Enhancement of Immune Response in Peyer's Patch Cells Cultured with *Bifidobacterium breve*

HISAKO YASUI and M. OHWAKI
Yakult Central Institute for Microbiological Research
Yaho Kunitachi
Tokyo 186, Japan

ABSTRACT

Bifidobacterium breve, included in fermented milk, was tested for adjuvanticity and mitogenicity using cells of mouse Peyer's patch, one of the gut-associated lymphoid tissues.

Addition of *B. breve* enhanced antilipopolysaccharide antibody production in Peyer's patch cells and also anti-sheep red blood cell plaque-forming cells in Peyer's patch cells cultured with sheep red blood cells.

Furthermore, addition of *B. breve* accelerated proliferation of Peyer's patch cells, particularly B cells. In BALB/c mice, enhancement of proliferation by *B. breve* was also found in Peyer's patch cells from nude mice and a B cell-enriched fraction, including both the B cell fraction and plastic-adherent cells. Enhancement was not found in the fraction in which Sephadex G10-adherent and carbonyl-iron phagocytic cells were excluded from Peyer's patch cells or in a pure B cell fraction in which plastic-adherent cells were excluded from the B cell-enriched fraction of Peyer's patch cells. The proliferation of B cells was enhanced when the supernatant of plastic-adherent cells cultured with *B. breve* was added. It is concluded that *B. breve* activated plastic-adherent cells and that these cells secreted a soluble factor that enhanced proliferation of B cells.

(Key words: *Bifidobacterium breve*, Peyer's patch cell, immunomodulation)

Abbreviation key: CI = carbonyl-iron, CW = cell walls, FBS = fetal bovine serum, G10 = Sephadex G10, LPS = lipopolysaccharide, MEM = Minimum Essential Medium, PAC = plastic-adherent cells, PFC = plaque-forming cells, PG = peptidoglycan, PP = Peyer's patch, SI = stimulation index, SRBC = sheep red blood cells.

Received July 13, 1990.
Accepted October 15, 1990.

INTRODUCTION

Antigen processing and initial cellular events of the immune response in the gut occur in Peyer's patch (PP), intestinal lymphoid tissues (21). The PP contain the precursor cells of IgA plasma cells that produce secretory IgA in the lumen. Secretory IgA provides an immunological barrier to foreign matter, particularly pathogenic microorganisms and allergenic food proteins, by preventing adsorption of such material to the mucosal epithelium and penetration into the body (1, 26).

Since first isolated by Tissier (27) from a breast-fed infant, bifidobacteria appear to be the predominant component of the intestinal flora of infants (19) and to play a role in resistance to infection in humans and animals (13, 24, 31). Hence, wide implantation of bifidobacteria in the intestine was made using yogurt and cultures of bifidobacteria, but the mechanism of host resistance to infection has still not been studied in detail. Therefore, our objective was to determine whether bifidobacteria enhanced immunological function.

In the present study we tested *B. breve* for adjuvanticity and mitogenicity in vitro PP cell cultures, and we discuss the relationship between enhancement of the immune response in PP cells and host resistance to infection.

MATERIALS AND METHODS

Mice

Male BALB/c and nude (*nu/nu*) mice were obtained from Japan (SLC Inc.) and bred in our

laboratories. The mice ranged in age from 7 to 12 wk when used.

Bifidobacterium

Bifidobacterium breve YIT 4010, isolated from the feces of healthy infants in 1968 by R. Tanaka (Yakult Central Institute for Microbiological Research) and kept frozen at –70°C, was used. *Bifidobacterium breve* was inoculated into prereduced modified VL medium (33) supplemented with .5% lactose. These cultures were incubated at 37°C for 18 h, and the organisms were harvested by centrifugation, washed three times with .15 *M* NaCl, and treated with .3% formalin at 37°C overnight. The cell walls (CW) of *B. breve* were prepared as described by Araki et al. (2).

Antigen

Sheep red blood cells (SRBC) were obtained from Kohjin-Bio Co., Ltd., Japan in sterile Alsever's solution. Before use, they were washed three times in cold phosphate-buffered saline.

Reagents

Escherichia coli lipopolysaccharide (LPS) (055:B5, Difco Laboratories, Detroit, MI) and Concanavalin A (Type IV, Sigma Chemical Co., St. Louis, MO) were used as mitogens at doses of 50 and 2 μg/ml, respectively. Various concentrations of polymyxin B (Sigma) were used as the LPS inhibitor.

Peyer's Patch Cells

Peyer's patch cells were prepared according to the method of Frangakis et al. (10) as described in Yasui and Ohwaki (32). Single-cell suspensions of PP were cultured by the method of Mishell and Dutton (18) with modification (32). For measurement of anti-SRBC plaque-forming cells (PFC), 5×10^5 PP cells in .2 ml of Eagle's Minimum Essential Medium (MEM) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 5% fetal bovine serum (FBS) were cultured in 96-well Nunc trays (Nunc Intermed., Kamstrup, DK-4000 Roskilde, Denmark) with 1×10^7 *B. breve* cells or 10 μg of *B. breve* CW and 1×10^5 SRBC in a 37°C, 5% CO_2 incubator. For measurement of anti-LPS antibody, 5×10^5 PP cells in .2 ml of MEM supplemented with 5% FBS were cultured with 1×10^7 *B. breve* cells in a 37°C, 5% CO_2 incubator. They were fed .02 ml of a nutrient mixture per well daily. Four days after initiation of the culture, anti-SRBC PFC were assessed, and 7 d after initiation of the culture anti-LPS antibody in the supernatants was assessed by ELISA (29). The composition of the nutrient mixture and MEM was as described in Yasuki and Ohwaki (32). For measurement of proliferation, 5×10^5 PP cells in .2 ml of RPMI 1640 medium (Boehringer Mannheim GmbH, Germany) supplemented with 5% FBS were cultured in 96-well Nunc trays with 1×10^7 *B. breve* cells or 10 μg *B. breve* CW in a 37°C, 5% CO_2 incubator and were pulsed with [^{3}H]thymidine (.5 μCi, 2.0 Ci/mmol, New England Nuclear Corp., Boston, MA) during the final 18 h of a 90-h culture period, and [^{3}H]thymidine uptake was counted with a liquid scintillation counter (Packard Instrument Co., Inc., Rockville, MD).

Purification of B Cells

The PP cells were resuspended in RPMI 1640 medium supplemented with 5% FBS. A B cell-enriched fraction was obtained from PP cells by panning on plastic dishes (15 × 100 mm; Falcon Labware, Oxnard, CA) coated with goat anti-mouse Ig (IgA, IgG, and IgM) (Cappel Laboratories, Cochranville, PA) (17). The adherent B cell-enriched fraction was removed from the dishes by vigorous pipetting. A T cell-enriched fraction was prepared by exclusion of the adherent B cell-enriched fraction from PP cells. A B cell fraction was prepared by exclusion of the plastic-adherent cells (PAC) from the B cell-enriched fraction and contained >93% immunoglobulin$^+$ cells and <1% Thy-1.2$^+$ cells and <1% PAC.

Isolation and Exclusion of Macrophage-Like Cells

A PAC fraction was isolated by incubation (2 h at 37°C) in uncoated plastic dishes.

Exclusion of Sephadex G10 (G10)-adherent and carbonyl-iron (CI)-phagocytic cells was accomplished according to the methods of Ly and Mishell (16) and Sjoberg et al. (25), respective-

ly. Cells that passed through a G10 column were pooled. Then these cells were cultured with CI at 37°C for 1 h, and free CI and CI-phagocytic cells were removed by using a magnet.

Enzyme-Linked Immunosorbent Assay

The method of Elson et al. (8) was used with modification. *Escherichia coli* LPS (1 μg/well) in PBS (.05 *M*, pH 7.5) was coated onto the wells of a 96-well ELISA plate (Nunc). Then a culture supernatant sample was added to each well and incubated at 37°C for 1.5 h. All samples were assayed in quadruplicate. Horseradish peroxidase-labeled rabbit anti-mouse Ig (IgM, IgG, and IgA) (1:1000, Zymed Laboratories, Inc., San Francisco, CA) were added to the wells. The wells were extensively washed between incubations. Then *o*-phenylenediamine solution (.4 mg/ml of citrate buffer pH 5.0) containing .02% H_2O_2 was added to each well, and the plates were left at 37°C for 10 min. After the reaction was stopped by addition of 2.5 *M* H_2SO_4, the absorbance of the contents of the wells was measured at 492 nm by using a Titertek Multiscan (Flow Laboratories, Inc., McLean, VA). Antibody values are expressed as absorbance at 492 nm.

Antibody-Forming Cells

Anti-SRBC PFC were determined by the technique of Cunningham and Szenberg (4). The number of PFC was expressed as the mean ± SD of four wells.

Statistical Analysis

The statistical significance of the difference between the experimental and the control groups was determined by Student's *t* test.

RESULTS

Enhancement of Antibody Production

Peyer's patch cells are always stimulated by LPS, a Gram-negative bacterial antigen, in the lumen. When PP cells were cultured with *B. breve* in the absence of LPS, the amount of anti-LPS antibody in the culture supernatant was greater than in the supernatant of a PP cell culture without *B. breve* (control) (Figure 1). When PP cells were cultured with SRBC, which are absent from the lumen, anti-SRBC PFC appeared 4 d after the initiation of culture. The addition of *B. breve* or *B. breve* CW to these cultures increased the number of anti-SRBC PFC (Figure 2).

Enhancement of Proliferation

In the next experiment, the mitogenicity of *B. breve* or *B. breve* CW for PP cells was tested (Figure 3). When PP cells were cultured with *B. breve* or *B. breve* CW, the uptake of [^{3}H]thymidine by PP cells was greater than in cell cultures without *B. breve* or *B. breve* CW. To determine whether the enhancement of proliferation was due to LPS in the *B. breve* suspension, polymyxin B (1 or 5 μg/ml), an LPS inhibitor, was added (Figure 4). With addi-

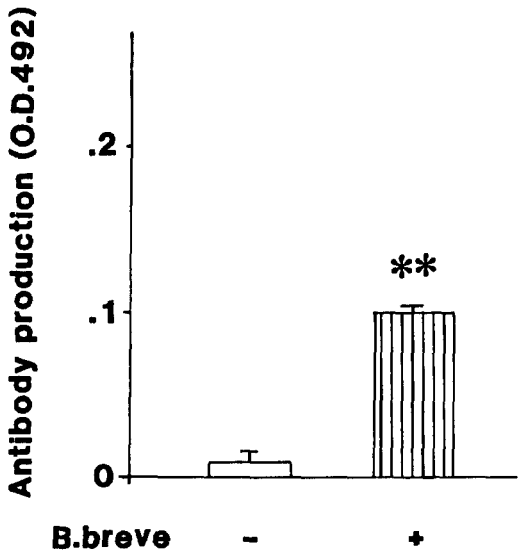

Figure 1. Effect of *Bifidobacterium breve* on anti-lipopolysaccharide (LPS) antibody production by Peyer's patch (PP) cells. The PP cells were cultured without (open bar) or with (striped bar) *B. breve* 4010 in the absence of LPS as antigen for 7 d at 37°C, and anti-LPS antibody levels in the culture supernatant were measured by enzyme-linked immunosorbent assay and are expressed as absorbance values at 492 nm (mean and SD) of four wells. Significant difference from control: $^{**}P < .01$.

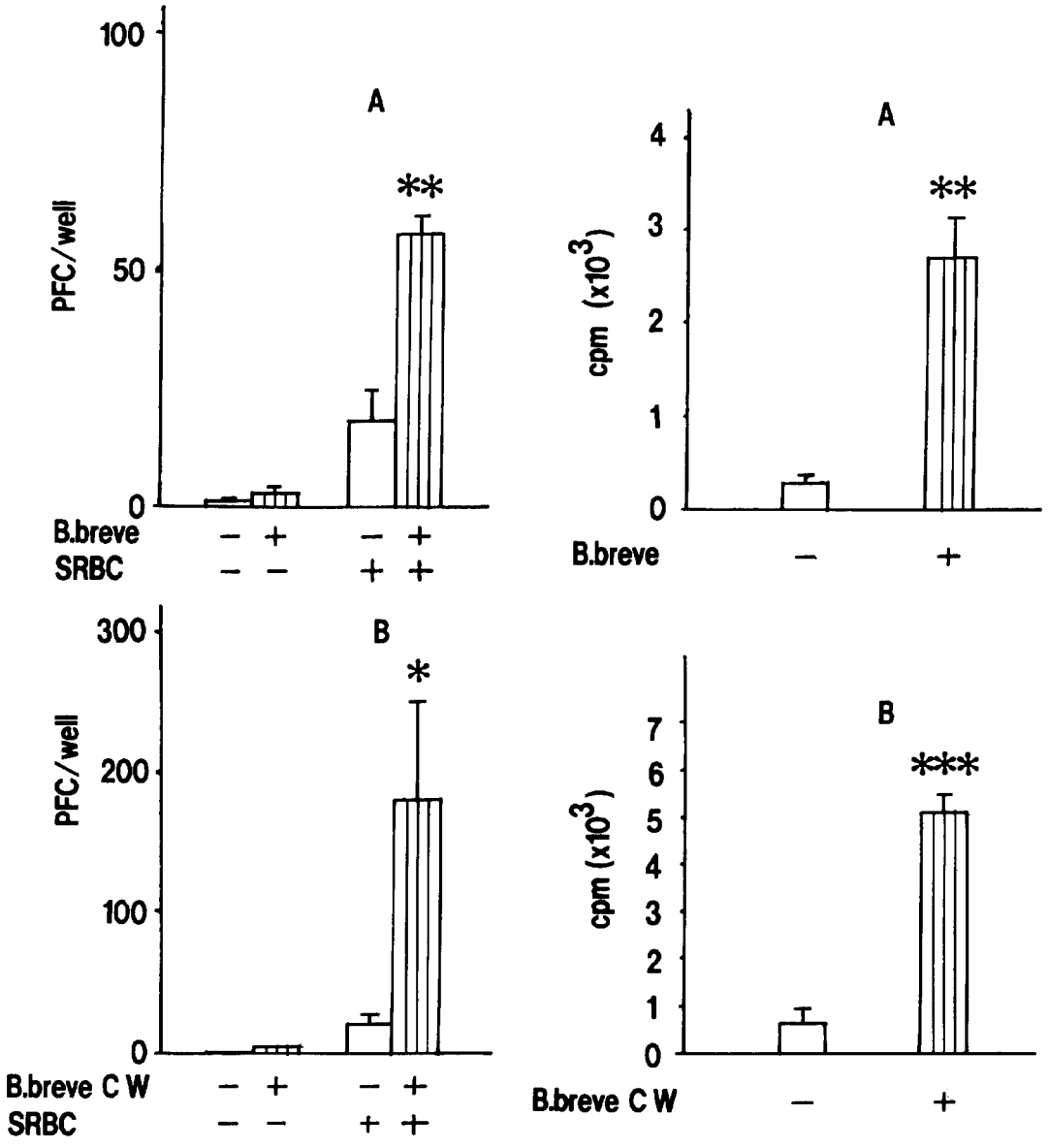

Figure 2. Effect of *Bifidobacterium breve* and *B. breve* cell walls on anti-sheep red blood cells plaque-forming cells (PFC) response of Peyer's patch (PP) cells. The PP cells to which SRBC had or had not been added were cultured without (open bar) or with (striped bar) *B. breve* (A) and *B. breve* CW (B), and the number of PFC was determined after 4 d of culture at 37°C. The number of PFC is expressed as the mean and SD of four wells. Significant difference from control: $*P < .05$; $**P < .01$.

Figure 3. Effect of *Bifidobacterium breve* and *B. breve* cell walls (CW) on the proliferation of Peyer's patch (PP) cells. The PP cells were cultured without (open bar) or with (striped bar) *B. breve* (A), and *B. breve* CW (B) were pulsed with [^{3}H]thymidine during the final 18 h of a 90-h culture period at 37°C, and [^{3}H]thymidine uptake was counted with a liquid scintillation counter. Results are expressed as the mean and SD of four wells. Significant difference from control: $**P < .01$; $***P < .001$.

tion of polymyxin B, the enhancement of proliferation by LPS (1, 5, or 50 μg/ml) was inhibited, but enhancement by *B. breve* (5×10^7/ml) was not inhibited. Therefore, the mitogenicity of *B. breve* was not due to LPS present in the *B. breve* suspension.

Mechanism of Enhancement of Proliferation

In the next experiment, we tried to determine the target cell for *B. breve* in the enhanced proliferation (Table 1). Uptake of [^{3}H]thymidine by unfractionated cells [stimulation index (SI) 5.9] and by the B cell-enriched fraction (SI 11.8) increased upon addition of *B. breve*, but proliferation of the T cell-enriched fraction (SI 1.5) did not increase. Furthermore, enhancement of proliferation of PP cells by *B. breve* was tested in nude, T cell-deficient mice (Table 2). Proliferation of PP cells in the mice was enhanced by addition of *B. breve*, the same as by addition of LPS, but was not enhanced by addition of Concanavalin A. Therefore, *B. breve* enhanced proliferation of the B cell-enriched fraction in BALB/c mice and PP cells from nude mice but did not enhance proliferation of the T cell-enriched fraction in BALB/c mice.

In the next experiment, we tested whether PAC participated in the enhancement of proliferation of the B cell-enriched fraction by *B. breve*. As shown in Table 3, [^{3}H]thymidine uptake by unfractionated cells (SI 6.8) and the B cell-enriched fraction (SI 6.9) was increased by addition of *B. breve*, but uptake by the pure B cell fraction (SI 1.3) and the T cell-enriched fraction (SI 1.5) was not increased. Furthermore, we tested whether G10-adherent cells and CI-phagocytic cells participated in enhancement of proliferation by *B. breve* (Table 4). The proliferation of cells that were prepared by exclusion of G10-adherent cells and CI-phagocytic cells was not enhanced by addition

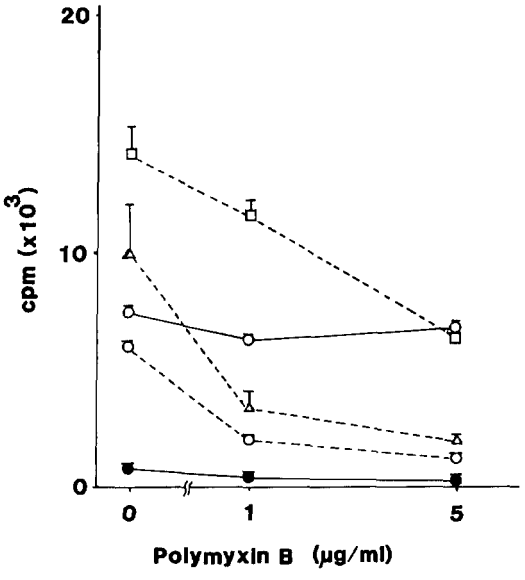

Figure 4. Effect of polymyxin B on the enhancement of proliferation of Peyer's patch (PP) cells by *Bifidobacterium breve*. The PP cells were cultured with *B. breve* (5×10^7/ml) (○—○) or lipopolysaccharide [1(○- - -○), 5(Δ- - -Δ), 50(□- - -□); μg/ml] and without mitogen (●—●) in various doses of polymyxin B.

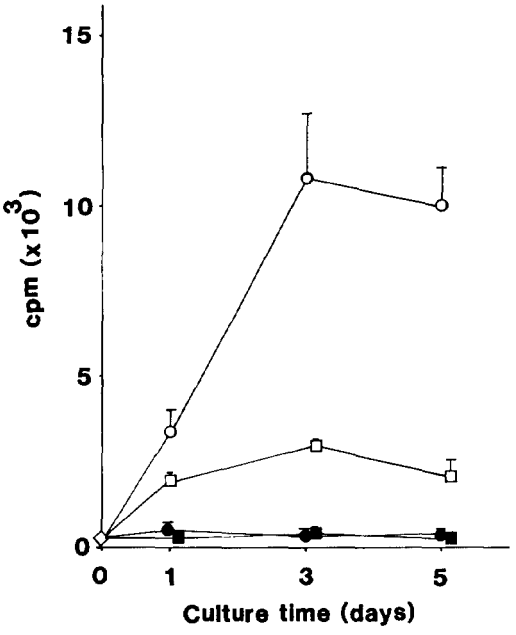

Figure 5. Effect on proliferation of Peyer's patch (PP) B cells of the culture supernatant of plastic-adherent cells cultured with *Bifidobacterium breve* for various periods of time. Plastic-adherent cells and nonadherent cells were isolated by incubation (2 h at 37°C) on uncoated plastic dishes. Plastic adherent cells with (○—○) or without (●—●) *B. breve* and plastic nonadherent cells with (□—□) or without (■—■) *B. breve* were cultured for various periods of time at 37°C. The culture supernatants were transferred to PP B cell cultures. The PP B cell fraction was isolated by the same method as in the footnote to Table 3.

TABLE 1. The types of cells whose proliferation was enhanced by *Bifidobacterium breve*.[1]

Mitogen	Unfractioned cells			B Cell-enriched fraction			T Cell-enriched fraction		
	$\bar{X}$	SD	SI	$\bar{X}$	SD	SI	$\bar{X}$	SD	SI
	(cpm)			(cpm)			(cpm)		
. . .	401	44	1.0	69	13	1.0	1299	195	1.0
		**			***				
B. breve	2376	261	5.9	815	69	11.8	1914	205	1.5
		***			***			*	
LPS	9228	381	23.0	3643	139	52.8	2272	130	1.7
		***			***			***	
Con A	83,329	6032	207.8	937	48	13.6	156,423	5868	120.4

[1]A B cell-enriched fraction and a T cell-enriched fraction were isolated, respectively, for adherent and nonadherent cells from Peyer's patch cells by panning on plastic dishes coated with goat anti-mouse Ig (IgA, IgG, and IgM). These cells were cultured with *B. breve* (5×10^7/ml), lipopolysaccharide (LPS) (50 μg/ml), or Concanavalin A (Con A) (2 μg/ml) and were pulsed with [^{3}H]thymidine as in the legend for Figure 3. Stimulation indices (SI) were calculated by dividing the counts incorporated in the presence of mitogen by those of control cultures without mitogen in each fraction. Significant differences from control: $^*P < .02$, $^{**}P < .01$, $^{***}P < .001$.

of *B. breve*. These observations suggested that macrophage-like cells participated in the enhancement of proliferation of PP cells by *B. breve*. To determine the mechanism of enhancement of proliferation in the B cell-enriched fraction by *B. breve*, an attempt was made to determine the relationship between B cells and macrophage-like cells. The culture supernatants of PAC or nonadherent cells cultured with or without *B. breve* for various periods of time were added to B cell cultures. As shown in Figure 5, the supernatant of PAC cultured for 3 d with *B. breve* enhanced the proliferation of B cells. Therefore, *B. breve* activated PAC and the PAC secreted soluble factor that enhanced the proliferation of B cells.

DISCUSSION

In the present study, we tested *B. breve*, which is present in fermented milk, for adjuvanticity and mitogenicity by an in vitro system using cells in mouse PP, one of the gut-associated lymphoid tissues.

Perdigon et al. (23) showed that oral administration of lactic acid bacteria (*Lactobacillus acidophilus* and *Streptococcus thermophilus*) augmented the production of antibody against SRBC through activation of the macrophages. In our study, *B. breve* augmented the production of antibody against LPS and SRBC. The production of antibody against LPS, which was present in the lumen and seemed to prime the PP cells under normal conditions, was augmented by addition of only *B. breve* (Figure 1). However, the production of antibody against SRBC, which was absent from the lumen and did not seem to prime the PP cells under normal conditions, was not augmented by addition of only *B. breve*, but it was augmented by addition of both SRBC and *B. breve* (Figure 2A). Therefore, we concluded that *B. breve* augmented the antibody production by cells primed by an antigen and did not augment the antibody production by virgin cells. Production of antibody against *B. breve* was not detected in the same conditions in an in vitro system (data not shown), and immunogenicity of *B. breve*

TABLE 2. Effect of *Bifidobacterium breve*, lipopolysaccharide (LPS), and Concanavalin A (Con A) on the proliferation of Peyer's patch (PP) cells in nude mice.[1]

Mitogen	$\bar{X}$	SD	SI
	(cpm)		
. . .	4529	585	1.0
		**	
B. breve	9119	432	2.0
		***	
LPS	19,588	1096	4.3
Con A	4815	925	1.0

[1]The PP cells from nude mice were cultured with *B. breve* (5×10^7/ml), LPS (50 μg/ml), or Con A (2 μg/ml) and were pulsed with [^{3}H]thymidine as in the legend for Figure 3. Significant difference from control: $^{**}P < .01$, $^{***}P < .001$.

TABLE 3. Participation of plastic-adherent cells in the enhancement of proliferation of Peyer's patch cells by *Bifidobacterium breve.*[1]

	Unfractioned cells			B Cell-enriched fraction			B Cell fraction			T Cell-enriched fraction		
Mitogen	$\bar{X}$	SD	SI[2]	$\bar{X}$	SD	SI	$\bar{X}$	SD	SI	$\bar{X}$	SD	SI
	(cpm)			(cpm)			(cpm)			(cpm)		
. . .	181	17	1.0	58	15	1.0	44	5	1.0	139	8	1.0
		**			**							
B. breve	1239	145	6.8	398	77	6.9	57	8	1.3	214	55	1.5

[1]A B cell fraction was isolated from the B cell-enriched fraction by exclusion of adherent cells after incubation (2 h at 37°C) on uncoated plastic dishes. Significant difference from control: $^{**}P < .01$.

[2]SI = Stimulation indices.

after oral administration had been found to be very weak in the mouse model in an in vivo system (32). Therefore, *B. breve* was a weakly immunogenic antigen and caused unlikely harmful effects as induction of type III hypersensitivity reaction.

Lipopolysaccharide of Gram-negative organisms can induce strong blastic transformation of murine spleen lymphocytes (14, 22). Regarding the mitogenic activity of components of Gram-positive organisms, peptidoglycan (PG) from *Micrococcus lysodeikticus* or *Staphylococcus aureus* did not induce blastogenic transformation of mouse and rabbit lymphocytes (5) or human peripheral blood lymphocytes (9), and, in contrast, PG from *Staph. aureus* is mitogenic for mouse splenocytes and human peripheral blood lymphocytes (7). We found that *B. breve* is mitogenic for mouse PP cells (Figure 3A) and demonstrated that, because the mitogenicity of *B. breve* was not inhibited by addition of polymyxin B, it was not due to LPS present in the *B. breve* suspension (Figure 4). Furthermore, we found that the fraction of *B. breve* that induced the adjuvanticity and mitogenicity was the CW fraction (Figures 2B and 3B).

The cells whose proliferation was enhanced by *B. breve* were present in the B cell-enriched fraction (Tables 1 and 2) but not in the T cell-enriched fraction (Table 1). Because the enhancement by *B. breve* was abolished from the B cell-enriched fraction by depletion of macrophage-like cells by means of plastic adherence (Table 3) or G10 adherence and CI phagocytosis (Table 4), macrophage-like cells were essential for the mitogenic activity of *B. breve.* B cells in the supernatant of PAC cultured with *B. breve* were more mitotic than those in the supernatant of nonadherent cells cultured with *B. breve* (Figure 5). It was concluded that the target cells for *B. breve* are macrophage-like cells that release the active factor in mitosis of B cells. The factor released by macrophage-like

TABLE 4. Participation of macrophage-like cells in the enhancement of proliferation of Peyer's patch (PP) cells by *Bifidobacterium breve.*[1]

PP cells	Mitogen	Mean	SD	SI[2]
		(cpm)		
Unfractionated	. . .	1699	181	1.0
			**	
	B. breve	6379	496	3.8
Sephadex G10-passed and CI-treated	. . .	35	5	1.0
	B. breve	49	3	1.4

[1]Exclusion of Sephadex G10-adherent and carbonyl-iron (CI)-phagocytic cells was accomplished by passage through a Sephadex G10 column and treatment with CI, respectively. Significant difference from control: $^{**}P < .01$.

[2]SI = Stimulation indices.

cells has a direct effect on mitosis of B cells (6). Various cytokines, including interleukin-1 and interleukin-6, which are produced by macrophages and support the growth or differentiation of B cells, have recently been reported (3, 11, 12, 15, 20, 28, 30). What factors are released from macrophage-like cells by addition of *B. breve* is not clear at present. Studies on the characterization of the factor are now in progress.

In this study, we showed that *B. breve* enhanced antibody production and proliferation of PP cells, that the initial target cells in the proliferation-enhancing activity of *B. breve* are macrophage-like cells, and that the cells released a soluble factor that was mitogenic to B cells. Therefore, we presume that *B. breve* implanted in the intestine stimulates the proliferation of B cells in PP and augments production of antibody against pathogens or food allergens and prevents the penetration of the pathogen or allergen into the body. We are now studying whether implantation of *B. breve* augments the production of antibody against pathogenic *E. coli* or ovalbumin, an allergen, and prevents infection with *E. coli* or absorption of ovalbumin.

ACKNOWLEDGMENTS

We wish to thank the animal care staff of our institute for their cooperation and Masato Nagaoka for his helpful distribution of *B. breve* CW. We appreciate the assistance provided by Noriko Kato.

REFERENCES

1 Andre, C., R. Lambert, H. Bazin, and J. F. Heremans. 1974. Interference of oral immunization with the intestinal absorption of heterologous albumin. Eur. J. Immunol. 4:701.

2 Araki, Y., T. Nakatani, K. Nakayama, and E. Ito. 1972. Occurrence of N-nonsubstituted glucosamine residues in peptidoglycan of lysozyme-resistant cell walls from *Bacillus cereus*. J. Biol. Chem. 247:6312.

3 Bazin, R., and R. Lemieux. 1987. Role of the macrophage-derived hybridoma growth factor in the in vitro and in vivo proliferation of newly formed B cell hybridomas. J. Immunol. 139:780.

4 Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody forming cells. Immunology 14:599.

5 Damais, C., C. Bona, L. Chedid, J. Fleck, C. Nauciel, and J. P. Martin. 1975. Mitogenic effect of bacterial peptidoglycans possessing adjuvant activity. J. Immunol. 115:268.

6 Damais, C., M. Parant, and L. Chedid. 1977. Nonspecific activation of murine spleen cells in vitro by synthetic immunoadjuvant (N-acetil-muramyl-L-alanyl-D-isoglutamine). Cell. Immunol. 34:49.

7 Dziarski, R., and A. Dziarski. 1979. Mitogenic activity of staphylococcal peptidoglycan. Infect. Immun. 23: 706.

8 Elson, C. O., W. Ealding, and J. Lefkowitz. 1984. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretion. J. Immunol. Methods 67:101.

9 Forsgren, A., A. Svedjelund, and H. Wigzell. 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus*. Eur. J. Immunol. 6:207.

10 Frangakis, M. V., W. J. Koopman, H. Kiyono, S. M. Michalek, and J. R. McGhee. 1982. An enzymatic method for preparation of dissociated murine Peyer's patch cells enriched for macrophages. J. Immunol. Methods 48:33.

11 Freedman, A. S., G. Freeman, J. Whitman, J. Segil, J. Daley, and L. M. Nadler. 1988. Pre-exposure of human B cells to recombinant IL-1 enhances subsequent proliferation. J. Immunol. 141:3398.

12 Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature (Lond.) 324:73.

13 Homma, N. 1974. Intestinal flora of infants and resistance to infection. Jpn. J. Pediatr. 27:1266.

14 Janossy, G., and M. F. Greaves. 1971. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clin. Exp. Immunol. 9:483.

15 Jelinek, D. F., and P. E. Lipsky. 1987. Enhancement of human B cell proliferation and differentiation by tumor necrosis factor and interleukin 1. J. Immunol. 139: 2970.

16 Ly, I. A., and R. Mishell. 1974. Separation of mouse spleen cells by passage through column of sephadex G-10. J. Immunol. Methods 5:239.

17 Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. J. Immunol. Methods 15:47.

18 Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:423.

19 Mitsuoka, T., and C. Kaneuchi. 1977. Ecology of the bifidobacteria. Am. J. Clin. Nutr. 30:1799.

20 Nordan, R. P., J. G. Pumphrey, and S. Rudikoff. 1987. Purification and NH_2-terminal sequence of a plasmacytoma growth factor derived from the murine macrophage cell line P388D1. J. Immunol. 139:813.

21 Owen, R. L. 1977. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. Gastroenterology 72:440.

22 Peavy, D. L., W. H. Adler, and R. T. Smith. 1970. The mitogenic effects of endotoxin and staphylococcal enterotoxin B on mouse spleen cells and human peripheral lymphocytes. J. Immunol. 105:1453.

23 Perdigon, G., M. E. Nader de Macias, S. Alvarez, G. Oliver, and A. A. Pesce de Ruiz Holgado. 1987. Enhancement of immune response in mice fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. J. Dairy Sci. 70:919.

24 Poupard, J. A., I. Husain, and R. F. Norris. 1973. Biology of bifidobacteria. Bacteriol. Rev. 37:136.

25 Sjoberg, O., J. Andersson, and G. Moller. 1972. Requirement for adherent cells in the primary and secondary immune response in vitro. Eur. J. Immunol. 20: 123.

26 Stokes, C. R., J. F. Soothill, and M. W. Turner. 1975. Immune exclusion is a function of IgA. Nature (Lond.) 255:745.

27 Tissier, M. H. 1899. La réaction chromophile d'Escherich et le *Bacterium coli*. C. R. Seances Soc. Biol. Fil. 51:943.

28 Tosato, G., K. B. Seamon, N. C. Goldman, K. D. Jones, and S. B. Pike. 1988. Monocyte-derived human B-cell growth factor identified as interferon-B_2 (BSF-2, IL-6). Science 239:502.

29 Viljanen, M. K., T. Nurmi, and A. Salminen. 1983. Enzyme-linked immunosorbent assay (ELISA) with bacterial sonicate antigen for IgM, IgA and IgG antibodies to *Francisella tularensis*: comparison with bacterial agglutination test and ELISA with lipopolysaccharide antigen. J. Infect. Dis. 148:715.

30 Vink, A., P. G. Coulie, P. Wanters, R. P. Nordan, and J. Van Snick. 1988. B cell growth and differentiation activity of interleukin-HP1 and related murine plasmacytoma growth factors. Synergy with interleukin 1. Eur. J. Immunol. 18:607.

31 Yamazaki, S., H. Kamimura, H. Momose, T. Kawashima, and K. Ueda. 1982. Protective effect of *Bifidobacterium*-monoassociation against lethal activity of *Escherichia coli*. Bifidobacteria Microflora 1:55.

32 Yasui, H., A. Mike, and M. Ohwaki. 1989. Immunogenicity of *Bifidobacterium breve* and change in antibody production in Peyer's patches after oral administration. J. Dairy Sci. 72:30.

33 Yasui, H., N. Yasutake, and M. Ohwaki. 1979. Immunogenicity of *Bacteroides* isolated from mice: relationship between immunogenicity and cell wall antigens. Infect. Immun. 24:39.