

## 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 IgG<sub>4</sub>, six had marked low or low normal levels of IgG<sub>3</sub>, two had marked low normal levels of both IgG<sub>2</sub> and IgG<sub>3</sub> and one had low levels of IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>. All these patients suffered from recurrent sinopulmonary infections. There were low percentages of IgG<sub>3</sub> and IgG<sub>4</sub> defects (about 15%) in the atopic patients, while a significant increase in the serum IgG<sub>4</sub> levels were observed (six patient out of 13 patients, 46.2%).

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### INTRODUCTION

Four subclasses of human IgG are currently recognized: IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>; 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.<sup>3,9,15</sup> IgG<sub>1</sub> to IgG<sub>4</sub> constitute 65%, 25%, 7%, and 3% of total serum IgG, respectively.<sup>6,12</sup> 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 IgG<sub>2</sub>, 3 or 4 may occur in the presence of normal concentration of total serum IgG. In this situation even IgG<sub>1</sub> deficiency may occur in the presence of a normal total IgG level.<sup>12</sup>

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.<sup>1</sup> Low serum levels of IgG and IgA have been associated with IgG subclass deficiencies in children with chronic intermittent or persistent chest symptoms.<sup>18</sup>

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.<sup>13</sup>

While the mechanism of action of IgG antibodies in atopy is unresolved, there are clear indications for the IgG<sub>4</sub> subclass in atopy. For example, raised levels of IgG<sub>4</sub> have been found in patients with a variety of atopic conditions. In particular it is well marked in atopic dermatitis.<sup>4</sup> IgG<sub>4</sub> differs

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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 IgG<sub>4</sub> concentrations may be reduced or raised, sometimes to a considerable degree.<sup>12</sup> Measurements of total IgE and IgG<sub>4</sub> shows a simple relationship.<sup>19</sup>

## MATERIALS AND METHODS

**Buffer and other reagents:** Immunon I 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 and stored at 4°C until used. The clones produced the antibodies and their respective specificities (shown in parentheses) were: SG-16 (IgG<sub>1</sub>), HP-6014 and GoM2 (IgG<sub>2</sub>), HP-6050 and ZG4 (IgG<sub>3</sub>), HP-6025 and RJ4 (IgG<sub>4</sub>). 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 H<sub>2</sub>O<sub>2</sub> at 100 ml sodium citric-buffer were prepared freshly and used as substrate for ELISA test. The stopping solution consisted of H<sub>2</sub>SO<sub>4</sub>, 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 bacteremic 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.

**Immunoassay protocol:** Concentrations of IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub> were measured by solid phase immunoenzymatic assay (ELISA).<sup>8</sup> In brief, for routine assays, we used the following protocol:

1) 100 µL anti-human IgG<sub>1</sub>, 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 µL 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.

## Statistical Analysis

Differences in the frequencies of dichotomous parameters such as the presence of infection, sinopulmonary or sinusitis, to IgG subclass deficiency, were analysed by Pearson chi square analysis and were statistically significant ( $\alpha=0.05$ ). To estimate the relationship between the concentration of IgG<sub>4</sub> and IgE in the atopic patients, we performed coefficient correlation on concentration of IgG<sub>4</sub> in the sera of atopic patients. Despite the small number of patients studied a positive correlation was found between concentration of IgG<sub>4</sub> and IgE in atopic patients ( $r=0.4$ ) but no correlation was found between concentration of IgG<sub>4</sub> and IgE in asthmatic patients.

## 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 ( $\alpha=0.05$ ).

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) asthmatic patients had high IgE levels and seven (7/13) atopic patients had high IgE 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 IgG<sub>3</sub>, four had low levels of IgG<sub>4</sub>, two had low levels of both IgG<sub>3</sub> and IgG<sub>4</sub>, and one had low levels of IgG<sub>2</sub>, IgG<sub>1</sub>, and IgG<sub>4</sub>.

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

In the atopic patients IgG<sub>4</sub> level was strikingly elevated and this increase was statistically significant. In these patients, also one case with low levels of IgG<sub>2</sub>, two cases with low levels of IgG<sub>3</sub>, and two cases with low levels of IgG<sub>4</sub> were

Table I. Clinical characteristics of asthmatic patients.

No	Age (yr)	Sex	Selective IgG subclass deficiency	Infections	IgG (mg/mL)	IgE (IU/mL)
1	1.5	M	N	P.S.I. (dyspnea)	7.80	21.8
2	2	F	IgG <sub>3</sub> , IgG <sub>4</sub>	pneumonia	18.20	0.6
3	3	M	IgG <sub>3</sub> , IgG <sub>4</sub>	sinopulmonary	14.15	44
4	4.5	F	IgG <sub>3</sub>	N.S.I.	11.52	930
5	4.5	F	IgG <sub>4</sub>	penumonia	6.60	65
6	5	M	N	N.S.I.	7.80	120
7	6	M	N	N.S.I.	18.96	70
8	6	M	N	dermatitis	19.50	800
9	7	F	IgG <sub>2</sub> , IgG <sub>3</sub> , IgG <sub>4</sub>	sinusitis, otitis	23.00	46
10	7.5	M	IgG <sub>3</sub>	sinusitis	13.30	242
11	8	M	IgG <sub>4</sub>	sinopulmonary	9.45	1000
12	8	M	N	sinusitis	10.30	192
13	9	M	IgG <sub>4</sub>	sinopulmonary	11.83	300
14	9	M	IgG <sub>3</sub> , IgG <sub>4</sub>	sinopulmonary	14.10	293
15	9.5	M	N	sinusitis	18.94	220
16	6	M	IgG <sub>3</sub>	sepsis	5.40	289

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

P.S.I.= possible sign of Infection

Table II. Clinical characteristics of atopic patients.

Age (yr)	Sex	Infections	IgG <sub>1</sub> (mg/mL)	IgG <sub>2</sub> (mg/mL)	IgG <sub>3</sub> (mg/mL)	IgG <sub>4</sub> (mg/mL)	Total IgG (mg/mL)	IgE (IU/mL)
1	M	dyspnea	7.60	1.20	1.50	0.56	10.00	49
1.5	M	urticaria	6.36	2.13	0.07	0.70	9.40	14
2	M	skin eruption	3.84	2.05	0.62	0.73	7.35	160
2	M	food allergy	7.30	2.26	0.85	0.05	10.50	35
2.5	F	itching, otitis	6.70	0.74	0.49	0.47	8.40	42
3.5	M	eczema	10.80	2.90	0.09	0.24	14.00	71
5	M	dyspnea	8.01	3.20	0.26	0.71	12.00	28
5	M	perianal thrush	12.85	3.80	0.74	0.92	17.80	465
6	F	cough, dyspnea	10.86	1.08	0.13	1.40	13.95	54
6	F	perianal eczema	5.45	1.06	0.03	1.59	740	178
7	F	rhinitis, sinusitis	11.50	3.15	0.38	0.04	15.20	82
7	F	dyspnea	8.01	2.60	0.69	0.02	11.70	6
14	M	pneumonia	8.02	4.40	0.37	0.96	13.54	360

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.<sup>1,6,7,18,19</sup> 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.<sup>1</sup> 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.<sup>2,17</sup>

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 ( $\alpha=0.05$ ). 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<sub>3</sub> was observed in most atopic patients (Table II). In 6 of 13 atopic patients (46%) the level of IgG<sub>3</sub> was higher than normal and the serum IgG<sub>3</sub> 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 Shakib.<sup>16,19</sup> In this study, most patients with high levels of IgG<sub>3</sub> showed manifestations such as urticaria, eczema, perianal lesions or itching.

IgG<sub>4</sub> 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 atopic patients have elevated serum IgE and IgG<sub>4</sub> levels, measurement of total IgE and IgG<sub>4</sub> shows no simple relationship.<sup>16,19</sup>

In our study IgG<sub>4</sub> 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.<sup>10</sup>

IgG<sub>4</sub> may contribute to allergic processes in two ways. Firstly, it may act as a blocking antibody; this is limited to animal models and IgG<sub>4</sub> probably does not operate in this way in man. Secondly, it may act as a blocking antibody: the evidence for this in man is based on clinical studies showing that IgG<sub>4</sub> levels rise on desensitization.<sup>19</sup>

The mechanism of IgG<sub>4</sub> elevation in atopic patients is not clear. It has been suggested that IgG<sub>4</sub> 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<sub>4</sub> synthesis. Since patients with atopy have a reduction of circulating CD8+ cytotoxic/suppressor cells, it has been suggested that an inadequate number of suppressor T cells results in increased IgE and IgG<sub>4</sub> production.<sup>14</sup> Recent studies have indicated that helper factors released by activated T lymphocytes play a major role in the regulation of IgE and IgG<sub>4</sub> secretion.<sup>11</sup> IL-4 is a T-cell-derived lymphokine that strikingly enhances the secretion of IgE and IgG<sub>4</sub>, and stimulates mast cell growth. IL-4 probably induces a switch in IgM-producing cells to IgE and IgG<sub>4</sub> production.<sup>11</sup>

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<sub>4</sub> levels and clinical manifestations.

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### REFERENCES

1. Beger W, Pollock J, Kiechel G: IgG subclass deficiency in children with chronic severe asthma. *Ann Allergy* 41: 67, 1978.
2. Bernatowska-Matuszkiewicz E, Pac M, Liszka K: IgG subclasses and antibody response to pneumococcal capsular polysaccharides in children with severe sinopulmonary infections and asthma. *Immunol* 20 (2): 173-85, 1991.
3. Ferrante A, Rowan-Kelly R, Beard LJ, Maxwell GM: An enzyme linked immunosorbent assay for the quantitation of human IgG subclasses using monoclonal antibodies. *J Immunol Methods* 93: 207, 1986.
4. Gwynn CN, Morrison-Smith J, Leon G, Staworth DR: Role of IgG<sub>4</sub> subclass in childhood allergy. *Lancet* 1, 910, 1978.
5. Hanilos DL, Young RM, Peter JB: Hypogammaglobulinemia in asthmatic patients. *Ann Allergy* 68 (6): 472-81, 1992.
6. Hamilton RG: Human IgG subclass measurements in the clinical laboratory. *Clin chem* 33 (10): 1707, 1987.
7. Hansom LA, Soderstrom R, Avanzini A, Bengtsson U, Bjorkander J: Immunoglobulin subclass deficiency. *Pediatr Infect Dis J* 7 (5 suppl): 517-21, 1988.
8. Hussain R, Poindexter RW, Wistar R, et al: Use of monoclonal antibodies to quantify subclasses of human IgG. *J Immunol Method* 93: 89, 1986.
9. Jefferis R, Kumaratne DS: Selective IgG subclass deficiency: quantification and clinical relevance. *Clin Exp Immunol* 81: 357-67, 1990.
10. Lilja G, Magnusson CG, Oman H, Johansson SG: Serum levels of IgG subclasses in relation to IgE and atopic disease in early infancy. *Clin Exp Allergy* 20 (4): 407-13, 1990.
11. Lundgren M, Person U, Larsson P, Magnusson C, Smith E, Hammarstrom L, Severinson E: Interleukin 4 induces synthesis of IgE and IgG<sub>4</sub> in human B cells. *Eur J Immunol* 19: 1311-5, 1989.
12. Morgan G, Levinsky RJ: Clinical significance of IgG subclass deficiency. *Arch Dis Child* 63: 771, 1988.
13. Parish WE: Short-term anaphylactic IgG antibodies in human sera. *Lancet* ii, 591, 1970.
14. Reinhold U, Pawelec G, Herold WM: Immunoglobulin E and immunoglobulin G subclass distribution in vivo and relationship

- to in vitro generation of interferon- gamma and neopterin in patients with severe atopic dermatitis. *Int Arch Allergy Appl Immunol* 87: 120-6, 1988.
15. Schur PH, Borel H, Rosen FS: Selective gamma-G globulin deficiencies in patients with recurrent infections. *N Engl J Med* 283: 631, 1970.
  16. Shakib FP, McLaughlan DR, Stanworth E, Fairburn E: Elevated serum IgE and IgG<sub>4</sub> in patients with atopic dermatitis. *Br J Dermatol* 97: 59, 1977.
  17. Stanley PJ, Corbo Cole PJ: Serum IgG subclasses in chronic and recurrent respiratory infections. *Clin Exp Immunol* 58: 703, 1984.
  18. Smith TF, Morris EC, Raymond PB: IgG subclasses in nonallergic children with chronic chest symptoms. *J Pediatr* 105: 896, 1984.
  19. Wilson PB, Wood KJ, Dore P et al: Investigation of IgG<sub>4</sub> levels in atopic patients using a competitive inhibition assay employing biotinylated IgG<sub>4</sub> myeloma and avidin peroxidase. *J Immunol Method* 87: 59, 1986.