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Probiotic supplement consumption alters cytokine production from peripheral blood mononuclear cells: a preliminary study using healthy individuals

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RESEARCH PAPER

Abstract

The objective of this study was to examine the effect of daily probiotic supplementation upon the immune profile of healthy participants by the assessment of *ex vivo* cytokine production. Twenty healthy adult volunteers received a multi-strain probiotic supplement consisting of two strains of *Lactobacillus acidophilus* (CUL60 and CUL21), *Bifidobacterium lactis* (CUL34) and *Bifidobacterium bifidum* (CUL20) and fructooligosaccharide for 12 weeks. Blood samples were collected at baseline, 6 and 12 weeks. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured *ex vivo* in the presence or absence of lipopolysaccharide and cytokine production was assessed. Post-intervention, a significant decrease in the production of interleukin-6 and interleukin-1β was apparent when PBMCs were incubated in the presence of lipopolysaccharide, whilst a significant increase in IL-10 and transforning growth factor-β production was seen when the cells were incubated without an additional stimulus. This preliminary study demonstrates the potential of a multi-strain probiotic supplement to alter the immune response as demonstrated by changes in *ex vivo* cytokine production. Such results demonstrate the potential benefit of probiotic supplementation for healthy individuals and warrants further investigation.

Keywords: Lactobacillus, Bifidobacterium, cytokines, immune modulation

1. Introduction

The Joint FAO/WHO Expert Consultation defines a probiotic as 'a live microorganism which when administered in adequate amounts confers a health benefit on the host' (FAO/WHO, 2001). In a recent review it was found that multi-strain probiotic products appeared to show greater efficacy for a range of beneficial health-related outcomes than single strain products (Chapman *et al.*, 2011). The benefits of probiotics include reduction in the incidence and/or severity of antibiotic-associated diarrhoea and *Clostridium difficile*-associated diarrhoea, reduction of symptoms in irritable bowel syndrome and prevention of atopic eczema in high-risk individuals (Avadhani and Miley, 2011; Pelucchi *et al.*, 2012; Ritchie and Romanuk, 2012; Williams *et al.*, 2009). The mechanisms by which probiotics exert these effects have not been fully elucidated but they have been shown to modulate the permeability of intestinal epithelial barriers, compete with pathogens for colonisation of the mucosa, alter the inflammatory potential of epithelial cells and modify the activity of immune cells (Boirivant and Strober, 2007; Rizzello *et al.*, 2011; Vanderpool *et al.*, 2008).

The immunological effects of probiotics include alterations of the inflammatory/anti-inflammatory response, the T-helper (Th)1/Th2 balance and the induction of a regulatory T cell (Treg) response. *Lactobacillus casei* NIZO B255 and *Lactobacillus reuteri* ASM2001 have been shown to interact with C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin expressed on co-cultured monocyte-derived dendritic cells in order to drive Treg development and interleukin (IL)-10 production *in vitro* (Smits *et al.*, 2005). *Ex vivo* analysis of immune cell activation following 3 weeks *Lactobacillus rhamnosus* HN001 supplementation in healthy volunteers showed enhanced activity of both phagocytes and natural

killer cells (Sheih *et al.*, 2001). Furthermore, there is evidence suggesting that probiotic supplementation can also augment the immune response *in vivo*. Rizzardini *et al.* (2012) observed a probiotic-induced enhancement of vaccine-specific antibody titres following seasonal flu vaccination during 6 weeks of supplementation with either *Bifidobacterium lactis* Bb-12 or *L. casei* 431. The immune-protective effect of probiotics is also apparent in murine models of rheumatoid arthritis, atopic dermatitis and colitis and has been attributed to reduced production of pro-inflammatory cytokines, increases in FoxP3+ CD4+ T cells and increases in tolerogenic markers on dendritic cells (Kwon *et al.*, 2010). Fructooligosaccharide (FOS), a selectively fermented prebiotic (Roberfroid *et al.*, 2010) that is often co-administered with probiotics can also induce an immune response as seen by increased expression of IL-10 positive lamina propria dendritic cells in FOS-treated Crohn's disease patients (Lindsay *et al.*, 2006) and reduced expression of pro-inflammatory cytokines by human epithelial colorectal cells exposed to FOS *in vitro* (Zenhom *et al.*, 2011).

To date, there are very few studies examining the immunological effect of probiotic and FOS supplementation in a healthy population. In this 12-week exploratory study, the participants received a daily dose of the LAB4 multi-strain probiotic consortium, which had previously proved effective in ameliorating symptoms in irritable bowel syndrome (Williams *et al.*, 2009), in combination with FOS and the impact of supplementation on pro-inflammatory and anti-inflammatory cytokine levels *ex vivo* was assessed.

2. Materials and methods

Participants

This study was approved by the University of Sheffield Research Ethics Committee (SMBRER130, Sheffield, UK). Volunteers from the general public aged 18-50 years were recruited in response to posters and e-mails. Exclusion criteria were body mass index >29.9, current smokers, history of abdominal surgery, pregnant or lactating, reported gastrointestinal disorder, receiving immune-modulating drugs or were already consuming a prebiotic or probiotic product. All participants provided written informed consent.

Study design

Twenty volunteers were recruited and the study was conducted over a 12-week period. The participants ingested, with food, the LAB4 supplement comprising two strains of *Lactobacillus acidophilus* CUL21 (NCIMB 30156) and CUL60 (NCIMB 30157), *Bifidobacterium bifidum* CUL20 (NCIMB 30153), *Bifidobacterium animalis* subsp. *lactis* CUL34 (NCIMB 30172) (total: 25×10^9 cfu/daily dose) and 2 g FOS. The LAB4 supplement was provided by Cultech Ltd. (Port Talbot, UK). Compliance was measured by the number of unused sachets returned.

Sample processing

10 ml blood samples were collected into EDTA-vacutainer® tubes (BD, Oxford, UK) by venous puncture at baseline, 6 and 12 weeks and processed within 30 min of collection. The blood was overlaid on 15 ml Histopaque-1077® (Sigma, Poole, UK), and the peripheral blood mononuclear cells (PBMCs) were purified by density centrifugation and re-suspended at 2×10^6 cells/ml in supplemented RPMI-1640 culture medium (Sigma) (containing 10% (v/v) heat inactivated foetal bovine serum (FBS), 1 mM sodium pyruvate and 2 mM glutamine (Sigma)) in standard 24-well culture plates.

Peripheral blood mononuclear cells incubation

Duplicate samples of PBMCs (1×10^6cells) were incubated in 0.5 ml supplemented RPMI-1640 culture medium with or without 10 μg/ml lipopolysaccharide (LPS; Sigma) for 72 h. Cell-free supernatants were stored at -80 °C until analysis.

Cytokine analysis

ELISAs were used to determine concentrations of IL-6, IL-10, tumour necrosis factor-α (TNF-α), IL-1β (OptEIA™; BD) and transforming growth factor-β (TGF-β; DuoSet®; RnD systems, Abingdon, UK) in accordance with the manufacturer's instructions. Cytokine levels present in FBS were determined and subtracted from all test samples. Cytokines levels are presented as a fold change compared to baseline.

Statistical analysis

Experimental data are expressed as mean ± standard deviation. Statistical comparison between intervention period (6 or 12 weeks) and baseline cytokine levels were performed using an ANOVA model with repeated measurements (STATS v9.2; Stata Corporation, TX, USA). *P*-values of less than 0.05 were considered statistically significant.

3. Results

Study compliance

20 people (age 42.1±8.4; 55% female) were enrolled in the trial. Six withdrew; 4 due to bloating, the remaining two for non-compliance reasons. Compliance to the product was greater than 90%.

Cytokine response in non-stimulated cultures

Incubation of PBMCs in media alone resulted in detectable levels of IL-10 and TGF-β in the supernatant. Concentrations of IL-10 increased during the course of the study reaching significance at 12 weeks compared to baseline (Figure 1A). At baseline, IL-10 concentrations were 7.6±3.6 pg/ml (difference: 4.3, 95% confidence interval (CI): -2.6 to 11.37), at 6 weeks they showed a 1.56-fold increase (difference: 15.9, 95% CI: 9.2 to 22.7) and at 12 weeks a 3.09-fold increase. Similarly, TGF-β production increased during the study (Figure 1B). TGF-β concentrations were 1,667.3±584.3 pg/ml at baseline (difference: 117.7, 95% CI: -289.3 to 524.6), at 6 weeks they showed a 1.07-fold increase (difference: 805.4, 95% CI: 398.4 to 1212.3), and at 12 weeks a 1.48-fold increase. Temporal changes in IL-10 and TGF-β levels for individual participants are shown in Supplementary Figure S1.

Cytokine response in stimulated cultures

In the presence of LPS, IL-6, IL-1β and TNF-α were detected in the supernatant from the PBMCs. IL-6 concentrations significantly decreased at 6 and 12 weeks compared to baseline (Figure 2A). IL-6 concentrations were 95.5±33.9 ng/ml at baseline (difference: -44.6, 95% CI: -66.0 to -23.1), at 6 weeks they showed a 0.53-fold decrease (difference: -36.6, 95% CI: -57.5 to -15.8), and at 12 weeks a 0.62-fold decrease. IL-1β production was also significantly lower at 12 weeks than at baseline (Figure 2B). At baseline IL-1 β concentrations were 1,621.7±724.9 pg/ml (difference: -356.0, 95% CI: -795.4 to 83.4), at 6 weeks they showed a 0.69-fold decrease (difference: -700.0, 95% CI: -1,124.3 to -275.8), and at 12 weeks a 0.63-fold decrease. No significant changes in TNF-α concentration were observed throughout the study period (Figure 2C). TNF-α concentrations were 52.8±39.9 pg/ml at baseline (difference: 18.8, 95% CI: -5.16 to 42.8), at 6 weeks they showed a 1.27-fold increase (difference: 6.0, 95% CI: -17.2 to 29.2), and at 12 weeks a 0.94-fold decrease. Temporal changes in IL-6, IL-1β and TNF-α level for individual participants are shown in Supplementary Figure S2.

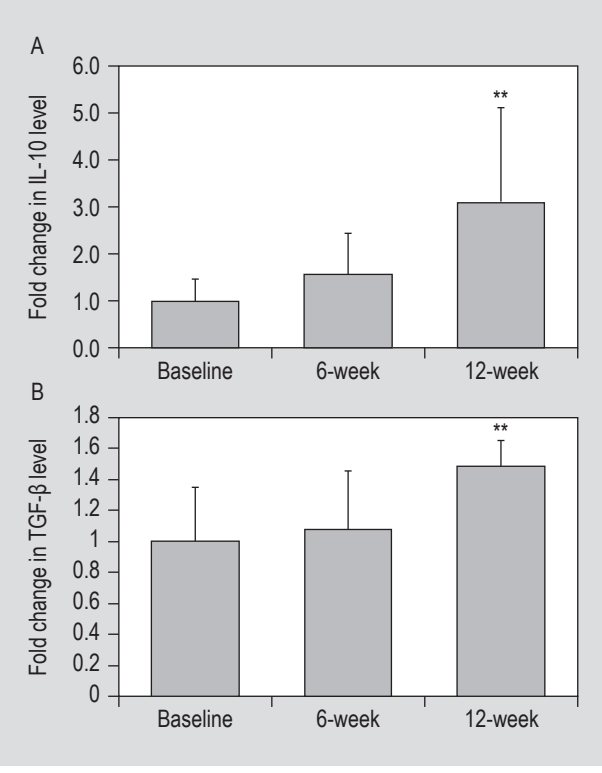

Figure 1. (A) Interleukin-10 (IL-10) and (B) transforming growth factor-β (TGF-β) production from non-stimulated peripheral blood mononuclear cell cultures. Data are presented as means and standard deviation. **P<0.0001. Representative graphs shown from duplicate ELISAs.

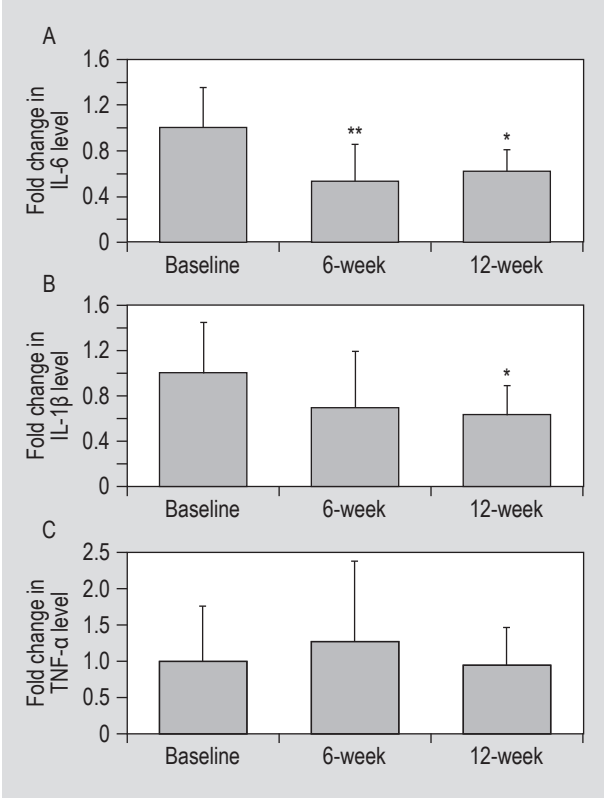

Figure 2. (A) Interleukin (IL)-6, (B) IL-1β and (C) tumour necrosis factor-α (TNF-α) production from lipopolysaccharide-stimulated peripheral blood mononuclear cell cultures. Data are presented as means and standard deviation. *P<0.01, **P<0.0001. Representative graphs shown from duplicate ELISAs.

4. Discussion

In the absence of immune challenge, *ex vivo* cytokine production by PBMCs showed significant enhancement of production of the anti-inflammatory cytokines IL-10 and TGF-β during the study period. LPS stimulation of the PBMCs resulted in significantly decreased production of the pro-inflammatory cytokines IL-1β and IL-6 over the study period, while no significant changes in TNF-α production were observed. These changes suggest that the LAB4 supplement is capable of having differential effects upon the immune system depending upon the nature of the immune stimulation. In the 'resting' immune state, the LAB4 supplement may contribute to immune regulation through enhancement of production of the regulatory cytokines IL-10 and TGF-β. However, under stimulating conditions, the LAB4 supplement prevents excessive immune activation by dampening the production of the pro-inflammatory cytokines IL-1β and IL-6.

Comparison with the limited amount of existing studies that examined probiotic-mediated PBMC *ex vivo* cytokine production suggest that the LAB4 supplement can modulate the immune response in a selective manner. To this end, PBMCs extracted from healthy volunteers subjected to 2 months treatment with another commonly administered probiotic, *L. reuteri* DSM 17938, showed no significant changes in IL-10, IL-1β or IL-6 production, although it should be noted that this study used a phorbol myristate acetate/ionomycin challenge and not an LPS-challenged system (Mangalat *et al.*, 2012). Furthermore, *ex vivo* PBMC cytokine analysis post *L. rhamnosus* GG or *B. lactis* Bb-12 administration showed a significant reduction in TNF-α production (Kekkonen *et al.*, 2008), an effect not apparent in our study.

Despite a lack of direct evidence showing that FOS treatment alone can regulate PBMC cytokine production in healthy subjects *ex vivo*, it seems likely that the changes observed during our study are likely to be at least in part mediated by FOS. A recent study that examined the immune profile of HIV-patients in response to combination therapy reported that treatment with complex FOS and probiotics (*L. rhamnosus* HN001 and *B. lactis* Bi-07) together resulted in a larger decrease of serum IL-6 levels when compared to those treated with FOS or probiotics individually (González-Hernández *et al.*, 2012). This suggests that both FOS and the probiotic component may be responsible for the immunological changes observed in our study. However, it does seem likely that the FOS component is responsible for the relatively high incidence of non-compliance observed in our study. FOS-related bloating has been reported on numerous occasions elsewhere (Bouhnik *et al.*, 2007; Briet *et al.*, 1995; Ten Bruggencate *et al.*, 2006).

In summary, this study has demonstrated that the LAB4 supplement has had a potentially beneficial immunomodulatory effect in healthy individuals, which is manifest as *ex vivo* alterations in cytokine production. Significant changes were observed despite the small number of participants and free-living setting of this study. The changes observed in this exploratory study warrant further investigation in a double-blind, placebo-controlled trial.

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Supplementary material

Supplementary material may be found online at http://dx.doi.org/10.3920/BM2013.0012.

Figure S1. Temporal changes in interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) production by non-stimulated peripheral blood mononuclear cell cultures.

Figure S2. Temporal changes in interleukin (IL)-6, IL-1β and tumour necrosis factor-α (TNF-α) production by lipopolysaccharide-stimulated peripheral blood mononuclear cell cultures.

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