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ORIGINAL ARTICLE

Effect of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5 on common cold episodes: A double blind, randomized, controlled trial

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Summary
Background & aims: The aim of this study was to investigate whether the consumption of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5 (5×10^7 cfu/tablet) during at least 3 months influences the severity of symptoms and the incidence and duration of the common cold.
Methods: A randomized, double-blind, placebo-controlled intervention study was performed over at least 3 months during two winter/spring periods. Four hundred and seventy nine healthy adults (aged 18–67) were supplemented daily with vitamins and minerals with or without the probiotic bacteria. Cellular immune parameters were evaluated in a randomly drawn subgroup of 122 volunteers before and after 14 days of supplementation. During common cold episodes, the participants recorded symptoms daily. Stool samples were collected before and after 14 days of probiotic supplementation to quantify fecal Lactobacilli and Bifidobacteria using qRT-PCR.

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Results: The total symptom score, the duration of common cold episodes, and days with fever during an episode were lower in the probiotic-treated group than in the control group: 79.3±7.4 vs. 102.5±12.2 points ($P = 0.056$), 7.0±0.5 vs. 8.9±1.0 days ($P = 0.045$), 0.24±0.1 vs. 1.0±0.3 days ($P = 0.017$). A significantly higher enhancement of cytotoxic plus T suppressor cells (CD8+) and a higher enhancement of T helper cells (CD4+) was observed in the probiotic-treated group. Fecal lactobacilli and bifidobacteria increased significantly after probiotic supplementation.

Conclusions: The intake of probiotic bacteria during at least 3 months significantly shortened common cold episodes by almost 2 days and reduced the severity of symptoms.

Introduction

Despite great advances in medicine, common virus infections such as the common cold or influenza continue to cause a considerable economic burden, due to loss in productivity and high medical costs.[1]

Several strains of probiotic microorganisms have a positive influence on a broad range of functions related to the host's defense system.[2,3] However, whereas many publications describe effects of probiotic microorganisms on intestinal pathogens,[4] there are few studies investigating their effect on infections in other organ systems, especially the respiratory tract.[5]

The first evidence that probiotic strains could be effective in respiratory tract infections came from mice, where the oral administration of *Bifidobacterium breve* YIT4064 augmented anti-influenza IgG production and protected against influenza infection.[6] A study on 129 children with acute respiratory tract infections showed that the administration of a bifidobacteria preparation stimulated T-cell and B-cell immunity, natural killer cells and interferon release.[7] Finnish children in day care centers who consumed milk containing *Lactobacillus rhamnosus* GG (ATCC 53103) during seven months in winter had 17% fewer respiratory tract infections than the control group.[8] The administration of an *Enterococcus faecalis* preparation (Symbioflor®) resulted in fewer relapses of chronic, recurrent, respiratory tract infections, especially bronchitis and sinusitis.[9,10] In a 3 weeks observation period in elderly people, the duration of gastrointestinal and respiratory illnesses was significantly lower in the group receiving *L. casei* DN-114001 than in the control group.[11] These studies, however, do not distinguish between bacterially and virally induced infections. In two of them gastrointestinal infections were considered together with respiratory infections in the primary parameter.

The aim of the present study was to investigate whether the consumption of certain probiotic bacteria during a period of at least 3 months in winter/spring affects the severity of symptoms, the incidence and duration of naturally acquired common cold infections, and the cellular immune response in otherwise healthy adults.

The dietary supplement used in the present study was thought to be an "all-in-one" product, which was designed to secure adequate vitamin and mineral intake according to the dietary allowances. Adequate supply was shown to have impact on immunity. Probiotics were added with the aim of enhancing immune defense. Since we wanted to investigate the probiotic effect, the control group was supplied with the same amount of vitamins and minerals as the probiotic-treated group.

Methods

Study design and ethics

The study was carried out double-blind, randomized, and placebo-controlled, with two parallel arms. It was approved by the ethics committee of the Medical Faculty of the Christian-Albrechts-University Kiel, Germany. All participants gave their written informed consent before inclusion in the study.

Participants

Volunteers were recruited by advertisements. A total of 479 healthy women and men were enclosed after physical examination. Four hundred and fifty-four of them completed the study. The exclusion criteria were: laboratory parameters outside the normal range, known congenital or acquired immune defects, allergies and other chronic or acute

diseases requiring treatment, alcohol or drug misuse or both, pregnancy or lactation, interfering dietary habits, or vaccination against influenza within the last 12 months.

During the study, neither immune stimulating medication nor abnormal physical exercise was allowed. The volunteers were asked to maintain their usual eating habits but to refrain from eating products containing prebiotics and/or probiotics according to labeling and the following fermented milk products: yogurts, kefir, crème fraiche, sour crème, and sour milk. Cheese was allowed for compliance reasons. Curd cheese and butter milk, which are fermented using mesophilic starter cultures, which do not include probiotic strains, were also permitted. The participants were briefed in an informative meeting and in written form before the study started.

Test preparations, blinding, and randomization

Test preparations were given in tablets with the same appearance, smell and taste. They contained either 5×10^7 cfu of the spray dried probiotic bacteria with vitamins and minerals (hereafter called probiotics) or just the vitamin mineral preparation (hereafter called control). The vitamins and minerals in this preparation were according the EC recommended daily allowance (RDA) as far as existing (for details see Table 1).

The probiotic strains used in this study were *L. gasseri* PA 16/8, *B. longum* SP 07/3, *B. bifidum* MF 20/5 (*Tribion harmonis*™). The number of viable probiotic bacteria declared for the product during the study period was guaranteed by long-term stability tests carried out by the supplier. These bacterial strains have been shown to help protect mice from intestinal infections with pathogenic *Escherichia coli* 0-136.[12]

All test preparations were prepared, packaged, and randomized by the supplier (Merck, Consumer Health Care) who labeled the packages with identification numbers in order to fulfill the criteria of a double-blind trial.

The volunteers were assigned to these identification numbers, according to recruitment.

Intervention

Two hundred and forty-four participants were observed during a 3 month period (between January and May 2001), and 237 participants during a 5.5 month period (between December 2001 and June 2002). The volunteers were asked to take one tablet of the test preparation per day, in addition to their usual diet. They collected the tablets from the Federal Research Centre for Food and Nutrition every 4 weeks. This ensured close contact to the volunteers in order to ascertain optimal compliance.

Cellular immune response was assessed by flow cytometry in a randomly drawn subgroup of 122 participants (61 per study group) before and after 14 days of supplementation in the first study period. As a randomized block design was used, we were ensured that the groups were of the same size.

During respiratory tract infection episodes, the participants used questionnaires to daily record the symptoms mentioned. An episode was defined as the appearance of at least one specific respiratory tract symptom (nasal, pharyngeal or bronchial). With the aim of excluding bacterial respiratory tract infections, episodes were not considered if a participant received antibiotic treatment. On day two of each episode, nasal secretions were collected for virus identification.

Outcome measures

The following parameters were calculated:

- single specific symptoms that appeared during common cold episodes,
- total symptoms score expressing overall severity of each episode (primary parameter),
- the duration and incidence of common cold episodes,
- the cellular immune response,
- the type of viruses identified, and
- fecal lactobacilli and bifidobacteria.

Clinical assessment of common cold episodes

The questionnaires for assessing the common cold episodes were based on studies by Stansfield et al. and Mossad et al.[13,14] Volunteers noted nasal symptoms (running nose, stuffed nose, blowing the nose, yellow secretion, bloody secretion, sneezing), pharyngeal symptoms (scratchy throat, sore throat, hoarseness), and bronchial symptoms (cough, secretion, yellow secretion), headache, myalgia, conjunctivitis (reddish eyes), fatigue, loss of appetite, and fever (oral temperature $>37.7\,^{\circ}C$) daily. Severity of nasal, pharyngeal, and bronchitis symptoms, as well as headache, myalgia, and conjunctivitis was each graded as: no symptoms = 0, mild symptoms = 2, moderate symptoms = 4, and severe symptoms = 6. Sneezing, fatigue, and loss of appetite were graded as: yes = 1, no = 0. Fever was

Table 1 Composition of the test preparations.

	Probiotics plus vitamins and minerals	Control (vitamins and minerals)
	Per tablet	
Probiotic bacteria (cfu):	5×10^7	
(*Lactobacillus gasseri* PA 16/8,	4×10^7	—
Bifidobacterium longum SP 07/3,	5×10^6	
B. bifidum MF 20/5)*	5×10^6	
Vitamin C (mg)	60	60
Vitamin E (mg)	10	10
Nicotinamide (mg)	18	18
Vitamin A (µg)	800	800
Vitamin K1 (µg)	30	30
Vitamin B6 (mg)	2	2
Vitamin B1 (mg)	1.4	1.4
Vitamin B2 (mg)	1.6	1.6
Vitamin D (µg)	5	5
Vitamin B12 (µg)	1	1
Folic acid (µg)	200	200
Biotin (µg)	150	150
Pantothenic acid (mg)	6	6
Calcium (mg)	40	40
Phosphorus (mg)	16	16
Potassium (mg)	5	5
Chloride (mg)	4.5	4.5
Magnesium (mg)	5	5
Iron (mg)	14	14
Zinc (mg)	15	15
Manganese (mg)	2	2
Selenium (µg)	30	30
Silicon (µg)	2	2
Chromium (µg)	25	25
Molybdenum (µg)	25	25
Iodine (µg)	150	150

Ingredients: Microcrystalline cellulose, Calcium ascorbate granulate, Lactose monohydrate, Tricalcium phosphate anhydrous. *Coating*: (Shellac, Hydroxypropyl methyl cellulose, Acetylated monoglycerides povidone glycerol), Iron (II) sulphate hydrate, Vitamin E preparation (DL-Alpha tocopheryl acetate), Glucose, Selenium yeast, Probiotic bacteria powder, Glycerol monostearate, Nicotinamide, Zinc oxide, Sodium carboxymethyl cellulose, Povidone, Potassium chloride, Magnesium oxide, Calcium pantothenate, Crospovidone, Vitamin A preparation, Manganese (II) sulphate monohydrate, Magnesium stearate, Iron oxide, Vitamin K1 glucose trituration, Vitamin D3 powder, Pyridoxine hydrochloride, Thiamine mononitrate, Riboflavin, Colloidal silicon dioxide, Cyanocobalamine glucose trituration, Folic acid, Potassium iodide, Biotin, Chromium (III) chloride hexahydrate, Sodium molybdate dihydrate.
*Tribion harmonis™.

always scored as 6. These symptom scores were combined to produce a daily symptom score with a maximum of 45 per day. The daily recorded scores were added up over the illness period to produce a total symptom score.

Flow cytometry

Blood cells were analyzed with the aid of a EPICS® XL-MCL flow cytometer (Beckman Coulter®, Krefeld, Germany). Lymphocytes (CD45+), B-lymphocytes (CD45+, CD19+), T-lymphocytes (CD45+, CD3+), T_H cells (CD45+, CD3+, CD4+), T_S plus T_C cells (CD45+, CD3+, CD8+), and natural killer cells (CD 45+, CD 56+) were differentiated using a Beckman Coulter tetraChrome test®. Cell populations were calculated by Coulter® System II Software, Version 3.0, and Coulter® tetraONE SYSTEMsoftware. In order to determine T-lymphocyte activation, whole blood was incubated with 5 µg PHA/ml or 20 µg PHA/ml for 2 h (the control was the same treatment with PBS). CD69

expression was chosen as an early activation marker.[15] The cells were stained with CD3, CD4, CD8, and CD69 monoclonal antibodies (IO Test® Coulter Immunotech). The number of granulocytes and monocytes was determinated using forward and sideward scatter. Phagocytic activity was investigated by the Orpegen PhagoTest®. This test kit determinates phagocytic function of granulocytes and monocytes in whole blood by measuring ingestion of fluorescein-labeled *E. coli*.[16]

Virus identification

For virus identification samples of nasal discharge were used. Knowing that this material warrants lower sensitivity than nasopharyngeal aspirate, we chose these samples for compliance reasons since aspiration is associated with some discomfort. The volunteers blew their noses into a cellulose wipe (Kimwipes® Lite 100© Kimberly-Clark Corporation). The samples were refrigerated and stored in lockable plastic containers at −20 °C until use. Viruses were identified after the extraction of nucleic acid (QIAamp® Viral RNA Mini Kit, Qiagen, Hilden, Germany) by means of RT-PCR or PCR. Reverse transcription and the first PCR for the detection of enterovirus, respiratory synzitial virus (RSV) and parainfluenza 3 virus RNA was performed in a one step reaction, using the cMasterRTplusPCR system (Eppendorf, Hamburg). Primers were supplied by Tib Molbiol (Berlin). In order to enhance the specificity, PCR products were used as templates in a second PCR round (nested PCR), using the Hot Master Taq polymerase (Eppendorf) in the corresponding buffer system. PCR products were analyzed by gel electrophoresis on 2% E-Gels (Invitrogen). The presence of parainfluenza-1, 2, and 4, metapneumo- A and B, influenza- A and B, and adenovirus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* was also sought.

PCR products from enterovirus RT-PCR were further characterized by cycle sequencing (performed by Sequa, Gutting). The obtained sequence data were analyzed using the software package Lasergene (DNA Star) and were identified by BLAST search.

Quantification of fecal lactobacilli and bifidobacteria

In order to assess the increase of fecal count of the bacteria administered, we collected stool samples of 20 volunteers (10 women and men each) from the study cohort in a separate trial. Before the first stool samples were collected, the volunteers were asked to refrain from eating the products mentioned above for 14 days. Thereafter, the volunteers took the dietary supplement containing the probiotic bacteria daily for 14 days still refraining from eating the mentioned edibles until stool samples were collected the second time. Samples were frozen in liquid nitrogen and stored at −80 °C until use.

DNA of the stool samples was isolated with the FastDNA SPIN Kit for Soil (BIO 101, Calsbad, USA) after mechanical homogenization (FastPrepTM FP 120 instrument, Bio 101, Calsbad USA) according to the manufacturer's instructions. The DNA was checked by 1.5% agarose gel electrophoresis. DNA concentrations were determined using PicoGreen™ (dsDNA Quantification Kit, Molecular Probes, Leiden, The Netherlands).

Quantitative real time PCR assays were performed and optimized to detect the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. in the stool samples. Primers and probes of the real time assays are based on the 16S rRNA gene (Table 2). All primers and probes used in this study hybridize to variable regions of the 16S rRNA gene specific for the bifidobacteria and lactobacillus group. The primer pair LAC_1_RT and LAC_2_RT produce a 16S rDNA fragment of ~320 bp length and

Table 2 Characteristics of primers and probes used for the real time PCR assays.

Primer/Probe	Position*	Direction	Dye	Sequence (5′–3′)	T_m (°C)	Reference
LAC_1_RT	350-370	Forward	—	GCAGCAGTAGGGAATCTTCCA	58.0	Modified from Walter et al.[17]
LAC_2_RT	677-690	Reverse	—	GCATTTCACCGCTACACATG	60.2	Modified from Walter et al.[17]
LACTO	455-471	—	FAM†	AGGCCAGTTACTACCT	66.0	Ott et al.[18]
Bif_164	164-181	Forward	—	GGGTGGTAATGCCGGATG	59.3	Langendijk et al.[19]
Bif_662	662-679	Reverse	—	TTCCACCGTTACACCGGGAA	62.0	Langendijk et al.[19]
Bifido_584	584-601	—	FAM†	TGAAAGTCCATCGCTTA	65.6	Modified from Bernhard et al.[20]

*According to the *Escherichia coli* reference numbering.
†FAM = 6-carboxyfluorescin.

includes bacterial species of the following groups: *Lactobacillus, Pediococcus, Leuconostoc,* and *Weissella.*[17] The probe LACTO is a minor groove binder (MGB) probe designed using the PROBE_Design tool of the ARB software package (ARB sofware package, www.arb-home.de).[18] The probes are labeled with the fluorescence dye FAM (6-carboxyfluorescin). The specificity of the LACTO probe encloses most species of the lactobacillus group, among them *L. acidophilus, L. delbrueckii, L. johnsonii, L. gasseri,* and *L. casei.* For optimization of the assay and generation of standard curves, serial dilutions of *L. acidophilus* and *L. gasseri* strains were used,[18] the latter cultivated from the probiotic compound. The primer pair Bif_164 and Bif_662 produces a fragment of ~500 bp length. The probe Bifido_584 is a FAM-labeled minor groove binder (MGB) probe modified from Bernhard and Field.[20] Both the primers and the probe hybridize to bifidobacterium specific regions of the 16S rRNA gene, including *B. longum, B. bifidum, B. adolescentis,* and *B. infantis.*[19,20] For optimization of the assay and generation of standard curves, serial dilutions of *B. adolescentis, B. longum,* and *B. bifidum* strains were used. The *B. bifidum* strain was cultivated from the probiotic compound. Real time PCR was performed with the aid of an ABI PRISM™ 7700 Sequence Detector using TaqMan™ Universal PCR 2x Master Mix (Applied Biosystems, Foster City, CA, USA), primer (0.4 μM), probe (0.2 μM), and 100 ng of sample DNA in a final volume of 50 μl per reaction with an initial hold of 50 °C for 2 min to activate No Amp Erase™ UNG, a hold of 95 °C for 10 min to activate AmpliTaq™ Gold Polymerase followed by 50 cycles for 30 s at 95 °C, 60 °C for 1 min, and 72 °C for 2 min. The PCR products were checked for size and side bands by gel electrophoresis using 1.5% agarose gels. The real time PCR results were averaged from two independent experiments and related to 1 g of stool.

Statistical power and analyses

Information on the duration of common cold episodes was obtained a year before from interviews with residents of Kiel and the surrounding area. A minimum of 61 participants per group was calculated from: a mean duration of 6.0 (SD 1.7) days, $\alpha = 0.05$, power $(1 - \beta) = 0.1$ and an expected reduction of 1 day in the duration of episodes in the probiotic-treated group, according to the results of a study on oseltamivir.[21] Assuming that only 60% of the participants would catch a cold and allowing for 20 withdrawals, a total of 120 participants was to be recruited per group and intervention period.

The minimum of 61 participants was in accordance with a study by Hoheisel et al., in which 60 volunteers with initial cold symptoms were necessary per group in order to demonstrate the significantly positive effect of an immune stimulating *Echinacea purpurea* extract on the duration of common cold episodes.[22]

The sample size calculation was based on an entire common cold season from autumn to spring. For technical reasons the study did not start before January 2001. Therefore, we decided to extend the study to a second period. In order to avoid bias new volunteers were recruited in the second period, who were randomized separately securing equal numbers in the probiotic-treated group and control group in each period.

Data are presented as mean ± standard error of the mean (SEM). Differences between the probiotic-treated group and the control group were analyzed by independent Student's *t*-tests for the duration of common cold episodes, days with fever, and differences in cell counts between day 14 and day 0. The incidence of infections was compared between the two groups using the χ^2 test. The differences in symptom scores were assessed by the non-parametric Mann-Whitney test. As far as the available literature shows that probiotic bacteria, if they have any effect at all, stimulate immune response, one-tailed tests were chosen. Bacterial counts in stool samples were compared by sign test. A value of $P \leqslant 0.05$ was regarded as statistically significant. All statistical analyses were performed using the software package Statgraphics Plus®, version 4.1 (Manugistics, Rockville, USA).

Results

Participants

Two hundred and forty-two participants started in the study period between January and May 2001: 121 in the probiotic-treated and 121 in the control group. There were 5 withdrawals in the probiotic-treated and 3 in the control group. Two hundred and thirty-seven participants started the study period between December 2001 and June 2002: 117 in the probiotic-treated and 120 in the control group. There were 8 withdrawals in the probiotic-treated and 9 in the control group. In the first study period one withdrawal was due to pregnancy, the reminding were due to non-compliance. There was no report of adverse events. The distribution of sex and age was equal in the verum and the control group (Table 3). Altogether data from the 454

Table 3 Age and sex of volunteers.

		Probiotics+vitamins and minerals	Control (vitamins and minerals)	Total
Age				
Period I		36±12	36±13	36±13
Period II		39±12	41±14	40±13
Total		37±12	38±14	38±13
Sex				
Period I	W	75	73	148
	M	46	48	94
	W+M	121	121	242
Period II	W	77	69	146
	M	40	51	91
	W+M	117	120	237
Total	W	152	142	294
	M	86	99	185
	W+M	238	241	479

W, women; M, men.
Period I lasted from January 2001 to May 2001.
Period II lasted from December 2001 to June 2002.

Table 4 Effect of probiotic bacteria (5×10^7 cfu/day) on symptoms of the common cold episodes with more than one symptom in detail during the observation period.

	Probiotics+vitamins and minerals	Control (vitamins and minerals)	*P*
Duration (days)	7.0±0.5	8.9±1.0	0.045*
Symptom scores (points)			
Nasal symptoms (inclusive sneezing)	29.5±2.7	36.8±3.7	0.053†
Pharyngeal symptoms	12.9±1.7	17.1±2.5	0.051†
Bronchial symptoms	13.2±2.1	19.1±2.7	0.011†
Headache	8.5±1.2	10.0±1.6	0.245†
Myalgia	5.3±1.2	5.5±1.4	0.142†
Conjunctivitis	3.5±0.9	2.8±1.0	0.925†
Fatigue	2.7±0.3	3.0±0.5	0.407†
Loss of appetite	2.3±0.3	2.5±0.3	0.511†
Days with fever‡	0.24±0.1	1.0±0.3	0.017*
Total symptom score	79.3±7.4	102.5±12.2	0.056†

Values are mean ± SEM.
*P-value from independent Student's *t*-test.
†P-value from Mann–Whitney test.
‡Oral temperature >37.7 °C.

participants who completed the study were available.

Symptoms

The sum of all symptoms recorded daily by questionnaires (total symptom score) was lower in the probiotic-treated group than in the control group. Differences were most marked in nasal, pharyngeal, and bronchial symptoms, as well as in the number of days with fever during a common cold episode. The other symptoms were also fewer but did not attain significance (Table 4).

Duration and incidence of episodes

The mean duration of common cold episodes was significantly shorter in the probiotic-treated group than in the control group (relative reduction of 21.5%, Table 3). The total number of common cold episodes was 158 in the probiotic-treated group and 153 in the control group (not significant).

Flow cytometric analysis

Volunteers with abnormal leucocyte counts before intervention were rejected from the analysis. After the consumption of probiotic bacteria for 14 days, a significantly higher enhancement, calculated as difference between day 14 and day 0, of cytotoxic T-cells plus T suppressor cells (CD8+) was observed compared to control. Also the enhancement of the other immune cells investigated was higher in the probiotic-treated group, but it was only marginally significant or not significant (Table 5). There was no change in T-cell activation and phagocytic activity during the observation period (data not shown).

Viral infections

Ninety-five samples of nasal secretion were obtained. Viruses were identified in 24 of these samples: rhinoviruses in 19, RS viruses in five and enterovirus (not specified) in one. In one sample, a dual viral infection was found.

Fecal lactobacilli and bifidobacteria

The mean absolute numbers of cells per g stool assessed in a separate trial were significantly increased from day 0 to day 14 days of probiotic supplementation for both the bifidobacteria ($3.14 \times 10^7 \pm 5.57 \times 10^6$ vs. $3.39 \times 10^8 \pm 5.53 \times 10^7$) and the lactobacilli ($1.04 \times 10^5 \pm 3.69 \times 10^4$ vs. $1.45 \times 10^5 \pm 2.48 \times 10^4$) (Fig. 1).

Discussion

In this randomized, placebo-controlled, double-blind intervention study the long term effect of probiotic bacteria on naturally acquired common respiratory tract infections was investigated. The winter/spring periods were chosen because of the enhanced risk of cold infections. Since viruses are known to vary between the common cold seasons the inclusion of two seasons, although done for technical reasons, served the aim of a representative study. The total number of common cold episodes registered in this study was 311 (0.7 episodes per participant within 4.3 months) which corresponds to the rule of thumb that adults catch two to four cold episodes per year.[1]

In this investigation, self-assessment based questionnaires were used.[13,14] This was justified by studies, which demonstrate a good concordance between the individual's own estimation of symptoms and an evaluation by a physician.[23]

Analysis of the questionnaires showed that the use of probiotic bacteria significantly shortened the mean duration of the episodes and reduced the

Table 5 Effect of probiotic bacteria (5×10^7 cfu/day) on cellular immune parameters. On day 0 and day 14 of the first intervention period immune cell counts were assessed by flow cytometry in blood samples from 122 participants (61 per study group). Volunteers with abnormal leucocyte counts before intervention were rejected from the analysis. Results are expressed as Δ (day 14–day 0) cell counts per µL blood (mean±SEM).

	(Δ Cell counts $\times 10^6$ per L blood)		P*
	Probiotics+vitamins and minerals	Control (vitamins and minerals)	
Leukocytes	1749±556	1322±304	0.251
Lymphocytes	263±68	192±61	0.219
B lymphocytes	18±10	12±10	0.332
T lymphocytes	131±43	72±45	0.175
T_H	82±31	21±30	0.081
T_C+T_S	64±15	19±19	0.035
Natural killer cells	114±27	108±21	0.428
Granulocytes	1241±495	960±251	0.307
Monocytes	177±45	137±36	0.257

T_H, T helper cells (CD4+); T_C+T_S, cytotoxic T-cells+T suppressor cells (CD8+).
*P-values from independent Student's t-test.

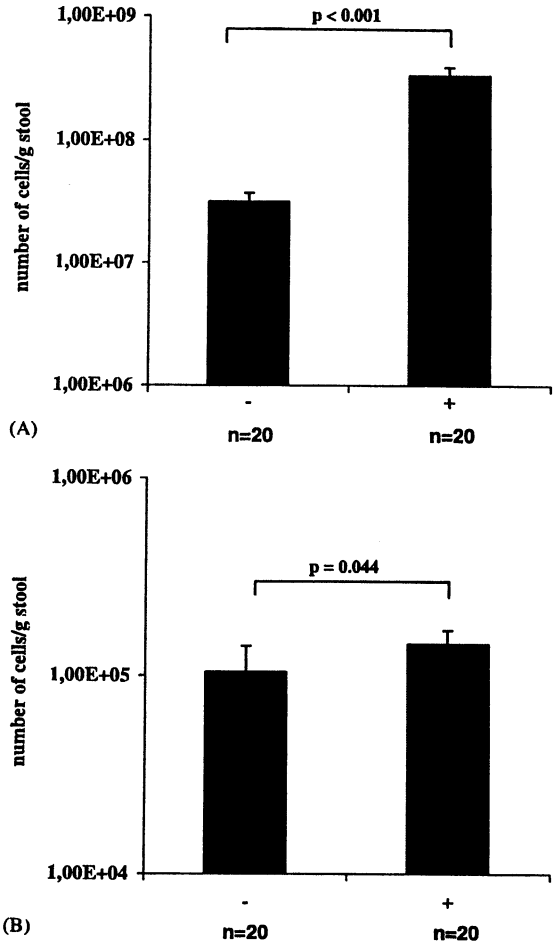

Figure 1 Effects on the number of bacterial cells of *Bifidobacterium* spp. (A) and *Lactobacillus* spp. (B) as assessed by quantitative real time PCR using group specific primers. Indicated are the mean absolute numbers of cells per g stool±SEM before (−) and after 14 days (+) of probiotic supplementation.

severity of symptoms. There was, however, no influence on the over all incidence of respiratory tract infections. The effects were similar to those of neuraminidase inhibitors in the case of acute influenza that, when administered within the first 48 h of the infection, reduced the severity of symptoms and shortened influenza by one day and, when inhaled prophylactically, by 2.5 days.[21,24] In the present study a reduction of about 2 days was found. In contrast to neuraminidase inhibitors, probiotics may be ingested as part of the daily diet and then do not induce extra costs to the health system.

The weak influenza-like symptoms and the mostly mild episodes of fever led to the conclusion that the infections were predominantly common cold episodes. This is also supported by the results of the PCR-analysis, which mainly detected rhinoviruses.

The reduction in the severity and duration of common cold episodes may be due to immune stimulatory effects. In response to certain probiotic strains, different cell lines such as human PBMCs, monocytes, dendritic cells, and human intestinal mucosa cells have been shown to release pro- and anti-inflammatory cytokines, as well as to express co-stimulatory molecules.[25–27] Such immunomodulation seems to be based on bacterial patterns, such as bacterial cell wall components (e.g. lipoteichoic acid) and CpG motifs of DNA (cytosine–phosphate–guanosine DNA, frequently found in bacterial and viral genome). They are recognized by toll-like receptors, which are expressed in intestinal and immune cells.[27–29] Moreover, several strains of *L. gasseri* have been shown to release a chemotactic factor acting on human monocytes.[30]

Cytotoxic T-cells plus T suppressor cells (CD8+) showed a significantly higher enhancement in the probiotic-treated group during the first 14 days of supplementation compared to the control group. By the probiotic bacteria used in the present study all immune cells investigated were enhanced. However, the enhancement was not significant or was only marginally significant. This may be due to the fact that both the probiotics and the control preparation contained supplemental vitamins and minerals, some of which have immunostimulatory properties; this could have blurred differences between the groups.[31]

The quantitative RT-PCR demonstrated a substantial increase of fecal lactobacilli and bifidobacteria after administration of the probiotic preparation. This may indicate survival during gastrointestinal transit, which is part of most of the definition proposed in literature.[32]

In conclusion, the present study presents evidence for the positive effects of consumption of *L. gasseri* PA 16/8, *B. longum* SP 07/3, *B. bifidum* MF 20/5 during at least 3 months in winter/spring on the severity of common cold episodes in otherwise healthy adults.

The growing number of studies in which favorable effects have been described, indicates some benefit of probiotics in respiratory tract infections. Until now the evidence, however, is based on one study for one strain or strain mixture, each. This has to be considered since probiotic effects are regarded as strain specific.

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