

Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway

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Background: Commensal gut bacteria are essential for the development and maintenance of the gut's immune system. Some bacteria strains, such as *Lactobacillus* and

Bifidobacterium species, have been reported to provide protection from allergic and inflammatory bowel diseases. However, the interactions between these commensal bacteria and the immune system are largely unknown.

Objective: We studied the effects of a supernatant from the culture of *B. breve* C50 (BbC50) on the maturation, activation, and survival of human dendritic cells (DCs).

Methods: DCs were differentiated from human monocytes with IL-4 and GM-CSF for 5 days and cultured with BbC50 supernatant (BbC50_{SN}) or LPS for 2 days.

Results: BbC50_{SN} induced DC maturation, with increase in CD83, CD86, and HLA-DR expression. We also showed, for the first time, that BbC50_{SN} prolonged DC survival, with high IL-10 and low IL-12 production compared with that seen in LPS-DCs. Moreover, BbC50_{SN} inhibited the effects of LPS on DCs, both in terms of IL-12 production and in terms of survival. The prolonged DC survival was independent of IL-10 production and nuclear factor κ B pathway but was associated with an upregulation of Bcl-x_L and Phospho-Bad. Finally, BbC50_{SN} induced activation of Toll-like receptor 2 (TLR2)-transfected cells in contrast to TLR4-, TLR7-, and TLR9-transfected cells.

Conclusion: The supernatant of *B. breve* C50 can induce DC maturation and prolonged DC survival through TLR2, with high IL-10 production. These properties might correspond to a regulatory DC profile, which could limit the excessive T_H1 response and control the excessive T_H2 polarization observed in atopic newborns. (J Allergy Clin Immunol 2006;117:696-702.)

Key words: *Bifidobacterium breve*, dendritic cells, maturation, cell survival, tolerance, Bcl-2 protein family, Toll-like receptor 2, IL-10, IL-12, allergy

Abbreviations used

7-AAD: 7-Amino actinomycin D

BbC50: *Bifidobacterium breve* C50

BbC50_{SN}: Supernatant of *Bifidobacterium breve* C50

BbC50_{SN}-DC: DC treated with BbC50_{SN}

DC: Dendritic cell

NF: Nuclear factor

PE: Phycoerythrin

TLR: Toll-like receptor

as “living microorganisms exerting health benefit,”² define the concept of probiotics. These bacteria have been reported to prevent and treat rotavirus infections and postantibiotic diarrhea,^{3,4} allergic diseases,⁵⁻⁷ and recurrence of inflammatory bowel disease.^{8,9} These studies suggest that the intestinal immune system could be a privileged target of probiotics. Nevertheless, despite several studies reporting modifications of immunologic parameters induced by probiotic bacteria, the interactions between the cells of the intestinal immune system and bacteria remain largely unknown.¹⁰ However, it has been shown that killed probiotic bacteria can affect the maturation and cytokine secretion profile of dendritic cells (DCs),^{11,12} which represent potent antigen-presenting cells. DCs also have properties to induce negative regulation, with generation of regulatory T cells.¹³ DCs can be activated by bacterial components through the interaction between pathogen-derived immunostimulatory molecules from bacteria and membrane receptors called pattern-recognition receptors, including the Toll-like receptor (TLR) family.¹⁴ The different TLRs recognize a broad spectrum of highly conserved microbial structures, such as proteins, lipids, glycoproteins, and nucleic acid motifs, contained in the wall, cytoplasm, and nucleus of bacteria. TLR engagement could therefore have different types of effects on DC activation according to the bacteria strain.¹⁵ Because of these properties, DCs represent a potential target of probiotic bacteria. Menard et al¹⁶ recently reported that active bacterial products from *Bifidobacterium breve* C50 (BbC50) could cross an intestinal monolayer of epithelial cells. We therefore studied the effects of a supernatant of BbC50 (BbC50_{SN}) on human monocyte-derived DCs *in vitro* to define the interactions with the immune system. In our model BbC50_{SN} was able to mature and fully activate DCs, inducing a particular cytokine synthesis profile and prolonged DC survival through a TLR2 pathway.

Live commensal microflora is essential for the development of the gut's immune system.¹ Some bacteria, such as *Lactobacillus* and *Bifidobacterium* species, described

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METHODS

Medium, cytokines, mAbs, and reagents of cell culture

The culture medium used was RPMI 1640 (Gibco, Cergy Pontoise, France) supplemented with 50 IU/mL penicillin (ICN, Orsay, France), 50 IU/mL streptomycin (ICN), 2 mM L-glutamine (Bio Whittaker, Vervier, Belgium), and 10% heat-inactivated FCS (Gibco). Recombinant human IL-4 was obtained from R&D Systems (Abingdon, United Kingdom). GM-CSF was obtained from Abcys (Reuil Malmaison, France). LPS (50 ng/mL), lactacystin, and polymyxin B (20 µg/mL) were obtained from Sigma-Aldrich (St Quentin Fallavier, France), and zymosan (0.5 µg/mL) was obtained from Invivogen (Toulouse, France). Anti-human IL-10 antibodies were obtained from R&D Systems, and anti-human TLR2 (20 µg/mL) was obtained from eBiosciences (Montrouge, France). The following mouse anti-human mAbs were used for cytometric analysis: phycoerythrin (PE)-anti-CD83 (IgG2b, HB15A), PE-anti-CD86 (IgG2b, HA5), and PE-anti-HLA-DR (IgG2b, B8.12.2) purchased from Immunotech (Marseille, France). Control cells were stained with corresponding isotype-matched control mAbs (Immunotech and Becton Dickinson, Rungis, France). Fluorescein isothiocyanate-labeled Annexin V (5 µL/1 × 10⁵ cells) and 7-amino actinomycin D (7-AAD; 10 µg/mL) were used for apoptosis analysis (Becton Dickinson and Sigma).

Production of *B breve* supernatant

Bifidobacteria were isolated from infant stools as *B breve*, and the strain was called C50. BbC50 was cultured in the presence of hydrolyzed cow's whey. Fermentation was carried out at 37°C under anaerobic conditions for 15 hours. The supernatant of the culture medium was collected by means of high-speed centrifugation after fermentation and concentrated by means of ultrafiltration (300 kd) and then dialysis on a 10-kd membrane. After concentration, the supernatant was lyophilized for use and called BbC50_{SN}. All of the results reported in the article were obtained with the same batch. First, BbC50_{SN} activity was evaluated on the basis of its ability to promote an increase in bifidobacteria and a reduction of *Clostridium* and *Bacteroides* species pullulation in mouse gut (evaluation by counting bacteria in mouse stools with the standard plate count method and statistical analysis after oral feeding with BbC50_{SN} vs control feeding).¹⁷

Differentiation and maturation of DCs

Blood of healthy volunteers was obtained from cytapheeresis. Human PBMCs were then isolated over Ficoll Hypaque (Abcys, France), and 10⁸ were plated in a 75-cm² flask (Falcon, Becton Dickinson) in complete culture medium. After 45 minutes at 37°C, nonadherent cells were discarded, and adherent cells were cultured in the presence of 25 ng/mL recombinant human IL-4 and 1000 IU/mL GM-CSF.¹⁸ After 5 days, 5 to 9 × 10⁶ cells were harvested, washed, and resuspended in culture medium with IL-4 and GM-CSF. DC purity was 97.3% (± 1.4 SD), as determined on the basis of CD1a-positive cells by means of flow cytometry. Polymyxin B, anti-IL-10, or anti-TLR2 mAbs were added 1 hour before BbC50_{SN}, LPS, or zymosan. After stimulation, DCs were harvested, washed, and used for cytometric analysis or functional assays.

Analysis of cell-surface molecules and measurement of apoptosis by means of flow cytometry

Monocyte-derived DCs were harvested, and 1 to 2 × 10⁵ cells per sample were resuspended in PBS. Cells were then incubated with

saturating concentrations of the different fluorochrome-conjugated mAbs for 30 minutes at 4°C. The stained cells were washed twice in PBS and fixed in 0.5% paraformaldehyde PBS solution until analysis by means of flow cytometry. Cell-surface expression was then analyzed with a 488-nm laser flow cytometer (FACSCalibur; Becton Dickinson, Mountain View, Calif). Analyses were performed with CELLquest software (Becton Dickinson). Data were analyzed for geometric mean fluorescence intensity (mean fluorescence intensity of antibody of interest/mean fluorescence intensity of isotype control) and for the percentage of marker-positive cells (at least 10,000 cells per sample were analyzed). For measurement of apoptosis, the proportions of positive and negative 7-AAD and fluorescein isothiocyanate-labeled Annexin V were determined by means of flow cytometry (FACSCalibur, Becton Dickinson). DCs (2 × 10⁵ for each sample) were incubated with Annexin V and 7-AAD.

CD4⁺ T-lymphocyte isolation

Human PBMCs from heparinized blood of healthy human volunteers were isolated with Ficoll Hypaque. PBMCs were first depleted of adherent cells by two 45-minute adhesion cycles on plastic. CD4⁺ T lymphocytes were isolated by using anti-CD4-coated Dynabeads, followed by Detachabeads (Dynal, Compiègne, France).

Mixed allogeneic culture

Two days after stimulation with BbC50_{SN} or LPS, DCs were harvested, washed, numerated, and then distributed in 96-well flat-bottom culture plates (Falcon, Becton Dickinson) at 3 × 10⁴ cells in 100 µL per well before CD4⁺ T cells (10⁵ cells/50 µL) were added to each well and cocultured with LPS-DCs or BbC50_{SN}-DCs in RPMI-10% FCS at 37°C in a humidified 5% CO₂ atmosphere at a total volume of 200 µL per well over a 5-day culture. Cell proliferation was assessed by means of the incorporation of 1 µCi of tritiated thymidine (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) during the last 18 hours of culture and measured by means of liquid scintillation counting (Tri-Carb 2550 TR/LL; Packard, Rungis, France). Results are expressed as mean ± SD counts per minute obtained from triplicate wells.

Western blot analysis

Monocyte-derived DCs were harvested after stimulation with BbC50_{SN} or LPS and were washed in cold PBS. Three million cells per sample were incubated with 60 µL of lysis buffer (1% Triton X100, 20 mM Tris-HCl pH8, 50 mM NaCl, 5 mM EGTA, 5 µg/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 4 mM orthovanadate) for 30 minutes at 4°C. The homogenates were centrifuged at 14,000g for 30 minutes at 4°C. Cell lysates were electrophoresed on 10% PAGE gels and transferred to nitrocellulose polyvinylidene difluoride membranes for Western blot analysis. After blocking with 5% fat-free dried milk for 30 minutes at room temperature, membranes were incubated overnight at 4°C with appropriate concentrations of antibodies raised against phospho-Bad (Mouse polyclonal antibody), Bad, Bcl-x_L, phospho-Bcl-2, and β-actin (Rabbit polyclonal antibodies) from Cell Signaling (Saint Quentin, France). The membranes were then washed and incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (Bio-Rad, Marnes la Coquette, France) for 60 minutes at room temperature. Immunoreactive bands were visualized by means of chemoluminescent detection (Perkin Elmer Life Science, France). ImageJ 1.3 (National Institutes of Health, Bethesda, Md) was used to measure the intensity of the signal and calculate the ratio of Bcl-2 family proteins to their controls.

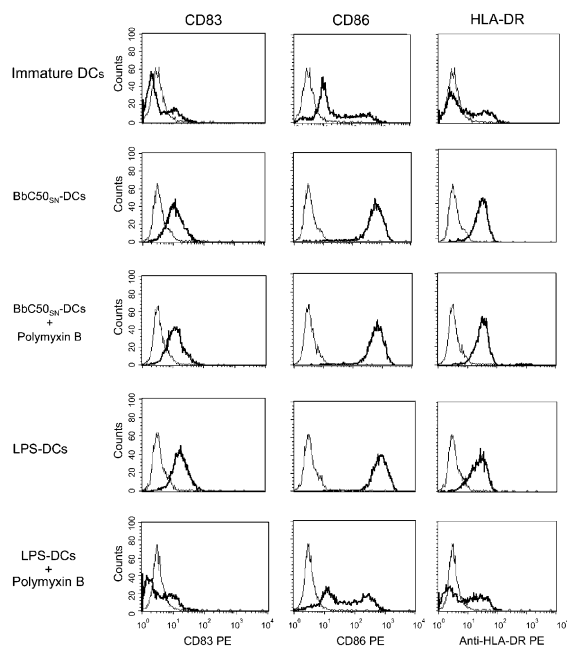


FIG 1. Expression of DC membrane markers in the presence of BbC50_{SN} or LPS or polymyxin B for 2 days. **Bold histograms** represent specific staining of the indicated cell-surface markers, and **thin histograms** correspond to isotype controls. These results are representative of one of 6 experiments.

Cytokine quantification in culture supernatant

Measurements of IL-12 (p70) and IL-10 levels were performed by using appropriate human ELISAs with commercially available antibodies and standards.

Cell line

293/TLR clones were obtained from HEK293 cells stably transfected with a plasmid carrying TLR2, TLR4, TLR6, TLR7, and TLR9 human genes (Invivogen). Plasmids were selected with the potent antibiotic blasticidin. Expression of the TLR gene was verified by means of Western blotting with available antibodies. TLR involvement was revealed by a nuclear factor (NF) κ B-inducible reporter plasmid (pNiFty-SEAP), which allowed the measurement of a colorimetric reaction in supernatants of transfected cells. 293/TLR clones were cultured in standard RPMI medium with 10% FCS supplemented with blasticidin (10 μ g/mL). After trypsin-EDTA treatment, 293/TLR clones were distributed in 48-well flat-bottomed culture plates (Falcon, Becton Dickinson) at 2×10^5 cells/mL per well. After 12 hours of culture, 293/TLR clones were pulsed with BbC50_{SN} or with specific TLR ligands: 10 ng/mL LPS for TLR4, 10 μ g/mL ODN for TLR9, 10 μ g/mL HKLM for TLR2, 1 μ g/mL R848 for TLR7. Changes in medium staining were read by means of spectrophotometry at 405 nm.

Statistical analysis

Results of proliferation analyses, presented as means $\pm$ SD, were compared with the Student *t* test.

RESULTS

BbC50_{SN} induced DC maturation

We first tested the ability of BbC50_{SN} to induce DC maturation. Immature monocyte-derived DCs were

TABLE I. Proliferation of CD4⁺ T cells in the presence of BbC50_{SN}-DCs

	CD4 ⁺ T cells	Polymyxin B	Mean (cpm)	$\pm$ SD
Immature DCs	+	—	15,688	1327
	—	—	340	95
BbC50 _{SN} -DCs	+	—	39,844*	3400
	+	+	41,966*	2079
LPS-DCs	+	—	44,417*	2645
	+	+	26,100†	2900

After 2 days of stimulation, DCs were cocultured with purified CD4⁺ T lymphocytes for 5 days. Cell proliferation was assessed by means of incorporation of tritiated thymidine (last 18 hours of the assay, mean $\pm$ SD of triplicate).

**P* < .001 (comparison with immature DC condition) and †*P* < .002 (with mature DC condition). These results are representative of one of 4 experiments.

stimulated in the presence or absence of BbC50_{SN}. As shown in Fig 1, BbC50_{SN} induced upregulation of surface expression of CD83, CD86, and HLA-DR similar to that observed with LPS. The effects of BbC50_{SN} on DC maturation were time and dose dependent and were maximal after 48 hours of coculture at 100 μ g/mL (data not shown). To determine the origin of the supernatant activity, we used (1) hydrolyzed cow's whey without fermentation by bifidobacteria and (2) supernatant of BbC50 bacteria cultured in the presence of casein instead of cow's whey. In these conditions no DC maturation was observed, indicating that the fermentation process by BbC50 in the presence of cow's whey was necessary to produce active molecules (data not shown). Moreover, the reduction in CCR5 expression and induction of CCR7 expression observed with BbC50_{SN} was similar to that observed on DCs matured with LPS (data not shown). To exclude a potential effect of contaminating endotoxin in BbC50_{SN}, we added polymyxin B to the culture medium. Polymyxin B reduced phenotypic expression of maturation markers induced by LPS but not by BbC50_{SN} (Fig 1).

BbC50_{SN} induced functional DCs

To study the functional capacity of DCs matured by BbC50_{SN}, we studied the ability of DCs cultured in the presence or absence of BbC50_{SN} to induce CD4⁺ T-lymphocyte proliferation. As shown in Table I, BbC50_{SN}-DCs increased CD4⁺ T-cell proliferation, as well as LPS-matured DCs. As in maturation experiments, polymyxin B did not reduce the functional properties of BbC50_{SN}-DCs. T cells stimulated by BbC50_{SN}-DCs or LPS-DCs produced similar levels of IL-2 (428 $\pm$ 18 pg/mL vs 415 $\pm$ 10 pg/mL) and INF- γ (25,300 $\pm$ 350 pg/mL vs 20,400 $\pm$ 1200 pg/mL) but reduced levels of IL-4 (2 $\pm$ 0.7 pg/mL vs 20 $\pm$ 5 pg/mL) and IL-5 (55 $\pm$ 7 pg/mL vs 94 $\pm$ 5 pg/mL).

BbC50_{SN} induced DC IL-10 synthesis

We then assessed cytokine synthesis of DCs in the presence of BbC50_{SN} or LPS. As shown in Fig 2, BbC50_{SN}-DCs strongly produced IL-10 compared with

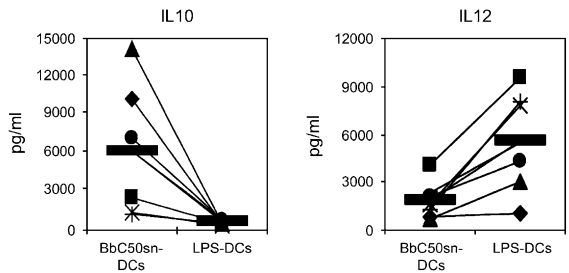


FIG 2. IL synthesis by DCs in the presence of BbC50_{SN} (100 μ g/mL) or LPS (50 ng/mL) over 48 hours was determined by means of ELISA. Each *point* connected with a *heavy line* represents the same donor; *bars* represent the means of measurements. Results are expressed in picograms per milliliter.

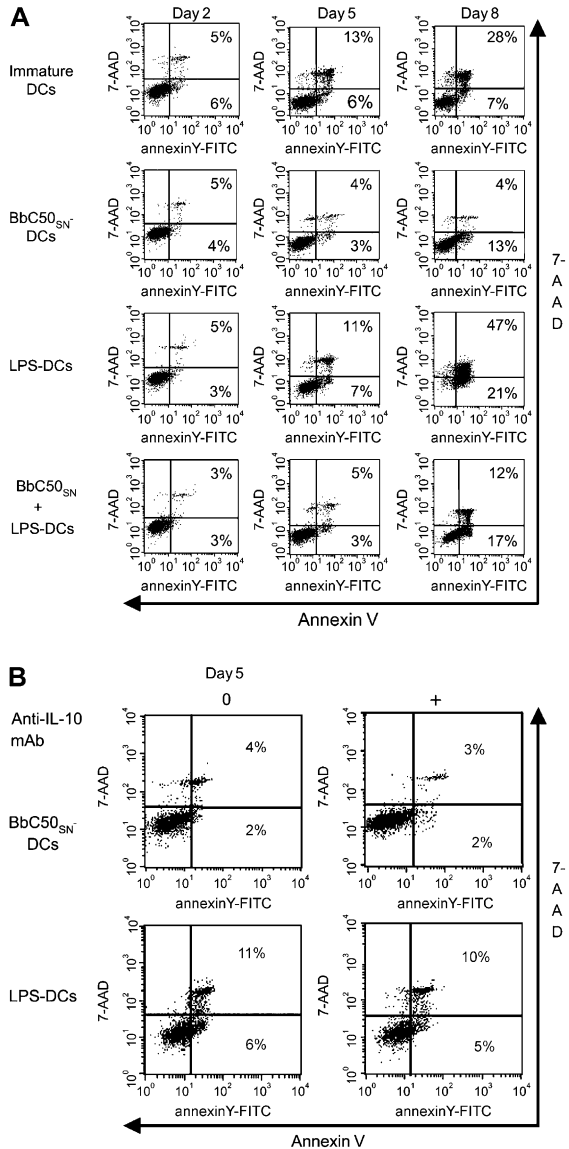


FIG 3. DC survival in the presence of BbC50_{SN} (100 μ g/mL) or LPS (50 ng/mL). **A**, Two, 5, or 8 days after stimulation. **B**, with or without anti-IL-10 mAb (10 μ g/mL). Results are representative of one of 6 experiments.

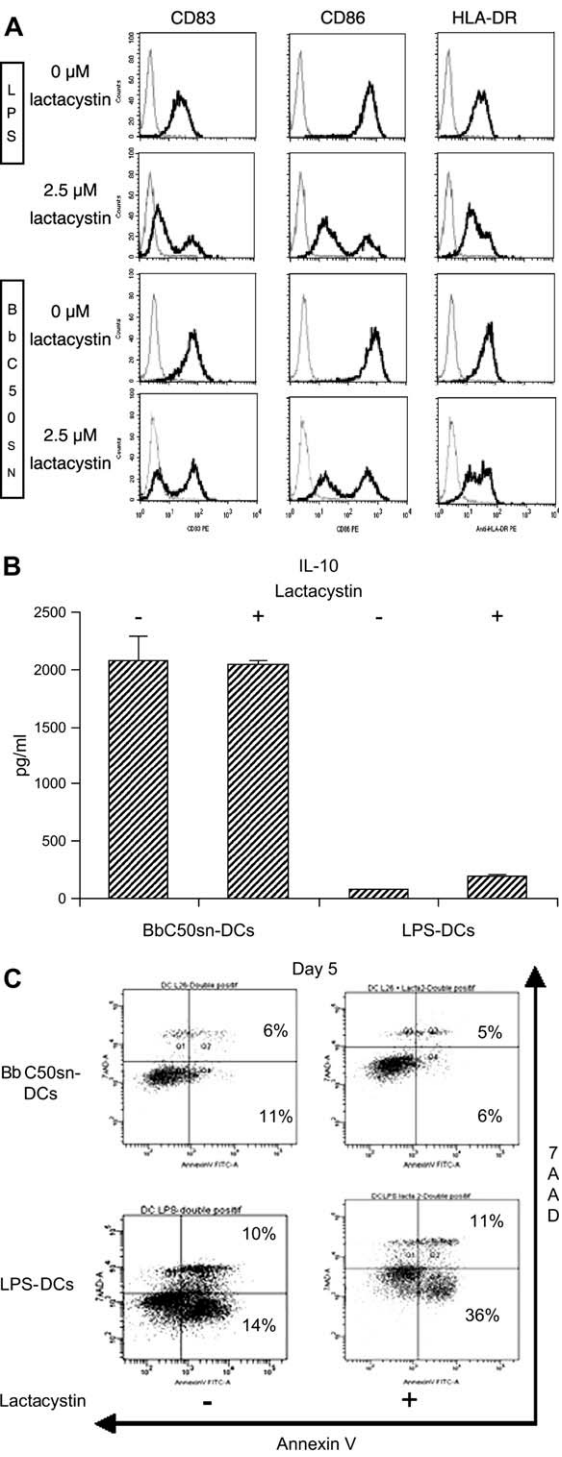


FIG 4. Role of lactacystin in DC maturation, survival, and activation 2 days after stimulation with BbC50_{SN} or LPS. **A**, *Bold histograms* represent specific staining, and *thin histograms* correspond to isotype controls. **B**, IL-10 production from supernatant was determined by means of ELISA. **C**, After 5 days, DCs were stained with 7-AAD and Annexin V. These results are representative of one of 3 experiments.

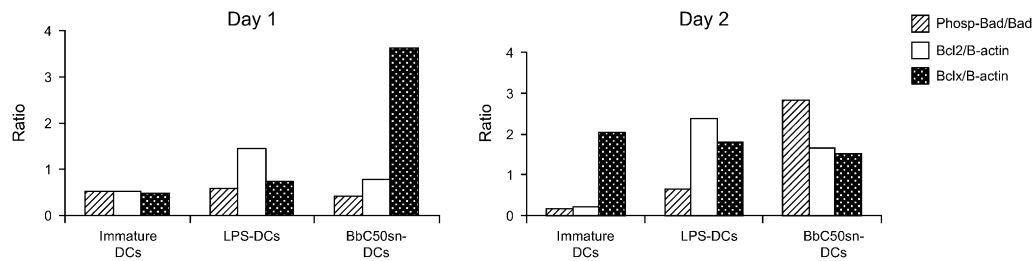


FIG 5. Production of phospho-Bad, Bcl-2, and Bcl-x_L proteins by DCs in the presence of BbC50_{SN} (100 μg/mL) or LPS (50 ng/mL) over 24 or 48 hours and detected by means of Western blotting. The ratios of Phospho-Bad/Bad, Bcl-x_L/β-actin, and Bcl-2/β-actin were measured by using the ImageJ program. Results are representative of one of 3 experiments.

LPS-DCs, although levels varied according to donors. Conversely, BbC50_{SN}-DCs produced 3-fold less IL-12 than LPS-DCs. Interestingly, in a concomitant association, BbC50_{SN} reduced IL-12 production of LPS-DCs by 60% (data not shown).

BbC50_{SN} induced survival of DCs independently of DC IL-10 synthesis

As for maturation and activation, survival of DCs is also involved in the control of the initiation and termination of specific immune responses. We then analyzed the survival of BbC50_{SN}-DCs and LPS-DCs by means of flow cytometry to measure the proportion of apoptotic cells that were positive for Annexin V, 7-AAD, or both. As shown in Fig 3, A, BbC50_{SN} induced survival of DCs: 83% of these cells remained alive in culture at day 8, whereas only 32% of LPS-DCs were still alive. Interestingly, addition of BbC50_{SN} to LPS-DCs increased survival (Fig 3, A). To determine the involvement of IL-10 in the survival of BbC50_{SN}-DCs, we analyzed the apoptosis of treated DCs in the presence of anti-IL-10 neutralizing antibodies. Addition of anti-IL-10 mAb (10 μg/mL) did not decrease survival of BbC50_{SN}-DCs (Fig 3, B). Similarly, addition of IL-10 (10 ng/mL) to LPS-DCs did not increase survival (data not shown).

DC maturation induced by BbC50_{SN} was dependent on the NF-κB pathway but not survival or activation

Lactacystin, an inhibitor of the NF-κB pathway, was used to investigate the transduction signals induced by BbC50_{SN}. As shown in Fig 4, A, lactacystin decreased the expression of CD83, CD86, and HLA-DR in both LPS-DCs and BbC50_{SN}-DCs. The NF-κB pathway is therefore involved in DC maturation induced by BbC50_{SN}. In contrast, lactacystin did not affect BbC50_{SN}-DC IL-10 production (Fig 4, B) or survival (Fig 4, C).

Bcl-x_L and phospho-Bad were involved in survival of BbC50_{SN}-DCs

To understand the mechanisms underlying the enhancement of DC survival by BbC50_{SN}, we investigated the involvement of the anti-apoptotic proteins Bcl-2 and Bcl-x_L and of a negative regulator of apoptosis, phospho-Bad.¹⁹

As shown in Fig 5, BbC50_{SN} strongly induced increased expression of Bcl-x_L at 24 hours in comparison with LPS. At 48 hours, both BbC50_{SN} and LPS induced an increase in Bcl-2 and Bcl-x_L expression, but only BbC50_{SN} induced increased expression of phospho-Bad.

BbC50_{SN} induced DC maturation and activation through a TLR2 pathway

To characterize the target receptor of BbC50_{SN}, we used a cell line transfected with different TLRs. We did not observe any activation of TLR4-, TLR7-, or TLR9-transfected cell lines with BbC50_{SN}, whereas these cells were activated by their specific TLR agonists (Fig 6, A). In contrast, activation of the TLR2-transfected cell line by BbC50_{SN} was similar to that observed with HKLM, a specific TLR2 agonist (Fig 6, A). We further characterized the role of TLR2 in BbC50_{SN} signaling. By using a blocking anti-TLR2 mAb, we demonstrated significant inhibition of DC maturation and DC IL-10 production induced by BbC50_{SN} (Fig 6, B and C).

DISCUSSION

DCs have been recognized as sensors of infection, generating specific immune responses with activation of effector T cells.²⁰ However, maturation, activation, and survival of DCs can be differentially affected according to the bacteria strain, the cytokine environment, and the presence or absence of inflammation.

In this study we first described upregulation of CD83, CD86, and HLA-DR expression on DCs activated by the supernatant of BbC50, in accordance with the findings of Hart et al¹² and Braat et al,²¹ who reported that a probiotic mixture of lactobacilli and bifidobacteria could increase expression of costimulatory molecules. However, the originality of our model is that we did not directly use bacteria (alive or killed) or bacterial components but rather a supernatant of a viable *B. breve* culture. LPS contamination of BbC50_{SN} can be excluded because polymyxin B had no effect on BbC50_{SN}-DCs and because the TLR4-transfected cell line was not activated by the supernatant. Moreover, our results suggest that soluble bacteria products presented in the intestinal lumen are able to interact with mucosal DCs.

We also demonstrated that BbC50_{SN}-DCs produced high IL-10 and low IL-12 levels in contrast to LPS-DCs. IL-12 is a proinflammatory cytokine that can induce a T_H1 response. IL-10, initially described as a cytokine produced by T_H2 cells, usually acts as a general inhibitor of proliferative and cytokine responses of both T_H1 and T_H2 cells. Moreover, IL-10 production by DCs can induce regulatory T cells.²² In rat, murine, and human models, some strains of probiotic bacteria have been reported to enhance IL-10 synthesis from PBMCs, splenocytes, Peyer's cells, and DCs.²³⁻²⁶ This property could explain the results of probiotic bacteria on allergic and inflammatory bowel diseases observed in clinical studies.^{6,7,9}

Another interesting feature of our study relates to the lifespan of treated DCs. The duration of DCs and T-cell contact is probably a key point in induction of immunity or tolerance.²⁷ BbC50_{SN} can induce prolonged DC survival, with oppositional action on the maturation-apoptosis program induced by LPS. This suggests that the anti-apoptotic BbC50_{SN} signal surpasses the proapoptotic effect of LPS on DCs. The lifespan of activated DCs is estimated to be a few days but might be affected by different activator signals from the innate or acquired immune system.²⁸ DCs undergoing apoptosis lose their ability to present antigens to T cells. Limiting or, in contrast, increasing the lifespan of DCs might also be useful to regulate the immune system.²⁷ By increasing DC survival, BbC50_{SN} might affect T-cell differentiation.

To determine the survival signal, we studied the involvement of NF- κ B. This nuclear factor is involved in maturation and activation of cells and regulation of apoptosis. The inhibition of NF- κ B by lactacystin reduced costimulatory molecule expression induced by both BbC50_{SN} and LPS but did not affect IL-10 production or survival of BbC50_{SN}-DCs. These results therefore suggest that BbC50_{SN} induces distinct transduction pathways for maturation, activation, and survival. Survival is regulated by the balance of proapoptotic and antiapoptotic Bcl-2/Bad family proteins. Bad is a proapoptotic protein, whereas Bcl-2 and Bcl-x_L are antiapoptotic proteins. Bad can bind to and inhibit Bcl-2 and Bcl-x_L. However, when phosphorylated, Bad is unable to bind to either protein. Bcl-2 and Bcl-x_L could therefore perform their antiapoptotic action.²⁹ The upregulation of Bcl-x_L and phospho-Bad observed in BbC50_{SN}-DCs might thus explain the prolongation of DC survival.

Finally, we demonstrated that TLR2 is involved in the induction of the different properties observed with BbC50 products. Gram-positive bacilli, such as *B brevis*, have been described as ligands of TLR2.³⁰ Their cell wall comprises a thick peptidoglycan layer and a bilayer phospholipid membrane with lipoproteins, which constitute the main ligands of TLR2. On the other hand, DC IL-10 secretion after TLR2 engagement has been previously reported by Agrawal et al³¹ and Dillon et al.³² However, our results confirm that IL-10 production induced by concomitant TLR2 engagement can block IL-12 production induced by TLR4 in human DCs.³³ Hou and Van Parijs³⁴ showed that TLR (TLR2, TLR4, and TLR9) activation induces

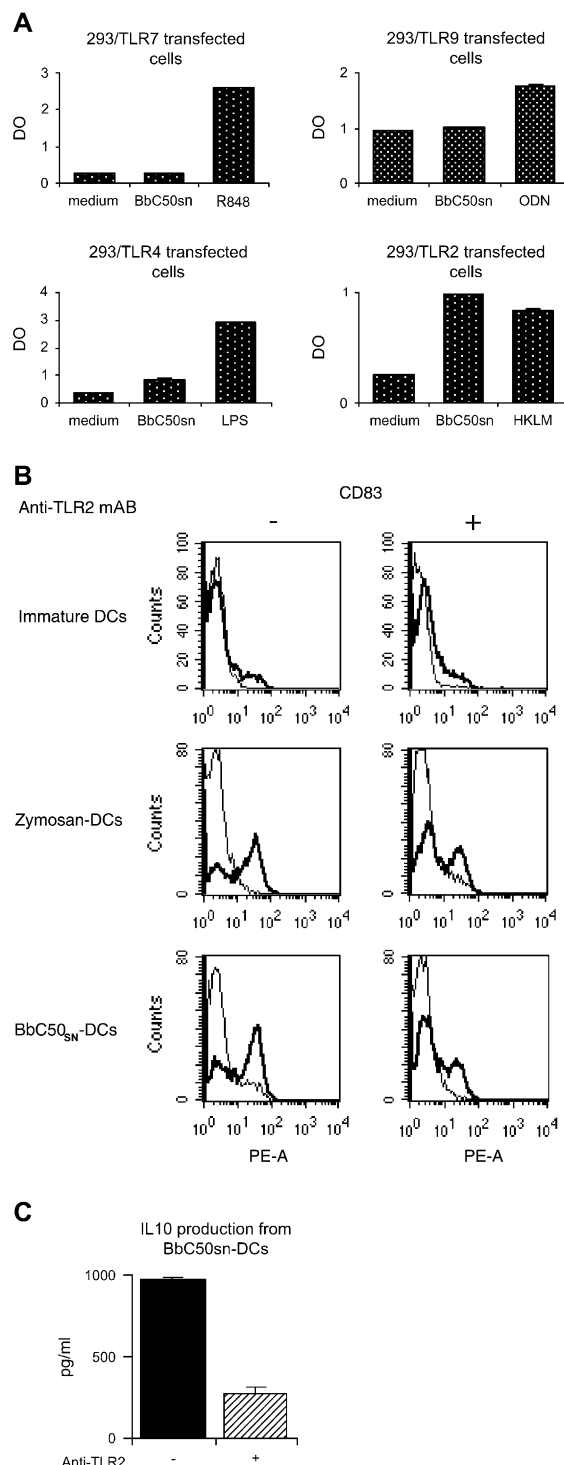


FIG 6. **A**, Activation of TLR-transfected cells in the presence of BbC50_{SN} or specific ligand. Results are expressed as mean optical density ($\pm$ SD of triplicate determination). **B**, DC membrane markers after 18 hours of BbC50_{SN} (5 μ g/mL) or zymosan. **C**, DC IL-10 production after 2 days of BbC50_{SN} (5 μ g/mL) stimulation in the presence or absence of anti-TLR2 mAb. These results are representative of one of 2 experiments.

DC survival through the Bcl-2 protein family. Finally, our results also suggested that TLR2 is not only involved in producing an inflammatory response but can also be involved in producing a regulatory DC profile.

Indeed, although initially described as being immature or semimature, regulatory DCs can also be fully mature.¹³ Moreover, Smits et al³⁵ recently reported that some probiotic strains could modulate DC function to induce regulatory T cells. Their results and ours therefore suggest that the microflora is present not only to initiate and develop the gut's immune system, but also to regulate the immune responses for some specific strains.

In conclusion, we demonstrated that the supernatant of *B. breve* (BbC50) could induce prolonged DC survival with full DC maturation and high IL-10 DC production through TLR2, suggesting that these cells could have regulatory functions.

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