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## Differential effect of *Bifidobacterium* species on cytokine production in a Caco-2/PBMC co-culture system

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*Bifidobacterium* species present in the gut microbiota have shown capacity to modulate cytokine production by intestinal epithelial cells, monocyte derived dendritic cells and peripheral blood mononuclear cells (PBMC) in *in vitro* experiments<sup>(1,2)</sup>. These findings may confer them the potential to be used as probiotics in the prevention and treatment of pathologies with underlying immune alterations, such as inflammatory bowel diseases, allergy and celiac disease.

The aim of this study was to evaluate the effect of different bifidobacterial species (*Bifidobacterium adolescentis* ATCC15703; *Bifidobacterium angulatum* ATCC27535; *Bifidobacterium breve* ATCC15700; *Bifidobacterium catenulatum* LMG; *Bifidobacterium longum* biovar infantis LMG 11046T; *B. longum* biovar longum ATCC15707) on cytokine production in a Caco-2/PBMC co-culture system. Caco-2 monolayers were grown on 12 mm inserts (1 µm pore size) in 24-well cell culture plate assemblies (Millipore) and challenged by apical addition of  $4 \times 10^6$  cfu/insert of a *Bifidobacterium* strain or a combination of strains corresponding to the species composition in faecal samples from breast fed (BF) and formula fed (FF) infants<sup>(3)</sup>, respectively. PBMCs isolated from healthy adult donors were added in the basal compartment of the culture well ( $1 \times 10^6$  cells/ml) for a 12-hour incubation. Thereafter, a further 36-h incubation was allowed after disassembly of the system in order to measure the cytokine production by the sensitised Caco-2 and PBMCs separately. IL-8 was measured in Caco-2 cells' basolateral medium by ELISA and IFN-γ was measured in the PBMCs supernatant by high sensitivity Immunoassay Xmap Technology (Linco). Samples from 5 volunteers and duplicates of each experimental condition were used. Mann-Whitney test was employed for statistical analysis. *B. breve*, *B. longum* and the BF mixture induced a higher IL-8 production by Caco-2 cells than *B. infantis* and FF mixture. The rest of the *Bifidobacterium* species assayed showed intermediate levels and no differences with other strains were observed. A correlation between IL-8 production stimulated by *B. breve* and by BF was found, which can be explained by the singularly high relative presence of *B. breve* in the BF combination (22%) compared to the FF mixture (7%). The production of IFN-γ by PBMCs was low in this system (range: 1–93 pg/ml and under the detection limit for one donor) and the effect of each strain seemed donor dependent. However, 3 donors showed stimulation of IFN-γ with BF mixture and 2 of them also with *B. breve*. Significant differences between IFN-γ production induced by BF mixture ( $349 \pm 161$  % control; Mean  $\pm$  SE) and *B. adolescentis* ( $-16 \pm 14$  % control) were observed. The results suggest that *B. breve* is the most stimulatory strain and more so when combined with other *Bifidobacterium* strains, probably indicating synergistic effects. The presence or absence of particular bifidobacterial species as well as the overall composition of the bifidobacterial population in the infant gut could be key factors defining the immunomodulator effect of the gut microbiota in early life.

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