Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019[1–3]

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ABSTRACT

Background: The aging process can lead to a decline in cellular immunity. Therefore, the elderly could benefit from safe and effective interventions that restore cellular immune functions.

Objective: We determined whether dietary supplementation with the known immunostimulating probiotic *Bifidobacterium lactis* HN019 could enhance aspects of cellular immunity in elderly subjects.

Design: Thirty healthy elderly volunteers (age range: 63–84 y; median: 69 y) participated in a 3-stage dietary supplementation trial lasting 9 wk. During stage 1 (run-in), subjects consumed low-fat milk (200 mL twice daily for 3 wk) as a base-diet control. During stage 2 (intervention), they consumed milk supplemented with *B. lactis* HN019 in a typical dose (5×10^{10} organisms/d) or a low dose (5×10^9 organisms/d) for 3 wk. During stage 3 (washout), they consumed low-fat milk for 3 wk. Changes in the relative proportions of leukocyte subsets and ex vivo leukocyte phagocytic and tumor-cell-killing activity were determined longitudinally by assaying peripheral blood samples.

Results: Increases in the proportions of total, helper (CD4+), and activated (CD25+) T lymphocytes and natural killer cells were measured in the subjects' blood after consumption of *B. lactis* HN019. The ex vivo phagocytic capacity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of natural killer cells were also elevated after *B. lactis* HN019 consumption. The greatest changes in immunity were found in subjects who had poor pretreatment immune responses. In general, the 2 doses of *B. lactis* HN019 had similar effectiveness.

Conclusion: *B. lactis* HN019 could be an effective probiotic dietary supplement for enhancing some aspects of cellular immunity in the elderly. *Am J Clin Nutr* 2001;74:833–9.

INTRODUCTION

The elderly represent an ever-increasing proportion of most Western populations (1). Many of the health issues faced by the elderly are the consequence of declining physiologic function with increasing age, which leads to a predisposition to infectious and noninfectious diseases and increased morbidity and convales-

cence. The immune defense system in particular is known to be adversely affected by the aging process (2, 3), and there is strong evidence that a poorly functioning immune system can contribute to decreased disease resistance and reduced life expectancy in the elderly (4, 5). Immunosenescence is characterized by suboptimal function of the cellular immune system in particular (6, 7). The most well-characterized age-related changes involve thymic (T) lymphocytes, including a decrease in the numbers of mature CD3+ T cells in peripheral circulation, a decrease in the pool of naive (CD45RA+) T cells, a predisposition toward T helper 2 phenotype expression, and poor capacity of peripheral blood T cells to proliferate and secrete interleukin 2 or to contribute to effective delayed-type hypersensitivity responses in vivo (8, 9).

Immunosenescence can also be expressed by changes in the function or proportions of leukocytes that contribute to innate immunity, such as phagocytes and natural killer (NK) cells (10–12), although far less research has been done on this topic than on the effects of aging on T cells. Both polymorphonuclear and mononuclear phagocytes expressed diminished in vitro activity in elderly subjects (13, 14), mostly through a decline in opsonin-mediated phagocytosis (11). NK cells also had diminished in vitro activity in the elderly (12). Up to 70% of subjects older than 70 y had low NK cell–mediated cytotoxicity responses against K562 tumor cells compared with 40% of young adults (15), although there are contradictory reports that elderly subjects selected on the basis of strict health-performance criteria do not show lower NK cell numbers or function (6, 16). Nevertheless, a decline in innate immune cell function is generally considered to be a contributing factor to decreased immunity in the elderly (11). Moreover, because both NK cells and phagocytes (particularly monocytes) secrete many immunoregulatory cytokines (17), their potentially diminished function in elderly individuals may have important downstream effects on

immune events in the integrated immune system, such as lymphocyte activation and differentiation. Accordingly, interventions that can combat immunosenescence by restoring cellular immune function are highly desirable.

An attractive means of restoring immune function is dietary intervention. Previous research in the elderly showed that dietary supplementation with micronutrients enhanced some aspects of cellular immunity, including the proportion of circulating T lymphocyte subsets and the in vitro activity of blood-derived NK cells (18–20). Another dietary regimen that could benefit the elderly is probiotic supplementation with immunostimulating strains of lactic acid bacteria. Certain well-defined lactic acid bacteria strains were shown to be potent enhancers of immunity and to offer clinical benefits to defined groups. For example, *Lactobacillus rhamnosus* strain GG has been used successfully as a dietary immunomodulator in pediatric care. When used as an oral adjuvant, *L. rhamnosus* GG increased antigen-specific immune responses in children administered rotavirus vaccine (21). In hospitalized children, *L. rhamnosus* GG enhanced rotavirus-specific antibody titers and promoted recovery from diarrhea (22, 23). In contrast, however, the ability of defined probiotic lactic acid bacteria strains to modulate immunity in the elderly has not been reported.

The present report describes the outcomes of a 9-wk dietary intervention trial designed to determine the effects of the immunomodulating probiotic *Bifidobacterium lactis* HN019 on cellular immunity in the elderly. This strain was previously shown to possess immune-enhancing properties in humans and animal models (24, 25).

SUBJECTS AND METHODS

Subjects

Elderly volunteers were invited to enroll in this dietary trial via newspaper advertisements in the Manawatu-Wanganui region of New Zealand during February and March 1999. Thirty healthy subjects aged 63–84 y (median: 69 y) were enrolled. The sample size was estimated to provide 98% and 91% statistical power for the phagocytosis and NK cell assays, respectively (MINITAB software, version 12; Minitab Inc, State College, PA) on the basis of preliminary immunologic data (B-L Chiang and HS Gill, unpublished observations, 1999). The subjects were 12 men (age range: 65–84 y; weight range: 61–96 kg) and 18 women (age range: 63–80 y, weight range: 50–97 kg). The enrolled subjects were all living independently (ie, not in residential care).

The eligibility of the subjects was assessed by interviewing them and consulting with their health care providers. The inclusion criteria were general good health and mobility and a willingness to either follow the trial guidelines (eg, avoid potentially conflicting nutritional or vitamin supplements) or provide notification of noncompliance. Exclusion criteria included any recent history of acute or chronic debilitating illness and any intolerance to milk products. Informed consent was obtained, and subjects were told that some of the milks that they would consume might contain microorganisms with health benefits. The trial protocol was approved by the Manawatu-Wanganui Ethics Committee, New Zealand.

Diets

Low-fat milk (LFM), which was also low in lactose, was supplied in powder form by the New Zealand Dairy Board (Welling-ton, New Zealand). *B. lactis* (strain HN019) was obtained from the microbial strain culture collection of the New Zealand Dairy Research Institute (Palmerston North, New Zealand). Lyophilized bacteria were incorporated into the LFM powder to provide 2 different doses: a typical dose (1×10^9 organisms/g powder) and a low dose (1×10^8 organisms/g powder). Subjects were allocated by random drawing into 2 groups of 15; 1 group received the typical dose and the other received the low dose of probiotic supplement during stage 2 of the trial.

The diets were vacuum-sealed inside sachets (25 g/sachet) containing either LFM powder or *B. lactis* HN019–supplemented LFM powder. The sachets were stored at room temperature and were supplied directly to the participants. The sachets were number-coded respective to each individual trial participant, but the participants did not know which treatment they were receiving. Each participant was given a supply of sachets sufficient to last throughout each stage of the trial; replenishments were given for the next stage at regular visits by their health care provider (every 3 wk). Sachets were reconstituted by the subjects immediately before consumption by mixing the powder with 200 mL cool drinking water according to the instructions.

Protocol

The trial had a 3-stage, pre-post intervention design to detect changes in immunity corresponding to changes in diet over time. Each subject served as his or her own internal control for the data analyses. During the first stage (run-in; weeks 1–3), all subjects consumed unsupplemented milk (25 g LFM powder per 200 mL) twice daily. During the second stage (intervention; weeks 4–6), subjects consumed milk supplemented with *B. lactis* HN019 as follows: 6 men and 9 women consumed the typical dose of *B. lactis* (total intake: 5×10^{10} organisms/d; typical-dose group) and 7 men and 8 women consumed a lower dose of *B. lactis* (total intake: 5×10^9 organisms/d; low-dose group). During the third stage (washout; weeks 7–9), all subjects consumed unsupplemented milk.

Throughout the trial, the subjects' general health was assessed at each immune measurement time point (*see* below) via direct interview conducted by the health care provider. Subjects were asked to confirm their compliance with, or deviation from, the dietary regimens.

Blood sampling and immune measurements

Peripheral blood samples were obtained from subjects by venipuncture at 4 time points: *1*) week 0 (baseline, immediately before the trial began), *2*) at the end of week 3 (after subjects had consumed unsupplemented milk for 3 wk), *3*) at the end of week 6 (after subjects had consumed *B. lactis* HN019–supplemented milk for 3 wk), and *4*) at the end of week 9 (after subjects stopped consuming *B. lactis* HN019 and consumed unsupplemented milk for the final 3 wk of the trial).

Blood samples were number-coded with respect to subject identification and time point; laboratory personnel did not know the meaning of these codes. Major leukocyte subset phenotypes were enumerated in EDTA-treated whole-blood samples via flow cytometry (FACScalibur CELLQUEST software, version 1.2.2; Becton Dickinson, Oxford, United Kingdom) by using the following fluorochrome-conjugated monoclonal antibodies (Becton Dickinson): anti-CD3+, -CD4+, -CD8+, -CD19+, -CD25+, -CD56+ and -HLA-DR+. The results are expressed as the percentage of mononuclear cells that stained positively for each cell surface marker.

TABLE 1
In elderly subjects participating in a probiotic supplementation trial, the percentage of mononuclear leukocytes that stained positively for major phenotype cell-surface markers at each of the 4 time points[1]

	Time point			
	1 (baseline)	2 (after 3 wk consumption of unsupplemented LFM)	3 (after 3 wk supplementation with *B. lactis* HN019)[2]	4 (3 wk after cessation of *B. lactis* HN019)
			%	
T cells				
Total (CD3$^+$)	65.1 ± 2.1[3]	64.9 ± 2.6[3]	67.9 ± 2.6	65.9 ± 2.7
CD4$^+$	41.0 ± 1.6[4]	41.7 ± 1.8[4]	45.2 ± 1.3	43.7 ± 1.9
CD8$^+$	19.2 ± 2.1	20.4 ± 2.0	19.6 ± 2.1	19.5 ± 2.1
CD25$^+$	6.4 ± 0.7[4]	6.9 ± 0.7[4]	11.0 ± 1.0	7.3 ± 0.7[4]
B cells				
CD19$^+$	10.7 ± 1.4	10.3 ± 1.2	11.2 ± 1.1	9.5 ± 1.1
NK cells				
CD56$^+$	14.8 ± 1.3[4]	15.9 ± 1.4[5]	18.2 ± 1.4	16.9 ± 1.6
APCs				
HLA-DR$^+$	13.7 ± 1.4	15.4 ± 1.3	15.1 ± 1.3	14.5 ± 1.1

[1] $\bar{x}$ ± SEM; n = 15. LFM, low-fat milk; NK, natural killer; APCs, antigen-presenting cells.
[2] Dosage was 5×10^{10} *Bifidobacterium lactis* HN019 organisms/d.
[3–5] Signficantly different from time point 3 (Dunnett's post hoc test): [3] $P < 0.05$, [4] $P < 0.001$, [5] $P < 0.01$.

In vitro phagocytic activity was determined in whole-blood samples after the uptake of fluoresceinated *Escherichia coli* (25). Phagocytically active monocytes and polymorphonuclear cells were differentiated by flow cytometry and the results are expressed as the percentage of each leukocyte sample showing phagocytic activity (25). To assess in vitro tumoricidal activity, mononuclear cells were separated from whole blood as described previously (24), and viable cells were resuspended to the desired concentration in RPMI 1640 medium (Sigma Chemical Co, St Louis) supplemented with 10% fetal calf serum. Tumoricidal activity was assessed by killing D275-stained K562 cells (an NK cell–specific target) at an effector-to-target ratio of 40 to 1 with an assay system that was developed in our laboratory to be optimal for determining cytotoxicity (25). The percentage of target cells killed was determined with propidium iodide exclusion by using flow cytometry (25).

Statistical analyses

Repeated-measures analysis of variance was used to identify changes in immune measurements associated with different treatment regimens over time; significant differences between time point 3 and all other time points were identified by using Dunnett's post hoc tests with week 6 as the reference week. $P < 0.05$ was considered sufficient to reject the null hypothesis of no treatment effect.

At the outset of the study, there was an a priori reason to believe that consumption of *B. lactis* HN019 could enhance some immune variables (24, 25). Therefore, additional analyses were undertaken to compare the magnitude of changes in *B. lactis*–induced immune responses between groups and subgroups. Thus, data were also expressed as the relative increase in immune response during consumption of *B. lactis* HN019, calculated as the percentage change in response between time points 2 and 3 for each subject. These changes were compared between the typical-dose and low-dose groups by using the two-sided Wilcoxon's rank-sum test.

In addition, the immune-response data of individual subjects obtained at time point 2 were ranked in descending order within each dose group (n = 15 per group). Ranked data were then stratified for each group: the lowest tertile of responses was designated as a subgroup with a poor pretreatment immune response (n = 5) and the highest and middle tertiles were combined and designated as a subgroup with an adequate pretreatment immune response (n = 10).

The percentage increases in responses resulting from consumption of *B. lactis* HN019 were subsequently compared between the subgroups with poor and adequate pretreatment immune responses by using Wilcoxon's rank-sum tests.

RESULTS

Clinical observations

Of the 30 subjects initially enrolled in the trial, 1 withdrew from the low-dose group before the intervention because of a dislike of the taste of reconstituted milk and some digestive discomfort. Follow-up studies indicated that these symptoms ended after the subject withdrew from the trial. All other subjects reported compliance with the dietary regimens. Throughout the 9-wk study, the subjects reported no other adverse effects on health or any general health problems associated with consumption of either the unsupplemented milk or the milk containing the probiotic supplement.

Overall changes in immune variables

Between-group analyses indicated no significant overall differences in immune variables between the low-dose and typical-dose groups. However, there were significant time-dependent treatment effects within both groups. There were no significant changes between time points 1 and 2. Significant changes were recorded for some immune variables between time points 1 or 2 (preintervention) and time point 3 (postintervention). The proportion of mononuclear leukocytes staining positively for CD3$^+$ (T lymphocytes), CD4$^+$ (MHC II–restricted T cells), CD25$^+$ (interleukin 2 receptor), and CD56$^+$ (NK cells) increased significantly after consumption of *B. lactis* HN019 (**Table 1**). After the
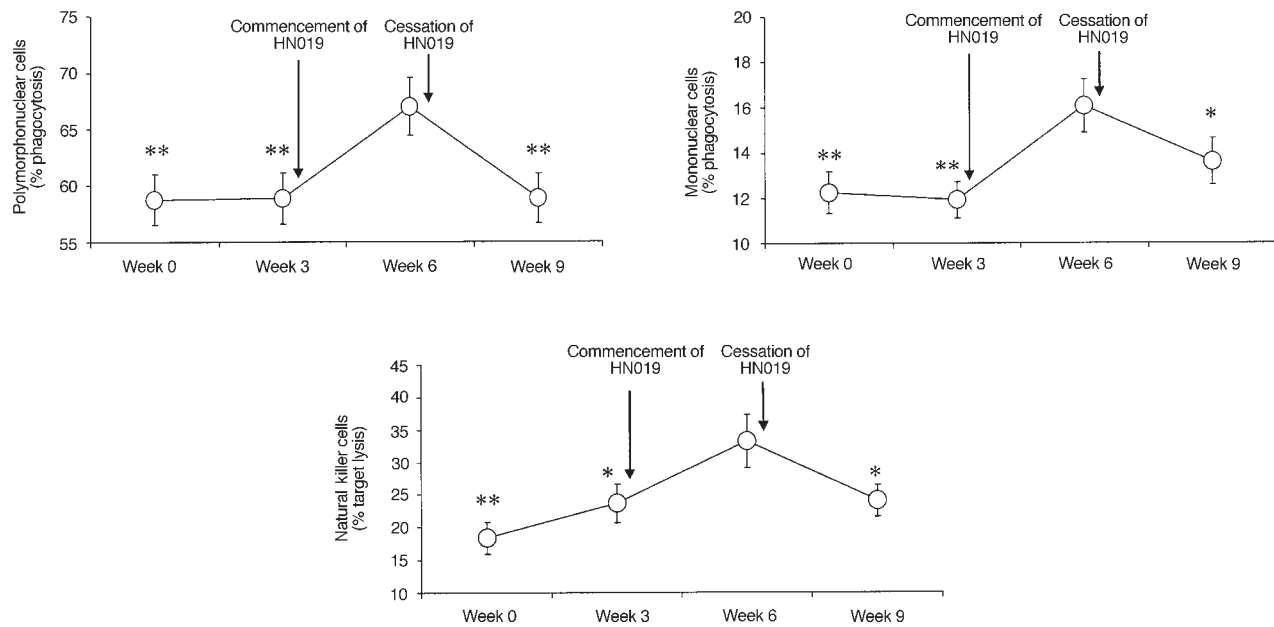
FIGURE 1. Mean (±SEM) phagocytic activity of polymorphonuclear and mononuclear cells and tumoricidal activity of natural killer cells after consumption of 5×10^{10} *Bifidobacterium lactis* HN019 organisms/d (in lactose-hydrolyzed low-fat milk) as a probiotic dietary supplement. Peripheral blood samples were obtained from 15 elderly subjects at 4 time points throughout the trial and immune responses were assessed via in vitro assays of leukocytes. **,*Significantly different from week 6: **$P < 0.005$, *$P < 0.01$.

3-wk washout period without probiotic supplementation (ie, at time point 4), these values declined (Table 1), although only in the case of CD25+ cells was this decline significant when compared with time point 3. The proportions of cells staining positively for CD8+ (MHC I–restricted T cells), CD19+ (B lymphocytes), and HLA-DR+ (MHC II–bearing antigen-presenting cells) remained unaltered throughout the trial.

The percentages of both mononuclear and polymorphonuclear cells showing phagocytic activity in vitro increased significantly after consumption of *B. lactis* HN019 (**Figure 1**). In addition, tumoricidal activity against K562 cells increased significantly

after consumption of *B. lactis*. In all cases, the values at time point 3 for in vitro cellular function were significantly greater than those at time points 1, 2, or 4 (Table 1 and Figure 1).

Relative increases in cellular immune function after *B. lactis* HN019 supplementation

The phagocytic and tumoricidal activities of mononuclear and polymorphonuclear cells were analyzed further by re-expressing the data as the relative increases in immune responses between time points 2 and 3 (ie, the period reflecting immune response changes resulting from *B. lactis* HN019 consumption) for each

TABLE 2
Percentage increases in in vitro immune responses between time points 2 and 3 after consumption of *Bifidobacterium lactis* HN019 by elderly subjects in the 2 dosage groups[1]

Immune variable and dosage group[2]	All subjects	Subjects with adequate preintervention immunity[3]	Subjects with poor preintervention immunity[3]
		%	
PMN cell phagocytosis			
Typical dose	14.5 (7.6, 21.4)	9[4]	21
Low dose	18.5 (9.5, 27.5)	16	23
Mononuclear cell phagocytosis			
Typical dose	38.2 (20.2, 56.2)	25[4]	53
Low dose	40.3 (20.8, 59.8)	30	58
NK cell tumoricidal activity			
Typical dose	52.1 (17.4, 86.8)	37	69
Low dose	61.8 (23.8, 99.8)	49	82

[1] $\overline{x}$; 95% CI in parentheses. $n = 15$ for entire typical-dose group and 14 for entire low-dose group. For subgroups with adequate and poor preintervention immunity, $n = 10$ and 5, respectively for typical-dose group and $n = 9$ and 5, respectively, for low-dose group. PMN, polymorphonuclear; NK, natural killer.
[2] Typical dose was 5×10^{10} *B. lactis* HN109 organisms/d and low dose was 5×10^9 *B. lactis* HN109 organisms/d.
[3] Preintervention immunity was defined on the basis of in vitro immune response; responses were stratified into poor and adequate subgroups (lowest tertile and middle and highest tertiles combined, respectively).
[4] Significantly different from group with poor preintervention immunity, $P \le 0.02$.
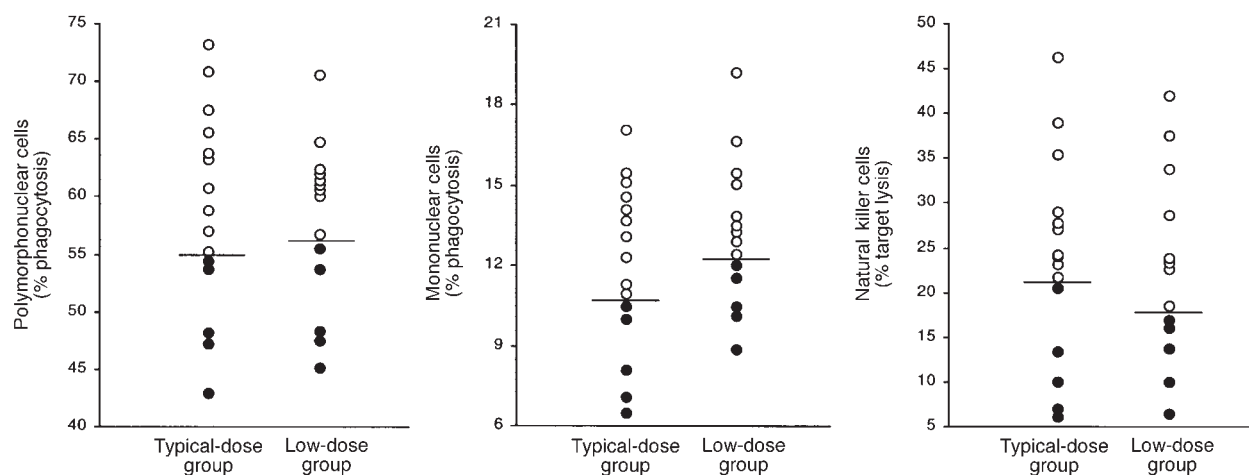
FIGURE 2. In vitro phagocytic activity of polymorphonuclear and mononuclear cells and tumoricidal activity of natural killer cells in individual elderly subjects in the low-dose (5×10^9 *Bifidobacterium lactis* organisms/d) and typical-dose (5×10^{10} *B. lactis* organisms/d) groups. These 2 dose groups were subgrouped on the basis of poor (●) and adequate (○) preintervention immune responses (*see* Results). Mean changes in immune responses after consumption of *B. lactis* HN019 were compared between the subgroups as described in Results and Table 2.

subject. Consumption of *B. lactis* HN019 led to average increases in cellular immune function of between 14.5% and 61.8%, with the greatest effect on NK cell activity (**Table 2**). There were no significant differences between the low-dose and typical-dose groups in the relative magnitude of immune enhancement, although increases tended to be greater in the low-dose group.

Subjects' preintervention immune responses were ranked and stratified into poor and adequate responses, as depicted in **Figure 2**. The percentage changes in immune responses observed between time points 2 and 3 were then compared between the poor and adequate subgroups. Individuals with poor preintervention immunity had consistently greater relative increases in immune function than did those with adequate preintervention immunity (Table 2). These differences were significant for mononuclear and polymorphonuclear cell phagocytosis in the typical-dose group.

DISCUSSION

The results of this study suggest that modifications in some aspects of cellular immunity can be achieved in the elderly by supplementation with an immunostimulating probiotic for 3 wk. This may have practical significance for enhancing immune function in the elderly; dietary intervention was proposed previously as a possible means of countering immunosenescence (26, 27). In previous studies, dietary supplements (single-nutrient or multivitamin and mineral) were effective at increasing the proportions of circulating T lymphocyte subsets and enhancing NK cell function in the elderly (18–20, 28, 29). The present study showed similar changes in cellular immunity after subjects consumed a milk-based dietary supplement containing *B. lactis* HN019. This reinforces the theory that dietary interventions could be a simple and effective means of achieving some improvements in health that depend on an optimally functioning immune system.

Consumption of *B. lactis* HN019 enhanced the proportions of total, helper, and activated T cells in the peripheral circulation.

Immunosenescence can be characterized by reduced proportions of each of these lymphocyte phenotypes (6, 7). Thus, in agreement with previous studies of micronutrient supplementation, *B. lactis* HN019 consumption in the present study conferred modest increases in immune cell populations that may be associated with health improvements in the elderly. Chandra (19) showed that increases in circulating lymphoid cells after multivitamin and mineral supplementation corresponded to enhanced antibody responses to the influenza vaccine and markedly reduced the duration of infection-related morbidity in elderly subjects.

B. lactis HN019 supplementation also enhanced cellular immune function, leukocyte phagocytosis, and tumoricidal activity. However, it is not certain whether the increases in tumoricidal activity were solely or partly the result of increased relative proportions of NK cells in the blood. Phagocytic and tumoricidal responses are relevant to providing protection against microbial infection and neoplasm development, respectively, and indicate a potential benefit of *B. lactis* HN019, especially because these diseases are of major importance in geriatric health. Previously, it was found that adults who consumed defined probiotic strains displayed enhanced natural (ie, non-lymphoid-mediated) cellular immunity (30, 31). Furthermore, both *L. rhamnosus* GG and *Lactobacillus casei* (Shirota strain) were shown to enhance protection against gastrointestinal infection and reduce tumor growth in adults (32–34), although it is not clear whether these physiologic processes were a direct consequence of enhanced immunity. It remains to be determined whether increases in innate cellular immune responses, conferred by *B. lactis* HN019 consumption, could promote enhanced disease resistance in the elderly.

The finding that the greatest relative increases in immune function (which ranged from 9% to 82%) after *B. lactis* HN019 consumption occurred in those individuals who had poor preintervention immune responses (Figure 2 and Table 2) implies that consumption of *B. lactis* offers the greatest potential benefit to individuals with poorly functioning immune systems. *B. lactis* HN019 may therefore offer the most promise as a dietary

supplement for optimizing or restoring effective immunity, a role that was suggested previously for immune-enhancing dietary supplements in the elderly (35, 36).

The present study also showed that *B. lactis* HN019 was efficient at enhancing the immune response at a comparatively low dose. Previous studies typically showed that daily doses of 1×10^{10} to 1×10^{11} organisms of probiotic lactic acid bacteria conferred physiologic benefits (22, 23, 30–34), whereas doses $< 1 \times 10^9$ organisms did not (37). In the present study, consumption of a relatively low dose of *B. lactis* HN019 (5×10^9 organisms/d) improved cellular immunity comparable with that observed previously with a 10-fold higher dose (38). There were no adverse reactions to continuous consumption of *B. lactis* HN019 for 3 wk, confirming previous studies of the biological safety of this strain (39, 40). *B. lactis* HN019 may therefore be a safe dietary supplement for enhancing innate cellular immune function and combating some of the deleterious effects of immunosenescence. Further clinical trials are necessary to determine whether *B. lactis*–mediated immunoenhancement can promote increased resistance to infection and disease in the elderly.

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