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Supplementation with a single and double strain probiotic on the innate immune system for respiratory illness

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SUMMARY

Background and aims: The immune mechanisms by which probiotics reduce susceptibility to upper respiratory tract illness is uncertain. The aim of this study was to examine purported cell-mediated immune mechanisms that might explain the reduction in respiratory illness observed following daily supplementation with *Bifidobacterium animalis* subsp. *lactis* Bl-04 (Bl-04) and a combined *Lactobacillus acidophilus* NCFM & *B. animalis* subsp. *lactis* BI-07 (NCFM & Bi-07).
Methods: A cohort of 144 healthy physically active individuals were allocated to daily supplementation consumed as a beverage with Bl-04 ($n = 46$) supplemented at a dosage of 2.0×10^9 colony forming units (cfu) per day, NCFM & Bi-07 ($n = 47$) at a dosage of 5.0×10^9 CFU per day each, or a placebo ($n = 51$) over 150 d. Markers included plasma cytokines, metalloproteinases and neurotrophins, peripheral blood leucocyte numbers, antibody-dependent and antibody-independent NK cell activity (NKCA), and peripheral blood mononuclear cell (PBMC) phagocytosis.
Results: A total of 125 subjects were included in the final analysis. No significant effects were observed on cytokines, on white cell differentials, NKCA or PBMC phagocytosis from pre- to post-supplementation. The biomarkers that increased significantly from pre- to post-supplementation were the concentration of plasma macrophage inflammatory protein (MIP)-1δ which was higher in the Bl-04 than placebo group (Bl-04 25% ± 11%, placebo −3.3% ± 9.4%; mean ± SD, $P = 0.003$) while the concentration of plasma matrix metallo-proteinase (MMP)-1 decreased by 11% ± 16% in the NCFM & Bi-07 group and increased by 21% ± 17% in the placebo group, which was a significant 26% difference (8–41%; $P = 0.02$).
Conclusion: Probiotic supplementation had little effect on parameters of the innate immune system. Mechanisms explaining the beneficial effect of Bl-04 or NCFM & Bi-07 supplementation on respiratory illness remain unclear.

1. Background & aims

Probiotic supplements are popular in the general population as a functional food supplement or ingredient to reduce susceptibility to illness. The concept that probiotics are beneficial to health relates to important roles that gut commensal microbiota play in human health. Dysbiosis in the host-microbiota interaction is linked to increased susceptibility to infectious illness, bowel disorders (such

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as Crohn's disease and ulcerative colitis), certain cancers, obesity, allergy, asthma and autoimmune disease [1]. Probiotic supplementation can reduce seasonal upper respiratory tract illness (URTI) episodes in various population cohorts, namely infants, the elderly, students and endurance-trained athletes [2]. A recent study by our group reported that probiotic supplementation elicited beneficial clinical effects with regard to seasonal winter colds in healthy active individuals. Daily consumption of *Bifidobacterium animalis* subsp. *lactis* (Bl-04) for 150 days by healthy active individuals yielded a 27% greater reduction in the risk of any upper respiratory tract illness (URTI) episode compared to placebo. The combination of *Lactobacillus acidophilus* NCFM & *B. animalis* subsp. *lactis* Bi-07 (NCFM & Bi-07) was associated with a non-significant 19% reduction in the risk of any URTI episode [3]. Both supplements were associated with substantial delays (~0.7–0.9 month) in the time to URTI. Although the physically active adult population has the lowest rate of URTI in the population [4] it appears that probiotic supplementation is of benefit in this group for the common cold.

The mechanism(s) by which probiotics mediate their benefits with regard to URTI is uncertain but thought to involve modulation of the immune system, modulation of microbiota, and competitive exclusion of pathogens from colonising mucosal surfaces. *In-vivo* investigations of probiotic-induced changes in the immune system have yielded conflicting results. In some studies daily probiotic supplementation alters activation markers on CD4+T and CD8+ T cells [5], maintains the concentration of secretory immunoglobulin A [6], and alters the concentration of cytokines [7] and innate immune effector cell functions, in particular natural killer cell activity and phagocytosis [8]. Variable outcomes between studies relate to methodological differences in the strain of bacterium ingested, dosage and route of administration, cohort under investigation and laboratory processing of samples [9]. More supplement and cohort-specific studies are required to identify the mechanisms of probiotics in reducing URTI. To gain further insight on the mechanisms resulting in the reduced URTI healthy active individuals we analysed the peripheral blood of a subset of this cohort for plasma cytokines, natural killer cell activity and phagocytosis capacity.

2. Methods

Full methodological details and results of the clinical trial are published elsewhere [3]. In brief, the study was a three-arm double-blind randomised controlled trial with participants centrally allocated to a group by simple randomisation. The trial examined the effect of daily supplementation with either a single strain or double strain probiotic over 150 days on episodes of respiratory and gastrointestinal illness, cold medication usage and frequency of doctor visits in 465 male and female participants. A subgroup of the participants (n = ~50 in each group) provided samples for secondary assessment of the effects of probiotics on white cell count, plasma cytokines, PBMC granulocyte phagocytosis and natural killer (NK) cell activity.

The first phase of the trial consisted of a 14 d wash-out period in which participants ceased consumption of probiotic/prebiotic supplements and fortified foods to allow previously ingested probiotics to wash out of the system [16]. Participants were also provided detailed instructions on use of an internet-based physical activity and illness questionnaire, had their height and body mass recorded, and given the full course of supplements following group allocation. Those participants providing samples for the assessment of supplementation on immune biomarkers did so at the end of the wash-out period and before starting supplementation (day 0). Participants then completed a 150 d supplementation phase in which either a sachet of the probiotic or placebo supplement in powder form was consumed. Samples were recollected at the end of supplementation (day 150). The study was conducted over an autumn to spring period. The trial was registered on the Australia New Zealand Clinical Trials Registry (ACTRN12611000130965). Ethics approval was obtained from the Griffith University Human Research Ethics Committee and the Australian Institute of Sport Human Research Ethics Committee.

2.1. Subjects

There were 144 participants recruited from Canberra, ACT, Australia and surrounding regions. Details on the participants undertaking the full probiotic trial are published [3]. Inclusion criteria for participants were a body mass index below 25 kg m^{-2} and undertaking a minimum of three sessions of exercise of at least 30 min duration per week. Participants were excluded if they were on immunomodulating drugs, suffered from an autoimmune or gastrointestinal disease, or unwilling to adhere to the study protocol.

2.2. Treatment

Participants consumed one sachet daily of either 2.0×10^9 CFU per day of *B. animalis* subsp. *lactis* Bl-04 (Bl-04; Danisco USA, Madison, WI) or *L. acidophilus* NCFM and *B. animalis* subsp. *lactis* Bi-07 (Danisco USA) 1.0×10^{10} CFU per day (5.0×10^9 CFU of each strain) in a 1 g sucrose base or placebo powder (sucrose base without the probiotic bacteria) dissolved in a cold non-alcoholic beverage during the intervention period of 150 days. The placebo was identical in packaging, appearance and taste to the probiotic supplement but did not contain any probiotic cells.

2.3. Measurement of plasma cytokines

Blood samples were drawn directly into a K^3EDTA tube (Greiner Bio-one; Frickenhausen, Germany) from an antecubital vein. For the plasma cytokine analysis, blood was separated by centrifugation at ~800 g for 5 min and stored at −80 °C until analysis. EDTA-plasma samples were analysed for brain-derived neurotrophic factor (BDNF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage -colony stimulating factor (GM-CSF), interferon (IFN)-γ, interleukin (IL)-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IL-17E, IL-23, inducible protein (IP)-10, lymphotactin, macrophage inflammatory protein (MIP)-1α, MIP-1β,MIP-3α, MIP-1δ, matrix metalloproteinase (MMP)-1, MMP-9, thymic stromal lymphopoietin (TSLP), neurotrophin (NT)3, Regulated on Activation, Normal T Expressed and Secreted (RANTES), transforming growth factor (TGF)-β1, TGF-β2, tumour necrosis factor (TNF-α), and tissue inhibitors of metalloproteinases (TIMP)-1, using an antibody array-based multiplex ELISA platform (Aushon Biosystems, MA, USA). TGF-β1 and TGF-β2 were chemically activated using HCl acidification and neutralisation before ELISA. Samples were diluted with an assay buffer 1:2, 1:50, or 1:200 and incubated for 1–3 h on 96-well plates depending on the manufacturer's protocol for the array. Biotinylated secondary antibody and streptavidin conjugated horse radish peroxidase were subsequently applied to wells and incubated for 1 h and 30 min, respectively. After each incubation step the plates were washed three times with ELx50 Automated Strip Washer (Biotek U.S., VT, USA). The substrate solution (Aushon Biosystems) was applied to wells and chemiluminescence detected using SignaturePlus imager (Aushon Biosystems). The results were analysed using ProArray software (Aushon Biosystems). Mean intra-assay and inter-assay coefficients of variation (CV) of the 34 biomarkers were 12% and 13% respectively.