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Effect of Bifidobacteria on Production of Allergy-Related Cytokines from Mouse Spleen Cells

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Abstract To study the effect of bifidobacteria on preventing allergy response, levels of IFN-γ, IgG2a, IL-4, and IgG1 were investigated in splenocytes isolated from ovalbumin (OVA)-sensitized allergic mice and BGN4-administered allergy-suppressed mice in the presence of various bifidobacterial strains. Most of the bifidobacteria, except 2A, increased production of Th1-associated immune markers, IFN-γ and IgG2a. In addition, most of the bifidobacteria, except 2A and 19A, decreased production of IL-4, whereas the differences in the production of IgG1 were less pronounced. These results suggest that some strains of bifidobacteria may have the potential to prevent the occurrence of allergy by switching Th1/Th2-type antibodies and/or related cytokines.

Key words: Allergy, *Bifidobacterium*, interferon-γ, interleukin-4, immunoglobulin IgG1, immunoglobulin IgG2a

Allergy, in the form of atopic diseases such as atopic eczema, allergic rhinitis, and asthma, has been increasing in well-developed countries [5]. The Centers for Disease Control and Prevention (CDC) of the U.S.A. estimated 16 million (7.5%) U.S. adults with asthma [3]. The International Study of Asthma and Allergies in Childhood investigated 11,607 Finnish children aged 13–14 years; 10–20% of the children had symptoms of asthma, 15–23% had allergic rhinitis, and 15–19% had atopic eczema [13, 14]. In the Republic of Korea, the prevalence of the symptoms of asthma, rhiniconjunctivitis, and flexural eczema were 8.7%, 10.5%, 7.3% in 6–12-yr-olds, and 8.2%, 10.0%, 3.9% in 12–15-yr-olds, respectively [9].

Differentiation of T-helper (CD4+) cells into two subsets, Th1 and Th2, each with a characteristic profile of cytokine production, is central to the understanding of the pathogenic mechanisms of allergy. Th1 cells produce IFN-γ, IL-2, and tumor necrosis factor-β, whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10. The balance of the two types of cells is considered to be important to maintain homeostasis of the host immune system. Once this balance becomes disturbed, various immunological diseases, such as allergies and infections, can occur due to the evasion of host defense mechanisms. Recently, some probiotic strains were reported to alter the balance of immune cell types and their cytokines. Shida *et al.* [15]. showed that *Lactobacillus casei* induced IFN-γ, but inhibited IL-4 and IL-5 secretion, and markedly suppressed total and antigen-specific IgE secretion by ovalbumin (OVA)-stimulated splenocytes. The production of Th1 cell-associated cytokines, IFN-γ and IL-2, by the spleen cells was higher than that by the spleen cells from the control group in the mice fed *L. casei* strain Shirota [12]. These results suggest that some strains of probiotic bacteria may be able to switch the balance of T-helper cells from Th2 to Th1.

Fang *et al.* [4] showed that there were differences in *Bifidobacterium* strains isolated from allergic and healthy infants. Allergic infants were found to have an adult type *Bifidobacerium* flora with a high level of *B. adolecentis*, while the healthy infants had a typical infant *Bifidobacterium* flora with high levels of *B. bifidum* and *B. infantis*. Furthermore, infants with food allergies have been reported to have a disturbed balance between beneficial and potentially harmful bacteria in the large intestine [1, 8], and the development of an aberrant microbial composition in the gut, such as inadequate bifidobacterial biota, is reported to deprive the developing immune system from counter-regulatory signals against Th2-mediated allergic responses [6]. In this context, supplementation of *Bifidobacterium* strains to human hosts may bear some relevance to preventing allergic reactions. The present study was conducted to

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investigate the effect of various *Bifidobacterium* strains on the patterns of T-helper cell-associated antibodies and cytokines using mice spleen cells *in vitro*.

MATERIALS AND METHODS

Mice

Three-week-old C3H/HeJ female mice, weighing 11-13 g, were purchased from Japan SLC (Hamamatsu, Japan) and maintained on ovalbumin-free chow conditions. The mice were kept in plastic cages and allowed free access to water. The temperature and humidity were controlled at 23±1°C and 55±10 %, respectively, and the animals were maintained on a 12:12 h light:dark cycle in an animal environmental control chamber. The animal experimentation guidelines of Seoul National University were followed.

Microorganisms

Bifidobacteria strains were provided by Maeil Co Ltd., and were cultured anaerobically in MRS media (Difco, Detroit, MI, U.S.A.) containing 0.05% L-cysteine (Sigma, St. Louis, MO, U.S.A.) at 37°C for 24 h [7]. For the preparation of the mice diet, all the bacterial cells were collected by centrifugation (Hanil, Seoul, Korea) at 4,000 ×*g* for 40 min at 4°C, and washed twice with sterile phosphate buffer saline. Then, the pellets were lyophilized by freeze-drier (Ilshin, Seoul, Korea), and lyophilized powders were suspended in RPMI medium and boiled at 100°C for 30 min to prepare cell cultures.

Intragastric Antigen Sensitization and Treatment

The mice were deprived of diet for 2 h. Sensitization was performed by intragastric (ig) administration of 50 μg of ovalbumin (OVA) (Sigma, St. Louis, MO, U.S.A.) with 10 μg of cholera toxin (CT) on days 0, 1, 2, 7, and 21 by means of a blunt stainless steel feeding needle. OVA was used as the antigen. The cholera toxin was purchased from Sigma (St. Louis, MO, U.S.A.). The mice were fed with 0.2% of lyophilized *Bifidobacterium* strains in the diet pellets. They were fed experimental bacteria powder 2 weeks before initial sensitization until sacrifice.

Enzyme-Linked Immunosorbent Assay (ELISA) for Cytokines and Chemokines

Cytokine sandwich ELISA was used to quantify the concentration of soluble cytokine and chemokine proteins according to the manufacturer's instruction. Antibodies for ELISAs were purchased from PharMingen (San Diego, CA, U.S.A.).

Preparation of Cells from Spleen

Splenocytes were isolated from each group of mice sacrificed at week 7. Cells from the spleen were prepared by gently teasing with glass slides and passed through a 200-gauge stainless steel mesh. Red blood cells in the spleen were lysed by 0.83% NH_4Cl-Tris buffer (pH 7.6). Cells were cultured in RPMI 1640 culture media (Gibco BRL, N.Y., U.S.A.) containing 10% fetal bovine serum (Gibco BRL) and 1% penicillin/streptomycin (Gibco BRL). The cells were stimulated with ConA (10 μg/ml, Sigma), lyophilized bifidobacteria powder (100 μg/ml) for 48 h in 24-well flat-bottom plates at a density of 5×10^6 cells/ml in RPMI 1640 culture media (Gibco BRL) containing 10% fetal bovine serum (Gibco BRL) and 1% penicillin streptomycin (Gibco BRL) under an atmosphere of 5% CO_2. The supernatant collected was used to measure cytokine production. All cultures were incubated at 37°C in humidified atmosphere with 5% CO_2 (Sanyo, Japan).

RESULTS AND DISCUSSION

In the present study to investigate the immunomodulatory effect of various bifidobacteria on the occurrence of allergy, we modified the peanut allergy murine model by Lee *et al.* [10] and instead employed OVA as a food allergen. Because this OVA-induced allergy murine model was sensitized by only oral challenge, but not by injection, the antigen will permeate only through the intestinal membrane. Therefore, the response of the OVA-induced allergy murine model was expected to be very close to food allergy response. Additionally, the immunomodulatory effect of bifidobacteria was also investigated in the spleen cells from BGN4-treated mice, in which the occurrence of OVA-induced allergy was suppressed by the simultaneous administration of probiotic strain *Bifidobacterium* sp. BGN4. The levels of IFN-γ, IL-4, IgG1, and IgG2a in the splenocyte culture supernatant were measured as immune markers for the study.

In mice, IL-4 is the most important signature cytokine of Th2 cells. It induces a class switch in B-cells to the antibody classes IgE and IgG1, while IFN-γ from Th1 cells stimulate the production of IgG2a antibodies [2]. Therefore, IgG2a is considered to be a Th1-associated antibody, while IgG1 is considered to be a Th2-associated antibody. The IgG1/IgG2a ratio has frequently been used to detect switching of Th1/Th2 balance. Indeed, it was reported that allergic mice possessed a high serum ratio of IgG1/IgG2a [9].

The production of IFN-γ and IgG2a in the experimental groups was higher than that of the control group, with the exception of 2A. Specifically, 6A, 7A, and 8A showed marked increases of IFN-γ levels in the spleen cells from both OVA-induced mice and BGN4-administered mice. In comparison, 2A showed the highest level of IgG2a in the splenocytes and highly stimulated the production of IL-4 and IgG1 in the splenocytes isolated from OVA-induced mice and BGN4-administered mice. The 19A highly stimulated

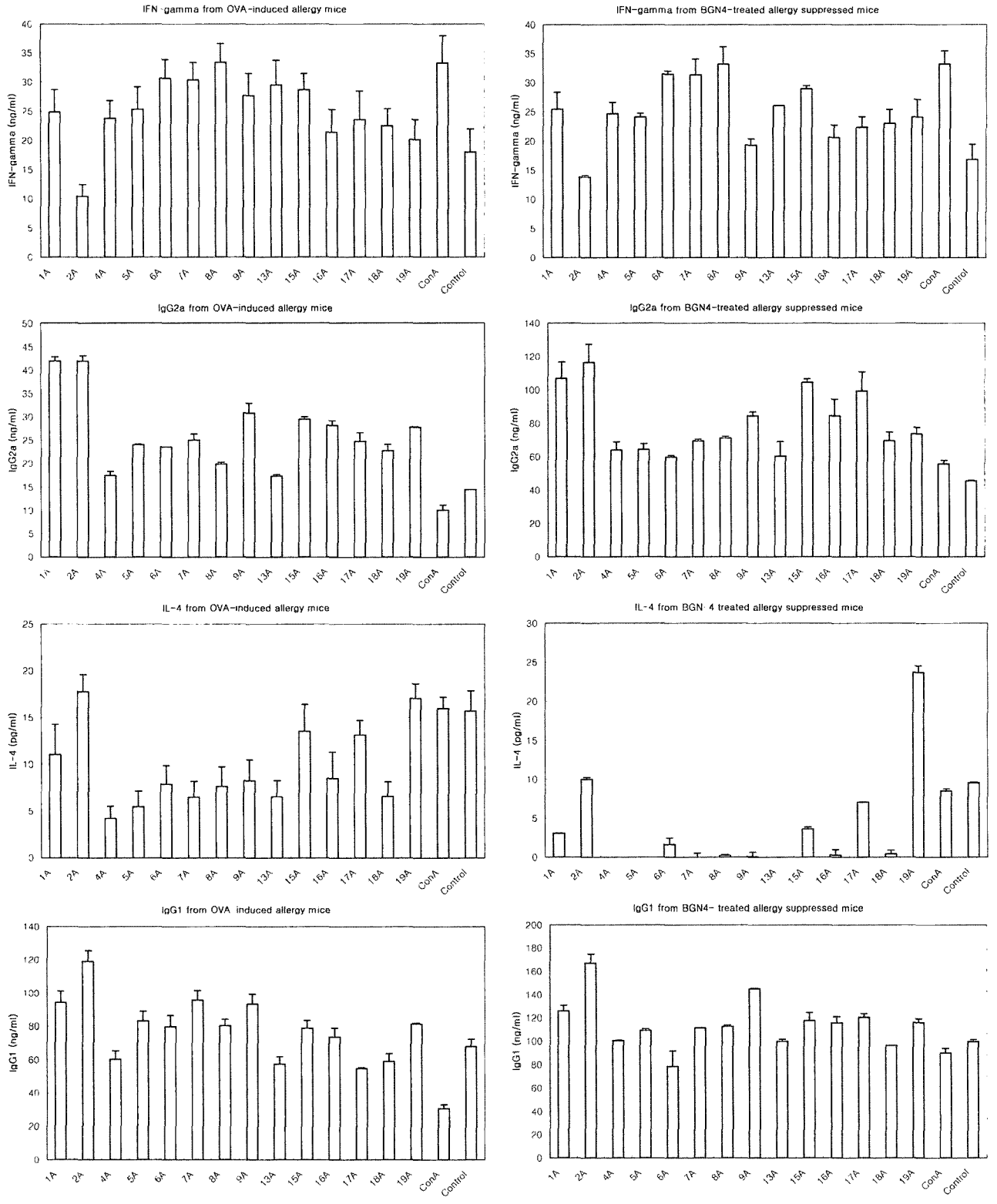

Fig. 1. The production of IFN-γ, IgG2A, IL-4, and IgG1 by spleen cells *in vitro*.
C3H/HeJ mice were orally sensitized on days 0, 1, 2, 7, 14, and 21 with 50 μg of ovalbumin and 10 μg of choleratoxin at a total volume of 0.2 ml. The mice were then fed a diet containing (wt/wt) 0.2% *Bifidobacterium bifidum* BGN4 for 21 days. Allergy mice were fed a diet containing (wt/wt) 0.2% cornstarch powder instead of bacteria powder. Spleen cells were collected on day 30 and were cocultured with various *Bifidobacterium* strains powder for 72 h. The amount of each of IFN-γ, IgG2A, IL-4, and IgG1 in the supernatant was measured by ELISAs. The data are representative of one of two independent experiments. Bars represent mean±SEM of triplicate cultures.

the production of IL-4 in the splenocytes from OVA-induced mice and BGN4-administered mice, but did not show any marked increase in the levels of IgG1, compared with other bifidobacteria.

Overall, most of the strains, except 2A and 19A, suppressed the production of IL-4, when compared to that of the control group. However, the differences in the production of IgG1 were less pronounced between the experimental groups.

The ratio of IgG1/IgG2a rather than individual levels of IgG1 and IgG2a is considered to be important to compare immunomodulatory effect on the occurrence of allergy and hypersensitivity. In correlation to the findings of Marinaro *et al.* [11], the results from the present study suggest that the reversal of IgG1/IgG2a ratio was due to markedly increased IgG2a synthesis rather than to the reduction of IgG1. This phenomenon was observed in both OVA-induced allergy mice and BGN4-treated allergy-suppressed mice. We also demonstrated that the degree of augmentation of IFN-γ and the reduction of IL-4 was dependent on the strain, in support of Fang *et al.* [4] who reported differences in *Bifidobacterium* strains isolated from allergic and healthy infants. The bifidobacteria strains in the present study that showed strong increases of Th1-type response might be possible candidates to be used as probiotics for allergy prevention. However, further experiments should be conducted to elucidate the detailed mechanisms by which *Bifidobacterium* inhibits allergy response in experimental animals and clinical human studies.

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