Ex vivo peripheral blood mononuclear cell response to R848 in children after supplementation with the probiotic *Lactobacillus acidophilus* NCFM/*Bifidobacterium lactis* Bi-07

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Abstract

Several studies have demonstrated a decrease in upper respiratory infection (URI) frequency and severity in subjects taking probiotic supplements. We hypothesised beneficial effects of probiotics on viral URI in children are due to modulation of inflammatory innate immune responses. We tested this hypothesis, providing children with a probiotic combination of *Lactobacillus acidophilus*/Bifidobacterium animalis ssp. lactis Bi-07 (NCFM/Bi-07) and measuring levels of cytokines in response to stimulation of peripheral blood mononuclear cells (PBMCs) to toll-like receptor (TLR) 7/8 agonist resiquimod (R848). In this open label study, 21 (2 dropouts) children received probiotic containing 5×10^9 cfu each of NCFM/(Bi-07) daily for 30 days. Whole blood was taken from each subject at study entry and 30 days for culture of PBMCs. PBMCs stimulated with resiquimod (R848) or unstimulated were incubated and a panel of immune markers was measured. There was a significant decrease in the net (stimulated-null) level of myeloid progenitor inhibitory factor 1 (MPIF-1) (mean decrease 0.1 ng/ml, 95% confidence interval 0.01-0.24, $P=0.032$) following probiotic supplementation. The change in immune marker levels after supplementation, when analysed together with respect to expected inflammatory/anti-inflammatory effects, was increased for interleukin (IL)-10 and decreased for MPIF-1, IL-8, interferon gamma induced protein 10, macrophage inflammatory protein 3 alpha (MIP-3α) and E-selectin ($P=0.01$). Adverse events were mild. In conclusion, supplementation with this probiotic combination was safe and resulted in significant modulation of PBMC limited immune response to TLR7/8 agonist R848 and in levels of MPIF-1 and MIP-3α. The anti-inflammatory effect may be one mechanism by which probiotics modulate the immune system however further study is needed.

Keywords: probiotic, upper respiratory infection, *Lactobacillus*, *Bifidobacterium*

1. Introduction

The acute upper respiratory infection (URI) is the most frequent infection experienced by children and adults. URI is frequently complicated by bacterial infections, such as acute otitis media, acute sinusitis or pneumonia, especially in children. Concern from practitioners for 'missing' important bacterial complications of URI leads to inappropriate antibiotic use and contributes to the local and global problems of antibiotic resistance. In addition,

viral URI may be the most important trigger for acute episodes of wheezing and exacerbations of reactive airways disease and asthma (Olenec *et al.*, 2010). The population of the US experiences approximately 500 million URIs per year with an economic impact of \$40 billion annually including a loss of an estimated 22 million school days and 20 million work days (Fendrick *et al.*, 2003). Despite such a high prevalence, serious health consequences and huge economic burden there are very few preventative or treatment options for URI.

There has been considerable interest in the use of probiotics to reduce the risk of URI and/or to shorten the duration of symptoms across all age groups. Several studies investigating various probiotics particularly in children have shown a decrease in the number of URIs (Hatakka *et al.*, 2001; Hojsak *et al.*, 2010; Lin *et al.*, 2009; Luoto *et al.*, 2014; Maldonado *et al.*, 2012; Merenstein *et al.*, 2010; Taipale *et al.*, 2011) and a reduction in duration and severity of symptoms (Giovannini *et al.*, 2007; Hatakka *et al.*, 2001; Hojsak *et al.*, 2010; Kukkonen *et al.*, 2008; Kumpu *et al.*, 2012; Leyer *et al.*, 2009; Rautava *et al.*, 2009; Rerksuppaphol and Rerksuppaphol, 2012; Sazawal *et al.*, 2010; Weizman *et al.*, 2005). In particular, a randomised, double-blind, controlled study (Gerasimov *et al.*, 2016) showed that *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium lactis* UABLA-12 shortened the duration of URI in children and a review of probiotics in children attending day care centres concluded that *Lactobacillus rhamnosus* GG had a modest effect on reducing the duration of URIs (Laursen and Hojsak, 2018). Additionally, meta-analyses including both children and adults (King *et al.*, 2014) or focusing only on children (Wang *et al.*, 2016) have shown that probiotic consumption leads to shorter duration of illness, fewer illness episodes, as well as fewer days of absence from day care/school. Although meta-analyses and multiple studies show efficacy of probiotics in general, several individual studies have found no impact of probiotics on URI in children. The current scientific consensus is that the effects of probiotics on immunomodulation are strain specific (Hill *et al.*, 2014). This underscores the need to understand the mechanisms of this phenomenon and the effect of strain or strain combinations on immune responses and clinical benefits.

The effects of a probiotic combination *L. acidophilus* NCFM (NCFM) and *Bifidobacterium animalis* spp. *lactis* Bi-07 (Bi-07) on URI has been investigated in clinical trials in children and in adults (Fisberg *et al.*, 2002; Leyer *et al.*, 2009; West *et al.*, 2014a,b). Leyer *et al.* (2009) evaluated the effect of NCFM and a combination of NCFM and Bi-07 (NCFM/Bi-07) on URI in healthy children 3 to 6 years of age in a double-blind, randomised, placebo-controlled clinical trial. Children who received NCFM/Bi07 had a reduction of fever, cough and rhinorrhoea, and shorter duration of symptoms, as well as reduced antibiotic use and days absent from group child care and work (for parents) compared to those who received placebo (Leyer *et al.*, 2009). In another clinical trial, a subgroup of 3- to 5-year old underweight children consuming NCFM/Bi-07 together with fructo-oligosaccharide in a nutritional supplement had fewer gastrointestinal and respiratory illness associated sick days compared with those receiving the nutritional supplement alone (Fisberg *et al.*, 2002). On the other hand, in a clinical study conducted in an active healthy adult population the efficacy of NCFM/Bi-07 on URI was more subtle than in children and NCFM/Bi-07 had no effect on peripheral

blood immune markers, reflecting potential differences in the immune system between children and adults (Cox *et al.*, 2014; West *et al.*, 2014a).

The development of the immune system throughout childhood is dynamic and dependent on the influence of gut microbiota and nutrition (Kollmann *et al.*, 2012). Therefore, probiotics with immunomodulatory properties may have a more pronounced impact on children than adults. However, there is limited evidence on how probiotics influence the immune system of children and whether alterations in the function of the immune system by the probiotic may account for the decrease in frequency and severity of viral URIs. We hypothesised that the beneficial effects of probiotics on viral URI in children are at least partly due to modulation of the inflammatory innate immune response which constitutes the initial host response to viral infection. We tested this hypothesis in a clinical trial by providing children with a combination of probiotics NCFM and Bi-07 for one month and then measuring the levels of cytokines/chemokines in response to stimulation of whole blood derived PBMCs to the toll like receptor 7/8 agonist resiquimod (R848) – a mimic of a viral infection.

2. Materials and methods

This study was an, open label clinical trial approved by the Institutional Review Board of the University of Wisconsin-Madison (approval date January 04, 2017). All procedures performed were in accordance with International Conference on Harmonization of Good Clinical Practice guidelines and in agreement with the Declaration of Helsinki (World Medical Association 2013) and registered in [Clinicaltrials.gov](https://clinicaltrials.gov) with identifier NCT02687503.

Healthy children, 13 to 36 months of age, were recruited from two paediatric primary care practices in Madison, WI, USA. A study nurse obtained written informed consent from the parents or legal representatives of the child before initiating study procedures. Children were excluded from this study if they had any of the following: asthma/allergic rhinitis, premature birth (<36 weeks gestation), immunodeficiency or any underlying problem requiring the use of steroids or other immunosuppressive agents, the use of nonsteroidal anti-inflammatory drugs in the last 2 weeks, any antibiotic treatment in the last 3 months, and conditions which might interfere with dispersion of the probiotic after oral administration, such as anomalies of the digestive tract. Also excluded were children who were currently receiving probiotics, having received probiotics in the previous two weeks or unwilling to refrain from the use of probiotics during the three months prior to study entry. In addition, children concurrently participating in another clinical study, were excluded from this study.

Children received a probiotic product consisting of 5×10^9 cfu of *L. acidophilus* NCFM and 5×10^9 cfu of *B. animalis* ssp. *lactis* Bi-07 in 1 g of sucrose as a carrier (HOWARU® Protect Kids, manufactured by DuPont, Madison WI, USA) given once a day orally in milk or juice for 30 days. The probiotic product was provided by the manufacturer in sachets which were dispensed by a central pharmacy and stored under refrigeration by the caregivers until used. Compliance with the study product was assessed from a diary completed by the parents/caregivers over the course of the study.

Sample analysis

Whole blood samples were taken from each subject at study entry (pre-supplementation) and 30 days after starting probiotic (post-supplementation) for culture of peripheral blood mononuclear cells (PBMCs) using the TruCulture® (RBM Myriad, Austin, TX, USA) system. PBMCs stimulated with R848 at a final concentration of $1 \mu\text{M}$ and unstimulated (null) were incubated for 24 hours at 37°C . A detailed description of these procedures is found elsewhere (Duffy *et al.*, 2014).

For determination of cytokine levels, samples were transported on dry ice to a commercial laboratory (Myriad RBM) and analysed for expression of cytokines and chemokines using a proprietary Luminex Bead technology and assay platform (Human CytokineMAP A v.1.0) which included E-selectin, interferon gamma induced protein (IP-10), chemokine (C-X-C motif) ligand 10 (CXCL10), tumour necrosis factor alpha (TNF- α), tumour necrosis factor beta (TNF- β), interleukin(IL)-6 receptor (IL-6r), macrophage inflammatory protein 3 alpha (MIP-3 α), chemokine (C-C motif) ligand 20 (CCL20), monokine induced by gamma interferon (MIG, CXCL9), myeloid progenitor inhibitory factor 1 (MPIF-1, CCL23), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN- γ), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-10, IL-18, macrophage inflammatory protein-1 alpha (MIP-1 α , CCL3), macrophage inflammatory protein-1 beta (MIP-1 β , CCL4), and monocyte chemoattractant protein 1 (MCP-1, CCL2).

Adverse effects

Potential adverse events were monitored on a weekly basis during the 30-day study period including fever, diarrhoea, loose stools, frequent stools, rash, abdominal pain/colic, constipation, diaper rash, other illness, and physician visits.

Statistics

As this was an exploratory study, a pre-determined statistical analysis plan was not applied. Changes in net cytokine/chemokine levels (R848 stimulated minus null value) from the pre- to the post-probiotic assessment were

evaluated using a paired t-test. Values were log-transformed before conducting the comparisons to satisfy the normality assumption. Absolute differences in net cytokine/chemokine levels were summarised in terms of means and 95% confidence intervals (CI). All reported *P*-values are two-sided and *P* < 0.05 was used to define statistical significance level. Due to the exploratory nature of the study, no false discovery adjustments were used when comparing changes in individual cytokine/chemokine levels.

As an additional exploratory analysis, the null hypothesis that net cytokine level following one month of probiotic supplementation does not result in a change in the panel of IL-10, IL-8, IP-10, MIP-3 α , MPIF-1 and E-selectin values was tested against the alternative that it does result in an anticipated increase in IL-10 levels combined with an anticipated decrease in IL-8, IP-10, MIP-3 α , MPIF-1, E-selectin marker levels (anti-inflammatory effect). A chi-square test of expected (under the null hypothesis) vs observed direction of changes in the cytokine/chemokine panel was then conducted to test whether net (R848 stimulated-null) PBMCs following one month of probiotic supplementation results in an anticipated increase in IL-10 levels combined with an anticipated decrease in IL-8, IP-10, MIP-3 α , MPIF-1, E-selectin marker levels.

Statistical analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, USA).

3. Results

Subjects

Twenty-three subjects were enrolled in the study over a six-month period. Two subjects were withdrawn as blood was not able to be obtained at the initial visit (pre-supplementation). Two additional subjects had successful venipuncture at the initial visit but blood was unable to be obtained at the second visit. Thus, a total of 21 pre-supplementation and 19 post-supplementation samples were used in the final analysis (Figure 1). The mean age of subjects was 19.9 months (range 12–36 months). Subjects identified as White, non-Hispanic (90%), Asian (5%), and Black (5%); 43% were female.

Immune marker analysis

The results of stimulated immune marker measurements are shown in Table 1. Taken individually, there was a significant decrease in net (R848 stimulated-null) measurements of MPIF-1 (-0.1 ng/ml , 95% CI: $-0.2 - -0.01 \text{ ng/ml}$, *P* = 0.032) in whole blood following 30 days of probiotic supplementation. Furthermore, a marginal decrease in MIP-3 α (-385.9 pg/ml , 95% CI: $-779.7 - 7.1 \text{ pg/ml}$, *P* = 0.05) was observed after probiotic supplementation. There was a trend for an increase in IL-10 (*P* = 0.1).

Figure 1. CONSORT 2010 flow diagram.

The distribution of immune marker levels is shown in box-whisker plots in Figure 2. The change in immune marker levels, post-probiotic supplementation, when analysed together with respect to expected pro-inflammatory/anti-inflammatory effects, was significantly increased for IL-10 and significantly decreased for MPIF-1, IL-8, IP-10, MIP-3 α and E-selectin ($P=0.01$).

Safety and adherence

Table 2 shows reported potential adverse events during the 30-day study. All adverse events were considered mild and no subject discontinued probiotic. In addition, a causal relationship between the events noted and probiotic was not established. One patient had a medically attended visit for rash not attributable to probiotic. No serious adverse events were observed. The adherence to the study product was as follows: no missed doses were reported in 13 subjects, 6 subjects reported a single missed dose and 1 subject each reported two and three missed doses.

Table 1. Mean net (R848 stimulated-null) cytokine/chemokine whole blood levels before and after 30-day probiotic intake.

Analyte ¹	Unit	Pre-probiotic (Mean)	Post-probiotic (Mean)	Mean change (95% CI)	P-value ²
E-selectin	ng/ml	0.4	0.7	0.2 (-0.9-1.3)	0.69
GM-CSF	pg/ml	12.9	11.3	-3.0 (-10.7-4.7)	0.43
IFN- γ	pg/ml	473.2	491.6	-7.2 (-176.1-161.5)	0.92
IL-2	pg/ml	0.0 ³	0.0 ³	0	NS
IL-3	ng/ml	0.0	0.0	0	NS
IL-4	pg/ml	4.4	0	-4.8 (-10.4-0.8)	0.09
IL-5	pg/ml	0.0 ³	0.0 ³	0	NS
IL-6	pg/ml	18,641.8	16,355.1	-2,670.2 (-9,194.7-3,854.2)	0.40
IL-6r	ng/ml	1.2	0.7	-0.4 (-2.6-1.9)	0.73
IL-7	pg/ml	25.1	22.0	-1.9 (-18.7-14.9)	0.82
IL-8	pg/ml	7,077.4	5,056.9	-2,003.2 (-4,438.3-432)	0.10
IL-10	pg/ml	2,687.2	3,110.9	501.1 (-104.6-1,106.8)	0.10
IL-18	pg/ml	49.6	58.6	-15.9 (-34.7-66.5)	0.52
IP-10	pg/ml	37,700.1	30,491.9	-5,863.0 (-14,182-2,456.2)	0.16
MCP-1	pg/ml	46,732.8	48,499.4	3,076.4 (-6,104.6-12,257.4)	0.49
MCP-2	pg/ml	1,5618.8	15,456.5	113.4 (-3,163.6-3,390.4)	0.94
MIG	pg/ml	2,467.9	2,024.3	-392.8 (-879.5-93.8)	0.11
MIP-1 α	pg/ml	43,030.7	44,897.6	1,030.3 (-10,973.5-13,034.2)	0.86
MIP-1 β	pg/ml	851,420.7	852,574.7	-6,348.3 (-201,413.9-188,717.4)	0.95
MIP-3 α	pg/ml	1,908.2	1,497.2	-385.9 (-779.7-7.1)	0.053
MPIF-1	ng/ml	0.3	0.2	-0.1 (-0.2-0.0)	0.03
TNF- α	pg/ml	22,565.7	21,810.6	-975.8 (-6,434-4,482.4)	0.71
TNF- β	pg/ml	3.5	6.6	2.7 (-6-11.3)	0.52

¹ GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IP = interferon γ -induced protein; MCP = monocyte chemotactic protein; MIG = monokine induced by gamma interferon; MIP = macrophage inflammatory protein; MPIF = myeloid progenitor inhibitory factor; TNF = tumour necrosis factor.

² P-value for evaluating change from between pre- and post-probiotic supplementation. NS = not significant.

³ Lower detection limit.

Figure 2. Box-whisker plots of (A) interleukin (IL)-10, (B) myeloid progenitor inhibitory factor 1, (C) IL-8, (D) interferon γ -induced protein 10, (E) macrophage inflammatory protein-3 α and (F) E-selectin from peripheral blood mononuclear cells after stimulation with R848 pre-supplementation and post-supplementation with *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07. Cross represents the mean value.

Table 2. Adverse events during 30-day study period in 21 children.

Symptom	Number of subjects	%
Diarrhoea	9	42.9
Constipation	7	33.3
Vomiting	0	0.0
Nausea	0	0.0
Gassy	4	19.0
Abdominal pain	1	4.8
Fussiness	7	33.3
Rash	4	19.0

4. Discussion and conclusions

We have demonstrated that daily supplementation with NCFM and Bi-07 has a limited but significant effect on peripheral blood innate immune function in response to TLR 7/8 agonist in children 13-36 months of age. After one month of supplementation, PBMC responses were significantly decreased for the cytokine MPIF-1 when stimulated with R848. When the immune markers were

statistically analysed by categorising them as pro- or anti-inflammatory, the impact of probiotic on R848 stimulated PBMCs was to exert an anti-inflammatory effect; namely an increase in IL-10 combined with a decrease in MPIF-1, MIP-3 α , IL-8, IP-10 and E-selectin.

Toll-like receptors (TLRs) are a class of highly conserved pattern recognition receptors that are responsible for the initiation of the innate immune response by sensing molecular patterns for early recognition of a pathogen such as bacteria, viruses and protozoa. (Walleet *et al.*, 2018). The natural ligands for TLR 7/8 are single stranded RNA molecules such as those found in viruses which are potent stimulators of the innate immune response (Gaajetaan *et al.*, 2012; Vasilakos and Tomai, 2013). In addition, TLR7 recognises rhinovirus and has been demonstrated to govern the inflammatory response to rhinovirus infections (Hatchwell *et al.*, 2015). TLR7 and TLR 8 are also involved in the recognition and response to other viral upper and lower respiratory infections including respiratory syncytial virus and influenza (Lukacs *et al.*, 2010; Scagnolari *et al.*, 2009; Stegemann-Koniszewski *et al.*, 2018; Wu *et al.*, 2013). We chose a stimulant, R848, that is a ligand of TLR 7/8 to model the inflammatory response to viral infection and determine the effect of probiotic supplementation on this

innate response. Our results suggest that one mechanism of the effect of NCFM and Bi-07 and potentially other probiotics on viral URI may be to modulate the TLR7/8 induced inflammatory cascade and innate immune response to viruses.

Our study provides a possible mechanistic explanation for the reduction in respiratory symptoms experienced by children with URI who receive supplementation with probiotic. Levels of IL-8 and IP-10 correlate closely with symptoms during URI (Gern *et al.*, 2002; Hayney *et al.*, 2017). Thus, the reduction in IL-8 and IP-10 following supplementation with probiotic may be the mechanism of reduction of symptoms seen in the study by Leyer *et al.* (2009). The role of E-selectin in URI has not been described though it is a potent recruiter of neutrophils to sites of inflammation and infection (Silva *et al.*, 2017). Our study suggests that NCFM/Bi07 may alter inflammation by decreasing expression of E-selectin. Turner *et al.* (2017) performed a study with another probiotic, *B. lactis* Bl-04, in experimental rhinovirus infection in human volunteers. In the probiotic group, higher levels of IL-8 in nasal lavage prior to virus challenge were observed. However, the baseline adjusted IL-8 response to rhinovirus infection in nasal lavage was significantly reduced in the probiotic group (Turner *et al.*, 2017). Although this study was in adults and used different methods (nasal lavage) it tends to lend support to our finding that probiotics may influence cytokine and chemokine levels associated with an initial innate immune response. Although the probiotics in our study and the clinical studies mentioned were administered orally, the effect on respiratory immune function may occur due to dissemination from the gastrointestinal immune effector cells via the so-called gut-lung axis (Brandtzaeg, 2009; Dumas *et al.*, 2018).

In contrast to the limited immunomodulatory effects that were observed in the present study in children, the effect of NCFM/Bi-07 supplementation on immune markers in adults has been less clear. West *et al.* (2014a,b, 2016) conducted a randomised, double-blind, placebo-controlled clinical study in healthy active adults and showed a delayed time to experiencing a first URI episode during the 'cold' season, but without effect on risk or duration of the symptoms with intake of NCFM/Bi-07. The different results observed between the present study in children and in the adult population may potentially reflect the differences in assaying the immune markers – challenged *ex vivo* PBMC cells vs plasma immune markers – or perhaps the developing immune system of children may be more prone to effects of probiotics.

A novel finding in our study was a significant reduction (in stimulated PBMCs) in levels of MIP-1 and a decrease in MIP-3 after supplementation with NCFM/Bi-07. MIP-1 is known to selectively recruit resting T-lymphocytes and

monocytes, inhibit proliferation of myeloid progenitor cells and promote angiogenesis. MIP-3 α is produced by human neutrophils and monocytes in response to R848 stimulation (Arruda-Silva *et al.*, 2017). MIP-3 α is responsible for the chemoattraction of immature dendritic cells (DC), effector/memory T-cells and B-cells and plays a role in inflammation in skin and mucosal surfaces. In an animal model of rotavirus infection, Zhou *et al.* (2019) demonstrated that neonatal rhesus monkeys showed elevated blood levels of the chemokines MIP-1 and MIP-3 α after infection with human rotaviruses suggesting they play an important role in the inflammatory response. A recent meta-analysis showed that probiotics exert a positive effect in reducing the duration of acute rotavirus diarrhoea compared with controls (Ahmadi *et al.*, 2015). The effect of NCFM/Bi-07 on these two chemokines may explain, in part, the mechanism by which probiotics moderate the symptoms of acute infectious diarrhoea from rotavirus.

The results of the present study showed that supplementation with a combination of NCFM and Bi-07 resulted in immunomodulatory effects towards a more anti-inflammatory response upon stimulation of PBMCs with R848 *ex vivo*. The direct effect of the combination of NCFM/Bi07 has not been investigated on PBMCs or immune cells *in vitro*, however, NCFM and Bi-07 have been investigated separately suggesting that they upregulate key inflammatory pathways for innate viral defence (Foligne *et al.*, 2007; Gad *et al.*, 2011; Konstantinov *et al.*, 2008; Weiss *et al.*, 2010a,b).

Our study did not demonstrate differences in some cytokines that are known to play a role in the inflammatory response following viral URI. Most notably, IL-6 levels have been associated with URI and correlate with symptom severity; these were not changed in our study (Alper *et al.*, 2010; Patel *et al.*, 2009). However, our sample size was small and we assayed whole blood which may not reflect the environment in the nasal mucosa. In addition, we used only one stimulant of PBMCs (R848) and focused on a TLR 7/8 response which may have limited the scope of our findings. In addition, some cytokines such as interferon gamma are limited in utility as a biomarker during URI (Turner *et al.*, 2017). Since other studies have failed to demonstrate an antiviral effect of a probiotic we did not assess levels of type I interferons in this study. It is difficult to assess the clinical significance of the immunomodulation as our study did not assess symptoms of URI. Further randomised double-blind placebo-controlled trials with larger numbers are required to verify the findings of this study and to connect immunomodulation to health benefits in children.

Overall, the probiotic product was well-tolerated by study subjects, similar to other studies (Cox *et al.*, 2014; Fisberg *et al.*, 2002; Leyer *et al.*, 2009; Schrezenmeir *et al.*, 2004; West *et al.*, 2014b). Potential adverse events were mild and

no serious adverse events were noted. Adherence to the study product, as measured by parent/caregiver report, was excellent.

In conclusion, we have demonstrated that the supplementation with the probiotic combination *L. acidophilus* NCFM and *B. lactis* Bi-07 resulted in modulation of the immune response to TLR7/8 agonist R848 and in levels of MIP-1 and marginally for MIP-3 α . This effect may provide a mechanism by which probiotics ameliorate symptoms in viral upper respiratory and gastrointestinal infections but further studies are needed to investigate this effect.

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