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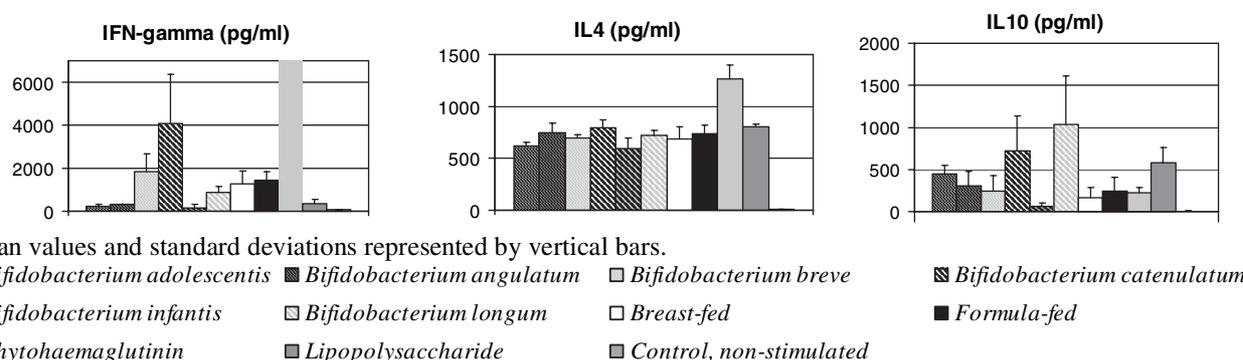
## Differential effect of *Bifidobacterium* species characteristic of the gut microbiota of breast-fed and formula-fed infants on *in vitro* cytokine production

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The importance of the bacterial colonization process of the newborn intestine in the maturation of the immune system has been recognized. The presence of a healthy microflora (adequate composition of species and their frequency) in the gut is thought to play a role in the balance of T-helper (Th) 1–Th2 immune responses. Some differences have been observed in the composition of bifidobacterial species in relation to the type of milk fed. While *B. breve* is one of the predominant species of the gut microbiota of breast-fed (BF) babies, *B. catenulatum* and *B. adolescentis* are characteristic of that of formula-fed (FF) infants<sup>(1)</sup>. The aim of the current study was to assess the differential effect of the bifidobacterial species identified in the intestinal microbiota of BF and FF infants on cytokine production by peripheral blood mononuclear cells (PBMC). The effects of different bifidobacterial species were assessed individually and in combinations representing their proportions in infants under both feeding types. Live bacterial cell suspensions (10<sup>7</sup> colony-forming units/ml) were incubated with PBMC in the proportion 10:1 for 48 h (5% CO<sub>2</sub>, 37°C) and cytokine concentrations were measured in the supernatant fraction by flow cytometry (CBA; BD Biosciences, Madrid, Spain). The experiments were performed with blood from four volunteers and duplicates were done of each experimental condition and analysed separately. Statistical analyses were carried out using ANOVA and Mann-Whitney tests. Results for representative cytokines of Th1 and Th2 responses (interferon-γ (IFN-γ) and IL-4 respectively) and the anti-inflammatory cytokine IL-10 are presented. Significant differences were found among different bifidobacterial species for the induction of IFN-γ and IL-10 production ( $P < 0.001$  and  $P = 0.003$ ). *B. catenulatum* was the strongest enhancer of IFN-γ production, followed by *B. breve*. *B. catenulatum* is more frequent in FF infants and *B. breve* is more frequent in BF infants<sup>(1)</sup>, but no differences were found in the induction of IFN-γ production by BF and FF mixtures of bifidobacteria used. *B. catenulatum* also induced significantly higher levels of IL-4 than *B. adolescentis* and *B. infantis* ( $P = 0.029$ ). *B. infantis* was a mild cytokine inducer, showing the lowest effect among the assayed species for all three cytokines. *B. longum*, which is the third most frequent bifidobacterial species in infant flora of both BF and FF infants, showed the strongest IL-10-inducing capacity. The effects of BF and FF bifidobacterial species combinations on cytokine production were not significantly different.



These results suggest that 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 immunomodulatory effect of the gut microbiota in early life.

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1. Haarman M & Knol J (2005) *Appl Environ Microbiol* 71, 2318–2324.