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Intestinal Bifidobacterium species induce varying cytokine production

To the Editor:

Bifidobacteria are among the predominant bacteria in the human intestinal microbiota; they colonize the neonatal intestine from the first days after birth and inhabit the gastrointestinal tract throughout life. They also act as important determinants influencing the development of the human immune system. An aberrant intestinal microflora, especially reduced numbers of fecal bifidobacteria and high numbers of fecal clostridia, has been reported as one of the important events related to allergic disease.^{1,2} Macrophages are tissue-based phagocytic cells that play an important role in innate and adaptive immune responses.

To assess the differences in cytokine secretion induced by bifidobacteria strains isolated from dairy foods, feces of allergic and healthy infants, and feces of healthy adults, murine macrophage-like cell line J774.1 was cultured in the presence of heat-inactivated cells of 27 strains of bifidobacteria. The concentration of bifidobacteria before inactivation was 108 CFU/mL, and 100 μ L was added to each incubation well. After 24 hours of incubation, the concentrations of IL-1, IL-6, IL-10, IL-12, and TNF- in the supernatant were analyzed through use of commercial cytokine ELISA kits.

The tested bifidobacteria stimulated cytokine production by J774.1 cells in a strain-specific manner. Bifidobacteria from allergic infants induced more IL-1, IL-6, IL-12, and TNF- secretion by J774.1 cells than did those isolated from healthy adults and infants and from dairy foods (Table I).

Table I. Cytokine secretion by murine macrophage-like J774.1 cells after exposure to bifidobacteria from dairy foods, healthy adults, healthy infants, or allergic infants

Origin	No. of strains tested	Cytokine production (pg/mL)				
		TNF- α	IL-1 β	IL-6	IL-10	IL-12
Dairy food	6	16005 $\pm$ 3719	4.4 $\pm$ 4.8	64160 $\pm$ 35622	25.8 $\pm$ 24.8	7780 $\pm$ 4752
Healthy adult	4	13626 $\pm$ 2943	0.9 $\pm$ 1.5	58034 $\pm$ 25222	13.6 $\pm$ 12.3	6284 $\pm$ 1611
Healthy infant	6	21062 $\pm$ 1650	7.8 $\pm$ 6.2	103603 $\pm$ 14505	21.8 $\pm$ 4.9	12221 $\pm$ 3663
Allergic infant	11	37634 $\pm$ 4508*	8.2 $\pm$ 1.7*	138171 $\pm$ 45934*	5.9 $\pm$ 12.9†	22989 $\pm$ 479*

Each value is a mean $\pm$ SD for at least 3 assays.

* $P < .05$.

† $P < .01$.

No significant differences were found among the bifidobacteria isolated from the feces of healthy adults, the feces of healthy infants, and dairy foods. Bifidobacteria from allergic infants stimulated less IL-10 secretion by J774.1 cells than did those from healthy adults, those from healthy infants, and those from dairy foods. Among the bifidobacteria from allergic infants, 8 strains did not induce IL-10 production by J774.1 cells.

Recent clinical and epidemiologic studies have indicated a higher incidence of allergy among children who have a smaller enteric population of gram-positive bacteria—in particular, of

lactobacilli and bifidobacteria. This supports a role for gut-colonizing bacteria in regulating atopy.^{1,2,4} Kalliomäki et al¹ have shown that infants with low numbers of bifidobacteria at 3 weeks of age and at 3 months of age have a increased risk of developing atopic disease later in life.

In addition to quantitative changes in the bifidobacteria flora, an alteration in the species composition of the bifidobacteria flora was recently observed in allergic infants.⁵ We have shown that allergic infants have mostly *B adolescentis*, a common resident in the adult intestine, in their fecal flora.⁵ Bifidobacteria from allergic infants have been reported to adhere less to human intestinal mucus than those from healthy infants.⁶ The present study extended these findings to functional alterations of bifidobacteria in allergic infants: 27 strains of bifidobacteria were tested for their ability to induce the production of cytokines from the macrophage-like cell line J774.1. The tested bifidobacteria differentially triggered proinflammatory TNF-, IL-1, and IL-6 production by J774.1 cells, indicating that specific strains activated macrophages. Our result is consistent with a previously published finding that heat-inactivated bifidobacteria markedly induced TNF-, IL-1, and IL-6 production in another murine macrophage model.⁷

Proinflammatory TNF- secretion caused by members of the microflora can influence the development of atopic disease. This is enhanced if the anti-inflammatory IL-10 secretion is reduced, as was observed, to counterregulate the TH2 responder phenotype. Thus, allergic infants might have a bifidobacteria microflora that can promote proinflammatory cytokine secretion, predisposing toward the expression of atopic disease.

Taken together, the results suggest that the cytokine induction by bifidobacteria is a strain-dependent property. A bifidobacteria flora from allergic infants that is dominated by *B adolescentis* might be more potent to amplify but less able to downregulate the intestinal inflammatory responses. Our results also suggest that a specific bifidobacteria microbiota is related to a different immunologic status. Thus, early development of the right type of intestinal microflora and the correct modification of the microflora composition might be important factors influencing the inflammatory processes in the gut.

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