel form of T cell hyporesponsiveness; this mechanism might be an explanation for the observed beneficial effects of probiotic treatment in clinical disease. Am J Clin Nutr 2004;80:1618–25.

KEY WORDS Lactobacillus rhamnosus, hyporesponsiveness, T cells, Crohn disease, dendritic cells

INTRODUCTION

Different microorganisms elicit widely divergent effects in the gastrointestinal tract, including an influence on the mucosal immune system (1). Whereas most bacteria are potentially dangerous for the mucosal homeostasis and activate deleterious immunologic responses, the so-called probiotics do not have this effect (2). Probiotics are a group of bacteria in which the Lactobacilli and Bifidobacteria are the most prominent members, and it is claimed that they have antiinflammatory properties. Accordingly, such probiotic bacterial strains protect against experimental colitis in rodents (3–5) and against exacerbations of inflammatory bowel disease (6) and topical allergy (7, 8) in human patients. In agreement, food supplements specifically enhancing the growth of probiotic bacteria are widely recognized to be beneficial in a variety of inflammatory conditions, including inflammatory bowel disease (1). Thus, in contrast with most microorganisms, some probiotic bacteria are capable of impairing the immunologic reaction to their mucosal presence in particular and have a dampening effect on the adaptive immune system in general. The immunologic details by which probiotic bacteria exert their immunosuppressive activity remains very poorly understood.

To a certain extent, probiotics exert their action by niche occupation and thus prevent colonization of the bowel by pathogenic bacterial species (9–11). Moreover, it has become clear that probiotic bacteria directly influence host physiology (12, 13), but their antiinflammatory effects in inflammatory bowel disease (14) and topical allergic disease (7, 8) suggest that a direct effect on the immune system is involved as well. Because inflammatory bowel disease displays mainly T helper subset 1 (Th1) characteristics and because topical allergies are characterized by a T helper subset 2 (Th2) phenotype, both benefit from probiotic supplementation (15); the immunologic effects of probiotic bacteria probably do not involve altered Th1/Th2 polarization. These observations prompt research in alternative directions like the induction of regulatory T cells or direct suppression of specific immune responses.

Interestingly, ex vivo coculture of mucosal specimens with various Lactobacillus species diminished CD4⁺ T cell outgrowth (16), which suggests that T cell activation itself may be a target of probiotic bacteria. T cell activation is usually a consequence of antigen presentation by dendritic cells (DCs) on major histocompatibility complex class II molecules in combination with proper expression of costimulatory molecules and the secretion of specific cytokines (17). Therefore, T cell–modulating...
The effects of probiotics may be mediated via altered DC function. Hence, to gain more insight into the effects of probiotic bacteria, we investigated the effects of L. rhamnosus on DC-dependent T cell responses in vitro. Moreover, we orally supplemented healthy volunteers and patients with Crohn disease with L. rhamnosus for 2 wk and assessed the immunomodulating effects of this probiotic on ex vivo–stimulated T cells.

METHODS

Dendritic cell culture and final maturation

All cultures were performed in Iscove’s modified Dulbecco’s medium (Iscove’s Modified Dulbecco’s Medium; Bio-Whittaker, Walkersville, MD) with 1% fetal calf serum (HyClone, Lagan, UT) and gentamycin (86 mg/L; Sigma, St Louis). Peripheral blood from healthy volunteers was used to generate immature DCs; monocytes were isolated after density gradient centrifugation (1000 × g, 30 min, room temperature) and cultured in the presence of recombinant human (rhu) granulocyte-macrophage colony-stimulating factor (500 U/mL; a gift from Schering Plough, Uden, Netherlands) and rhu interleukin (IL) 4 (250 U/mL; Pharma Biotechnology Hannover, Hannover, Germany). After 6 d, cultures consisted of CD14−, HLA-DR+, CD83−, CD86low, and CD40high immature DCs. Final maturation was achieved on day 6 by adding 1 × 107 colony-forming units (CFUs) of L. rhamnosus (Numico Research B.V., Wageningen, Netherlands) to immature DCs derived from 5 × 105 monocytes. Maturation factors, rhu IL-1β (ng/mL; Boehringer Mannheim, Germany), rhu tumor necrosis factor α (TNF-α; 25 ng/mL; Pharmacia Biotechnology Hannover), and lipopolysaccharide (LPS; 100 ng/mL; Sigma) were used when indicated. On day 8, DCs were harvested, washed twice, and subsequently used for fluorescence activated cell sorting (FACS) analysis, cytokine production, and mixed lymphocyte reactions.

FACS analysis and cytokine production of dendritic cells

DCs were labeled with mouse anti-human CD83-APC (Caltech, Burlingame, CA) and mouse anti-human CD86-PE (BD Pharmingen, San Jose, CA) and subsequently analyzed with the use of a FACS Vantage (FACScan; Becton Dickinson, Mountain View, CA). Immature DCs (2 × 105 cells in 200 μL) were washed and stimulated for 48 h as earlier described. IL-6 and IL-8 were measured in supernatant fractions with the use of Cytometric Bead Array (BD Biosciences, Mountain View, CA) and Rantes (regulated upon activation, normal T cell expressed and secreted; R&D Systems, Minneapolis) was measured with the use of an enzyme-linked immunosorbent assay. Mature DCs were washed and stimulated (2 × 105 cells in 200 μL) with CD40L-transfected J558 plasmacytoma cells and interferon γ (IFN-γ) as indicated. After 24 h of stimulation, supernatant fractions were used for cytokine detection with the following assays: IL12p70 (detection limit: 31.2 pg/mL; R&D Systems), TNF-α (CLB, Amsterdam), and IL-10 (CLB).

Mixed lymphocyte reaction and [3H]thymidine incorporation

Highly purified CD4+CD45RA+CD45RO− naïve T cells (>90% as assessed by flow cytometry) were purified from peripheral blood mononuclear cells (PBMCs) with the use of a MACS separation system (Miltenyi Biotech GmbH, Gladbach, Germany).

For cytokine measurement, naïve CD4+ T cells (2 × 105 cells/200 mL Iscove’s Modified Dulbecco’s Medium with 10% fetal calf serum) were cocultured with 5 × 105 mature DCs in 96-well flat-bottom culture plates (Costar, Cambridge, MA). Around day 5, when T cells were optimally proliferating, cells were restimulated for cytokine production (see Stimulation and cytokine analysis below).

For the measurement of T cell proliferation after DC stimulation, naïve CD4+ T cells (2.5 × 105 cells/200 μL) were cocultured in 96-well flat-bottom culture plates (Costar) with different amounts of distinctively treated DCs. Fully proliferated T cells, after DC stimulation, were serially diluted (1.0 × 104−1.6 × 106 cells/200 μL) in 96-well round-bottom culture plates (Costar) and stimulated with rhu IL-2 (10 U/mL; Cetus, Emeryville, CA) or soluble mouse anti-human-CD3 and mouse anti-human-CD28 (both from CLB) with an end concentration of 1:1000. T cell proliferation was finally assessed by the incorporation of [3H]thymidine (Radiochemical Centre, Amersham, Little Chalfont, United Kingdom) after a pulse with 13 kBq/well during the last 16 h of a 5-d culture after stimulation and was then measured by liquid scintillation spectroscopy.

Design of the human study

The Medical Ethical Committee of the Academic Medical Center in Amsterdam approved the study. Six patients with quiescent Crohn disease (n = 2 men and 4 women) and 6 healthy control subjects (n = 3 men and 3 women) were included in the study after informed consent had been given (Table 1). The mean age of the healthy volunteers was 37 y and of the patients was 35.8 y. Four patients had ileocolonic disease, whereas 2 patients had involvement of the colon only (Table 1). Two of the 6 patients used azathioprine, 1 was treated with budesonide, and 3 received no maintenance treatment; no changes in medication occurred during the supplementation period (Table 1). All patients had a Crohn disease activity index (18) <150 (median: 46), and this index did not change during the study period (Table 1). L. rhamnosus (Numico Research B.V.), conserved as a lyophilized powder, was administered as a single daily oral preparation at a concentration of 5 × 1010 CFUs for 2 wk. On day 0, immediately before supplementation, and on day 14 after supplementation of L. rhamnosus, venous blood samples were obtained.

Isolation of PBMCs and cell sorting

Whole blood was collected aseptically, and PBMCs were isolated with the use of density gradient centrifugation (Ficoll...
According to their CD45RB expression: the lowest (CD45RBlow) and highest (CD45RBhigh) 40% of total CD4+ T cells, 92% express CD45RO, a memory T cell marker; 91% of all CD45RBhigh T cells express CD45RA, which is characteristic of naive T cells. Both populations were 92% and 95% pure, respectively, on reanalysis (B). Of all CD4+ T cells, 92% express CD45RO, a memory T cell marker; 91% of all CD45RBhigh T cells express CD45RA, which is characteristic of naive T cells (C).

Paque; Pharmacia Biotechnology, Uppsala, Sweden). The PBMCs were washed twice and resuspended in sterile phosphate-buffered saline containing 5% bovine serum albumin (Sigma Aldrich Chemie, Steinheim, Germany).

Cells were stained with one of the following antibodies: unconjugated mouse anti-human CD45RB (Southern Biotechnology), goat F(ab)’ anti-mouse IgG+IgM/Fitc (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA), and mouse anti-human CD4/Cy-chrome (CLB). Cells were subsequently sorted on a FACS Vantage (Becton Dickinson) to yield a CD4+CD45RBlow and CD4+CD45RBhigh subset, defined as the lowest and highest 40% of the CD4+CD45RB cell population (Figure 1). Both populations were >90% pure on reanalysis (Figure 1) and mainly expressed CD45RO and CD45RA, respectively (Figure 1).

**Stimulation and cytokine analysis**

Before stimulation, T cells were washed twice in and diluted to a concentration of 1 × 10^6/mL in Iscove’s Modified Dulbecco’s Medium with 10% fetal calf serum. After transfer of 100 μL of the cell suspension to round-bottom 96-well culture plates (Costar) cells were stimulated for 72 h with soluble mouse anti-human-CD3 and mouse anti-human-CD28 (both from CLB); the end concentration was 1:1000. Supernatant fractions of the stimulated cells were collected and frozen at −20 °C until cytokine analysis was performed.

Cytokine production by T cells was measured with a Cytometric Bead Array (BD Biosciences). In short, beads with distinct fluorescence intensities and coated with capture antibodies specific for different cytokines were incubated with the stimulated T cell supernatant fractions. The bound cytokines were detected with an antibody coupled to a second fluorochrome. Bead suspensions were analyzed with a FACS Vantage, and the fluorescence intensity of the second fluorochrome was used to calculate cytokine concentrations. The following cytokines were measured for 6 healthy volunteers and 6 Crohn disease patients: IL-2, IL-4, IL-10, IFN-γ, and TNF-α. The detection limit for all cytokines was 20 pg/mL. Transforming growth factor β (TGF-β) in T cell supernatant fractions from 6 healthy volunteers and from 6 Crohn disease patients was determined with the use of a standard enzyme-linked immunosorbent assay (R&D Systems); the detection limit was 31 pg/mL.

**CD25 expression**

The expression of CD25 by unstimulated CD4+ T cells was determined before and after probiotic supplementation in 4 healthy volunteers and in 4 patients with Crohn disease. PBMCs (2 × 10^5 cells) were stained with unconjugated mouse anti-human CD45RB (Southern Biotechnology), goat F(ab)’, anti-mouse IgG+IgM/Fitc (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA), and mouse anti-human CD4/Cy-chrome (CLB), and mouse anti-human CD25/Phyco-erythrin (BD Biosciences). The mean fluorescence intensity of CD25/PE was measured with the FACS Vantage.

**Statistical analysis**

DC-, IL-2-, and CD3/CD28-stimulated T-cell proliferation of various conditions was compared by using a multiple regression analysis. For comparison of cytokine production from in vitro and ex vivo experiments, we performed a heteroscedastic Student’s t test. When multiple groups were present, a Kruskall-Wallis one-factor analysis of variance or two-factor analysis of variance for differences over time and between health status (data not shown) was also performed (SPSS software, version 11.01; SPSS Inc, Chicago). The comparison between cytokine production by naive- and memory T cells was not performed. Statistical significance was defined as a P value < 0.05.

**RESULTS**

*L. rhamnosus* inhibits the responsiveness of T cells via modulation of DC function

The observation that probiotics have immunomodulatory effects on adaptive immune-mediated disease suggests that such bacteria may directly influence the maturation of DCs, because these cells are the main driving force of specific T cell responses in immunity. Thus, to test the functional consequences of *L. rhamnosus*–dependent DC maturation, we compared the stimulatory effects of DCs in a mixed lymphocyte reaction. To this end, monocytes isolated from peripheral human blood were exposed to a DC differentiation protocol that involved the sequential application of IL-4 and granulocyte macrophage colony-stimulating factor followed by the addition of LPS, TNF-α, and...
**LACTOBACILLUS RHAMNOSUS INDUCES HYPORESPONSIVENESS**

Ingestion of *L. rhamnosus* by healthy subjects induces T cell hyporesponsiveness in vivo

Subsequent experiments were performed to determine the in vivo relevance of *L. rhamnosus*-dependent T cell hyporesponsiveness; a group of 6 human volunteers was orally treated with $1 \times 10^{10}$ CFU of viable *L. rhamnosus* per day for 2 wk. Subsequently, sorted naive and memory T cells were stimulated with CD3/CD28 to assess T cell responsiveness. The production of IL-2 and IL-4 decreased in naive and memory T cells in most of the subjects; moreover, a decrease in IL-10 in memory T cells was detected after oral supplementation with *L. rhamnosus* (Figure 6; *P* < 0.05).

Ingestion of *L. rhamnosus* by patients with Crohn disease induces T cell hyporesponsiveness in vivo

The T cell hyporesponsiveness described above was observed in healthy subjects, which raises the question as to the validity of these findings in a proinflammatory environment. Nevertheless, the effects described with probiotics in clinical disease suggest that these microorganisms have potent antiinflammatory effects.
in chronic inflammatory bowel disease. Hence, it was important to investigate the effects of *L. rhamnosus* on T cell responsiveness in patients with established Crohn disease. As expected, T cell responsiveness before supplementation of *L. rhamnosus* and after T cell receptor stimulation (CD3/CD28) in patients compared with healthy subjects was skewed toward Th1 cytokines and in general more pronounced. At baseline, naïve T cells from patients with Crohn disease produced more IL-2 than did naïve T cells from healthy subjects (*P* < 0.04; Figure 6), and after 2 wk of oral supplementation with *L. rhamnosus*, IL-2 production decreased in this T cell subset in most of the patients (Figure 6). Stimulated naïve T cells from patients with Crohn disease produced less IL-4 than did stimulated naïve T cells from healthy volunteers (Figure 6; *P* < 0.01), and probiotic therapy did not significantly affect IL-4 production by either T cell subpopulation in patients (Figure 6). The production of IFN-γ by memory T cells was initially higher in Crohn disease patients than in healthy volunteers (Figure 6; *P* < 0.01), and probiotic supplementation decreased IFN-γ production by this T cell population in most of the patients (Figure 6). Strikingly, the production of IL-10 by memory T cells and the expression of CD25 by CD4<sup>+</sup> T cells (both characteristics of regulatory T cells) were significantly lower after oral supplementation of *L. rhamnosus* in

**FIGURE 4.** Mean (±SEM) [3H]thymidine incorporation of naïve T cells. Immature dendritic cells (DCs) received no stimulation or were matured with tumor necrosis factor α and interleukin 1β (maturation factors (MF)), MF and lipopolysaccharide (LPS) (MF/LPS), or MF, LPS, and *Lactobacillus rhamnosus* (MF/LPS/Lr). Subsequently, mature DCs were washed twice, counted, and serially diluted in a range of 20-10 000 DCs/well in triplicate. Naïve T cells (25 000 cells/well) were added, and T cell proliferation was measured after 5 d. T cell proliferation induced with DCs matured with MF/LPS/Lr was significantly higher than that induced with DCs matured with MF/LPS (*P* = 0.05) or MF (*P* < 0.03) by multiple regression analysis. Moreover, T cell proliferation induced with MF/LPS was significantly higher than that induced with MF, *P* = 0.05 (multiple regression analysis).

**FIGURE 5.** A: Immature dendritic cells (DCs) were stimulated with tumor necrosis factor α (TNF-α) and interleukin (IL) 1β (maturation factors (MF)), MF and lipopolysaccharide (MF/LPS), or MF, LPS, and *Lactobacillus rhamnosus* (MF/LPS/Lr) for 48 h and subsequently analyzed for CD83 and CD86 expression by fluorescence activated cell sorting. B: Supernatant fractions of the stimulated immature DCs (20 000/well, in triplicate) were taken after 48 h for IL-6, IL-8, and Rantes (regulated upon activation, normal T cell expressed and presumably secreted) measurement (upper panels). Mature DCs (20 000/well) were restimulated with CD40L-transfected J558 plasmacytoma cells and interferon γ for IL-12p70 and TNF-α measurements and with CD40L-transfected J558 plasmacytoma cells alone for IL-10 measurement (lower panel). *Significantly different from MF/LPS: *P* < 0.05 (heteroscedastic Student’s *t* test), *P* < 0.03 (Kruskall-Wallis one-factor ANOVA).
healthy subjects and Crohn disease patients (Figure 6; $P < 0.05$ for both IL-10 and CD25).

**DISCUSSION**

Most bacteria are potent activators of mucosal immunologic responses, but the so-called probiotics have antiinflammatory properties. Hence, such probiotic bacterial strains are increasingly being used for the treatment of a variety of inflammatory disorders. The immunologic details by which these protective organisms mediate their effects remain poorly understood. In the present study we observed that *Lactobacillus rhamnosus* influenced DC maturation in a manner that leads to optimal T cell stimulation. However, T cells expanded in the presence of DCs matured with *L. rhamnosus* were severely impaired with respect to T cell receptor activation as measured by cytokine production and proliferation. Surprisingly, the proliferation of these T cells (in the absence of DC) to IL-2 is normal and hence the biological behavior of these T cells is consistent with certain aspects of anergic T cells (19). Importantly this T cell hyporesponsiveness was not due to a generalized suppressive effect on DC physiology because DCs were potent T cell stimulators in a mixed lymphocyte reaction. The effects observed are likely to be specific to probiotic bacteria and do not reflect a general effect of the presence of microorganisms during DC maturation. Using an experimental setup similar to that used in the present study, we recently characterized the effects of commensal *Klebsiella pneumoniae* on DC maturation and subsequent T cell responses in vitro (20). In contrast with the action of *L. rhamnosus*, the presence of *K. pneumoniae* during DC maturation produced strong Th1-polarized responses. The DCs exposed to *L. rhamnosus* showed attenuated cytokine production and lacked the ability to induce Th1 or Th2 polarization of naive T cells (20). Thus *L. rhamnosus* seems to have specific effects on DC immunology, which leads to the generation of T cells with hyporesponsive characteristics and possibly explains the immunologic effects of probiotics during human disease.

The in vitro effect of *L. rhamnosus* on DC and subsequent T cell hyporesponsiveness was reflected in our in vivo studies. At baseline, peripheral T cells from patients with Crohn disease produced high amounts of IL-2 (predominantly the naive population) and IFN-γ (predominantly the memory population). Probiotic supplementation strikingly reduced IFN-γ and IL-2 production by peripheral T cells in patients with quiescent Crohn disease, proposing a possible antiinflammatory mechanism of *L. rhamnosus*. The CD4+ IFN-γ–producing T cells are a hallmark of Th1 immune responses ubiquitous in Crohn disease (21, 22), and IL-2 is of crucial importance for the survival and proliferation of effector T cells. Probiotic supplementation also affected Th2 immune responses because the healthy subjects showed a
remarkable reduction in IL-4 production. Hence, the effects of probiotic supplementation on peripheral blood T cell responses were unlikely to result from a mere skewing of the Th1-Th2 balance. These observations agree with the results of clinical trials that report beneficial effects of probiotics in diseases characterized by exaggerated Th1 and Th2 immune responses (6–8).

Adaptive immune responses are controlled by regulatory T lymphocytes, of which ≥3 functional phenotypes have been reported. Regulatory T cells type 1 generated from naive T cells cultured in the presence of IL-10 are able to suppress experimental colitis in an IL-10–dependent manner (23) and IL-10–producing regulatory T cells with a memory phenotype (low expression of CD45RB) prevent colitis (24, 25). In our study, IL-10 production by memory T cells from patients with Crohn disease and healthy subjects decreased after probiotic therapy. Therefore, our data do not support the hypothesis that L. rhamnosus supplementation generates IL-10–producing regulatory T cells. Alternatively, CD4+CD25+ T cells are potent regulatory lymphocytes that are able to prevent T cell activation and proliferation via cell-cell interactions but not through production of known negative regulatory cytokines (26–29).

In the present study, L. rhamnosus supplementation decreased the expression of CD25 in unstimulated CD4+ T cells, which suggests that L. rhamnosus did not lead to an increase in the number CD4+CD25+ regulatory T cells, although we cannot formally exclude the increased activation of this cell type by this microorganism. Finally, T cell hyporesponsiveness may be obtained via a third type of regulatory T cell described that induces its regulatory effects via the production of TGF–β (30, 31). In our experiments, TGF–β production by T cells from patients with Crohn disease and healthy subjects was not affected by supplementation with L. rhamnosus. We believe that our in vivo data are best explained by the L. rhamnosus–specific modulation of DC function, which resulted in the induction of T cell hyporesponsiveness. The phenotype of these T cells does not have the characteristics of known regulatory T cells, which suggests a novel mechanism of immune regulation. The immunomodulatory effects of L. rhamnosus are most likely not limited to this strain and it is therefore encouraging to test different probiotic and or commensal organisms in a clinical setting comparable with ours. For example, a placebo-controlled study in patients with allergy that allows sensitive clinical testing for disease activity seems very promising.

The molecular basis of the immunologic modulation of DCs by L. rhamnosus was not addressed in this study, but it may lie in either the secretion of soluble factors interfering with DC-signaling (eg, nuclear transcription factor κB or signal transducer and activator of transcription activation), thereby modulating DC-function (32). This may provide the bacterium with an evasive strategy with respect to the immune system and hence a competitive advantage at the mucosal surface and thus provides the bacterium with a niche for cell growth in the intestinal bowel. How bacteria prevent other microorganisms from profiting from this niche is unclear, but evidence exists that L. rhamnosus is able to suppress the growth of a variety of other bacteria via a different strategies (33–35). Disregarding, however, the exact evolutionary advantages that have led to L. rhamnosus–dependent modulation of DC immunologic responses, the present study showed that L. rhamnosus mediates its effects on T cell responses via modulation of monocyte-derived DCs in vitro. Moreover, the observation that comparable effects on T cell function can be achieved by oral supplementation with L. rhamnosus suggests that similar events take place in vivo. Of course, the in vivo situation is more complicated with different DC lineages and the important influence of the environmental make-up on DC maturation. However, we propose a model in which probiotic bacteria might have a direct effect on DC function, which is supported by recent findings that some probiotic organisms can bind to cell receptors specific for DCs (36). The resulting DC-primed, hyposensitive T cells down-modulate both Th1 and Th2 immune responses, which in turn may well explain the remarkable effects of probiotics on adaptive immunity and autoimmune disease.

HB and JvdB participated in the conception, design, execution, analysis, interpretation, and draft of the study. EvT and DH participated in the conception and design of the clinical trial. MP and SvD participated in the conception, design, interpretation, and revision of the study. None of the authors had a conflict of interest.

REFERENCES