

INTERNATIONAL ARTICLE

Allergic Symptoms and Microflora in Schoolchildren

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We studied 867 junior high school children and administered a questionnaire documenting allergic symptoms and environmental variables, and measured Immunoglobulin E serum levels and the immunoglobulin G titers of serum antibody to microflora. A total of 716 subjects were ultimately used for statistics; those with at least two of the following allergic symptoms: asthma, rhinitis, eczema, or food allergy, showed significantly higher IgG titers to *Bacteroides vulgatus* than other groups. This finding suggests that a species of the *Bacteroides* genus of the intestinal microflora tends to affect the gut issues, but further studies are needed to clarify this. © Society for Adolescent Medicine, 2004

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The prevalence of allergic diseases has been increasing in developed countries. However, many studies have demonstrated that the prevalence of these diseases is lower in the formerly socialist countries of Central and Eastern Europe than in Western European countries [1]. One factor associated with the rise of allergic diseases may be the decline of many infectious diseases in developed countries as the

result of improved living standards and vaccinations [2]. In modern societies, it has been noted that allergies may be “mapped” according to gradients dictated by hygiene and by the individual degree of genetic predisposition to allergy [3]. These concepts are known as the “Hygiene hypothesis” [3–5]. It has been suggested that a reduced microbial stimulation during infancy and early childhood would result in slower postnatal maturation of the immune system and development of an optimal balance between Th1- and Th2-like immunities [6].

To clarify what and when bacterial species of commensal intestinal microbes are recognized as antigens that induce serum antibody responses in allergy patients, we studied the serum antibody responses to microflora in atopic school-aged children.

Methods

From a population of approximately 1000 12–13-year-old schoolchildren attending the 18 junior high schools in the country in 1995, we did a retrospective study of records of tuberculin responses in 867 children. They had been immunized with bacillus Calmette-Guerin (BCG) at 6 and 12 years of age and they showed negative tuberculin responses. Shirakawa et al showed the strong inverse association between delayed hypersensitivity to tuberculin and atopy [7]. The Institutional Review Board of RIKEN approved this study and 787 of all recruited subjects gave informed consent. We administered a questionnaire documenting atopic symptoms; the

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Table 1. The Relationship Between Allergy Symptoms and IgG Titers of Serum Antibody Microflora in School Children (Mean $\pm$ SE)

Microflora or IgE	Nonallergy (n = 433)	One allergy symptom (n = 200)	2 or more allergy symptoms (n = 83)	F	p
<i>E. coli</i>	3.04 $\pm$ 0.01	3.07 $\pm$ 0.02	3.04 $\pm$ 0.03	0.62	.54
<i>B. vulgatus</i>	2.94 $\pm$ 0.01	2.91 $\pm$ 0.01	2.97 $\pm$ 0.02	3.00	.05
<i>E. faecalis</i>	2.99 $\pm$ 0.01	2.99 $\pm$ 0.02	3.03 $\pm$ 0.03	1.11	.33
<i>B. longum</i>	3.02 $\pm$ 0.01	3.01 $\pm$ 0.02	3.06 $\pm$ 0.02	1.38	.25
IgE	2.19 $\pm$ 0.03	2.25 $\pm$ 0.04	2.41 $\pm$ 0.08	4.23	.02

All were analyzed by one-way analysis of variance. Each gut flora or IgE values were converted to $\log_{10}$ because they didn't show normal distribution. SE = means standard error; *E. coli* = *Escherichia coli*; *B. vulgatus* = *Bacteroides vulgatus*; *E. faecalis* = *Enterococcus faecalis*; *B. longum* = *Bifidobacterium longum*; IgE = Immunoglobulin E.

questions were: "Do you have asthma?," "Do you have rhinitis?," "Do you have eczema?," "Do you have food allergy?" Also included were environmental variables, family allergy history, tuberculin response, etc., and we also measured immunoglobulin E (IgE) serum levels (measured by Mitsubishi BCL, Inc., Kyoto, Japan) and the immunoglobulin G (IgG) titers of serum antibody to *Escherichia coli* (*E. coli*), *Bacteroides vulgatus* (*B. vulgatus*), *Enterococcus faecalis* (*E. faecalis*), and *Bifidobacterium longum* (*B. longum*). This assay was described in detail in a previous article [8]. The titers of IgG class antibodies reacting with bacteria in serum were measured by enzyme-linked immunosorbent assay, and the absorbance was read at 495 nm using a plate reader.

We avoided subjects who did not complete questions about atopic symptoms and whose serum sample could not be measured for technical reasons. Ultimately we used 716 subjects for statistics. The values of the IgG titers of serum antibody to microflora and the level of IgE were converted to $\log_{10}$. Differences in IgG titers to *E. coli*, *B. vulgatus*, *E. faecalis*, and *B. longum* between nonallergic schoolchildren with no allergic symptoms, and symptomatic schoolchildren with more than one allergic symptom among asthma, rhinitis, eczema, and food allergy, were analyzed by two-tailed Student's *t*-test. We also tested the relation in IgG titers by each symptom; asthma, rhinitis, eczema, and food allergy by two-tailed Student's *t*-test. Then we divided subjects into three groups; "nonallergy," who have no allergy symptoms; "one allergy," who have one allergic symptom among asthma, rhinitis, eczema, and food allergy; and "two or more allergy," who have at least two allergic symptoms among asthma, rhinitis, eczema, and food allergy. We examined the differences in IgG titers to *E. coli*, *B. vulgatus*, *E. faecalis*, and *B. longum* among the three groups by one-way analysis of variance. The statistical analysis for this study was done using SPSS 10.0J for Win.

Results

IgG titers to *E. coli*, *B. vulgatus*, *E. faecalis*, and *B. longum* showed no significant difference between nonallergic schoolchildren ($n = 433$) with no allergic symptoms, and symptomatic schoolchildren ($n = 283$) who have more than one allergic symptom among asthma, rhinitis, eczema, and food allergy. We also tested the relation by each symptom; asthma, rhinitis, eczema, and food allergy; there were, however, no differences between nonallergic children and children with one or more allergic symptoms. The subjects were divided into three groups; "nonallergy" ($n = 433$), who have no allergy symptoms; "one allergy" ($n = 200$), who have one allergic symptom among asthma, rhinitis, eczema, and food allergy; and "two or more allergy" ($n = 83$) who have at least two allergic symptoms among asthma, rhinitis, eczema, and food allergy. In the "two or more allergy" group, IgG titers to *B. vulgatus* were significantly higher than in the other two groups (Table 1) ($F = 3.00$ $df = 2$, $p = .05$). IgG titers to *E. coli*, *B. vulgatus*, and *E. faecalis* showed no significant difference among three groups. Also in the "two or more allergy" group, subjects showed the highest mean IgE levels (2.41 ± 0.08 vs. 2.19 ± 0.03 , 2.25 ± 0.04 ; $F = 4.23$ $df = 2$, $p = .02$) among three groups (Table 1).

Discussion

The children with two or more of the allergic symptoms showed higher IgG titers to *B. vulgatus* than the groups of nonallergic or one-allergic-symptom schoolchildren. This finding suggests that a species of the *Bacteroides* genus of the intestinal microflora might be an organism that affects the gut issues and thus, induces inflammation accompanied by an elevation of serum antibodies to this bacterium [8], although the exact mechanism of this remains un-

clear. Some studies reported that there was a difference in the composition of intestinal microflora between allergic and nonallergic infants. They compared 1-year-old infants living in two countries with a low (Estonia) and a high (Sweden) prevalence of atopy, and found that *Lactobacilli* and *Eubacteria* were more frequently found in the intestinal microflora of Estonian infants, whereas *Clostridia* was more prevalent in Swedish infants [9]; 2-year-old allergic children were colonized less often by *Lactobacilli*, and harbored higher counts of aerobic bacteria (coliforms, *Staphylococcus aureus*) than nonallergic children [10]. These subjects were, however, all infants, and the number of samples was small. Kirjavainen et al recently showed that, in 4.2–10.5-year-old children, serum total IgE concentration correlated with bacteroides in heightened risk of subjects with atopic disorders, and those subjects also showed a greater number of lactobacilli/enterococci than those in the low-risk group [11]. Our data is partly consistent with this.

This is the first study to clarify the relationship between atopic symptoms and bacterial species of predominant commensal intestinal microflora in a large group of junior high school children. Further studies are needed to clarify when bacterial species of commensal microbes in the gut are recognized as the antigens that induce serum antibody responses in allergic subjects.

References

1. Bjorksten B, Dumitrascu D, Foucard T, et al. Prevalence of childhood asthma, rhinitis and eczema in Scandinavia and Eastern Europe. *Eur Respir J* 1998;12:432–7.
2. Doll R. Health and the environment in the 1990s. *Am J Public Health* 1992;82:933–41.
3. Matricardi PM, Bonini S. High microbial turnover rate preventing atopy: A solution to inconsistencies impinging on the Hygiene hypothesis? *Clin Exp Allergy* 2000;30:1506–10.
4. Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989;299:1259–60.
5. Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;354(Suppl 2):SII12–5.
6. Bottcher MF, Nordin EK, Sandin A, et al. Microflora-associated characteristics in faeces from allergic and nonallergic infants. *Clin Exp Allergy* 2000;30:1590–6.
7. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77–9.
8. Saitoh S, Noda S, Aiba Y, et al. Bacteroides ovatus as the predominant commensal intestinal microbe causing a systemic antibody response in inflammatory bowel disease. *Clin Diagn Lab Immunol* 2002;9:54–9.
9. Sepp E, Julge K, Vasar M, et al. Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997;86:956–61.
10. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999;29:342–6.
11. Kirjavainen PV, Arvola T, Salminen SJ, Isolauri E. Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? *Gut* 2002;51:51–5.