Original

Elevation of Serum IgG Antibody to Enteric Bacteria in Allergic Children

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Abstract : Intestinal microflora play a key role in the maturation of the intestinal immune system. In addition, immunological tolerance to commensal enteric bacteria (CEB) is thought to exist, although abnormal responses to CEB occur under some pathological conditions. This paper examines whether allergic conditions can induce such responses to CEB. We examined serum IgG titers of *Escherichia coli*, *Bacteroides vulgatus*, *Bifidobacterium longum*, and *Lactobacillus gasseri* by ELISA in an allergic (n=25) and non-allergic group (n=22). The titres for *Escherichia coli* (p<0.05) and *Bacteroides vulgatus* (p<0.05) were abnormal in the allergic group as compared with the control, non-allergic group, similar to the non-IgE mediated allergy responses to commensal intestinal flora. We conclude that there are abnormal responses to CEB under allergic condition, and that these responses may be influenced by changes in lifestyle.

Key words : atopic disease, intestinal microflora, oral tolerance, IgG antibody

Introduction

The role of intestinal microflora in the maturation of the mucosal immune barrier is well described. Such flora can potentiate the infant intestinal barrier not only by contributing a non-immunological defense but also by stimulating and directing the development of the intestinal immune system¹⁾. Indeed, a genotobiotic murine model was used to show that, intestinal bacteria play a crucial role in generating an immune system that is fully susceptible to oral tolerance induction, probably by affecting the development of gut-associated lymphoid tissue at the neonatal stage²⁾. Due to the immature stage of the intestinal barrier and immunity, infants are probably vulnerable to allergic sensitization by the intestinal lumen contents during the first few months of life until tolerance to such allergens is established after about one year³⁾. In some cases, however, an atopic status characterized by a high IgE response can occur, potentially developing into an atopic disease such as food allergy, bronchial asthma and atopic dermatitis.

Microbial colonization of the intestine commences just after birth, although development of the normal adult flora is a gradual process. Several factors such as the composition of the maternal intestinal microflora, hygiene status, mode of delivery, use of antibiotics and genetic predisposition can all affect the establishment of the commensal microflora. In a conventional delivery, the first bacteria to colonize the infant intestine are those present

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during passage through the birth canal (i.e., bacteria associated with the mother's vaginal and intestinal flora such as Bifidobacteria, Lactobacilli and Enterobacteria). Subsequently, anaerobes such as Bacteroides appear⁴⁾. The lack of any immune response to such autochthonous microflora is maintained in an intact mucosal system that can discriminate pathogenic bacteria from tolerogenic, indigenous bacteria. In fact, Duchmann *et al*⁵⁾ reported that T cells from the lamina propria of an individual mouse do not respond to their own bacterial flora, although they do respond to bacteria from other hosts. Here, we directly test the hypothesis that allergic patients show an abnormally increased immune response against non-pathogenic commensal intestinal bacteria. The pathogenic role of this response in allergic diseases is also discussed.

Materials and Methods

Subjects

This study involved 47 subjects, comprising infants and children from 3 months up to 10 years of age. They were divided into allergic (n=25) and non-allergic groups (n=22). The allergic patients were diagnosed with one or more of bronchial asthma, atopic dermatitis, allergic rhinitis, and food allergies. Bronchial asthma was diagnosed according to the criteria of the Asthma Prevention and Management Guidelines⁶⁾. Atopic dermatitis was diagnosed based on the criteria reported by Hanifin & Rajka⁷⁾. Allergic rhinitis was diagnosed based on symptoms, including sneezing, watery rhinorrhea, nasal obstruction, and an itchy nose. Food allergies were diagnosed on the basis of history of a temporal relationship between the ingestion of a suspected food and the onset of allergic symptoms. The control, non-allergic group had no sign or history of these allergic disorders. For the allergic group, the mean age, age range, and gender ratio (male/female) were 3.3, 1 to 8 and 1.2, respectively. For the non-allergic group, these profiles were 3.1, 1 to 10 and 1.4, respectively. The study was approved by the Ethics Committee of our institute.

Serum IgE and bacteria-specific antibodies

Serum IgE levels were determined by ELISA, as previously described²⁾. Serum IgG titres for the antibodies raised against the bacteria investigated were measured also by ELISA. The serum was diluted appropriately so that the absorbance readings were within a range that provided a linear dose-absorbance relationship. Nonpathogenic *Escherichia coli* ATCC 5922 and *Bacteroides vulgatus* JCM 5826 were obtained from the American Type Culture Collection, Rockville, Maryland and Japan Collection of Microorganisms, Wako, Saitama, Japan, respectively. *Bifidobacterium longum* T1015 and *Lactobacillus gasseri* T1016 were cultivated in our laboratory (unpublished data). Bacterial lysates to use as antigen in the ELISA were prepared by sonication in PBS (BIORUPTURE, CosmoBio Inc., Tokyo, Japan) using a high setting for 30 min. A microtiter plate (Costar 3590 96 Well Assay Plate, Corning Incorporated, Corning, NY) was coated with 50 μ l of bacterial lysate per well at a concentration of 10 μ g/ml in carbonate/bicarbonate coating buffer by incubation at 4 $^{\circ}$ C overnight. The plate was washed with PBS containing 0.05% (v/v) Tween 20, before blocking with 10% (w/v) bovine serum albumin in PBS for 2 hrs at room temperature. Serum samples were serially diluted in PBS-Tween, and 50 μ l of each was added in duplicate to the microtiter plate wells. After two hours incubation at room temperature, the plate was washed, then 50 μ l of 1 μ g/ml peroxidase-conjugated goat anti-human IgG

fraction (ICN Pharmaceu. ticals, Inc., Aurola, Ohio) was added to each well and incubated for an additional two hours. Unbound reagent was washed off with PBS-Tween, and the enzyme was detected by the addition of o-phenylenediamine dihydrochloride. This reaction was stopped after 5 min by the addition of 3 M sulphuric acid, and the absorbance in each well was read at 495 nm using a plate reader. The results were expressed as mean values $\pm$ SE. A statistical analysis was done using the Student's t test.

Results

Assay of serum IgG antibody response to bacterial antigens

The antibody response to non-pathogenic commensal bacteria colonizing the intestine, was examined by measuring the serum titers of IgG antibodies to the prevailing fecal bacterial groups, such as *Bacteroides*, *Bifidobacteria*, *Lactobacilli* and *Enterobacteria*. The representative bacterial species employed in this study were *Bacteroides vulgatus*, *Bifidobacterium longum*, *Lactobacillus gasseri* and *Escherichia coli* (Fig. 1). The serum IgG antibody titer to *Bacteroides vulgatus* (Fig. 1A, $p < 0.05$) and, in particular, to *Escherichia coli* (Fig. 1D, $p < 0.05$) was significantly higher in allergic subjects than in non-allergic, control subjects, with similar bacterial counts for these species recorded in both of the subject groups. The antibody titer for either *Bifidobacterium longum* (Fig. 1B, $p > 0.05$) or *Lactobacillus gasseri* (Fig. 1C, $p > 0.05$) was not significantly different between the two groups. Total serum IgE was measured in all samples, as shown in Fig. 1E. As expected, the total IgE level was much higher in allergic subjects than in non-allergic controls ($p < 0.001$). Notably, no significant correlation was found between the levels of total IgE, an indication of allergy, and the antibody titers to the bacteria such as *Bacteroides vulgatus* ($R = 0.16$, Fig. 2A) and *Escherichia coli* ($R = 0.07$, Fig. 2B). Thus, these results suggest that the elevation of IgG antibodies to the commensal enteric bacteria was caused by a non-allergic immune response devoid of excessive IgE production.

Discussion

Oral tolerance is acknowledged as a form of peripheral tolerance in which mature lymphocytes from the peripheral lymphoid tissue are rendered non-functional or hypo-responsive by prior oral administration of antigen. Such tolerance is underscored by evidence that most populations show lifelong clinical and immunological tolerance, both to food antigens and to their own intestinal microflora⁸. Although the dominant response to CEB is this immunological tolerance, the gut-associated lymphoid tissue can initiate a regional and systemic immune response to these commensal bacteria in some pathological conditions such as inflammatory bowel disease (IBD). Emerging evidence indicates that T cells from IBD patients respond to resident luminal bacteria of the intestine^{9,10}. These studies suggest that IBD patients exhibit defective tolerance to CEB, which provides the constant antigenic stimuli for relapsing chronic inflammation. While bacterial antigen-specific humoral immune responses are clearly evident in IBD patients, B cells have been shown to play an important role in the development of colitis in an animal model¹¹. Therefore, serum antibody responses to intestinal bacterial flora may be markers of enhanced permeability or elevated immuno-responsiveness rather than being directly involved in disease pathogenesis.

In this study, a high level of serum IgG antibody to enteric commensal bacteria such as

Fig. 1. Serum IgG antibody titres to major fecal bacteria groups, *Bacteroides vulgatus* JCM 5826 (1A), *Bifidobacterium longum* T 1015 (1B), *Lactobacillus gasseri* T 1016 (1C), *Escherichia coli* ATCC 5922 (1D), and serum IgE (1E) levels in allergic and non-allergic subjects. In the allergic group, the serum IgG antibody titres to *B.vulgatus* JCM 5826 (1A, $p < 0.05$) and *E.coli* ATCC 5922 (1D, $p < 0.05$) were significantly higher than those of the non-allergic group.

Escherichia coli and *Bacteroides vulgatus* was found in allergic patients. Furthermore, it indicated the presence of such abnormal levels in allergy patients as evidence of CEB tolerance. Allergic diseases, especially food-mediated ones, are thought to be caused by a breakdown or failure of oral tolerance induction or maintenance of the corresponding food antigens¹². It appears unlikely that an elevation in the levels of antibodies specific to *Bacteroides vulgatus* and *Escherichia coli* in allergic patients was merely a result of predominant colonization of these bacteria, as the counts of these bacterial groups in the feces of the allergic subjects were less than in the non-allergic group¹³. However, our subjects' conditions included atopic dermatitis, showing the possibility of an immune response to *Escherichia coli* as one of the components of skin flora^{14, 15}. In addition, there was no significant correlation between total serum IgE and the levels of specific IgG to those bacteria. Considering that IgE plays a crucial role in the pathophysiology of allergies, the elevation of such IgG antibodies (irrelevant of the total IgE level) is not reflective of a heightened allergic state. As recently indicated, allergic disorders may consist of exclusively IgE-mediated, partially IgE-mediated, or exclusively cell-mediated reactions¹⁶. Allergens responsible for non-IgE-mediated reactions are yet to be identified by laboratory tests¹⁷. In this respect, it is of note that a bacteria-specific IgG antibody response provides a clinical index of a non-IgE mediated allergic condition.

Fig. 2. Correlation between the levels of total IgE and IgG titres to the bacteria, *Bacteroides vulgatus* (2A) and *Escherichia coli* (2B). No significant difference was shown between total IgE and these bacteria.

Immediately after birth, the immune system starts receiving immunological stimuli from the commensal flora, environment, and sporadic infection. This immunological input not only provides antigens but also promotes production of cytokines that affect the maturation of neonatal T cells. Without these correct inputs, the immune system would not function or develop efficiently¹⁸⁾.

We have demonstrated through animal models that naturally occurring antigens fail to develop under germ-free condition²⁾. While modern human infants are not germ-free, they have increasingly limited or aberrant immunological experiences, such as a decreased frequency of breast-feeding, consumption of pasteurized foods, environmental sanitation and intermittent exposure to antibiotics. These events may alter the nature of intestinal flora in infants, resulting in a distorted mucosal immune response to CEB, as supported by this study.

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[Received December 12, 2001 : Accepted January 19, 2002]