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Lactobacillus delbrueckii subsp *lactis* CIDCA 133 modulates response of human epithelial and dendritic cells infected with *Bacillus cereus*

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RESEARCH ARTICLE

Abstract

It is known that probiotic microorganisms are able to modulate pathogen virulence. This ability is strain dependent and involves multiple interactions between microorganisms and relevant host's cell populations. In the present work we focus on the effect of a potentially probiotic lactobacillus strain (*Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133) in an *in vitro* model of *Bacillus cereus* infection. Our results showed that infection of intestinal epithelial HT-29 cells by *B. cereus* induces nuclear factor kappa B (NF-κB) pathway. Noteworthy, the presence of strain *L. delbrueckii* subsp. *lactis* CIDCA 133 increases stimulation. However, *B. cereus*-induced interleukin (IL)-8 production by epithelial cells is partially abrogated by *L. delbrueckii* subsp. *lactis* CIDCA 133. These findings suggest that signalling pathways other than that of NF-κB are involved. In a co-culture system (HT-29 and monocyte-derived dendritic cells), *B. cereus* was able to translocate from the epithelial (upper) to the dendritic cell compartment (lower). This translocation was partially abrogated by the presence of lactobacilli in the upper compartment. In addition, infection of epithelial cells in the co-culture model, led to an increase in the expression of CD86 by dendritic cells. This effect could not be modified in the presence of lactobacilli. Interestingly, infection of enterocytes with *B. cereus* triggers production of proinflammatory cytokines by dendritic cells (IL-8, IL-6 and tumour necrosis factor alpha (TNF-α)). The production of TNF-α (a protective cytokine in *B. cereus* infections) by dendritic cells was increased in the presence of lactobacilli. The present work demonstrates for the first time the effect of *L. delbrueckii* subsp. *lactis* CIDCA 133, a potentially probiotic strain, in an *in vitro* model of *B. cereus* infection. The presence of the probiotic strain modulates cell response both in infected epithelial and dendritic cells thus suggesting a possible beneficial effect of selected lactobacilli strains on the course of *B. cereus* infection.

Keywords: probiotics, epithelial cells, immunomodulation, virulence, dendritic cells

1. Introduction

Probiotic microorganisms have long been proposed to promote human health (Ashraf and Shah, 2014; Marchesi *et al.*, 2015; Martin *et al.*, 2014). Lactobacilli and bifidobacteria species are widely used to prevent and treat allergy and intestinal disorders (Tojo *et al.*, 2014). Although several authors have shown favourable effects of probiotics in human health, their mechanism of action is not completely understood. Some proposed mechanisms are modulation of enzymatic activities (Hugo *et al.*, 2006; Parvez *et al.*, 2006), inhibition of intestinal pathogens (Franco *et al.*, 2013), modulation of host defence mechanisms (Dogi *et al.*, 2010; Galdeano and Perdigón, 2006; Kang and Im, 2015; Linares *et al.*, 2015; Riedel *et al.*, 2006; Zeuthen *et al.*, 2006), immune regulation trough balance of pro-inflammatory and anti-inflammatory cytokines (Hua *et al.*, 2010), and competition

for adhesion sites at the intestinal epithelium (Jung *et al.*, 2015; Scaldaferri *et al.*, 2012; Viggiano *et al.*, 2015).

Intestinal epithelial cells (IECs) represent an important barrier between lamina propria cells and the potentially harmful luminal contents. IECs are important immunoeffector cells with the capacity to release cytokines, chemokines, and other molecules involved in antigen presentation and immune defence (Canny *et al.*, 2006). IECs and antigen presenting cells such as dendritic cells (DC) and macrophages play a key role in the orchestration of immune responses. Mucosal DCs are highly specialised in function and display a unique response to toll-like receptor ligands, are capable of driving immunoglobulin isotype switching to IgA, can imprint gut-homing receptors on T and B cells, and drive either T regulatory or Th17 cells depending on the analysed subtype. In the last years, there are several reports describing how the local microenvironment can shape DC function (Rescigno, 2010). Immunomodulation by probiotics can be achieved by modification of the Th1/Th2 balance (Hua *et al.*, 2010). Furthermore, they can induce B cells migration and increase specific immunoglobulin A (IgA) secretion by modulation of intestinal DC (Macpherson and Uhr, 2004), thus facilitating antigen uptake and presentation in Peyer's patches.

The nuclear factor kappa B (NF-κB) is a transcription factor which plays a key role in regulating biological processes. NF-κB is typically present and resides in the cytoplasm of most cells as a complex with members of the IκB inhibitor protein family. In response to signals, NF-κB activation occurs via phosphorylation of its inhibitor, which dissociates from the NF-κB dimer allowing the translocation to the nucleus, inducing gene expression. Over 200 physiological stimuli are known to activate NF-κB, for instance, proinflammatory cytokines; bacterial toxins; viral products; and cell death stimuli (Baldwin, 1996; Tergaonkar, 2006). In this context, commensal bacteria or probiotic microorganisms, mostly through Toll like receptor-mediated signalling, could modify activation of NF-κB of intestinal epithelial cells (Cerf-Bensussan and Gaboriau-Routhiau, 2010). On the other hand, intestinal infections caused by enterobacteria, induce activation of an inflammatory cascade that lead to an increase of proinflammatory mediators (e.g. interleukin (IL)-8) that in turn enhances cell recruitment to the infection zone. Interestingly, some probiotic microorganisms are capable to inhibit IL-8 production from intestinal epithelial cells through modulation of NF-κB (Sokol *et al.*, 2008). IL-8 is a pro-inflammatory interleukin that plays a pivotal role in cell recruitment to the site of infection. It is a relevant marker of intestinal inflammation (Roberts-Thomson *et al.*, 2011) and IL-8 levels has been assessed in studies of the immunomodulatory effect of potentially probiotic strains (Kechaou *et al.*, 2013; Torres-Maravilla *et al.*, 2015). Interestingly, even proinflammatory strains lead to protective effects (Kechaou *et al.*, 2013). Other authors propose that probiotics contribute to intestinal homeostasis trough stimulation of tumour necrosis factor alpha (TNF-α) production, whereby beneficial effects would be associated with immunological stimulation process (Mizoguchi *et al.*, 2008; Pagnini *et al.*, 2010).

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 is a potentially probiotic strain. Indeed, this strain has the ability to inhibit microorganisms involved in food-spoilage (Kociubinski *et al.*, 1996), to resist bile (Kociubinski *et al.*, 1999), to antagonise biological effects of enterohaemorrhagic *Escherichia coli* (EHEC) on cultured eukaryotic cells (Hugo *et al.*, 2008) and to inhibit harmful enzymatic activities (Hugo *et al.*, 2006). Furthermore, this strain has differential susceptibility to enterocyte-derived antimicrobial peptides (Hugo *et al.*, 2010).

Bacillus cereus is a spore-forming microorganism responsible for foodborne illness, i.e. emetic and diarrheic syndromes (Bottone, 2010). Virulence of *B. cereus* is a multifactorial process that involves regulation of the expression of relevant virulence coding genes for extracellular factors (Gohar *et al.*, 2002) as well as the ability of adhesion/invasion events (Minnaard *et al.*, 2001, 2004, 2007; Rowan *et al.*, 2001). We have previously isolated four *B. cereus* strains that show differences in the presence of sequence of virulence genes as well as different biological effects on eukaryotic cells (Minnaard *et al.*, 2004, 2007, 2013).

In this work, we studied the effect of *L. delbrueckii* subsp. *lactis* CIDCA 133 on *B. cereus* infection of human epithelial and DC. In this context, HLA and CD86 markers from DC, cytokine production and NF-kB activation on epithelial cells were evaluated.

2. Materials and methods

Bacterial strains and culture conditions

L. delbrueckii subsp. *lactis* strain CIDCA 133 belongs to the CIDCA culture collection. Lactobacilli were grown in De Man, Rogosa and Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France) at 37 °C for 16 h in anaerobic conditions. Microorganisms, stored frozen at -80 °C, were reactivated twice in liquid medium before the assays. *B. cereus* strains were cultured in brain hearth infusion (BHI) broth (Biokar Diagnostics) supplemented with glucose 0.1% (w/v) (BHIG). Bacteria were cultured for 16 h at 32 °C under orbital agitation and afterwards, they were inoculated (4% v/v) in 5 ml of BHIG and incubated with agitation at 32 °C for 3 h. Microorganisms were harvested by centrifugation (900×*g* for 10 min). *B. cereus* strains 2 and M2, were isolated from infant formula (Minnaard *et al.*, 2001), T1 (Buchanan and

Schultz, 1992) and B10502 (Minnaard *et al.*, 2004) were involved in different foodborne outbreaks.

A correlation curve between absorbance measured at 600 nm (A_{600nm}) versus cfu was plotted for each strain. The A_{600nm} values were employed to calculate the bacterial number used in each experiment.

Labelling of bacteria

Bacteria labelling was carried out with carboxyfluorescein diacetatesuccinimidyl ester (CFSE; Molecular Probes, Eugene, OR, USA) at a final concentration of 5 µmol/l (Tuominen-Gustafsson *et al.*, 2006). Microorganisms were incubated for 30 min at 37 °C in the dark, and washed twice with phosphate-buffered saline (PBS).

Cell lines and culture medium

HT-29 (ATCC® HTB-38™) and HT-29-NF-κB-hrGFP reporter cells (Guimarães *et al.*, 2010) were cultured in DMEM or RPMI 1640 (Life Technologies, Carlsbad, CA, USA) supplemented with 10% (v/v) foetal bovine serum (FBS) (Life Technologies). HT-29-NF-κB-hrGFP cells were obtained after stable transfection of HT-29 cells with the pNF-kB-hrGFP plasmid from the PathDetect Signal Transduction Pathway cis-Reporting Systems Kit (Stratagene, Santa Clara, CA, USA). Briefly, subconfluent HT29 cells were transfected with pNF-kB-hrGFP plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and selected with hygromycin. After two weeks of selection, cells were stimulated for 24 h with a pro-inflammatory cocktail (25 ng/ml TNF-α, 1.25 ng/ml IL-1b and 3.75 ng/ml interferon gamma (IFN-γ)) and GFP positive cells were sorted with a MoFlo cell sorter (Dako, Carpinteria, CA, USA).

Cells were routinely propagated in 25 or 75 cm^2 tissue culture flasks at 37 °C, 5% CO_2 in a humidified incubator. Subsequently, cells were trypsinised and concentration was adjusted in order to obtain the required cell concentrations (see below). In all the assays cells were cultured for less than twenty passages.

Generation of human monocyte-derived dendritic cells

Peripheral blood mononuclear cells (PBMC) were obtained as described by Tiscornia *et al.*, (Tiscornia *et al.*, 2012) by Ficoll-Hypaque density-gradient centrifugation and stored in liquid nitrogen before use. For DCs differentiation, monocytes were incubated during 48 h in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FBS, 1000 U/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) (PeproTech, Rocky Hill, NJ, USA) and IL-4 (1% of a conditioned supernatant from the IL-4 transfected J588L cell line). Monocyte-derived DCs were harvested and cultured in 24-well tissue culture plates for experiments. Cell purity was assessed by cell surface staining and flow cytometry using antibodies (described below in flow cytometry and antibodies). Viability was determined by trypan blue exclusion. The selection criterion for sample acceptance was established in order to choose donors presenting more than 85% of CD11c+/CD14- cells and less than 5% of lymphocyte contamination (CD3+ and/or CD19+ cells) after the DC-differentiation process. Cells with the following phenotype (evaluated by flow cytometry): $CD11c^{+}CD14^{-}MHC\text{-}II^{low}CD86^{low}$ were considered immature DCs, and cells expressing $CD11c^{+}CD14^{-}MHC\text{-}II^{high}CD86^{high}$ were considered mature DCs.

NF-κB activation assay using HT-29-NF-κB-hrGFP reporter cells

250 µl of cell suspension containing 2.5×10^5 cells/ml were added per well in 24-well tissue culture plates. Stimulation with *B. cereus* and *L. delbrueckii* subsp. *lactis* CIDCA 133 was performed using a multiplicity of infection of 20 for 18 h. Culture medium (DMEM) was supplemented with the bacteriostatic antibiotic chloramphenicol (100 µg/ml) to prevent bacterial growth during the experiment (Minnaard *et al.*, 2004). TNF-α (3 ng/ml) stimulated cells were used as a positive control. Following infection, supernatant was obtained, clarified by centrifugation and stored at -80 °C for IL-8 determination. Cells were washed twice with PBS and detached with trypsin. Green fluorescent protein (GFP) expression was analysed by flow cytometry (Cyan™ ADP Analyzer; Beckman Coulter, Brea, CA, USA). For each sample, 10,000 counts were recorded, which were gated on a forward scatter (FSC) versus side scatter (SSC) dot plot. Doublets were excluded from analysis.

Co-culture assays

In the present study we have used an experimental setting previously optimised in our lab (Grompone *et al.*, 2012; Tiscornia *et al.*, 2012) and by other groups (Mileti *et al.*, 2009; O'Hara *et al.*, 2006). Briefly, human monocyte-derived DCs were seeded (1.25×10^5 cells/well) in 12-well tissue culture plates (Corning Inc., Corning, NY, USA) using 500 µl of culture medium (RPMI 1640). Non-polarised (HT-29 or HT-29-NF-κB-hrGFP according to the experiment) cells were grown in the upper chamber of a transwell filter (3 µm diameter of pores; Corning Costar, Cambridge, MA, USA) and incubated for 2 days in RPMI 1640 supplemented with 10% heat-inactivated FCS. Inserts containing epithelial cells (HT-29 or HT-29-NF-κB-hrGFP according to the experiment, see below) monolayer were transferred to the 12-well plates containing DCs. Fresh complete culture medium was added (500 µl) to the upper compartment and the cells were incubated for further 6 h.

The apical surface of the monolayers was stimulated with a bacteria-cell ratio of 20:1. Non-stimulated cells and cells

treated with 0.5 µg/ml of lipopolysaccharide (LPS) from *E. coli* serotype O26:B6 (Sigma-Aldrich, St. Louis, MO, USA) were used as controls. Plates were incubated for 18 h at 37 °C in a 5% CO_2 atmosphere. Culture supernatants from the basal compartment were clarified by centrifugation and stored at -80 °C for cytokines determination. Monocyte-derived DCs were harvested and stained for flow cytometry analyses.

In order to evaluate the effect of *B. cereus* in a co-culture system experiments with DCs and HT-29 cells were performed. HT-29 cells cultured in the upper compartment were apically infected with CFSE-labelled *B. cereus* strain B10502 and incubated for 18 h in RPMI 1640 with 100 µg/ml chloramphenicol. After washing with PBS, cells were detached with trypsin and analysed by flow cytometry to assess association. Similarly, association with DC in the lower compartment was determined.

All the experiments were performed in the presence or absence of non-labelled strain CIDCA 133 in the upper compartment.

Flow cytometry and antibodies

The following antibodies were used for flow cytometry: B-ly6 (anti-human CD11c, allophycocyanin (APC)-conjugated), 2331 (anti-human CD86, phycoerythrin (PE)-conjugated), TU36 (anti-human HLA-DR fluorescein isothiocyanate (FITC)-conjugated), M5E2 (anti-human CD14 FITC-conjugated), HIB19 (anti-human CD19 PE-conjugated), HIT3a (anti-human CD3 FITC-conjugated). The corresponding isotype controls were used. All antibodies were used according to manufacturer's instructions (BD Pharmingen, San Jose, CA, USA). Surface markers (HLA-DR and CD86) were expressed as the median of the fluorescence intensity (MFI) according to Tiscornia *et al.* (2012). For sample analysis, 10,000 counts, gated on FSC vs SSC dot plot, were recorded. Acquisitions were performed using a Cyan™ ADP (Beckman Coulter) flow cytometer and Summit 4.3 software.

Cytokine quantification

IL-6, IL-8, IL-10, IL-12 p70 and TNF-α levels were determined by FlowCytomix™ technology (Bender MedSystems, Vienna, Austria) and analysed by flow cytometry (BMS FlowCytomix Software version 2.2.1).

Statistical analysis

Results were obtained from 2 or 3 independent experiments. Student t-test and median analysis were performed with InfoStat (InfoStat, Version 2008, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Cordoba, Argentina). Differences were considered statistically significant when $P<0.05$.

3. Results

Bacillus cereus induced NF-κB activation in HT-29-NF-κB-hrGFP reporter cells

In order to study the effects of *B. cereus* strains on NF-κB activation, HT-29-NF-κB-hrGFP reporter cells were incubated with microorganisms for 18 h and the activation of NF-κB was determined by flow cytometry (% of GFP^+ cells). A significant increase ($P<0.05$) of GFP^+ cells was found in cells infected with *B. cereus* (Figure 1); 4.1±0.1%; 3.9±0.5%; 3.6±0.3% and 4.0±0.0% for strain B10502, T1; M2 and 2, respectively. Interestingly, the co-incubation of *L. delbrueckii* subsp. *lactis* strain CIDCA 133 with *B. cereus* (strains B10502, T1 and M2) led to a further increase of GFP^+ cells ($P<0.005$) compared with cells infected only with *B. cereus* strains (6.9±0.3%; 9.8±0.1% and 8.3±0.2% for strains B10502, T1 and M2, respectively). On the other hand, the *Lactobacillus* had no effect on cells infected with *B. cereus* strain 2 (3.8±0.7%). The positive control (TNF-α-stimulated cells) led to 33.4±1.5% of GFP^+ cells (Figure 1).

Bacillus cereus induced IL-8 production by HT-29-NF-κB-hrGFP reporter cells

Since several cis elements, including a binding site for the inducible NF-κB, have been identified in the regulatory region of the IL-8 gene, we have examined the ability of different *B. cereus* strains to stimulate IL-8 secretion. This cytokine production was significantly increased when HT-29-NF-κB-hrGFP reporter cells were infected with B10502, T1 and 2 strains (Figure 2). IL-8 levels after stimulation with B10502 strain (2,009.7±131.8 pg/ml) were comparable to those obtained in TNF-α-stimulated cells (2,063.8±141.6 pg/ml), while after T1 and 2 strains stimulation were 796.8±64.0 pg/ml and 782.7±44.4 pg/ml, respectively. In contrast, IL-8 levels produced by cells incubated with *B. cereus* M2 strain (197.5±29.6 pg/ml) or *L. delbrueckii* subsp. *lactis* strain CIDCA 133 (143.7±18.9 pg/ml) were slightly higher than levels produced by control unstimulated cells (52.7±11.0 pg/ml) (Figure 2).

Interestingly, the presence of the *Lactobacillus* strain significantly ($P<0.05$) reduced the IL-8 production of *B. cereus* infected cells. Indeed, 562.2±27.7, 193.6±2.3 and 232.8±8.6 pg/ml were found for strains B10502, T1 and 2, respectively (Figure 2). On the other hand, *Lactobacillus* did not change the IL-8 production of M2-stimulated cells (166.6±72.6 pg/ml).

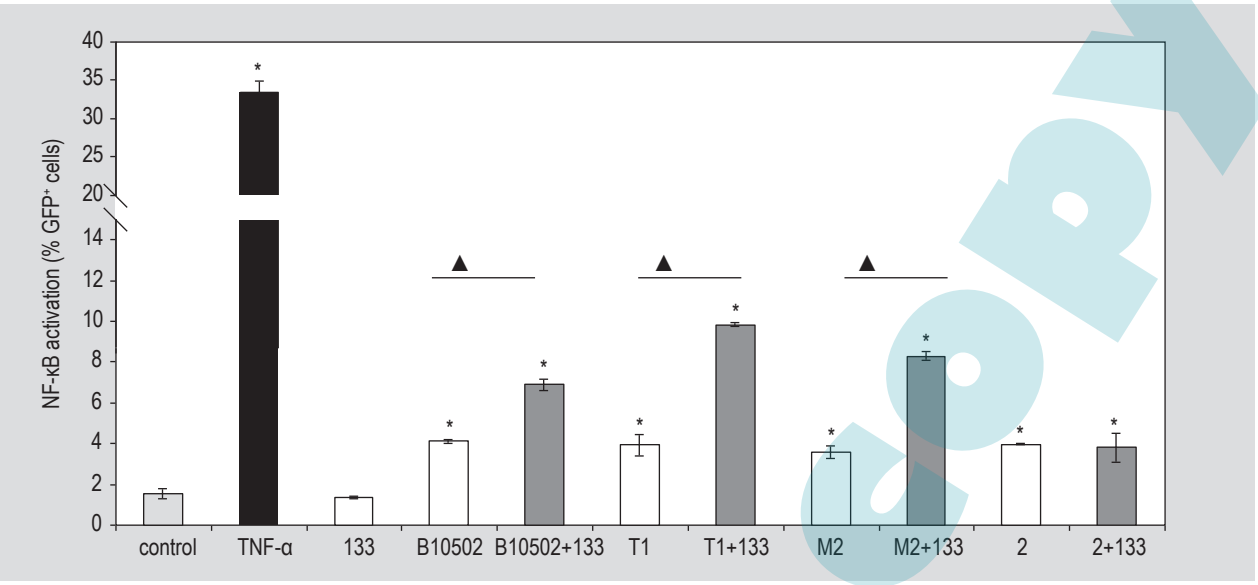

Figure 1. NF-κB activation in HT-29-NF-κB-hrGFP cells infected with different strains of *Bacillus cereus* in the presence or not of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133. Different microorganisms (*L. delbrueckii* subsp. *lactis* CIDCA 133 or *B. cereus* strains: B10502, T1, M2 and 2) were added to the cells and incubated for 18 h. NF-κB activation (measured by the percentage of GFP+ cells) was analysed by flow cytometry. Stimulation with TNF-α was used as a positive control. Bars represent means ± standard deviation from three independent experiments. ▲ indicate significant differences (*P*<0.005) between the bars; * indicate significant differences with control.

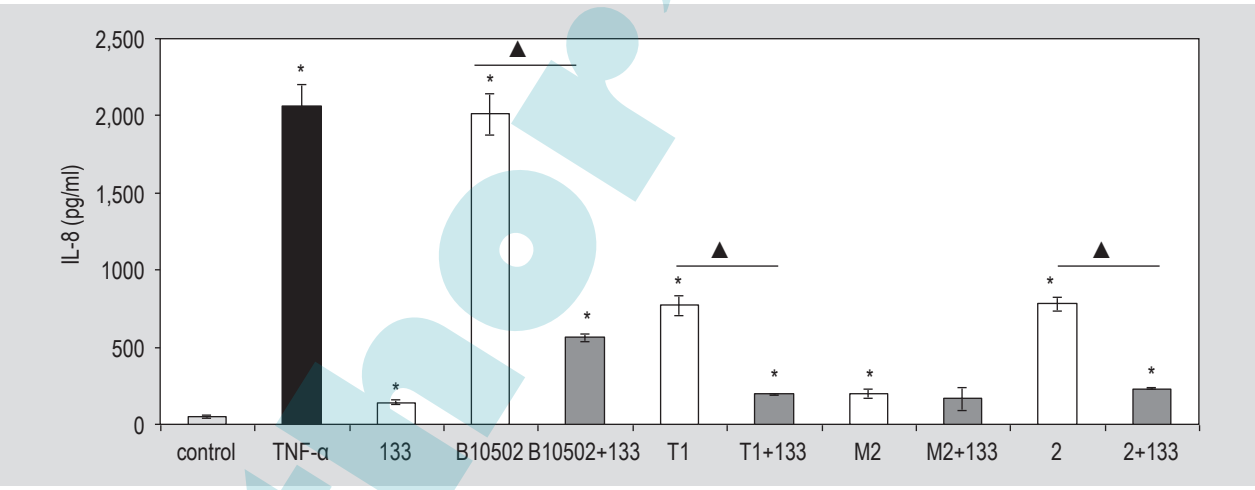

Figure 2. Effect of *Lactobacillus delbrueckii* subsp *lactis* CIDCA 133 on IL-8 production induced by *Bacillus cereus* strains in HT-29-NF-κB-hrGFP cells. Different microorganisms (*L. delbrueckii* subsp *lactis* CIDCA 133 or *B. cereus* strains: B10502, T1, M2 and 2) were added to HT-29-NF-κB-hrGFP cells and incubated for 18 h. IL-8 production was measured on supernatants by flow cytometry. Stimulation with TNF-α was used as a positive control. Bars represent means ± standard deviation from three independent experiments. ▲ indicate significant differences (*P*<0.005); * indicate significant differences with control.

Lactobacillus delbrueckii modifies *Bacillus cereus* interaction with dendritic and HT-29 cells

Infection of HT-29 cells (upper compartment) in co-culture with monocyte-derived DC (lower compartment) was performed in order to assess association of bacteria to eukaryotic cells. These studies were performed with strain B10502 since this strain was able to induce strong inflammatory response in HT-29 cells (IL-8 production) and lactobacilli were capable to significantly decrease this effect.

As depicted in Figure 3, *B. cereus* strain B10502 was able to migrate from the upper compartment to the lower compartment. In addition, the presence of lactobacilli modified the percentage of *B. cereus* associated to DC. Indeed, 42.7±2.0% of the DC were associated to *B. cereus*

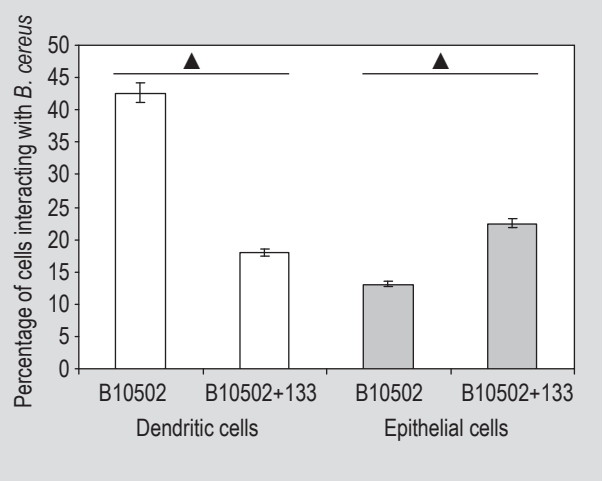

Figure 3. Association of CFSE-labelled *Bacillus cereus* B10502 to monocyte-derived dendritic cells and epithelial cells (HT-29). Association of *B. cereus* B10502 to monocyte-derived dendritic cells (white bars) and epithelial cells (grey bars) after 18 h of incubation in the presence or not of *Lactobacillus delbrueckii* subsp *lactis* CIDCA 133. Microorganisms were added to the upper compartment (HT-29 cells) in a co-culture system with dendritic cells (DC) in the lower compartment. Bars represent means ± standard deviation from two independent experiments. ▲ indicate significant differences between compared treatments (*P*<0.05).

whereas these values decreased to 18.0±0.6% in the presence of strain 133. In contrast, HT-29 cells (upper compartment) revealed different association pattern: 13.1±0.4% of cells interacting with *B. cereus* while 22.4±0.7% of cells were found when the lactobacilli was present during infection.

CD86 and HLA-DR expression markers on dendritic cells

In order to determine the DC response in co-culture with HT-29 cells to the bacteria, CD86 and HLA-DR expression markers were assessed after 18 h of incubation with *B. cereus* strain B10502 alone or in combination with *L. delbrueckii* subsp. *lactis* strain CIDCA 133. As shown in Figure 4, even though the system was responsive to LPS stimulation, no differences in HLA-DR expression levels (control MFI: 89.1±18.1) were observed in cells stimulated with *B. cereus* B10502 (MFI: 108.9±24.8), lactobacilli (MFI: 114.6±23.3), or both microorganisms (MFI: 131.2±6.7) when compared to the control (cells without stimulation). In contrast, expression of the co-stimulatory marker CD86 induced by *B. cereus* B10502 was similar to the positive control stimulated with LPS (MFI 611.1±77.8 and 703.3±18, respectively). Incubation of cells with *L. delbrueckii* subsp. *lactis* strain CIDCA 133 alone lead to a trend of higher expression of CD86 (*P*=0.11) as compared with control unstimulated cells.

Cytokine production by dendritic and HT-29 cells in co-culture with *Bacillus cereus*

To determine secretion of cytokines, supernatants of co-cultures were analysed after 18 h of incubation with *B. cereus* strain B10502 alone or in combination with *L. delbrueckii* subsp. *lactis* strain CIDCA 133. Incubation with single strains as well as with combined strains lead to a significant stimulation of IL-8 production in co-cultures as compared with unstimulated control (885.66±19.28 pg/ml). Although these values were lower than those obtained for the LPS control (6,181.4±65.6 pg/ml) there were no

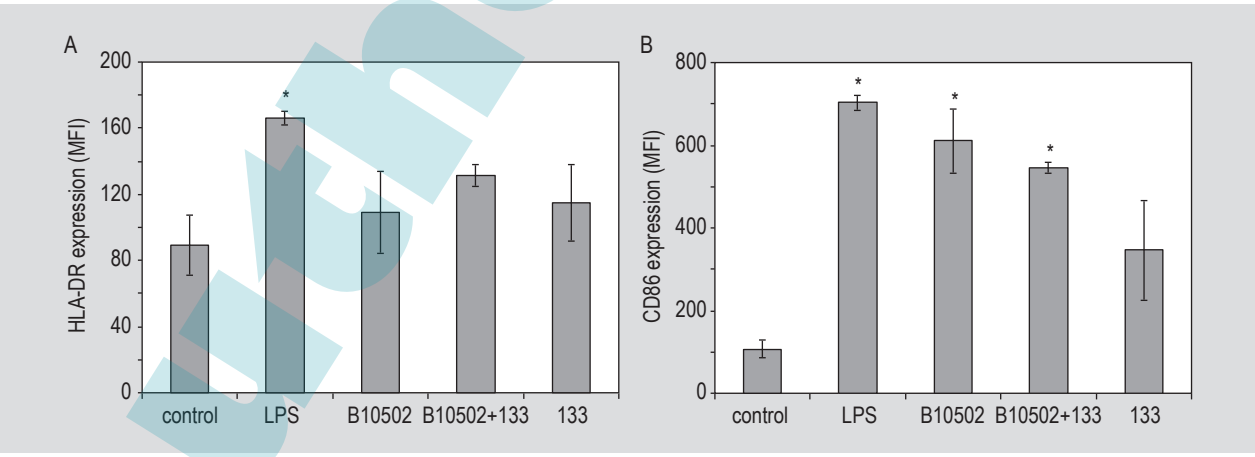

Figure 4. Expression of (A) HLA-DR and (B) CD86 in monocyte-derived dendritic cell (DC) co-cultured with HT-29-NF-κB-hrGFP cells, *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 and *Bacillus cereus* B10502. Epithelial cells (upper compartment) were infected with *B. cereus* B10502 in the presence or absence of *L. delbrueckii* subsp *lactis* CIDCA 133. Surface markers on DC (lower compartment) were determined by flow cytometry after 18 h incubation. Controls: untreated cells (control) and lipopolysaccharide-stimulated cells (LPS). Results were expressed as the medians of fluorescence intensity (MFI) ± standard deviation from two independent experiments. * indicate significant differences with control (*P*<0.05).

significant differences for any of the treatments tested (Figure 5A).

Production of IL-6 showed different pattern of stimulation as compared with IL-8. Values similar to basal levels were observed when cells were stimulated with either LPS (128.3±11.6 pg/ml) or strain CIDCA 133 (87.6±123.9 pg/ml), while a significant increase was observed when cells were incubated with strain B10502 (890.6±263 pg/ml). The presence of *Lactobacillus* in combination with *B. cereus* did not modify IL-6 production (1,091.52±55.73 pg/ml) as compared to stimulation with *B. cereus* alone (Figure 5B).

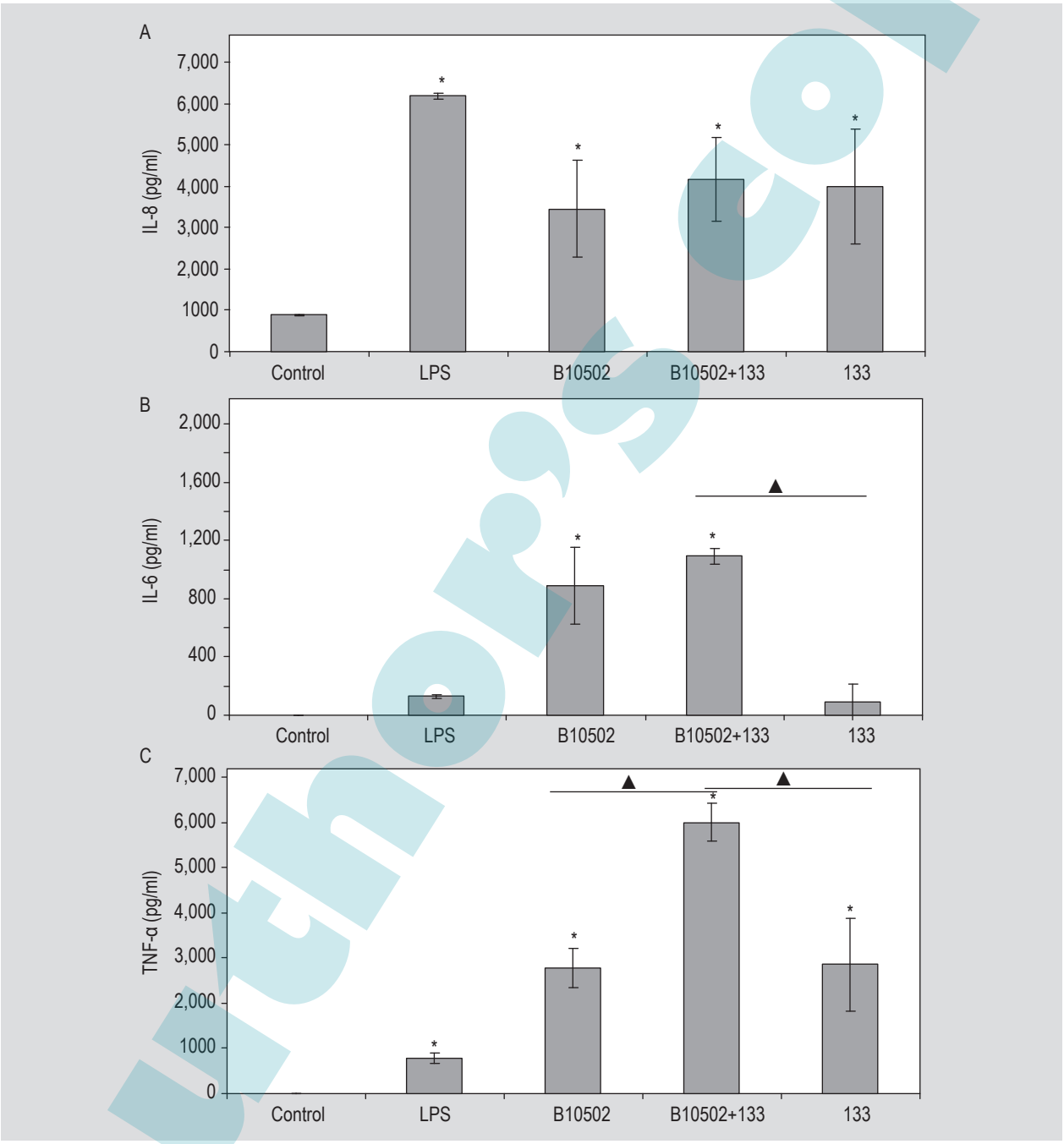

Figure 5. Cytokine production (A – IL-8; B – IL-6; and C – TNF-α) by monocyte-derived dendritic cells co-cultured with HT-29 cells, *Lactobacillus delbrueckii* subsp *lactis* CIDCA 133 and *Bacillus cereus* B10502. Epithelial cells (upper compartment) were infected with *B. cereus* B10502 in the presence or absence of *L. delbrueckii* subsp *lactis* CIDCA 133. After 18 h incubation, cytokines were determined in the lower compartment (DC). Controls: untreated cells (basal) and lipopolysaccharide-stimulated cells (LPS). Results were expressed in pg/ml. Data are shown as the mean ± standard deviation from three independent experiments. * indicate significant differences (*P*<0.05) with control. ▲ indicate significant differences between compared treatments (*P*<0.05).

Lactobacilli and *B. cereus* in single cultures were able to increase TNF-α production ($P<0.05$) in co-cultures as compared to uninfected control. Of note, when both lactobacilli and *B. cereus* were added to the epithelial compartment, a significant increase in TNF-α production was observed (5,990.5±421.3 pg/ml, $P<0.05$) as compared with cells treated with individual strains (Figure 5C). Production of IL-10 and IL-12p70 was not detectable under any of the experimental conditions of the present study (data not shown).

4. Discussion

In the present study the immunomodulatory effect of a potentially probiotic strain on *B. cereus*-infected cells was demonstrated through *in vitro* studies with cultured human epithelial and DC. By using cultures of HT-29 cells, we demonstrate that infection with *B. cereus* leads to the production of IL-8 following induction of NF-kB pathway. Studies with co-cultures of epithelial and DC allowed us to get a further insight and show that infection of human epithelial cells induces phenotypic and functional maturation of DC and production of pro-inflammatory cytokines. Interestingly, the presence of *L. delbrueckii* subsp. *lactis* CIDCA 133 modifies cell response.

Adhesion of microorganisms to enterocytes and secretion of extracellular factors can induce IL-8 production in epithelial cells. This has been demonstrated for example for *Helicobacter pylori* (Papadakos *et al.*, 2013), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) (Edwards *et al.*, 2011), enteroinvasive *E. coli* (Jung *et al.*, 1995), *Yersinia enterocolitica* (Jung *et al.*, 1995; Schulte *et al.*, 1996), *Salmonella dublin, Shigella dysenteriae* and *Listeria monocytogenes* (Jung *et al.*, 1995). In contrast, potentially probiotic microorganisms belonging to genera *Bifidobacterium* and *Lactobacillus* not only do not induce IL-8 production in epithelial cells (Imaoka *et al.*, 2008; Lammers *et al.*, 2002) but also have the ability to reduce the activation of the NF-κB pathway in epithelial cells (Chen, 2013; Grompone *et al.*, 2012). This potentially anti-inflammatory effect was also demonstrated for yeasts isolated from kefir that were also able to decrease flagellin-mediated activation of NF-κB (Romanin *et al.*, 2010). Agonists involved in these effects comprise genomic DNA and cell wall components (Hiramatsu *et al.*, 2013). Lipoteichoic acid (LTA) is a key cell wall component of Gram (+) bacteria. However, even though LTA from pathogenic and non-pathogenic bacteria share many common structures, it has been demonstrated that biological responses are different. Indeed, whereas LTA from pathogens induce inflammation, those from non-pathogens can even decrease inflammation (Kim *et al.*, 2012a).

We determined that the presence of strain CIDCA 133 did not modified viability of *B. cereus* (data not shown). In contrast, in our experimental conditions, there was an effect of chloramphenicol on the viability of all the *B. cereus* strains under study. Even if this is a bacteriostatic agent that does not modified *B. cereus* viability in short experiments (Minnaard *et al.*, 2004, 2013), there was a significant reduction of *B. cereus* viability (4 log) after 18 h incubation with 100 μg/ml chloramphenicol (data not shown). Since no bacterial lysis was observed, the number of total bacteria remained unchanged. It is important to point out that all the *B. cereus* strains under study decreased to similar values of cfu and that the presence of strain 133 did not modify these values. On the other hand, viability of strain 133 did not change in the presence of chloramphenicol in the 18 h incubation period. Therefore, the effect of strain 133 cannot be ascribed to a reduction of total *B. cereus* concentrations. The supplementation of culture media with antibiotics is mandatory in cell stimulation experiments with *B. cereus* since even short incubation periods (3 h) in antibiotic-free media lead to complete destruction of the cell monolayers due to bacterial growth. Supplementation of culture media with antibiotics is a common practice in the studies of interaction between bacteria and eukaryotic cells. Furthermore, many studies have been performed in the presence of bactericidal agents, such as penicillin and streptomycin (Carasi *et al.,* 2015; Huang *et al.,* 2015; Klingspor *et al.,* 2015; Mastropietro *et al.,* 2015). It is worth noting that it has long been demonstrated that stimulation of cell response occurs even when cells are incubated with non-viable bacteria or cellular fractions (Jeon *et al.,* 2015; Kaji *et al.,* 2010).

Probiotic lactobacilli can modulate immune responses by signalling through NF-κB and mitogen-activated protein kinase (MAPK) pathways (Van Baarlen *et al.*, 2009). Of note, live *Lactobacillus rhamnosus* increases activation of NF-κB in human bladder cells but decreases production of TNF-α, IL-6 and CXCL8 (Karlsson *et al.*, 2012). In the present study, we show that activation of NF-κB triggered by *B. cereus* in HT-29 cells increased in the presence of *L. delbrueckii* subsp. *lactis* CIDCA 133 but with a decrease of IL-8 production. These findings emphasise the relevance of alternative transcription factors other than NF-κB (e.g. MAPK) and in addition the effect of post-transcriptional regulation of NF-κB. Furthermore, effect of extracellular factors produced by *L. delbrueckii* subsp. *lactis* CIDCA 133 cannot be ruled out (Garrote *et al.*, 2015; Iraporda *et al.*, 2015; Karlsson *et al.,* 2012; Tanaka *et al.*, 2015).

Co-cultures of epithelial and DC allow for the study of the cross talk between cell populations relevant for the host's response and pathogenic or beneficial microorganisms. Since early studies on the immune response elicited by probiotic microorganisms (Haller *et al.*, 2000) this *in vitro* model has demonstrated to be useful for the study

of the interaction of microorganisms in the context of intestinal infections (Fang *et al.*, 2010; Haller *et al.*, 2000; Pozo-Rubio *et al.*, 2011). This experimental approach allowed the demonstration of the immunomodulatory effect of *L. rhamnosus,* which decreases HLA-DR and CD86 expression marker on DC in a LPS-stimulated co-culture system (Grompone *et al.*, 2012). In contrast, other *Lactobacillus* strains induced HLA-DR and CD86 expression in DC from human origin (Mohamadzadeh *et al.*, 2005). Interestingly, other potentially probiotic strains modified the expression of MHCII, CD86 and CD40 and the production of inflammatory cytokines (IL-6 and TNF-α) by murine DC depending on the presence of epithelial cells in normal and inverted co-culture systems (Kim *et al.*, 2012b).

Presence of *B. cereus* or *L. delbrueckii* subsp. *lactis* CIDCA 133 alone, lead to stimulation of CD86 in the absence of LPS. This suggests a signalling pathway other than Toll-like receptor (TLR)-4-mediated. The presence of lactobacilli does not modifies CD86 expression. It is worth to note that epithelial cells (HT-29) in the upper compartment of co-cultures, were stimulated at the apical domain and that translocation of *B. cereus* to the lower compartment was reduced by the presence of *L. delbrueckii* subsp. *lactis* CIDCA 133.

In spite that number of DC associated to *B. cereus* in the lower compartment significantly decreases when epithelial cells at the upper compartment were incubated with both lactobacilli and *B. cereus*, there were no changes in CD86 expression in DC. These findings support the hypothesis that stimulation of epithelial cells in the upper compartment generates a cross-talk with DC of the lower compartment through soluble factors.

Concerning functional maturation, determination of cytokines demonstrates that both strains were able to induce IL-8 and TNF-α production by DC but only *B. cereus* was able to trigger IL-6 production. Interestingly, our results showed that whereas presence of *L. delbrueckii* subsp. *lactis* CIDCA 133 did not modify the production of IL-8 nor IL-6 by *B. cereus*-infected DC, this *Lactobacillus* significantly increased TNF-α production. The ability of lactobacilli to trigger TNF-α production by macrophages through TLR-2 signalling has been demonstrated (Ditu *et al.*, 2014; Matsuguchi *et al.*, 2003). In addition, spent culture supernatants of *B. cereus* induce TNF-α production in holoxenic mice (Ditu *et al.*, 2014). Furthermore, in a murine model of infection (Rolny *et al.*, 2014), mice infected with *B. cereus* strain B10502 show slight increases in expression of genes associated to TNF-α production. In the present work, the addition of *L. delbrueckii* subsp. *lactis* CIDCA 133 potentiates TNF-α production by DC in the lower compartment of a co-culture system with *B. cereus*-infected epithelial cells. Although TNF-α is a pro-inflammatory cytokine, it has been shown to play a role in the containment of *B. cereus* in a murine model of intraocular infection. Production of TNF-α at early stages of infection leads to the recruitment of immune cells that in turn contribute to the control of pathogen multiplication (Ramadan *et al.*, 2008).

Surprisingly, strain 133 was unable to induce neither IL-10 nor IL-12. Induction of these interleukins is a typical biological response following interaction between probiotics and host's cells. This strain-dependent response is related to differential activation of the ERK pathway by different bacterial components such as CpG motifs and cell wall components (Kaji *et al.*, 2010). Strong inducers of the ERK pathway lead to high levels of IL-10 and low levels of IL-12 whereas those bacteria that shows a weak ability to induce ERK pathway lead to high values of IL-12 and low values of IL-10. However, other signalling pathways are involved in the production of IL-10/IL-12 and some strains that share many common agonists (e.g. lypoteichoic acids) are unable to induce these interleukins. Interestingly, these microorganisms have shown faster intracellular digestion by macrophages (Kaji *et al.*, 2010).

The present work demonstrates for the first time the effect of *L. delbrueckii* subsp. *lactis* CIDCA 133, a potentially probiotic strain, in an *in vitro* model of *B. cereus* infection. Our results showed that the presence of the lactobacilli lead to the modulation of the cell response of infected cells and an enhancement of previously demonstrated protective response such as TNF-α production by DC.

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References

Ashraf, R. and Shah, N.P., 2014. Immune system stimulation by probiotic microorganisms. Critical Reviews in Food Science and Nutrition 54: 938-956.

Baldwin, A.S.J., 1996. The NF-κB and IκB proteins: new discoveries and insights. Annual Review of Immunology 14: 649-681.

Bottone, E.J., 2010. *Bacillus cereus*, a volatile human pathogen. Clinical Microbiology Reviews 23: 382-398.

Buchanan, R.L. and Schultz, F.J., 1992. Evaluation of the oxoid BCET-RPLA kit for the detection of *Bacillus cereus* diarrheal enterotoxin as compared to cell culture cytotonicity. Journal of Food Protection 55: 440-443.

Canny, G., Swidsinski, A. and McCormick, B.A., 2006. Interactions of intestinal epithelial cells with bacteria and immune cells: methods to characterize microflora and functional consequences. Methods in Molecular Biology 341: 17-35.

Carasi, P., Racedo, S.M., Jacquot, C., Romanin, D.E., Serradell, M.A. and Urdaci, M.C., 2015. Impact of kefir derived *Lactobacillus kefiri* on the mucosal immune response and gut microbiota. Journal of Immunological Research 2015: 361604.

Cerf-Bensussan, N. and Gaboriau-Routhiau, V., 2010. The immune system and the gut microbiota: friends or foes? Nature Reviews Immunology 10: 735-744.

Chen, K., 2013. *Lactobacillus acidophilus* strain suppresses the transcription of proinflammatory-related factors in human HT-29 cells. Journal of Microbiology and Biotechnology 23: 64-68.

Ditu, L.M., Chifiriuc, M.C., Bezirtzoglou, E., Marutescu, L., Bleotu, C., Pelinescu, D., Mihaescu, G. and Lazar, V., 2014. Immunomodulatory effect of non-viable components of probiotic culture stimulated with heat-inactivated *Escherichia coli* and *Bacillus cereus* on holoxenic mice. Microbial Ecology in Health and Disease 2014: 25.

Dogi, C.A., Weill, F. and Perdigón, G., 2010. Immune response of non-pathogenic Gram(+) and Gram(–) bacteria in inductive sites of the intestinal mucosa: study of the pathway of signaling involved. Immunobiology 215: 60-69.

Edwards, L.A., Bajaj-Elliott, M., Klein, N.J., Murch, S.H. and Phillips, A.D., 2011. Bacterial-epithelial contact is a key determinant of host innate immune responses to enteropathogenic and enteroaggregative *Escherichia coli*. PloS One 6: e27030.

Fang, H.W., Fang, S.B., Chiang Chiau, J.S., Yeung, C.Y., Chan, W.T., Jiang, C.B., Cheng, M.L. and Lee, H.C., 2010. Inhibitory effects of *Lactobacillus casei* subsp. *rhamnosus* on *Salmonella* lipopolysaccharide-induced inflammation and epithelial barrier dysfunction in a co-culture model using Caco-2/peripheral blood mononuclear cells. Journal of Medical Microbiology 59: 573-579.

Franco, M.C., Golowczyc, M.A., De Antoni, G.L., Perez, P.F., Humen, M. and Serradell Mde, L., 2013. Administration of kefir-fermented milk protects mice against Giardia intestinalis infection. Journal of Medical Microbiology 62: 1815-1822.

Galdeano, C.M. and Perdigón, G., 2006. The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. Clinical and Vaccine Immunology 13: 219-226.

Garrote, G.L., Abraham, A.G. and Rumbo, M., 2015. Is lactate an undervalued functional component of fermented food products? Frontiers in Microbiology 6: 629.

Gohar, M., Okstad, O.A., Gilois, N., Sanchis, V., Kolsto, A.B. and Lereclus, D., 2002. Two-dimensional electrophoresis analysis of the extracellular proteome of *Bacillus cereus* reveals the importance of the PlcR regulon. Proteomics 2: 784-791.

Grompone, G., Martorell, P., Llopis, S., Gonzalez, N., Genoves, S., Mulet, A.P., Fernandez-Calero, T., Tiscornia, I., Bollati-Fogolin, M., Chambaud, I., Foligne, B., Montserrat, A. and Ramon, D., 2012. Anti-inflammatory *Lactobacillus rhamnosus* CNCM I-3690 strain protects against oxidative stress and increases lifespan in *Caenorhabditis elegans*. PloS One 7: e52493.

Guimarães, F.S.F., Andrade, L.F., Martins, S.T., Abud, A.P.R., Sene, R.V., Wanderer, C., Tiscornia, I., Bollati-Fogolín, M., Buchi, D.F. and Trindade, E.S., 2010. *In vitro* and *in vivo* anticancer properties of a Calcarea carbonica derivative complex (M8) treatment in a murine melanoma model. BMC Cancer 10: 113.

Haller, D., Bode, C., Hammes, W.P., Pfeifer, A.M., Schiffrin, E.J. and Blum, S., 2000. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut 47: 79-87.

Hiramatsu, Y., Satho, T., Irie, K., Shiimura, S., Okuno, T., Sharmin, T., Uyeda, S., Fukumitsu, Y., Nakashima, Y., Miake, F. and Kashige, N., 2013. Differences in TLR9-dependent inhibitory effects of H_2O_2-induced IL-8 secretion and NF-kappa B/I kappa B-alpha system activation by genomic DNA from five *Lactobacillus* species. Microbes and Infection 15: 96-104.

Hua, M.-C., Lin, T.-Y., Lai, M.-W., Kong, M.-S., Chang, H.-J. and Chen, C.-C., 2010. Probiotic bio-three induces Th1 and anti-inflammatory effects in PBMC and dendritic cells. World Journal of Gastroenterology 16: 3529-3540.

Huang, I.F., Lin, I.C., Liu, P.F., Cheng, M.F., Liu, Y.C., Hsieh, Y.D., Chen, J.J., Chen, C.L., Chang, H.W. and Shu, C.W., 2015. *Lactobacillus acidophilus* attenuates *Salmonella*-induced intestinal inflammation via TGF-β signaling. BMC Microbiology 15: 203.

Hugo, A.A., De Antoni, G.L. and Pérez, P.F., 2006. *Lactobacillus delbrueckii subsp lactis* strain CIDCA 133 inhibits nitrate reductase activity of *Escherichia coli*. International Journal of Food Microbiology 111: 191-196.

Hugo, A.A., De Antoni, G.L. and Pérez, P.F., 2010. *Lactobacillus delbrueckii subsp lactis* (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like Caco-2 cells. Letters in Applied Microbiology 50: 335-340.

Hugo, A.A., Kakisu, E., De Antoni, G.L. and Pérez, P.F., 2008. Lactobacilli antagonize biological effects of enterohaemorrhagic *Escherichia coli in vitro*. Letters in Applied Microbiology 46: 613-619.

Imaoka, A., Shima, T., Kato, K., Mizuno, S., Uehara, T., Matsumoto, S., Setoyama, H., Hara, T. and Umesaki, Y., 2008. Anti-inflammatory activity of probiotic Bifidobacterium: enhancement of IL-10 production in peripheral blood mononuclear cells from ulcerative colitis patients and inhibition of IL-8 secretion in HT-29 cells. World Journal of Gastroenterology 14: 2511-2516.

Iraporda, C., Errea, A., Romanin, D.E., Cayet, D., Pereyra, E., Pignataro, O., Sirard, J.C., Garrote, G.L., Abraham, A.G. and Rumbo, M., 2015. Lactate and short chain fatty acids produced by microbial fermentation downregulate proinflammatory responses in intestinal epithelial cells and myeloid cells. Immunobiology 220: 1161-1169.

Jeon, J.H., Lee, H.R., Cho, M.H., Park, O.K., Park, J. and Rhie, G.E. 2015. The poly-γ-d-glutamic acid capsule surrogate of the *Bacillus anthracis* capsule is a novel Toll-like receptor 2 agonist. Infection and Immunity 83: 3847-3856.

Jung, H.C., Eckmann, L., Yang, S.K., Panja, A., Fierer, J., Morzycka-Wroblewska, E. and Kagnoff, M.F., 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. Journal of Clinical Investigation 95: 55-65.

Jung, T.H., Park, J.H., Jeon, W.M. and Han, K.S., 2015. Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway. Nutrition Research and Practice 9: 343-349.

Kaji, R., Kiyoshima-Shibata, J., Nagaoka, M., Nanno, M. and Shida, K., 2010. Bacterial teichoic acids reverse predominant IL-12 production induced by certain lactobacillus strains into predominant IL-10 production via TLR2-dependent ERK activation in macrophages. Journal of Immunology 184: 3505-3513.

Kang, H. and Im, S., 2015. Probiotics as an immune modulator. Journal of Nutritional Science and Vitaminology 61: 103-105.

Karlsson, M., Scherbak, N., Reid, G. and Jass, J., 2012. *Lactobacillus rhamnosus* GR-1 enhances NF-kappaB activation in *Escherichia coli*-stimulated urinary bladder cells through TLR4. BMC Microbiology 12: 15.

Kechaou, N., Chain, F., Gratadoux, J.J., Blugeon, S., Bertho, N., Chevalier, C., Le Goffic, R., Courau, S., Molimard, P., Chatel, J.M., Langella, P. and Bermudez-Humaran, L.G., 2013. Identification of one novel candidate probiotic *Lactobacillus plantarum* strain active against influenza virus infection in mice by a large-scale screening. Applied and Environmental Microbiology 79: 1491-1499.

Klingspor, S., Bondzio, A., Martens, H., Aschenbach, J.R., Bratz, K., Tedin, K., Einspanier, R. and Lodemann, U., 2015. *Enterococcus faecium* NCIMB 10415 modulates epithelial integrity, heat shock protein, and proinflammatory cytokine response in intestinal cells. Mediators of Inflammation 2015: 304149

Kim, H., Jung, B.J., Jung, J.H., Kim, J.Y., Chung, S.K. and Chung, D.K., 2012a. *Lactobacillus plantarum* lipoteichoic acid alleviates TNF-alpha-induced inflammation in the HT-29 intestinal epithelial cell line. Molecules and Cells 33: 479-486.

Kim, J.Y., Park, M.S. and Ji, G.E., 2012b. Probiotic modulation of dendritic cells co-cultured with intestinal epithelial cells. World Journal of Gastroenterology 18: 1308-1318.

Kociubinski, G., Perez, P. and De Antoni, G., 1999. Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. Journal of Food Protection 62: 905-912.

Kociubinski, G.L., Pérez, P.F., Añón, M.C. and De Antoni, G.L., 1996. A method of screening for highly inhibitory lactic acid bacteria. Journal of Food Protection 59: 739-745.

Lammers, K.M., Helwig, U., Swennen, E., Rizzello, F., Venturi, A., Caramelli, E., Kamm, M.A., Brigidi, P., Gionchetti, P. and Campieri, M., 2002. Effect of probiotic strains on interleukin 8 production by HT29/19A cells. American Journal of Gastroenterology 97: 1182-1186.

Linares, D.M., Ross, P. and Stanton, C., 2015. Beneficial microbes: the pharmacy in the gut. Bioengineered 28: 1-28.

Macpherson, A.J. and Uhr, T., 2004. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303: 1662-1665.

Marchesi, J.R., Adams, D.H., Fava, F., Hermes, G.D., Hirschfield, G.M., Hold, G., Quraishi, M.N., Kinross, J., Smidt, H., Tuohy, K.M., Thomas, L.V., Zoetendal, E.G. and Hart, A., 2015. The gut microbiota and host health: a new clinical frontier. Gut 65: 330-339.

Martin, R., Miquel, S., Ulmer, J., Langella, P. and Bermudez-Humaran, L.G., 2014. Gut ecosystem: how microbes help us. Beneficial Microbes 5: 219-233.

Mastropietro, G., Tiscornia, I., Perelmuter, K., Astrada, S. and Bollati-Fogolín, M., 2015. HT-29 and Caco-2 reporter cell lines for functional studies of nuclear factor kappa B activation. Mediators of Inflammation 2015: 860534.

Matsuguchi, T., Takagi, A., Matsuzaki, T., Nagaoka, M., Ishikawa, K., Yokokura, T. and Yoshikai, Y., 2003. Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. Clinical and Diagnostic Laboratory Immunology 10: 259-266.

Mileti, E., Matteoli, G., Iliev, I.D. and Rescigno, M., 2009. Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for *in vivo* efficacy. PloS One 4: e7056.

Minnaard, J., Delfederico, L., Vasseur, V., Hollmann, A., Rolny, I., Semorile, L. and Perez, P.F., 2007. Virulence of *Bacillus cereus*: a multivariate analysis. International Journal of Food Microbiology 116: 197-206.

Minnaard, J., Humen, M. and Perez, P.F., 2001. Effect of *Bacillus cereus* exocellular factors on human intestinal epithelial cells. Journal of Food Protection 64: 1535-1541.

Minnaard, J., Lievin-Le Moal, V., Coconnier, M.H., Servin, A.L. and Perez, P.F., 2004. Disassembly of F-actin cytoskeleton after interaction of *Bacillus cereus* with fully differentiated human intestinal Caco-2 cells. Infection and Immunity 72: 3106-3112.

Minnaard, J., Rolny, I.S. and Perez, P.F., 2013. Interaction between *Bacillus cereus* and cultured human enterocytes: effect of calcium, cell differentiation, and bacterial extracellular factors. Journal of Food Protection 76: 820-826.

Mizoguchi, E., Hachiya, Y., Kawada, M., Nagatani, K., Ogawa, A., Sugimoto, K., Mizoguchi, A. and Podolsky, D.K., 2008. TNF receptor Type I-dependent activation of innate responses to reduce intestinal damage-associated mortality. Gastroenterology 134: 470-480.

Mohamadzadeh, M., Olson, S., Kalina, W.V., Ruthel, G., Demmin, G.L., Warfield, K.L., Bavari, S. and Klaenhammer, T.R., 2005. Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. Proceedings of the National Academy of Sciences of the USA 102: 2880-2885.

O'Hara, A.M., O'Regan, P., Fanning, A., O'Mahony, C., Macsharry, J., Lyons, A., Bienenstock, J., O'Mahony, L. and Shanahan, F., 2006. Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. Immunology 118: 202-215.

Pagnini, C., Saeed, R., Bamias, G., Arseneau, K.O., Pizarro, T.T. and Cominelli, F., 2010. Probiotics promote gut health through stimulation of epithelial innate immunity. Proceedings of the National Academy of Sciences of the USA 107: 454-459.

Papadakos, K.S., Sougleri, I.S., Mentis, A.F., Hatziloukas, E. and Sgouras, D.N., 2013. Presence of terminal EPIYA phosphorylation motifs in *Helicobacter pylori* CagA contributes to IL-8 secretion, irrespective of the number of repeats. PloS One 8: e56291.

Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.Y., 2006. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology 100: 1171-1185.

Pozo-Rubio, T., Mujico, J.R., Marcos, A., Puertollano, E., Nadal, I., Sanz, Y. and Nova, E., 2011. Immunostimulatory effect of faecal *Bifidobacterium* species of breast-fed and formula-fed infants in a peripheral blood mononuclear cell/Caco-2 co-culture system. British Journal of Nutrition 106: 1216-1223.

Ramadan, R.T., Moyer, A.L. and Callegan, M.C., 2008. A role for tumor necrosis factor-alpha in experimental *Bacillus cereus* endophthalmitis pathogenesis. Investigative Ophthalmology and Visual Science 49: 4482-4489.

Rescigno, M., 2010. Intestinal dendritic cells. Advances in Immunology 107: 109-138.

Riedel, C.U., Foata, F., Goldstein, D.R., Blum, S. and Eikmanns, B.J., 2006. Interaction of bifidobacteria with Caco-2 cells – adhesion and impact on expression profiles. International Journal of Food Microbiology 110: 62-68.

Roberts-Thomson, I.C., Fon, J., Uylaki, W., Cummins, A.G. and Barry, S., 2011. Cells, cytokines and inflammatory bowel disease: a clinical perspective. Expert Reviews on Gastroenterology and Hepatology 5: 703-716.

Rolny, I.S., Minnaard, J., Racedo, S.M. and Perez, P.F., 2014. Murine model of *Bacillus cereus* gastrointestinal infection. Journal of Medical Microbiology 63: 1741-1749.

Romanin, D., Serradell, M., Gonzalez Maciel, D., Lausada, N., Garrote, G.L. and Rumbo, M., 2010. Down-regulation of intestinal epithelial innate response by probiotic yeasts isolated from kefir. International Journal of Food Microbiology 140: 102-108.

Rowan, N.J., Deans, K., Anderson, J.G., Gemmell, C.G., Hunter, I.S. and Chaithong, T., 2001. Putative virulence factor expression by clinical and food isolates of *Bacillus* spp. after growth in reconstituted infant milk formulae. Applied and Environmental Microbiology 67: 3873-3881.

Scaldaferri, F., Pizzoferrato, M., Gerardi, V., Lopetuso, L. and Gasbarrini, A., 2012. The gut barrier: new acquisitions and therapeutic approaches. Journal of Clinical Gastroenterology 46: 12-17.

Schulte, R., Wattiau, P., Hartland, E.L., Robins-Browne, R.M. and Cornelis, G.R., 1996. Differential secretion of interleukin-8 by human epithelial cell lines upon entry of virulent or nonvirulent *Yersinia enterocolitica*. Infection and Immunity 64: 2106-2113.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L.G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottière, H.M., Doré, J., Marteau, P., Seksik, P. and Langella, P., 2008. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proceedings of the National Academy of Sciences of the USA 105: 16731-16736.

Tanaka, K., Fujiya, M., Konishi, H., Ueno, N., Kashima, S., Sasajima, J., Moriichi, K., Ikuta, K., Tanabe, H. and Kohgo, Y., 2015. Probiotic-derived polyphosphate improves the intestinal barrier function through the caveolin-dependent endocytic pathway. Biochemical and Biophysical Research Communications 467: 541-548.

Tergaonkar, V., 2006. NFκB pathway: a good signaling paradigm and therapeutic target. International Journal of Biochemistry and Cell Biology 38: 1647-1653.

Tiscornia, I., Sanchez-Martins, V., Hernandez, A. and Bollati-Fogolin, M., 2012. Human monocyte-derived dendritic cells from leukoreduction system chambers after plateletpheresis are functional in an *in vitro* co-culture assay with intestinal epithelial cells. Journal of Immunological Methods 384: 164-170.

Tojo, R., Suarez, A., Clemente, M.G., De los Reyes-Gavilan, C.G., Margolles, A., Gueimonde, M. and Ruas-Madiedo, P., 2014. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. World Journal of Gastroenterology 20: 15163-15176.

Torres-Maravilla, E., Lenoir, M., Mayorga-Reyes, L., Allain, T., Sokol, H., Langella, P., Sanchez-Pardo, M.E. and Bermudez-Humaran, L.G., 2015. Identification of novel anti-inflammatory probiotic strains isolated from pulque. Applied Microbiology and Biotechnology.

Van Baarlen, P., Troost, F.J., Van Hemert, S., Van der Meer, C., De Vos, W.M., De Groot, P.J., Hooiveld, G.J., Brummer, R.J. and Kleerebezem, M., 2009. Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. Proceedings of the National Academy of Sciences of the USA 106: 2371-2376.

Viggiano, D., Ianiro, G., Vanella, G., Bibbò, S., Bruno, G., Simeone, G. and Mele, G., 2015. Gut barrier in health and disease: focus on childhood. European Review for Medical and Pharmacological Sciences 19: 1077-1085.

Zeuthen, L.H., Christensen, H.R. and Frøkiær, H., 2006. Lactic acid bacteria inducing a weak Interleukin-12 and tumor necrosis factor alpha response in human dendritic cells inhibit strongly stimulating lactic acid bacteria but act synergistically with gram-negative bacteria. Clinical and Vaccine Immunology 13: 365-375.