Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers  

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ABSTRACT
Background: Aging is associated with reduced numbers of beneficial colonic bifidobacteria and impaired immunity. Galactooligosaccharides (GOSs) stimulate the growth of bifidobacteria in younger adults, but little is known about their effects in the elderly and their immunomodulatory capacity.
Objective: We assessed the effect of a prebiotic GOS mixture (B-GOS) on immune function and fecal microflora composition in healthy elderly subjects.
Design: In a double-blind, placebo-controlled, crossover study, 44 elderly subjects were randomly assigned to receive either a placebo or the B-GOS treatment (5.5 g/d). Subjects consumed the treatments for 10 wk, and then went through a 4-wk washout period, before switching to the other treatment for the final 10 wk. Blood and fecal samples were collected at the beginning, middle (5 wk), and end of the test period. Predominant bacterial groups were quantified, and phagocytosis, natural killer (NK) cell activity, cytokine production, plasma cholesterol, and HDL cholesterol were measured.
Results: B-GOS significantly increased the numbers of beneficial bacteria, especially bifidobacteria, at the expense of less beneficial groups compared with the baseline and placebo. Significant increases in phagocytosis, NK cell activity, and the production of antiinflammatory cytokine interleukin-10 (IL-10) and significant reduction in the production of proinflammatory cytokines (IL-6, IL-1β, and tumor necrosis factor-α (TNF-α)) by peripheral blood mononuclear cells (PBMCs), as well as diminished phagocytic and natural killer (NK) cell activities (6–8).
Dietary substrates reaching the large intestine are able to influence the composition and activities of bacteria present through their fermentation capacities. Metabolic products from colonic bacteria may affect the immune system. Modulation of the intestinal microflora by dietary means is the basis for the probiotic (9) and prebiotic (10) concepts. Various strains of bifidobacteria and lactobacilli as probiotics were also shown to exert immunostimulatory properties (11, 12).
It is well known that prebiotics, namely inulin-type fructooligosaccharides (FOSs) and galactooligosaccharides (GOSs), support the growth of beneficial bacteria in younger adult populations. In general, studies report increased numbers of lactobacilli, bifidobacteria, or both and decreased enterobacteria after FOS supplements (13, 14). However, studies have not fully determined their effect on the gut microflora of elderly persons.
The immunomodulatory potential of prebiotics has not been adequately investigated, and most data originate from animal models. For elderly human subjects, data are scarce and contradictory for modulation of the gut microflora. In one study, FOS administration to frail elderly subjects in a nursing home for 3 wk resulted in increased bifidobacteria, reduced phagocytic activity, and reduced expression of IL-6 mRNA in PBMCs (15). However, in another study, the same researchers reported that 12 wk

INTRODUCTION
A progressive increase in the proportion and number of persons >60 y old has led to heightened attention to their physiologic and health needs, as well as to increased costs to the health care system. Aging is associated with changes in the function of many organs and tissues, including the gastrointestinal tract (GIT). The composition of the elderly intestinal microflora differs from that of younger adults. Studies investigating these differences report increases in putrefactive bacteria, such as clostridia (especially Clostridium perfringens) and enterobacteria at

of FOS administration to malnourished elderly subjects diminished IL-6 and TNF-α mRNA without any effect on the fecal microflora (16).

The effects of GOSs as potential modulators of both the elderly gut microflora and the immune system have not been reported thus far, although some reports suggest that these prebiotics have a good prebiotic effect in younger adults (17). In the present study, we have investigated the effect of administering a GOS mixture (B-GOS, Bi2muno) on immune function and fecal microflora composition of healthy elderly subjects in a double-blind, randomized, placebo-controlled crossover study.

SUBJECTS AND METHODS

Materials

Unless otherwise stated all chemicals and reagents were obtained from Sigma-Aldrich Co Ltd (Poole, United Kingdom) or BDH Chemicals Ltd (Poole, United Kingdom). Fluorescent probes, the K562 cell line and cell culture media, and supplements were also obtained from Sigma-Aldrich Co Ltd.

Subjects and study design

Forty-four, free-living, elderly volunteers [28 women and 16 men; average (±SD) age: 69.3 ± 4.0 y; range: 64–79 y; body mass index (BMI; in kg/m²) range: 22–31] were enrolled into the study to provide 99% statistical power (MGH BIOSTATISTICS SOFTWARE, Massachusetts General Hospital, Boston, MA) for the studied parameters on the basis of the data reviewed in the literature. Of these, 3 volunteers did not complete the study (1 fatality was unrelated to the study, 1 volunteer was reluctant to provide fecal samples regularly and was withdrawn, and 1 volunteer experienced tolerance problems and withdrew after 3 wk).

Subjects were asked to reconstitute sachets immediately before each visit (2 wk before the start; on day 0; and after 5, 10, 14, 19, and 24 wk). During all (except 2 wk before the start) visits, blood was sampled, and blood pressure, weight, use of any medication (including vitamin-mineral supplements), and adverse events were also recorded. In addition, subjects were asked to confirm their compliance with, or deviation from, the study protocol and to keep daily diaries recording bowel movements, stool consistency, and incidences of abdominal pain, flatulence, and bloating (data not presented).

Preparation and collection of fecal samples

Fecal samples were collected in plastic pots lined with a sterile plastic bag at the University of Reading, and they were immediately diluted in anaerobic sterile phosphate buffer [phosphate-buffered saline (PBS), 0.1 mol/L, pH 7.0, (1:10, wt:vol), Oxoid Ltd, Basingstoke, United Kingdom], homogenized in a Stomacher 400 (Seward, Norfolk, United Kingdom), and used for fluorescent in situ hybridization (FISH). A portion of each fecal sample was removed before dilution and used to freeze-dry and measure the dry weight.

FISH analysis

Differences in bacterial populations were assessed with the use of FISH analysis with oligonucleotide probes designed to target specific diagnostic regions of 16S rRNA. The probes were synthesized commercially and labeled with the fluorescent dye Cy3. The probes used were Bif164 (18), Bac303 (19), Lab158 (20), Ere482 and His150 (21), Ec1531 (22), and Srb687 (23), specific for Bifidobacterium spp., Bacteroides spp., Lactobacillus-Enterococcus spp., the Clostridium coccoide–Eubacterium rectale group, the Clostridium histolyticum group, Escherichia coli, and Desulfovibrio spp., respectively. For total bacterial counts, the nucleic acid stain 4’,6-diamidino-2-phenylindole (DAPI) was used. Fecal homogenates from the volunteers were diluted in 4% (wt:vol) paraformaldehyde and fixed overnight at 4 °C. Cells were then centrifuged at 1500 × g for 5 min, washed twice with PBS (0.1 mol/L, pH 7.0), resuspended in a mixture of PBS and 99% ethanol (1:1, wt:vol), and stored at −20 °C for at least 1 h. Hybridization of cell suspensions was performed with the use of the modified well method described by Daims et al (24). Briefly, the cell suspension was diluted appropriately for each probe and added to the well of a Teflon- and poly-L-lysine-coated 6-well slide (Tekton Inc, Myakka City, FL). Slides were dried in a desktop plate incubator.
PBMCs (10×10^6 cells/mL) were resuspended in RPMI-glutamine-antibiotics medium supplemented with autologous plasma (5% by vol). Cells were then plated onto 24-well plates in the presence of lipopolysaccharide (10μg/mL) and incubated at 37 °C in an air to carbon dioxide (19:1) atmosphere for 24 h. After incubation, the plates were centrifuged at 400×g for 5–6 min, and the supernatant fluids were collected and frozen in aliquots (200μL). The concentration of cytokines (IL-6, IL-10, IL-1β, TNF-α, and IL-8) was measured by enzyme-linked immunosorbent assay with the use of commercially available kits (R&D Systems Inc, Minneapolis, MN) and the instructions provided by the manufacturer. The limits of detection for these assays were as follows: IL-6, 2.5 ng/mL; IL-10, 3.3 ng/mL; IL-1β, 7.2 ng/mL; TNF-α, 3.7 ng/mL; and IL-8, 3.6 ng/mL (data were supplied by the manufacturer of the kits).

Measurement of phagocytic activity
Phagocytosis was determined with the use of Phagotest kits (Orpegen Pharma, Heidelberg, Germany). Before use, blood was cooled on ice for 10 min and mixed by vortexing. Aliquots (100μL) of blood were incubated on ice (control) or at 37 °C in a preheated water bath for 10 min with opsonized fluorescein isothiocyanate-labeled E. coli (20μL). The reaction was stopped by adding ice-cold quenching solution (100μL) and vortexing. Samples were then washed several times in solution provided by the manufacturer, erythrocytes were lysed, leukocytes were fixed, and the DNA was stained. Cell preparations (200μL) were transferred onto glass slides containing wells and covered with a cover slip. Slides were protected from light and examined with the use of phase-contrast and fluorescence microscopy within 1 h. At least 10–15 random fields of view were examined, and the number of leukocytes (monocytes and neutrophils), number of leukocytes that had internalized bacteria, and the number of bacteria engulfed by these cells were recorded.

Statistical analysis
All statistical tests were performed with the use of SPSS version 15.0 (SPSS Inc, Chicago, IL), and a value of P < 0.05 was taken to indicate statistical significance. All data were analyzed by an analysis of variance model with repeated measurements.
RESULTS

Effect of B-GOS on bacterial populations

The fecal microflora composition of the elderly volunteers who participated in this study was determined by the use of FISH and probes targeting bacterial groups of interest. No differences were observed in bacterial populations in the 2 treatment groups at baseline (Table 2). The 2 treatments administered did not appear to have an effect \((P > 0.14)\) on the total bacterial count during the study. After 5 wk, the placebo treatment did not alter the numbers of \textit{Bifidobacterium} spp., the \textit{C. coccosoides–E. rectale} group, the \textit{C. histolyticum} group, or \textit{Desulfovibrio} spp., whereas those of \textit{Bacteroides} spp. \((P < 0.001)\) and \textit{E. coli} \((P < 0.01)\) increased and \textit{Lactobacillus-Enterococcus} spp. \((P < 0.05)\) decreased (Table 2). At the same time, B-GOS had a significant effect on all bacterial groups measured compared with the baseline and placebo groups. Higher numbers of \textit{Bifidobacterium} spp., \textit{Lactobacillus-Enterococcus} spp., and the \textit{C. coccosoides–E. rectale} group were observed compared with both baseline and the placebo treatment (Table 2). In contrast, numbers of \textit{Bacteroides} spp., the \textit{C. histolyticum} group, \textit{E. coli}, and \textit{Desulfovibrio} spp. decreased compared with both baseline and the placebo treatment (Table 2).

By the end of the trial period (10 wk), both treatments had exerted a significant effect on all bacterial groups monitored in this study compared with baseline (Table 2). The placebo treatment resulted in increased numbers of \textit{Bifidobacterium} spp. \((P < 0.001)\), \textit{Bacteroides} spp. \((P < 0.001)\), the \textit{C. histolyticum} group \((P < 0.01)\), \textit{E. coli} \((P < 0.001)\), and \textit{Desulfovibrio} spp. \((P < 0.05)\), and decreased numbers of \textit{Lactobacillus-Enterococcus} spp. \((P < 0.05)\) and the \textit{C. coccosoides–E. rectale} group \((P < 0.05)\) (Table 2). Furthermore, numbers of \textit{Bifidobacterium} spp., \textit{Bacteroides} spp., the \textit{C. histolyticum} group, and \textit{Desulfovibrio} spp. were significantly higher than after 5 wk of the placebo treatment \((P < 0.01, P < 0.05, P < 0.01, \text{and } P < 0.001)\) for \textit{Bifidobacterium} spp., \textit{Bacteroides} spp., the \textit{C. histolyticum} group, and \textit{Desulfovibrio} spp., respectively (Table 2). The effect of B-GOS treatment observed at 5 wk was also further enhanced by 10 wk. There were higher numbers of \textit{Bifidobacterium} spp. \((P < 0.001)\) for all, \textit{Lactobacillus-Enterococcus} spp. \((P < 0.001)\) for all, and the \textit{C. coccosoides–E. rectale} group \((P < 0.001)\) for all and lower numbers of \textit{Bacteroides} spp. \((P < 0.001)\) for all, the \textit{C. histolyticum} group \((P < 0.001)\) for all, \textit{E. coli} \((P < 0.001)\) for all, and \textit{Desulfovibrio} spp. \((P < 0.001)\) for all) compared with baseline, the 5-wk B-GOS treatment, and the 10-wk placebo treatment (Table 2).

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial populations of the monitored fecal microflora components for the placebo and prebiotic galactooligosaccharide mixture (B-GOS) treatments used in elderly volunteers during the study period as determined by fluorescent in situ hybridization.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Bacterial group</strong></td>
</tr>
<tr>
<td><strong>log10 cells/g feces</strong></td>
</tr>
<tr>
<td><strong>Bifidobacterium spp.</strong></td>
</tr>
<tr>
<td><strong>Bacteroides spp.</strong></td>
</tr>
<tr>
<td><strong>Lactobacillus-Enterococcus spp.</strong></td>
</tr>
<tr>
<td><strong>Clostridium coccosoides–Eubacterium rectale</strong></td>
</tr>
<tr>
<td><strong>Clostridium histolyticum</strong></td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td><strong>Desulfovibrio spp.</strong></td>
</tr>
</tbody>
</table>

\(^1\)All values are \(\bar{x} \pm SD; n = 41\). Values were analyzed by ANOVA with 3 fixed effects (treatment, period of treatment, and sequence of treatment). There were no significant interactions between sequence and treatment or between period and treatment.

\(^2\)Significantly different from baseline, \(P < 0.001\) (Tukey’s test).

\(^3\)Significantly different from 5 wk, \(P < 0.01\) (Tukey’s test).

\(^4\)Significantly different from placebo, \(P < 0.001\) (Tukey’s test).

\(^5\)Significantly different from 5 wk, \(P < 0.001\) (Tukey’s test).

\(^6\)Significantly different from baseline, \(P < 0.05\) (Tukey’s test).

\(^7\)Significantly different from placebo (Tukey’s test): \(^8\)\(P < 0.05\), \(^9\)\(P < 0.01\).

\(^10\)Significantly different from baseline, \(P < 0.01\) (Tukey’s test).

\(^11\)Significantly different from 5 wk, \(P < 0.05\) (Tukey’s test).
Bifidobacterium spp., C. coccoides–E. rectale were no significant interactions between sequence and treatment or between period and treatment; there were no significant differences between groups at the study period (ANOVA with 3 fixed effects: treatment, period of treatment, and sequence of treatment). No significant effects were found.

The association between the effect of placebo and B-GOS, at the end of the trial period, on bacterial groups measured in this study and NK cell activity was determined by bivariate correlation analysis (Table 5). This analysis showed that the numbers of Bifidobacterium spp., Lactobacillus–Enterococcus spp., and the C. coccoides–E. rectale group were significantly positively (P < 0.01) correlated, and the numbers of Bacteroides spp. were significantly negatively (P < 0.01) correlated with NK cell activity (Table 5).

**Effect of B-GOS on cytokine production by PBMCs**

The effects of the 2 treatments on the production of cytokines (IL-10, IL-1β, TNF-α, IL-6, and IL-8) are shown in Table 5. Baseline TNF-α concentration was significantly higher (P < 0.05) before B-GOS supplementation (Table 6). No significant effects of time or treatment were observed on the production of IL-8 (Table 6). However, differences were observed in the production of other cytokines. At 5 wk, the placebo treatment resulted in increased production of IL-1β (P < 0.05), TNF-α (P < 0.001), and IL-6 (P < 0.05), whereas production of IL-10 remained unchanged compared with baseline (Table 6). B-GOS supplementation, however, resulted in decreased production of TNF-α (P < 0.01) and IL-6 (P < 0.05), increased production of IL-10 (P < 0.05), and no change in the concentration of IL-1β compared with the baseline at this time point (Table 6). TNF-α and IL-6 production after B-GOS treatment was significantly lower (P < 0.05 for both cytokines) at this time point than that observed after placebo treatment, whereas IL-10 and IL-1β concentrations did not differ significantly from placebo (Table 6). After 10 wk of the placebo treatment, no further increases were observed in the production of IL-1β, TNF-α, and IL-6, but the values were still significantly higher than those observed at baseline (P < 0.05, P < 0.001, and P < 0.05 for IL-1β, TNF-α, and IL-6, respectively). In the case of IL-10 production, the placebo treatment resulted in a further reduction at 10 wk, which was significant when compared with baseline (P < 0.01) and 5 wk (P < 0.05). At the same time, B-GOS treatment resulted in further decreases in the production of IL-1β (P < 0.01), TNF-α (P < 0.001), and IL-6 (P < 0.001) compared with both baseline (P < 0.05, P < 0.05, and P < 0.001 for IL-1β, TNF-α, and IL-6, respectively) and 5 wk (Table 6). Production of IL-10 was further significantly increased compared with both baseline (P < 0.01) and 5 wk (P < 0.01) after the B-GOS treatment (Table 6). Furthermore, production of IL-1β (P < 0.05), TNF-α (P < 0.01), and IL-6 (P < 0.001) was significantly lower and production of IL-10 (P < 0.01) was significantly higher after 10 wk of B-GOS treatment than was the placebo treatment at the same time (Table 6).

### Table 3

Effect of the placebo and prebiotic galactooligosaccharide mixture (B-GOS) treatments on total cholesterol and HDL cholesterol during the study period

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.94 ± 0.88</td>
<td>1.29 ± 0.32</td>
</tr>
<tr>
<td>Week 5</td>
<td>4.93 ± 0.96</td>
<td>1.26 ± 0.30</td>
</tr>
<tr>
<td>Week 10</td>
<td>4.97 ± 0.97</td>
<td>1.29 ± 0.31</td>
</tr>
<tr>
<td>B-GOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.02 ± 0.93</td>
<td>1.31 ± 0.27</td>
</tr>
<tr>
<td>Week 5</td>
<td>4.99 ± 0.91</td>
<td>1.28 ± 0.29</td>
</tr>
<tr>
<td>Week 10</td>
<td>5.07 ± 0.99</td>
<td>1.28 ± 0.27</td>
</tr>
</tbody>
</table>

1 All values are ± SD; n = 41. Values were analyzed by ANOVA with 3 fixed effects: treatment, period of treatment, and sequence of treatment. There were no significant interactions between sequence and treatment or between period and treatment; there were no significant differences between groups at baseline. 

### Table 4

Effect of placebo and prebiotic galactooligosaccharide mixture (B-GOS) treatments on natural killer cell activity of peripheral blood mononuclear cells

<table>
<thead>
<tr>
<th>Ratio of effector to target cells</th>
<th>Placebo</th>
<th>B-GOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 5</td>
</tr>
<tr>
<td>100:1</td>
<td>49.8 ± 18.4</td>
<td>52.9 ± 18.9</td>
</tr>
<tr>
<td>50:1</td>
<td>25.6 ± 9.0</td>
<td>26.9 ± 9.2</td>
</tr>
<tr>
<td>25:1</td>
<td>12.9 ± 5.1</td>
<td>14.5 ± 5.4</td>
</tr>
</tbody>
</table>

1 All values are ± SD; n = 41. Values were analyzed by ANOVA with 3 fixed effects: treatment, period of treatment, and sequence of treatment. There were no significant interactions between sequence and treatment or between period and treatment; there were no significant differences between groups at baseline.

2 Significantly different from baseline, P < 0.001 (Tukey’s test).

3 Significantly different from 5 wk, P < 0.001 (Tukey’s test).

4, 5 Significantly different from placebo (Tukey’s test): 4P < 0.05, 5P < 0.001.

6 Significantly different from baseline, P < 0.05 (Tukey’s test).

7 Significantly different from 5 wk, P < 0.01 (Tukey’s test).

8 Significantly different from placebo, P < 0.01 (Tukey’s test).
The change in the production of IL-10, IL-1β, TNF-α, and IL-6 at 10 wk of placebo and B-GOS treatments from baseline is shown in Figure 1. This figure summarizes clearly opposite and significantly different effects of the 2 treatments on the production of these cytokines.

**Effect of B-GOS on phagocytosis**

Total phagocytic activity of leukocytes (neutrophils and monocytes) is expressed as the percentage of cells able to internalize fluorescently labeled E. coli (number of cells involved in phagocytosis) and as the quantity of internalized bacteria per active cell (level of activity). As shown in Figure 2, the baseline samples did not differ significantly (P > 0.8) between the 2 treatments. The placebo treatment had no effect on either the percentage of cells engaged in phagocytosis or the amount of phagocytic activity, regardless of time (Figure 2). B-GOS, however, resulted in a significant increase in the percentage of cells engaged in phagocytosis of E. coli after 5 wk of treatment (P < 0.001 compared with baseline and placebo), and this effect was further enhanced after 10 wk (P < 0.001 compared with baseline, placebo, and 5 wk; Figure 2). Furthermore, B-GOS treatment also increased the phagocytic capacity, as assessed by the average number of phagocytosed bacteria per active cell (Figure 2). This increase was significant after 5 wk of treatment compared with baseline (P < 0.001) and with the placebo treatment (P < 0.05) at the same time point and was further enhanced after 10 wk of the B-GOS treatment (P < 0.001 compared with baseline, 5 wk, and placebo).

The association between the effect of placebo and B-GOS on bacterial groups measured in this study and phagocytosis as determined by bivariate correlation analysis is shown in Table 5. The numbers of Bifidobacterium spp., Lactobacillus-Enterococcus spp., and the C. coccoides–E. rectale group were significantly positively (P < 0.01) correlated, and the numbers of Bacteroides spp. and E. coli were significantly negatively (P < 0.01) correlated with both the percentage of cells involved in phagocytosis and the amount of phagocytic activity. In addition, the numbers of the C. histolyticum group and Desulfovibrio spp. were also significantly negatively (P < 0.01) correlated with the percentage of cells involved in phagocytosis (Table 5).

**TABLE 5**

Relation between bacterial groups measured and natural killer (NK) cell activity and phagocytosis at 10 wk of the treatments as determined by bivariate correlation analysis

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>NK cell activity</th>
<th>Cells engaged in phagocytosis</th>
<th>Phagocytic capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% active cell</td>
<td>bacteria/active cell</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>0.546</td>
<td>0.288</td>
<td>0.496</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>−0.309</td>
<td>−0.635</td>
<td>−0.429</td>
</tr>
<tr>
<td>Lactobacillus-Enterococcus spp.</td>
<td>0.337</td>
<td>0.353</td>
<td>0.317</td>
</tr>
<tr>
<td>Clostridium coccoides–Eubacterium rectale</td>
<td>0.394</td>
<td>0.375</td>
<td>0.450</td>
</tr>
<tr>
<td>Clostridium histolyticum group</td>
<td>NS</td>
<td>−0.504</td>
<td>NS</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>NS</td>
<td>−0.405</td>
<td>−0.350</td>
</tr>
<tr>
<td>Desulfovibrio spp.</td>
<td>NS</td>
<td>−0.455</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 All values are Pearson’s correlation coefficient (r) and are significant at P < 0.01 (1-tailed).
2 Percentage of cytolysis at an effector-to-target cell ratio of 100:1.

**TABLE 6**

Production of cytokines by peripheral blood mononuclear cells with placebo and prebiotic galactooligosaccharide mixture (B-GOS) treatments during the study period

<table>
<thead>
<tr>
<th></th>
<th>IL-10</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/mL</td>
<td>pg/mL</td>
<td>pg/mL</td>
<td>pg/mL</td>
<td>pg/mL</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>412.7 ± 297.1</td>
<td>5375 ± 3098</td>
<td>490.9 ± 267.6</td>
<td>41 750 ± 27 084</td>
<td>115 499 ± 42 729</td>
</tr>
<tr>
<td>Week 5</td>
<td>367.0 ± 229.9</td>
<td>5823 ± 3036</td>
<td>678.7 ± 377.7</td>
<td>47 899 ± 25 189</td>
<td>141 554 ± 117 808</td>
</tr>
<tr>
<td>Week 10</td>
<td>329.3 ± 220.3</td>
<td>6203 ± 2913</td>
<td>719.7 ± 368.6</td>
<td>49 518 ± 24 402</td>
<td>117 483 ± 57 498</td>
</tr>
<tr>
<td>B-GOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>366.7 ± 228.7</td>
<td>5090 ± 2670</td>
<td>679.9 ± 414.5</td>
<td>39 796 ± 20 766</td>
<td>126 615 ± 80 466</td>
</tr>
<tr>
<td>Week 5</td>
<td>400.5 ± 223.3</td>
<td>4815 ± 3216</td>
<td>527.3 ± 364.0</td>
<td>34 028 ± 16 610</td>
<td>124 750 ± 62 659</td>
</tr>
<tr>
<td>Week 10</td>
<td>522.5 ± 314.9</td>
<td>4394 ± 3020</td>
<td>447.5 ± 282.3</td>
<td>26 238 ± 13 928</td>
<td>132 555 ± 86 038</td>
</tr>
</tbody>
</table>

1 All values are x ± SD; n = 41. IL-10, interleukin-10; TNF-α, tumor necrosis factor-α. Values were analyzed by ANOVA with 3 fixed effects (treatment, period of treatment, and sequence of treatment). There were no significant interactions between sequence and treatment or between period and treatment.
2–4 Significantly different from baseline (Tukey’s test): 2 P < 0.05, 4 P < 0.001, 6 P < 0.01.
5 Significantly different from 5 wk, P < 0.05 (Tukey’s test).
6 Significantly different from placebo, P < 0.05 (Tukey’s test).
7 Significantly different from 5 wk, P < 0.01 (Tukey’s test).
8 Significantly different from placebo, P < 0.01 (Tukey’s test).
9 Significantly different from 5 wk, P < 0.001 (Tukey’s test).
10 Significantly different from placebo, P < 0.001 (Tukey’s test).
tumor necrosis factor-α (TNF-α), and IL-6. The mean for the B-GOS group was significantly different from that for the placebo group for all cytokines (IL-6: *P* < 0.001; IL-10 and TNF-α: *P* < 0.01; IL-1β: *P* < 0.05). Values were analyzed by ANOVA with 3 fixed effects (treatment, period of treatment, and sequence of treatment). No significant interactions were observed between sequence and treatment or between period and treatment.

### DISCUSSION

The intestinal microflora can be considered as an organ composed of a large diversity of bacterial cells that can perform different functions for the host. Specific components of this microflora, including bifidobacteria and lactobacilli, are associated with beneficial effects for the host, such as promotion of gut maturation and integrity, antagonism against pathogens, and immune modulation (9, 10, 12, 17). The principal aim of this study was to assess the effect of a prebiotic, B-GOS, compared with placebo on the major bacterial groups found in feces of elderly persons and to assess the effect of this change in the microflora components on immune function of the host.

Age-related changes in GIT physiology and function, such as greater permeability of mucosal membrane, reduced transit times, and secretion of acids by the gastric mucosa, result in a significant change in the composition of the intestinal microflora, marked by a decline in bifidobacterial numbers and an increase in putatively detrimental populations such as clostridia and enterobacteria (2, 3, 25). These bacteriologic and other physiologic changes may result in increased putrefaction in the colon and greater susceptibility to disease. In this sense, any dietary supplement, such as prebiotics, that could counteract these changes is of value. However, the effect of prebiotics on the fecal microflora of elderly has not been extensively investigated. GOSs are generally recognized prebiotics (17, 26), and the specific GOS ingredient used in this study, B-GOS, produced with the use of bifidobacterial enzymes (27), was shown to promote the selective growth of bifidobacteria in vitro mixed culture systems (28) and in vivo in younger healthy adults (29). Furthermore, B-GOS was shown in ex vivo experiments to selectively promote the growth of *Bifidobacterium bifidum* and *Bifidobacterium longum* (29), 2 species reported to have good immunomodulatory properties (30, 31).

In the present study, the placebo treatment increased fecal populations of bacteroides and *E. coli* and decreased lactobacilli, but it had no effect on the other bacterial groups tested at 5 wk. B-GOS, however, reduced numbers of less beneficial bacteria (bacteroides, the *C. histolyticum* group, *E. coli*, and *Desulfovibrio* spp.) and increased numbers of beneficial bacteria (bifidobacteria, lactobacilli, and the *C. coccoides-E. rectale* group). These effects became statistically significant by 5 wk of treatment (*P* < 0.05), but they were further enhanced at 10 wk (*P* < 0.001), showing that the bifidogenic effect of B-GOS was not lost or reduced over this time course. Although there were some increases in bifidobacteria after 10 wk of placebo treatment compared with the baseline sample, this was offset by significant increases in less beneficial bacterial groups and decreases in lactobacilli and the *C. coccoides-E. rectale* group. The effect of B-GOS treatment on host immune function was assessed with the use of a range of biomarkers of the innate and adaptive immune system. Probiotic bacteria, such as lactobacilli and bifidobacteria, were shown in vivo to interact with mouse epithelial cells of the small intestine and be internalized with the use of distinct pathways into the epithelial cells of the large intestine by the follicle-associated epithelium cells (32). They make contact with underlying immune tissues, and, through these interactions, they were suggested to bring about strain-specific immunomodulation (33), such as differentially induced cytokine production by macrophages in a concentration-dependent manner (34). Probiotics were also shown to regulate the balance between necessary and excessive immune response to support an adequate immune response to infection by enhancing phagocytosis, NK cell activity, and TH1 responses associated with the release of proinflammatory cytokines (IL-10 and transforming growth factor-β) through the regulatory Tr1 and Tfh3 cells (33).

Orally applied prebiotics were also associated with immunomodulating effects encompassing both innate and specific immunity, although evidence was mostly derived from animal
Many of the proinflammatory cytokines showed to be suppressed in chronic enterocolitis similar to inflammatory bowel disease (44). With potent antiinflammatory and immunosuppressive activities for the importance of IL-10 as a significant immunoregulator, the effect of the B-GOS treatment being significantly greater than that after the placebo treatment \((P < 0.05)\).

B-GOS unequivocally enhanced phagocytic activity in a time-dependent manner, whereas the placebo treatment had no such effect. Although, mechanisms by which prebiotics are able to influence these activities are not understood, we have shown a clear positive correlation between numbers of beneficial bacteria and both NK cell activity and phagocytosis, whereas some less beneficial bacteria (ie, bacteroides) were negatively correlated. This suggests that, at least in part, the observed modulating effects of the 2 treatments on the microflora were responsible for the changes in NK and phagocytic activities.

Cytokines have a range of important roles in the regulation of immunity. Elevated concentrations of proinflammatory cytokines are associated with both aging (7, 8, 39) and chronic inflammatory disease (40). In the present study, there was considerable intersubject variation in production of the cytokines studied, some of which could be attributed to factors such as sex, genetic polymorphisms, smoking status, BMI, and dietary habits, as well as early life events, hormonal status, and gut microflora (41, 42). Nevertheless, B-GOS significantly decreased the production of both TNF-\(\alpha\) and IL-6 by 5 wk, whereas the placebo treatment significantly increased production of the 2 cytokines. B-GOS also decreased the production of IL-1\(\beta\) at 10 wk, whereas the placebo treatment resulted in an initial increase at 5 wk and no further effect at the end of the treatment. In the case of IL-10, the antiinflammatory cytokine, B-GOS significantly increased production by 5 wk and further production by 10 wk, whereas the placebo treatment decreased production of this cytokine at 10 wk. These data clearly show opposite effects of the 2 treatments on the proinflammatory and antiinflammatory cytokine profiles, with the B-GOS treatment resulting in an overall antiinflammatory effect and the placebo treatment showing a tendency to produce a proinflammatory effect.

The antiinflammatory effect of IL-10 is partly achieved through suppression in the production of macrophage inflammatory proteins IL-1, IL-6, IL-12, and TNF-\(\alpha\) (43). In vivo evidence for the importance of IL-10 as a significant immunoregulator with potent antiinflammatory and immunosuppressive activities comes from the observation that IL-10–deficient mice develop chronic enterocolitis similar to inflammatory bowel disease (44). Many of the proinflammatory cytokines showed to be suppressed by IL-10 are known to be regulated by the nuclear transcription factor \(\kappa B\) (45), which also has a role in IL-10 gene expression (46, 47). In this study we have shown that B-GOS treatment resulted in a clearly less inflammatory profile, and it could be speculated that the mechanism of action involves the nuclear transcription factor \(\kappa B\) pathway.

To date, the immunomodulatory potential of GOS has not been directly shown. Two studies looked at the effect of combined application of probiotics with GOS on gut health and immunity (48, 49). In the first study, combination of GOS with Bifidobacterium lactis (HN019) enhanced NK cell activity compared with probiotic alone (48), and, in the other, the effect of GOS in combination with Lactobacillus rhamnosus (HN001) was not significantly different from the probiotic alone (49).

Several animal studies have shown that administration of probiotics, prebiotics, or fermented milk products is effective in lowering blood cholesterol concentrations. However, in vivo results are variable, with some studies reporting lowering effects (50) and increasing HDL cholesterol (51, 52) and others without any effect (53, 54). Most studies reporting decreased concentrations of serum cholesterol or increased HDL cholesterol or both, after either probiotic or prebiotic administration, have used subjects with elevated serum cholesterol concentrations. Results from our study showed that B-GOS had no effect on total serum cholesterol and HDL cholesterol concentrations. The reason for this might be because our subjects had normal serum cholesterol concentrations before the start of the trial.

In conclusion, this study showed that B-GOS administration to healthy elderly subjects led to a significant decrease in less beneficial bacteria and a significant increase in beneficial bacteria, especially bifidobacteria. We also found significant positive effects on the immune response, evidenced by an improvement in NK cell activity and phagocytosis, increased secretion of the antiinflammatory cytokine IL-10, and decreased secretion of proinflammatory cytokines (IL-6, IL-1\(\beta\), and TNF-\(\alpha\)) by stimulated PBMCs. Therefore, dietary intervention that uses B-GOS is an attractive option for enhancement of both the GIT and immune system, which could be of particular importance for the elderly.

The author’s responsibilities were as follows—JV, GT, and GRG: designed the study; PY: helped with a choice of immune methods; JV: prepared protocols, selected and recruited volunteers, analyzed data, and wrote the manuscript; AD: collected and processed all the samples; JV and AD: performed the experimental work; PY, GT, and GRG: reviewed the manuscript. GT is employed by Clasado Ltd. None of the other authors had a personal or financial conflict of interest.

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