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Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination

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■ **Summary** *Background* Orally ingested probiotic bacteria may modulate the immune response and increase antibody titers against enteric infections by bacteria or viruses. Even though positive effects of probiotics on respiratory tract infections have been reported, overall only few studies have examined effects on virus infections concerning organs other than the gastrointestinal tract. *Aim of the study* It was the aim of the study to investigate whether and how probiotics affect the immune response to a standardized enterovirus challenge (polio) and infections not limited to the gastrointestinal tract in healthy adults. *Methods* In a randomized, controlled and double-blind study 64 volunteers consumed for 5 weeks chemically acidified clotted milk without bacteria or with 10^{10}/serving *Lactobacillus rhamnosus* GG or *Lactobacillus acidophilus* CRL431 added. In the second week subjects were vaccinated orally against polio 1, 2 and 3. Polio virus neutraliz- ing serum activity, the primary parameter, was determined by the standard neutralization test (WHO) before and three times after vaccination. Polio-specific IgA, IgG and IgM were detected by ELISAs. *Results* Probiotics increased poliovirus neutralizing antibody titers (NT) and affected the formation of poliovirus-specific IgA and IgG in serum. The maximum increase after immunization was about 2, 2.2, or 4-fold higher, respectively, for NT, IgG or, IgA, in volunteers consuming probiotics instead of placebo. No consistent difference was noted between bacterial strains. *Conclusions* Probiotics induce an immunologic response that may provide enhanced systemic protection of cells from virus infections by increasing production of virus neutralizing antibodies.

■ **Key words** immune response – neutralizing antibodies – poliovirus – probiotics – vaccination

Introduction

A number of *in vivo* studies in animals and humans [1–4] have demonstrated that probiotic bacteria may modulate the immune response and increase antibody titers against enteric infections by bacteria (*S. typhimurium, Shigella, E. coli, Clostridium difficile*, Cholera- toxin) or viruses (rotavirus-induced diarrhea). Although the term "probiotic" is not limited to health effects concerning the gastrointestinal tract [5], only a few studies have examined the effect of probiotics on systemic infections, or virus infections concerning organs other than the gastrointestinal tract, respectively. Lykova et al. [6] observed enhanced immunity (T/B-cell immunity, NK-cells, γ-Interferon) in children with acute respi-

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ratory tract infections consuming a bifidobacteria preparation. A randomized, controlled study demonstrated that long-term consumption of the probiotic strain *Lactobacillus rhamnosus* GG (LGG) was associated with reduced absence from day-care centers due to common gastrointestinal and respiratory tract infections in otherwise healthy children [7].

Oral polio vaccination can be used as a model for systemic virus infections. Oral application of the attenuated poliovirus (Sabin vaccine) was authorized for use in Germany at the time of the study. Poliomyelitis viruses naturally infect via the oral route and multiply either in the intestinal mucosa or in lymphatic tissues of the small intestine. While most infections are asymptomatic, about 0.1 % of infections result in paralytic poliomyelitis characterized by lower motor neuron damage. IgM antibodies indicate an early humoral immune response to polio infection [8]. Their serum concentration peaks 2 weeks after infection and declines after 3–4 weeks. IgG antibodies appear later. Both types of antibody prevent polioviruses from spreading to the central nervous system and protect against paralytic disease [9–12]. In contrast to circulating neutralizing antibodies and specific IgG in serum, secretory IgA (sIgA) reflects mucosal immunity in the intestine, protects from infection, reduces virus excretion and limits poliovirus propagation in a community [9, 11].

The poliovirus neutralization test is the standard test for measuring immunity to and protection from the three poliovirus serotypes after vaccination. This test was chosen by the WHO as its reference method.

To investigate whether and how consumption of probiotics affects the immune response to a standardized enterovirus challenge in healthy adults, we vaccinated volunteers orally against polio and determined the response of protective neutralizing antibodies chosen as the primary parameter. Secondary immunological parameters were virus-specific IgG, IgA and IgM in serum.

Subjects and methods

■ Subjects

Sixty-six healthy male volunteers aged 20 to 30 years (mainly university students) were included after having given their informed written consent. Each volunteer fulfilled the recommendations of the German Federal Standing Committee on Vaccinations (STIKO) and had been immunized against polioviruses more than 15 years ago.

The exclusion criteria accorded with the guidelines of the STIKO: chronic diseases, acute diseases requiring treatment, known congenital or acquired immune defects either in the applicants themselves or in persons in close contact with them. Health care professionals being at risk of polio infection to their patients after vaccination were also excluded. Sixty-four volunteers completed the study; two dropped out due to scheduling difficulties.

■ Bacterial strains

The bacterial strains used in the present study were *Lactobacillus rhamnosus* strain LGG (ATCC 53103, provided by MONA, Woerden, The Netherlands), and *Lactobacillus paracasei* subspecies *paracasei* (strain CRL431, provided by Christian Hansen, Hørsholm, Denmark).

■ Diets

In order to exclude any effect from living or inactivated yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbruckii* subspecies *bulgaricus*) on the immune system, chemically acidified milk products with the appearance of yogurt were prepared as follows: low-fat milk (1.5 % fat) was inoculated with either LGG (test product 1) or CRL431 (test product 2) and incubated for 7 h at 37 °C, or the milk was used without inoculation to produce the placebo products. D-gluconic acid-δ-lactone (1.7 %) was then added and the pH reduced to 4.5 within 5–6 h by hydrolysis of the lactone. After inoculation the lactobacilli in the test products were metabolically active as indicated by a 10- to 50-fold increase in bacterial counts, estimated before and after acidification. The flavored test and control products were coded by the supplier (MONA, Woerden, The Netherlands) to fulfill the criteria of a double-blind study design. 10^{10} living bacteria per serving (100 g) in the test products were guaranteed.

■ Vaccine

Commercially available live attenuated poliomyelitis viruses of type 1 strain LSc_1, type 2 strain P2712, and type 3 strain Leon $12a_1b$ (Behring-Werke, Marburg, Germany) were applied orally at dosages guaranteed by the manufacturer to provide immune protection (NT > 1:4) in 95 % of vaccinated subjects. Although lower dosages would have improved the chances of detecting potential increases in the response to vaccination due to consumption of probiotics, the applied dosages were chosen to ensure that vaccination conferred sufficient protection for the participants.

■ Experimental protocol

The study protocol was approved by the Ethics Committee of the Medical Faculty of Christian-Albrechts Uni-

versity in Kiel, Germany. The study followed a controlled, randomized and double-blind design with 3 parallel branches: The volunteers were given 100 g/day acidified milk products without (placebo, n = 22) or with 10^{10} cfu/serving LGG (verum group 1, n = 21) or CRL431 (verum 2, n = 21) over 5 weeks. At day eight the subjects were vaccinated orally against polio. During two 3-week periods immediately before and after the intervention period and during intervention, the volunteers were asked to adhere to their usual eating habits but to exclude fermented food, namely yogurt and yogurt-like products, cheese, raw sausages, and fermented vegetables.

Blood samples of 4 ml each were taken for antibody determinations 4 weeks before, immediately before, and 2, 4 and 7 weeks after vaccination. Serum samples from all subjects were refrigerated and stored at –20 °C until use.

■ Antibody titration

Antibodies were determined after the samples of all volunteers had been collected. The samples from a single individual were measured by a single assay kit employing the same cell culture set used in the poliovirus neutralization test.

■ Neutralizing antibodies

Poliovirus serotype 1, 2 and 3 neutralizing antibody titers (NT) in sera were determined by the standard neutralization test following the recommendations of the World Health Organization [13, 14]. Identical strains as for vaccination were used as challenge viruses. Serial 2-fold dilutions of the volunteers' sera and 50 TCID$_{50}$ infectious doses of the viruses were incubated for 3 h at 37 °C and Vero cell suspensions added. The titers are expressed as the reciprocal of the highest dilution showing complete neutralization of the cytopathic effect. Samples were considered positive if Log$_2$ (NT) were ≥ 3.

■ Poliovirus binding-inhibition assay

An optimized ELISA-based poliovirus binding-inhibition test (PoBI) was performed in addition to the conventional poliovirus neutralization test to detect and quantify IgG antibodies to poliovirus serotypes 1, 2 and 3, which are predominantly exerting the neutralizing effect of serum [15, 16]. Briefly, serial 2-fold dilutions (1:4096) of serum samples were incubated for 2 h at 37 °C with 20, 4, and 16 D-antigen units/ml of monovalent, formaldehyde-inactivated poliovirus (Mahoney, MEF and Saukett strain for serotypes 1, 2 and 3, respectively,

produced at the RIVM, Bilthoven). Free viral epitopes were detected by a double antibody sandwich ELISA using specific polyclonal antisera (IgG fraction of bovine anti-poliovirus hyperimmune serum 1:500, 1:250 and 1:250 for serotypes 1, 2 and 3, respectively) and serotype-specific monoclonal antibodies (14D2E9, 1:3000, 6-15C6, 1:10000 and 2-13D9, 1:10000 for serotypes 1, 2 and 3, respectively) coupled to goat anti-mouse IgG alkaline phosphatase conjugate (1:500, Sigma, Zwijndrecht, The Netherlands). Titers are expressed as the reciprocal of the lowest dilution showing a ≥ 50 % reduction in OD$_{405}$ of added p-nitrophenylphosphate. The assay included appropriate blocking and control procedures.

■ Determination of poliovirus-specific IgA

Poliovirus serotype-specific IgA were detected by ELISA as described elsewhere [17]. Briefly, microtiter plates were coated with 5-18D8, 1-10C9E6 and 2-13D9 serotype-specific monoclonal antibodies for poliovirus serotypes 1, 2 and 3, respectively. 40–70 D-antigen units of formaldehyde-inactivated poliovirus (inactivated Mahoney, MEF and Saukett strains for serotype 1, 2 and 3 assays), IgG depleted serum dilutions (1:50), and finally an optimal dilution (1:8000) of goat anti-human IgA labelled with α-chain-specific alkaline phosphatase (Sigma, Zwijndrecht, The Netherlands) were added sequentially, incubated and washed appropriately. p-Nitrophenylphosphate was added and the developed color of p-nitrophenol was measured at 405 nm. Results are presented in OD$_{405}$. The assay included appropriate blocking and control procedures.

■ Determination of poliovirus-specific IgG

The poliovirus type-specific IgG assay was based on the IgA ELISA [17] by replacing the IgA conjugate with an antihuman IgG-alkaline phosphatase conjugate. Sera were not depleted in IgA, as effects on IgG response are unlikely due to the low serum IgA concentration compared to IgG,

■ Determination of poliovirus-specific IgM

Poliovirus serotype-specific IgM were detected using an antibody capture ELISA [8]. Microtiter plates were coated with μ-chain-specific monoclonal antibody to human IgM (Sanbio BV, Uden, The Netherlands). Serum dilutions, an inactivated poliovirus vaccine containing 40–70 D-antigen units, and finally horse-radish peroxidase-labeled serotype-specific monoclonal antibodies were added, incubated and washed. Tetramethylbenzi-

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dine was used as a substrate and color development was measured at 450 nm. Results are presented in OD_{450}. The assay included appropriate blocking and control procedures

■ Statistics

Efficacy of vaccination was determined by a chi-square analysis of the number of subjects with protective neutralizing antibody titers before and after vaccination, whereby Log_2 titers ≥ 3 on sampling days 1 and 2 (four weeks and immediately before vaccination) and on sampling day 5 (four weeks after vaccination) were considered protective.

Time courses within groups are presented as mean ± SEM and the pre- and post-vaccination titers were compared by paired t-tests.

The other examinations were performed by statistical tests by ranks of untransformed data because normal distribution of data was not sufficiently certain according to the non-parametric Kolmogorov-Smirnov one-sample test for the overall goodness-of-fit. Comparisons between placebo and each of the probiotic strains were done separately by single-sided Mann-Whitney rank tests. This approach was chosen because the immunological effects of probiotic bacteria are strain specific and the available literature shows that probiotic bacteria, if they have any effect at all, increase the antibody response. The difference in antibody titers between sampling day 1 (i. e. before consumption of the probiotic or placebo products and before vaccination) and the respective maximum values after vaccination was regarded as the clearest indicator of the effects of probiotic action on immune response and was used for statistical analysis.

Statistical analyses were performed using the software package "Statgraphics Plus for Windows" (version 2, Manugistics, 20852 Rockville, USA).

Results

■ Protective effect of vaccination

The number of volunteers having protective polio 1- and 2-specific NT already before vaccination were comparable in each dietary group, whereas more subjects in the LGG group had protective polio 3-specific NT than in the placebo and CRL431 groups. Vaccination significantly increased the percentage of subjects whose neutralizing antibody titers indicated protection against polio from 37 %, 51 % and 15 % of all subjects to 81 %, 80 % and 47 % for polio types 1, 2 and 3, respectively (Table 1). The increase of the proportion of protected persons after vaccination was not significantly different between

Table 1 Efficacy of polio vaccination in humans consuming a chemically acidified milk product either inoculated with probiotic lactobacilli (LGG or CRL431) or without bacteria (placebo). Log_2 (NT titers) 3 were considered protective. Differences between dietary groups were tested by chi-square analysis and Fisher's exact test. A significant increase ($p < 0.05$) in protected persons after immunization ("after" vs. "before") is indicated by asterisks. There were no significant differences between groups

| | n | Number of volunteers with protective neutralizing antibody titers against | | | | | |
| | | polio 1 | | polio 2 | | polio 3 | |
		before[1]	after[2]	before[1]	after[2]	before[1]	after[2]
Total	64	24	52*	33	51*	10	30*
Placebo	22	8	18*	12	15	2	8
LGG	21	8	19*	10	18*	7	12
CRL431	21	8	15*	11	18*	1	10*

[1] Mean of blood samples taken 4 weeks and immediately before vaccination
[2] Seven weeks after vaccination

the groups, whether the evaluation comprised all subjects or if the evaluation was limited to the participants with originally fairly low, non-protective NT-titers (NT(polio1) < 2.5).

■ Time course of antibody titers

The time course of poliovirus type 1-specific neutralizing antibodies (NT and PoBI) and IgG, IgM, IgA before and after vaccination (weeks –4 through +7) is shown in Fig. 1. In most cases consumption of the acidified milk did not significantly increase serum titers of neutralizing antibodies (NT and PoBI) and IgG and IgA before vaccination. Vaccination induced a much greater and significant increase in serum antibody titers. For all antibody classes this increase was most pronounced against poliovirus serotype 1 and least pronounced against serotype 3 (Table 2). As in other studies [18], IgA plots peaked sharply in week two after vaccination, whereas the other plots had a more sigmoidal shape within the observation period.

■ Effect of LGG and CRL431 on neutralizing antibodies

LGG and CRL431 equally enhanced the vaccination-induced titer increase in neutralizing antibodies (see Table 2). The response was most pronounced for poliovirus serotype-1-specific neutralizing antibodies. When determined by the standard neutralization test, the 2-log titer increase after vaccination was doubled in both probiotic groups. There was even a 6-fold titer increase when using the poliovirus binding-inhibition assay.

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Fig. 1 Time course of polio 1-specific humoral antibody titers (mean ± SEM) before and after polio vaccination in humans consuming a chemically acidified milk product either inoculated with probiotic lactobacilli (LGG: –●●– or CRL431: –▲▲–) or without bacteria (placebo: –○–○–). Antibody titers are expressed as the number of serum 2-fold dilution steps (NT and PoBi) or $OD_{405/450}$, (IgA, IgG or IgM) in the respective ELISA. Significant differences between pre- and post-vaccination titers within dietary groups are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$, paired t-test)
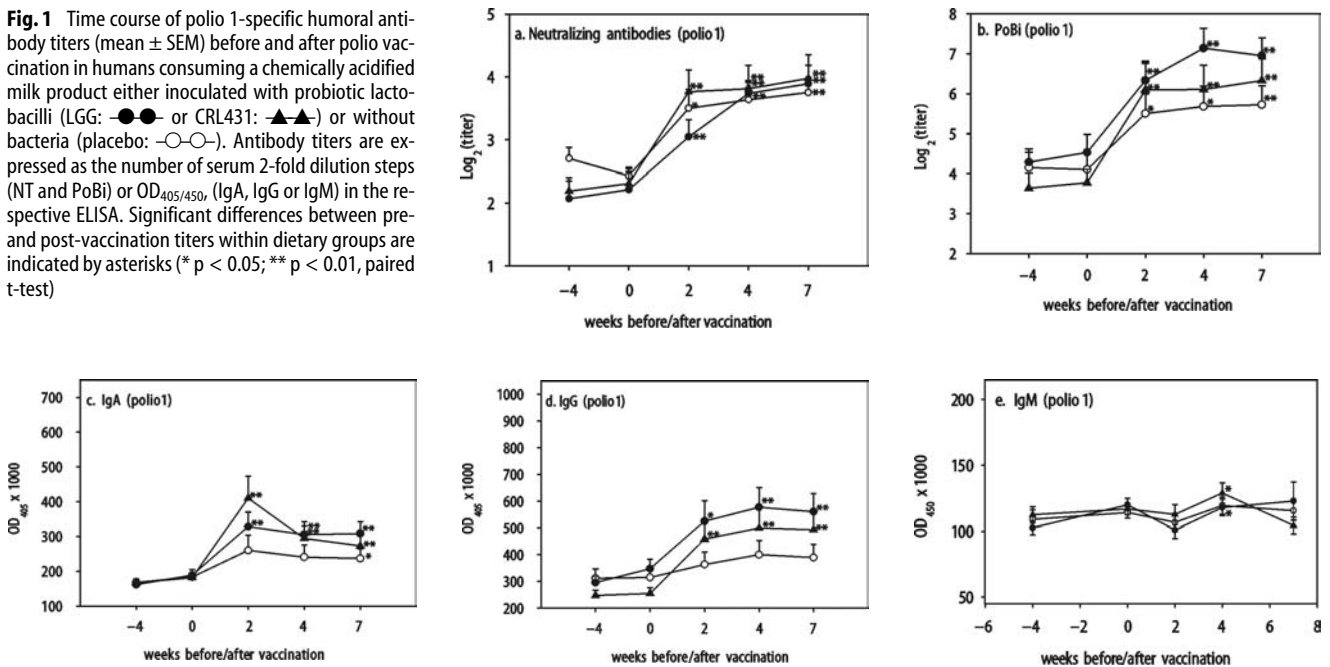

Table 2 Influence of LGG and CRL431-containing acidified milk on poliovirus serotype-specific neutralizing antibody response to polio vaccination. Subjects consumed during one week before and four weeks after polio vaccination a chemically acidified milk product either without bacteria (placebo) or inoculated with probiotic lactobacilli (LGG or CRL431). Medians and lower and upper quartiles of the difference (ΔTiter) between maximum antibody titers[1] after vaccination and prevaccination titers are given. Also shown are the ΔTiter ratios[2] and the between all pairs of groups (single-sided Mann-Whitney rank tests). significance (p) of the ΔTiter differences

Antibody	Serotype	ΔTiter[1]			ΔTiter ratio[2, 3]		p		
		I (placebo)	II (LGG)	III (CRL431)	II : I	III : I	II vs. I	III vs. I	III vs. II
NT	1	1.0 (0.5/2)	2.0 (1/3)	2.0 (0/3)	2.0	2.0	0.048	0.140	0.910
	2	0.75 (0/1.5)	1.0 (1/2)	1.0 (0/1.5)	1.3	1.3	0.014	0.401	0.051
	3	0.0 (0/1)	0.5 (0/1)	1.0 (0.5/2)	–	–	0.245	0.011	0.083
PoBI	1	0.5 (0/4)	3.0 (0/6)	3.0 (1/5)	6	6	0.100	0.050	0.741
	2	1.0 (1/3)	2.0 (0/3)	2.0 (1/3)	2	2	0.377	0.198	0.539
	3	0.0 (0/3)	1.0 (0/1)	1.0 (0/3)	–	–	0.197	0.204	0.431

[1] Antibody titers are expressed as the number of serum 2-fold dilution steps; [2] ΔTiter (verum group) divided by ΔTiter (placebo group); [3] when the increase in neutralizing antibodies was zero in the placebo group, a "multifold" could not be defined

■ Influence of LGG and CRL431 on virus-specific IgA, IgG and IgM in serum

Consumption of probiotics exerted a minor influence on poliovirus serotype-1-specific IgG and had no appreciable effect on serotype-2 and -3-specific IgG (Table 3).

Probiotic consumption led to a markedly enhanced IgA titer increase after vaccination ($p < 0.036$ in case of LGG). Here the influence of the probiotics was most obvious for poliovirus serotype-1-specific IgA, while the vaccination response itself (i. e. maximum titers or titer differences in the placebo group) was most pronounced for serotype-2-specific IgA (Table 3).

Though IgM titers and titer changes were only small, LGG and particularly CRL431 enhanced vaccination-induced poliovirus serotype-2- and -3-specific IgM titer increase. The effect of CRL431 on serotype-2-specific IgM was statistically significant (Table 3).

With the exception of the stimulation by CRL431 of poliovirus serotype-2-specific IgM, statistical comparison of both probiotics revealed no significant difference in effectiveness.

Discussion

We investigated the effect of two probiotic lactobacilli strains (LGG and CRL431) on the immune response of healthy subjects to an oral polio vaccine. The poliovirus

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Table 3 Influence of LGG and CRL431-containing acidified milk on poliovirus serotype-specific antibody response to polio vaccination. Subjects consumed for 1 week before and 4 weeks after polio vaccination a chemically acidified milk product either without bacteria (placebo) or inoculated with probiotic lactobacilli (LGG or CRL431) . Medians and lower and upper quartiles of the difference (ΔTiter) between the maximum increase in antibody titers[1] after vaccination and prevaccination titers are given. Also shown are the ΔTiter ratios[2] and the significance (p) of the ΔTiter differences between all pairs of groups (single-sided Mann-Whitney rank tests)

Antibody	Serotype	ΔTiter[1]			ΔTiter ratio[2, 3]		p		
		I (placebo)	II (LGG)	III (CRL431)	II : I	III : I	II vs. I	III vs. I	III vs. II
IgG	1	51.5 (−8/692)	116.0 (14/689)	86.0 (−8/612)	2.2	1.7	0.083	0.218	0.678
	2	252.0 (−69/938)	198.0 (−82/538)	115.0 (−33/420)	–	–	0.291	0.242	0.960
	3	60.0 (−25/166)	65.0 (−10/116)	0.0 (−31/121)	1.1	–	0.211	0.222	0.450
IgA	1	47.5 (−4/222)	183.0 (36/390)	122.0 (6/528)	3.9	2.6	0.036	0.124	0.860
	2	65.5 (−19/468)	159.5 (45/473)	116.0 (−36/542)	2.4	1.8	0.208	0.447	0.651
	3	29.0 (−26/45)	45.0 (16/145)	19.0 (2/314)	1.6	–	0.076	0.284	0.715
IgM	1	41.0 (3/64)	29 (10/47)	28.0 (7/58)	–	–	0.353	0.728	0.660
	2	6.5 (−3/28)	13 (−19/25)	29.0 (17/61)	2	3.9	0.558	0.040	0.047
	3	12.5 (3/31)	16 (6/21)	22.0 (8/40)	1.3	1.8	0.490	0.181	0.186

[1] Antibody titers are expressed as $OD_{405/450}$ in the respective ELISA; [2] ΔTiter(verum group) divided by ΔTiter (placebo group); [3] when the increase in neutralizing antibodies was zero in the placebo group, a "multifold" could not be defined

was chosen as a model of an orally ingested enterovirus whose actual site of pathogenicity is not limited to the intestine. This approach was taken to examine whether and to what extent probiotics may be protective against infectious diseases affecting other organ systems besides the gastrointestinal tract.

The main result of the study was that the vaccination-induced increases in neutralizing antibodies (measured as NT and by PoBI) were two- to six-fold in the two groups consuming probiotics compared with the placebo group. The enhancement of neutralizing antibody response as determined in this study indicates a systemic protection of cells against infections by viruses which do not only affect the gastrointestinal tract.

Although relevant and indicating in the same direction, these differences were not in all cases significant. This may be due to the following reasons:

- For ethical reasons the vaccine had been administered in fairly high doses which were recommended by the manufacturer, to ensure that every volunteer received adequate protection. This may have made it more difficult to find statistically significant differences between the groups than it would have been the case with low dosages.
- Most of the subjects studied had detectable neutralizing and anti-poliovirus specific antibody titers already before vaccination, and these initial titers varied considerably between the volunteers, who represented a normal population segment not preselected for low titers. Since wild-type poliovirus infections are rare in adults, these titers were most probably due to the polio vaccination usually performed during early childhood, although the most recent polio vaccination should have been more than 15 years ago. Thus, subjects had been given a booster polio vaccination – which induces a less pronounced in-

crease in antibody titres – rather than a very first vaccination.
- The vaccination response considerably varies between the subjects. "Responders" may show increases which are several orders higher that those of "non-responders". Therefore high initial titers and the observed extreme antibody titer increases in some subjects were regarded as physiological and not excluded as outlier values. This caused broad, right-skewed, non-normal distributions, which in most cases could not be normalized by logarithmic transformation.

Both probiotic strains enhanced not only circulating neutralizing antibody titres, but also affected poliovirus-specific IgA, IgG and IgM in the blood, as demonstrated by ELISA (Table 3).

Consumption of LGG or CRL431 nearly doubled the increase in polio-1-specific IgG titers after vaccination (p < 0.1; Table 3). However, the data do not indicate an effect of probiotics on the time course or the vaccination response of poliovirus serotype-2- and -3-specific IgG titers.

Post-vaccination enhancement of IgM titers was either low (significant in the case of CRL431 with respect to poliovirus serotype 2) or nonexistent, both in response to vaccination and to probiotics. It is reasonable to assume that this was because the vast majority of volunteers showed poliovirus serotype-specific antibody titers even before vaccination. In such cases of a booster reaction the absence of an IgM response and immediately stimulated IgG secretion is expected.

Comparison of the effectiveness of the 2 strains disclosed no consistent differences. The tendency of LGG to induce an IgA response greater than that induced by CRL431 was countered by the slightly greater poliovirus serotype-3-specific IgM response induced by CRL431.

In line with current immunological knowledge, oral vaccination with live attenuated virus induced not only a systemic humoral response, but also a mucosal response in the intestine, mainly represented by IgA, which contributes to mucosal immunity by preventing the virus from entering the blood circulation involved in the excretion and primary proliferation of polioviruses in the intestine. Whether and to which extent circulating IgA titers in serum correlate with mucosal production is an open question, and it is therefore not clear, whether the positive effects of both probiotic strains on serum anti-poliovirus IgA reflect enhanced mucosal immunity in the intestine.

The fate of the ingested probiotics or their effect on the intestinal microflora lie beyond the scope of the present study. The strains used in the study, however, were shown to survive gastrointestinal transit and exert probiotic health effects [19, 20].

The mechanisms by which lactobacilli modulate the immune response to a pathogen are not fully understood. Adjuvant effects may be mediated by components of the protoplast and the cell wall-like lipoteichoic acid and polysaccharide-peptidoglycan complexes. Muramyl peptides, which are components of peptidoglycans, are known to stimulate endogenous secretion of cytokines [21–28]. Lipoteichoic acid and polysaccharide-peptidoglycan complexes are ligands of toll-like receptors, particularly TLR_2, which are expressed by intestinal epithelial cells and immunocytes. Lipoteichoic acid from *L. casei* and *L. fermentum* were shown to stimulate TNFα secretion from macrophages and splenocytes expressing TLR2, but not from TLR2 -/- splenocytes [26]. *L. casei* shirota stimulated IL_{12}, IL_2 and IFNγ production in spleen [29], which favor TH1 cells and antigen-specific IgG production by B-cells in response to bacterial and viral antigens.

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