EVALUATION OF SERUM IgG SUBCLASS LEVELS IN ASTHMATIC AND ATOPIC CHILDREN WITH RECURRENT INFECTION

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ABSTRACT

Serum IgG subclass levels were measured using an indirect immunoenzymatic assay (ELISA) with monoclonal antibodies in 16 children with asthma and 13 children with atopy who had mostly recurrent infections. Seven of the asthmatic children had marked low or low normal levels of IgG1, six had marked low or low normal levels of IgG3, two had marked low normal levels of both IgG2 and IgG3, and one had low levels of IgG2, IgG3, and IgG4. All these patients suffered from recurrent sinopulmonary infections. There were low percentages of IgG1 and IgG4 defects (about 15%) in the atopic patients, while a significant increase in the serum IgG4 levels were observed (six patient out of 13 patients, 46.2%).

INTRODUCTION

Four subclasses of human IgG are currently recognized: IgG1, IgG2, IgG3, and IgG4. Antibody responses to certain antigens may be limited to one or some of the IgG subclasses. In addition, some of the biological activities attributed to the constant region (Fc fragment) are restricted to some of the subclasses. It is generally believed that IgG subclass deficiency is associated with increased susceptibility to infections. In normal human IgG, IgG1 constitutes 65%, IgG2 25%, IgG3 7%, and IgG4 3% of total serum IgG, respectively. A deficiency in the IgG subclasses may not be detected by measuring total serum IgG since some of the subclasses are present in very low concentrations. Therefore, deficiencies of IgG1, 3 or 4 may occur in the presence of normal concentration of total serum IgG. In this situation even IgG1 deficiency may occur in the presence of a normal total IgG level.

Studies of humoral immune function in children with chronic respiratory symptoms have provided conflicting results because of confusion in diagnostic criteria and variability in patient selection. Abnormal levels of one or more serum immunoglobulins have been reported in children with severe, chronic asthma and in children with asthma associated with severe respiratory tract infections. Low serum levels of IgG and IgA have been associated with IgG subclass deficiencies in children with chronic intermittent or persistent chest symptoms.

Since IgE antibodies are not elevated in all atopic individuals, it has been proposed that other immunoglobulin classes may contribute to the hypersensitivity reaction. Evidence for this mechanism was first presented by Parish who showed that human IgG antibodies were able to bind to monkey mast cells and function as short-term sensitizing antibodies.

While the mechanism of action of IgG antibodies in atopy is unresolved, there are clear indications for the IgG4 subclass in atopy. For example, raised levels of IgG4 have been found in patients with a variety of atopic conditions. In particular, it is well marked in atopic dermatitis.
from the other IgG subclasses in its inability to bind to complement effectively. In addition, this particular immunoglobulin class is preferentially elevated in atopic dermatitis and hay fever although its significance is not fully understood. Morgan and Levinsky reported that in atopic patients IgG₂ concentrations may be reduced or raised, sometimes to a considerable degree. Measurements of total IgE and IgG shows a simple relationship.

**MATERIALS AND METHODS**

**Buffer and other reagents:** Immunlon 1 microtiter plates (M 129/A) were purchased from Dynatech. O-phenylenediamine hydrochloride (OPD) and anti-human IgG peroxidase conjugates and monoclonal antibodies to human IgG subclasses 1-4 were purchased stored at 4°C until used. The clones produced the antibodies and their respective specificities (shown in parentheses) were: SG-16 (IgG₁), HP-6014 and GoM2 (IgG₂), HP-6050 and ZG4 (IgG₃), HP-6025 and RJ4 (IgG₄). All measurements were performed using phosphate buffered saline (PBS) 0.1 M, pH 7.4. The washing buffer and dilution buffer used was PBS 0.1 with Tween 20, 0.05%. Sodium citric, pH 5.0 was used as peroxidase substrate buffer. 40 mg OPD and 40 μl H₂O₂ at 100 ml sodium citric-buffer were prepared freshly and used as substrate for ELISA test. The stopping solution consisted of H₂SO₄, 2N.

**Serum:** The sera were collected from 29 patients with recurrent infections from January 1989 to June 1990. These patients were referred from another hospital to this department for more investigations. They had normal Ig levels (IgG, IgA, and IgM) but several episodes of the bacterial and viral infections and allergic manifestations. The 16 of these patients who suffered from asthma had multiple episodes of pneumonia or bacillary infections and recurrent sinusitis. The remaining 13 patients had atopic disease, recurrent minor upper respiratory tract infections, or otitis media.

Blood samples were obtained from these children and then serum was separated and stored in aliquots at -70°C. Measurements of serum immunoglobulins G,A,M, and E were determined routinely as part of the evaluation of recurrent infection in children referred to the above center.

**Immunoaosay protocol:** Concentrations of IgG₁, IgG₂, IgG₃, and IgG₄ were measured by solid phase immunoenzymatic assay (ELISA). In brief, for routine assays, we used the following protocol:

1) 100 μl anti-human IgG 2,3, or four was diluted 1/5000 in coating buffer. They were incubated for 2 hours at 37°C and then overnight at 4°C.

2) The microplates were washed four times with washing solution, and 100 mL serum, standard or control were appropriately added at relevant dilutions. These were followed by an incubation for 2 hr at 37°C.

3) They were washed and 100 μL of conjugate at 1/3500 dilution was added. The plates were incubated for 2 hr at 37°C.

4) The plates were washed three times with washing solution; and 100 μL of peroxidase substrate was added; followed by an incubation for 30 min at room temperature.

5) The reaction was stopped with 50 μL of stopping solution and optical density (OD) was read at 492 nm.

**RESULTS**

The mean age of the 29 patients, 16 asthmatic patients and 13 atopic patients, was 5.5 years (ranging from 1.5 to 14). The sex distribution was 68.9% males and 31.1% females. About 62% of patients had a history of recurrent infection with more than two episodes per year.

These patients showed a significant difference between IgG subclass deficiency and frequency of infection (α = 0.05).

The immunological data for the 16 asthmatic patients and 13 atopic patients are summarized in Tables I and II. All 29 patients had normal levels of three major immunoglobulin classes for their age, but ten (10/16) atopic patients had high IgE levels and seven (7/13) atopic patients had high IgG levels.

Measurement of IgE subclasses in 16 asthmatic patients were found to be markedly low or near normal level in 11 patients; three had low levels of IgG₁, four had low levels of IgG₂, two had low levels of both IgG₁ and IgG₃, and one had low levels of IgG₃, IgG₄, and IgG₅.

Significantly decreased IgG subclasses in the asthmatic patients relate to the frequency of infections such as sinusopulmonary infection. Using chi-square analysis, this difference was significant (P = 0.005).

In the atopic patients IgG₄ level was strikingly elevated and this increase was statistically significant. In these patients, one case had levels of IgG₂ cases with low levels of IgG₄, and two cases with low levels of IgG₂ were
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Table I. Clinical characteristics of asthmatic patients.

<table>
<thead>
<tr>
<th>No</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Selective IgG subclass deficiency</th>
<th>Infections</th>
<th>IgG (mg/mL)</th>
<th>IgE (IU/mL)</th>
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</thead>
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<tr>
<td>1</td>
<td>1.5</td>
<td>M</td>
<td>N</td>
<td>P.S.I. (dyspnea)</td>
<td>7.80</td>
<td>21.8</td>
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<td>2</td>
<td>2</td>
<td>F</td>
<td>IgG₂, IgG₄</td>
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<td>0.6</td>
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<tr>
<td>3</td>
<td>3</td>
<td>M</td>
<td>IgG₂, IgG₄</td>
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<td>14.15</td>
<td>44</td>
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<tr>
<td>4</td>
<td>4.5</td>
<td>F</td>
<td>IgG₄</td>
<td>N.S.I.</td>
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<td>930</td>
</tr>
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<td>65</td>
</tr>
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<td>M</td>
<td>N</td>
<td>N.S.I.</td>
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<td>120</td>
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<td>8</td>
<td>6</td>
<td>M</td>
<td>N</td>
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<tr>
<td>9</td>
<td>7</td>
<td>F</td>
<td>IgG₂, IgG₄, IgG₄</td>
<td>sinusitis, otitis</td>
<td>23.00</td>
<td>46</td>
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<tr>
<td>10</td>
<td>7.5</td>
<td>M</td>
<td>IgG₂</td>
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<td>11</td>
<td>8</td>
<td>M</td>
<td>IgG₂</td>
<td>sinopulmonary</td>
<td>9.45</td>
<td>1000</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>M</td>
<td>N</td>
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<tr>
<td>13</td>
<td>9</td>
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<td>16</td>
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<td>M</td>
<td>IgG₄</td>
<td>sepsis</td>
<td>5.40</td>
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</table>

N= normal N.S.I.= no sign of infection
P.S.I.= possible sign of infection

Table II. Clinical characteristics of atopic patients.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Infections</th>
<th>IgG₁ (mg/mL)</th>
<th>IgG₂ (mg/mL)</th>
<th>IgG₃ (mg/mL)</th>
<th>IgG₄ (mg/mL)</th>
<th>Total IgG (mg/mL)</th>
<th>IgE (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>dyspnea</td>
<td>7.60</td>
<td>1.20</td>
<td>1.50</td>
<td>0.56</td>
<td>3.00</td>
<td>10.00</td>
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<td>1.5</td>
<td>M</td>
<td>urticaria</td>
<td>6.36</td>
<td>2.13</td>
<td>0.07</td>
<td>0.70</td>
<td>0.74</td>
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<td>2</td>
<td>M</td>
<td>skin eruption</td>
<td>3.84</td>
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<td>0.73</td>
<td>0.73</td>
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<td>food allergy</td>
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<td>0.85</td>
<td>0.05</td>
<td>0.85</td>
<td>10.50</td>
</tr>
<tr>
<td>2.5</td>
<td>F</td>
<td>itching, otitis</td>
<td>6.70</td>
<td>0.74</td>
<td>0.49</td>
<td>0.47</td>
<td>1.16</td>
<td>8.40</td>
</tr>
<tr>
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<td>M</td>
<td>eczema</td>
<td>10.80</td>
<td>2.90</td>
<td>0.09</td>
<td>0.24</td>
<td>1.15</td>
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<td>8.01</td>
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<td>M</td>
<td>perianal thrush</td>
<td>12.85</td>
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<td>0.92</td>
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<tr>
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<td>F</td>
<td>cough, dyspnea</td>
<td>10.86</td>
<td>1.08</td>
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<td>perianal eczema</td>
<td>5.45</td>
<td>1.06</td>
<td>0.03</td>
<td>1.59</td>
<td>1.59</td>
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<tr>
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<td>F</td>
<td>rhinitis, sinusitis</td>
<td>11.50</td>
<td>3.15</td>
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<td>0.96</td>
<td>1.34</td>
<td>13.54</td>
</tr>
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</table>

found. Recurrent infection in atopic patients who had lower IgG subclass levels was observed, but compared with those patients who had normal levels of IgG subclasses, this difference was not statistically significant.

DISCUSSION

Numerous reports on the IgG subclass deficiency in asthmatic and atopic patients have been published.16,7,16,19 These investigators showed the IgG subclass deficiency and the manifestations and the severity of infection.

Low serum IgG values have been noted previously in children with severe chronic asthma, in children with "intractable" asthma, and in those who did not respond well to vigorous treatment.1 Low levels of IgG have also been noted in many children who had severe chronic asthma. In another study Smith et al. (1984) reported low levels of IgG subclasses in many asthmatic children. IgG subclass deficiency was related to recurrent pulmonary infections.5,17

In this study, generally the level of serum IgE showed a significant increase in asthmatic patients but level of IgG
was normal or higher than normal (Table I). A correlation between the IgG subclass deficiency and the manifestations of infection was also present (α = 0.05). Almost all patients who had low levels of IgG subclasses had experienced chronic pulmonary and sinus infections, which were resistant to treatment. These patients suffered a frequent cough while patients who had normal levels of IgG subclasses had not experienced any infection.

In the present study a high level of IgG\textsubscript{2} was observed in most atopic patients (Table II). In 6 of 13 atopic patients (46%) the level of IgG\textsubscript{2} was higher than normal and the serum IgG\textsubscript{2} in atopic patients was 0.64 mg/mL and the mean average of control subjects was 0.25 mg/mL. This finding is similar to those reported by Wilson and Shakih\textsuperscript{18,19}. In this study, most patients with high levels of IgG\textsubscript{2} showed manifestations such as urticaria, eczema, perianal lesions or itching.

IgG\textsubscript{2} differs from the other IgG subclasses in its inability to bind complement effectively. In addition, this immunoglobulin is preferentially elevated in atopic dermatitis and hay fever although the significance of this is not fully understood. While the majority of atopie patients have elevated serum IgE and IgG\textsubscript{1} levels, measurement of total IgE and IgG\textsubscript{2} shows no simple relationship\textsuperscript{18,19}.

In our study IgG\textsubscript{2} levels also showed a weak positive correlation with serum IgE (r = 0.42). This finding is consistent with a previous study by Lilja et al\textsuperscript{18}.

IgG\textsubscript{2} may contribute to allergic processes in two ways. Firstly, it may act as a sensitizing antibody: the evidence for this is based on clinical studies showing that IgG\textsubscript{2} levels rise on desensitization.\textsuperscript{19}

The mechanism of IgG\textsubscript{2} elevation in atopic patients is not clear. It has been suggested that IgG\textsubscript{2} levels are raised due to prolonged exposure to an allergen which initiated the IgE response. A constant finding in 60-70% of patients with atopy is defective regulation of IgE and IgG\textsubscript{2} synthesis. Since patients with atopy have a reduction of circulating CD4\textsuperscript{+} cytotoxic/suppressor cells, it has been suggested that an inadequate number of suppressor T cells results in increased IgE and IgG\textsubscript{2} production.\textsuperscript{14} Recent studies have indicated that helper factors released by activated T lymphocytes play a major role in the regulation of IgE and IgG\textsubscript{2} secretion.\textsuperscript{11} IL-4 is a T\textsuperscript{+} cell-derived lymphokine that strikingly enhances the secretion of IgE and IgG\textsubscript{2}, and stimulates mast cell growth. IL-4 probably induces a switch in IgM-producing cells to IgE and IgG\textsubscript{2} production.\textsuperscript{11}

Based on the results of this study, we conclude that IgG subclass deficiency may account for severity and recurrence of infections in the population studied. Although in some patients level of IgG subclass was normal but they suffered from recurrent infection. In atopic patients, however, no significant relationship was noticed between IgG\textsubscript{2} levels and clinical manifestations.

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