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Original Article

Comparison of IgG, IgG1 and IgG2 immune responses to pneumococcal polysaccharide in atopic and nonatopic children

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ABSTRACT

The levels of naturally occurring IgG, IgG1 and IgG2 antibodies against polyvalent pneumococcal capsular polysaccharide antigen (Pneumovax®) were compared between atopic and nonatopic children with different ages, 6–12 months and 1, 2, 3, 4, 5–9 and 10–15 years, by enzyme-linked immunosorbent assay. Children with asthma, atopic dermatitis, food allergy or a combination of these, and those having serum IgE levels exceeding 50 IU/mL at 6–12 months old and 100 IU/mL at more than 1-year-old were included as atopic groups. Asymptomatic children whose serum IgE levels were less than these atopic standards and those not having detectable IgE antibodies to *Dermatophagoides farinae* comprised the nonatopic groups. Geometric mean levels of IgG and IgG_1 antibodies against pneumococcal antigen increased steadily with age, and that of IgG2 antibodies was low until 3 years of age and then gradually increased age-dependently up to 15 years of age. The levels of IgG antibody as well as IgG1 and IgG2 antibodies were not significantly different between atopic and nonatopic children in any age group. This suggests that the immune response to the most common bacterial pathogen in the respiratory tract does not influence atopic status.

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INTRODUCTION

The increased prevalence of atopic diseases such as asthma, pollenosis, and atopic dermatitis has been documented in many parts of the world over the past several decades.[1,2] Several explanations for this phenomenon are possible; for example, air-pollution, increased indoor exposure to inhaled allergens, decreased ventilation in modern dwellings, and dietary changes.[3] A diminished exposure to infection is speculated as promoting the prevalence of atopic diseases.[4] There is evidence to suggest that a predominant activation of Th1-like T-helper cells in the course of recurrent viral or bacterial infections including *Mycobacterium tuberculosis* might prevent the proliferation of Th2 clones, resulting in decreased incidence of allergic diseases.[5,6]

The incidence of pneumococcal pneumonia has declined drastically during the past several decades in developed countries. However, scarcely any data are available regarding the influence of the pneumococcus infection on atopic status. In this study, to further evaluate the relations between antipneumoccal immunity and atopy, we compared naturally occuring IgG, IgG1, and IgG2 antibody levels against pneumococcal capsular polysaccharide antigen in a series of age-matched atopic and nonatopic groups of children.

METHODS

Subjects

Atopic sera were obtained from patients aged 6 months to 15 years who visited the Pediatric Allergy Clinic of the

University Hospital of Tsukuba. The patients had either asthma, atopic dermatitis, food allergies or a combination. A diagnosis of asthma was based on the definition to the criteria of the National Institutes of Health, USA, with minor modifications.[7] Patients must have shown the following characteristics: (i) recurrent episodes of wheezing and shortness of breath during the past year; and (ii) wheezing and dyspnea which was reversible, either spontaneously or with bronchodilator treatment. Atopic dermatitis was diagnosed based on the criteria established in 1980 by Hanifin and Rajka.[8] Children who suffered episodes of anaphylactic reaction to the ingestion of food and showed an IgE antibody against the food responsible were diagnosed with a food allergy. Non-atopic sera were collected from two populations. In younger children aged less than 6 years, sera were obtained from patients with acute illness whose blood was drawn for diagnostic purposes unrelated to allergy. In older children aged 6 years or more, sera were collected as part of an annual health check-up. The possibility of allergic symptoms in these subjects was carefully examined by a pediatric allergist, and only children confirmed by clinical history and physical examination as not having allergic symptoms were selected for the groups. Parents gave informed consent for their children to be included in this study. Serum samples from atopic and nonatopic children were divided into seven groups according to age: 6–12 months and 1, 2, 3, 4, 5–9 and 10–15 years. Serum IgE levels were determined by RIST kits (Pharmacia, Uppsala Sweden). Specific IgE levels to *Dermatophagoides farinae* (*D. farinae*) were also measured using the Pharmacia CAP system (Uppsala, Sweden). The sera which had IgE levels exceeding 50 IU/mL in atopic children aged 6–12 months and 100 IU/mL in children over 1 year of age were further selected for atopic sera, and the sera which had IgE levels less than those atopic standards for age in nonatopic children and those not having detectable IgE to *D. farinae* (< 0.35 UA/mL) were selected for nonatopic sera. Finally, 27–30 samples of each age group were selected. The average total serum IgE levels ± SE in atopic subjects aged 6–12 months and 1, 2, 3, 4, 5–9 and 10–15 years, respectively, were 240 ± 40, 262 ± 29, 565 ± 105, 954 ± 182, 506 ± 71, 754 ± 91 and 1266 ± 297 IU/mL, and those in nonatopic subjects were 12 ± 2, 37 ± 5, 38 ± 5, 27 ± 4, 28 ± 5, 38 ± 5 and 43 ± 9, respectively. More than 90% of subjects in each allergic group had asthma, except infants aged 6–12 months and 1 year. No subject had a history of prior pneumococcal immunization.

Antigen-specific ELISA

The assay was performed as described previously.[9] Microtiter plates (No. 69620, Nunc, Roskilde Denmark) were coated overnight with 100 μL/well of 1:100 diluted 23-valent pneumococcal vaccine (Pneumovax®, Merck, Sharp & Dohme, NJ, USA) that was dissolved in a carbonate coating buffer (sodium carbonate/bicarbonate 0.1 mol/L, pH 9.6). The plates were washed three times with washing buffer, (PBS containing 0.1% Tween 20). The buffer of the last wash was left in the wells for 30 min to ensure complete inhibition of non-specific binding. Blocking of C-polysaccharide (C-Ps, Statens Seruminstitut, Copenhagen, Denmark) anti-bodies was performed by adding 50 μL of a 0.2% C-Ps solution to 1 mL serum diluted 1/25, as described by Koskela.[10] The samples were incubated at 37°C for 2 h, then at 4°C overnight and finally stored at –20°C. The sera were diluted to 1:100, 1:25 and 1:50 in the IgG, IgG1 and IgG2 assays, respectively. On each plate the reference serum was tested in eight two-fold dilutions starting at 1:100, 1:25 and 1:50 in the IgG, IgG1 and IgG2 assays, respectively. A total of 100 μL of the samples were applied to each well. The plates were incubated for 1.5 h at room temperature, washed four times, and 100 μL of horseradish peroxidase (HRP)-conjugated antihuman IgG (Cappel, Durham, NC, USA), IgG1 and IgG2 antibodies (Binding site, Birmingham, UK) diluted to 1:2500, 1:250 and 1:500, respectively, were added. The plates were incubated at room temperature for another 1.5 h, washed four times, and then 100 μL of 0.2 mol/L citrate-phosphate buffer (pH 5.0) containing 0.1% (w/v.) o-phenylenediamine dihydrochloride (OPD; Sigma Chemical Company, St Louis, MO, USA) and 0.01% H_2O_2 was added to each well. After exactly 30 min, the reaction was stopped by adding 50 μL of 2 mol/L H_2SO_4. The absorbance was measured with an immunoreader (Japan Intermed Co. Ltd, Tokyo, Japan) using a test wavelength of 490 nm and a reference wavelength of 630 nm.

As a reference on each plate, International Pneumococcal Antibody Standard (PSAb90) consisting of a pool of post-vaccination sera from 17 healthy adults (Carl E Frasch, Center for Biologics, Evaluation and Research, Food and Drug Administration, Washington, USA), was assayed in eight two-fold dilutions starting at 1/100, 1/25 and 1/50 in the IgG, IgG1 and IgG2 measurements, respectively. The absorbancy at a serum dilution of 1:400 in IgG, 1:100 in IgG1 and 1:200 in IgG2 was arbitrarily defined as 320 ELISA units (EU)/mL, and IgG concentrations of the samples were quantitated by means of a regression line calculated for the standard serum.

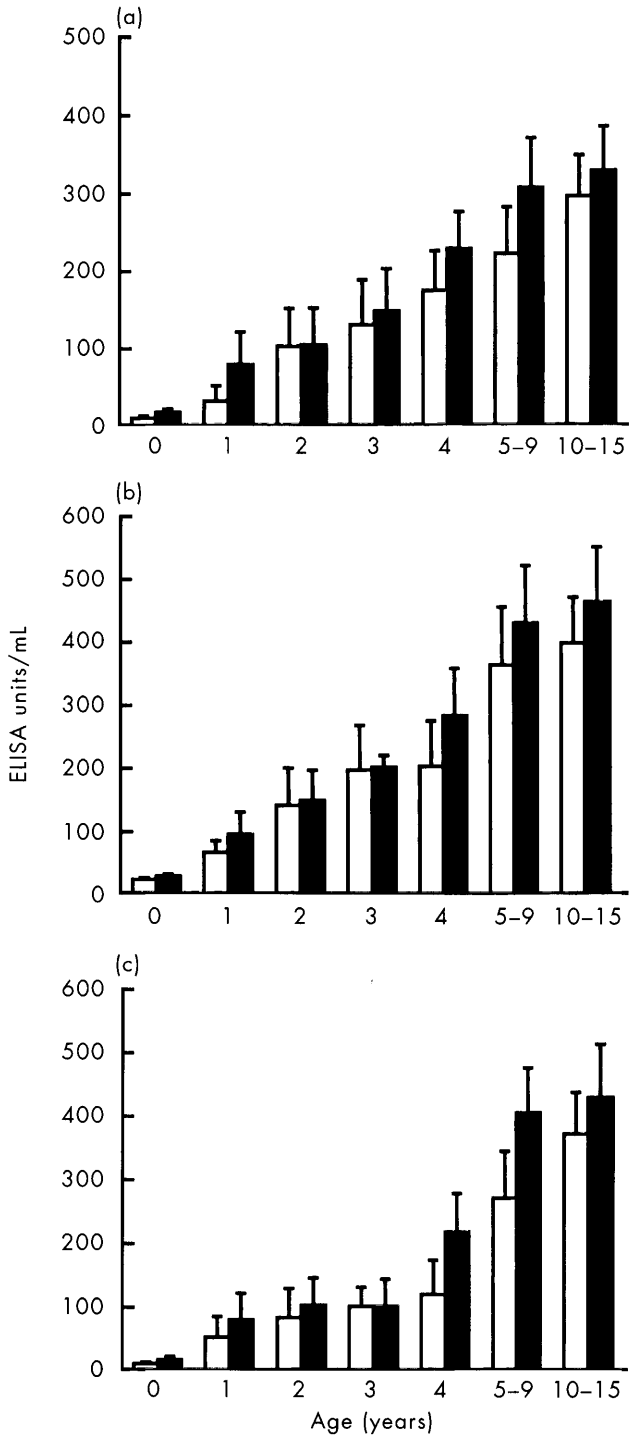

Fig. 1. Comparison of (a) IgG, (b) IgG1 and (c) IgG2 levels against pneumococcal capsular polysaccharide antigen between atopic (■) and nonatopic (□) children. Results are expressed as the mean ELISA units ± SEM for each group including 29–30 subjects. The differences in IgG, IgG1 and IgG2 levels to pneumococcus polysaccharide between atopic and nonatopic groups were not significant ($P > 0.05$) in each age groups by Student's t-test.

Statistical Analysis

Statistical analysis was performed using Student's *t*-test and the Pearson's correlation coefficient by STATVIEW 4.1 on an Apple Macintosh computer. Prevalence < 0.05 was considered significant.

Results

The levels of IgG, IgG1, and IgG2 antibodies against polyvalent pneumococcal capsular polysaccharide antigen were compared between atopic and nonatopic children in different age groups. The distributions of IgG, IgG1 and IgG2 antibody levels against pneumococcal polysaccharide in atopic and nonatopic children are shown in the Fig. 1 panels A, B and C, respectively. Geometric mean levels of IgG and IgG1 antibodies against pneumococcal antigen increased steadily with age, and that of IgG2 antibodies was low until 3 years of age and then gradually increased age-dependently up to 15 years of age. Although atopic children tended to have higher antibody levels against pneumococcal polysaccharide than nonatopic children in each age group, there were no significant differences between any two age-matched groups ($P > 0.05$).

The levels of IgG, IgG1, and IgG2 antibodies to polyvalent pneumococcal capsular polysaccharide antigen were compared in atopic and nonatopic groups by the Pearson's correlation coefficient. The correlation coefficients between IgG and IgG1, between IgG and IgG2 and between IgG1 and IgG2 in atopic groups were 0.50, 0.59 and 0.49 and those in nonatopic groups 0.62, 0.50 and 0.59, respectively. No significant difference was found in comparison of correlation coefficients between atopic and nonatopic groups ($P > 0.05$).

Discussion

In this study, the levels of naturally occurring IgG, IgG1, and IgG2 antibodies against polyvalent pneumococcal capsular polysaccharide antigens (Pneumovax®) were compared between atopic and nonatopic children in age-matched groups. The results indicate that the levels of IgG antibody as well as IgG1 and IgG2 antibody are not significantly different between atopic and nonatopic children in any age group.

CD4 + T-helper cells, type 1 and type 2 (Th1 and Th2), play a crucial role in facilitating immune responses.[11] In allergic responses, the Th2 subset of T cells is central in mediating IgE production and in developing immediate hypersensitivity.[12,13] Shirakawa *et al.* recently reported a strong inverse association between delayed hypersensitivity

to *M. tuberculosis* and atopy among Japanese school children, suggesting that exposure to *M. tuberculosis* may inhibit atopic disorders by promoting Th1 immunity.[5] Since the responses to pneumococcal antigen are thymus-independent,[14–16] it is difficult to directly compare our results with the thymus-dependent responses. However, our findings suggest that the immune response to pneumococcus, the common bacterial pathogen in the respiratory tract, does not influence atopic status.

The pneumococcal capsular polysaccharide antigen used, Pneumovax®, includes multiple serotypes with poor, intermediate, or good immunogenicity in infants.[17] It is likely that high levels of anti-pneumococcal antibodies may reflect a strong response to a particular serotype. However, recent studies have shown that antibody titers against polyvalent pneumococcal capsular antigens correlate well with the resistance to pneumococcal infection.[18,19]

Pneumococcal infection frequently follows a viral respiratory tract infection,[20] which may produce mucosal damage,[21] diminish the epithelial ciliary activity,[22] and depress the function of the alveolar macrophages.[23] Asthmatic patients also have damaged airway epithelium, often accompanied by depressed ciliary function.[24] Although no significant difference was seen, the atopic children did appear to have higher levels of pneumococcal antibody titers than did the controls. This may be influenced by the fact that more than 90% of our atopic children were asthmatics.

The present study did not show a significant association between anti-pneumococcal immunity and allergy. Further study is required to evaluate the role of infection in the development of allergic diseases.

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