

Immunoglobulin G subclass concentrations and infections in children and adolescents with severe asthma

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It was the aim of this study to investigate possible dysfunctions of the humoral immune system in asthmatic children with frequent respiratory infections. Forty-one severe asthmatics (7–15 years of age), classified according to the Second Brazilian Consensus in Asthma (1998), were divided into two groups: group I (n = 12) had recurrent respiratory infections; and group II (n = 29) were without recurrent respiratory infections. Immunoglobulin (Ig)G, IgA and IgM levels (nephelometry), and IgE (radioimmunoassay) and IgG subclasses (enzyme-linked immunosorbent assay), were evaluated using standard methods.

Asthmatics with recurrent infections presented with worse clinical evolution, an increased number of hospital admissions, and a higher need of medication than the children without recurrent infections. There were no significant differences between the mean values of IgG, IgA or IgM levels, or IgE or IgG subclasses, in patients of both groups. A complete IgA deficiency was detected in two patients of group I (one was associated with IgG subclass deficiency). Deficiency of one or more IgG subclasses was verified in eight of 12 (66%) children from group I and in 16/29 (55%) from group II. The following deficiencies were found in both groups: IgG₃ (10/41), IgG₄ (three of 41), IgG₂ (two of 41), IgG₁ (one of 41), IgG₃-IgG₄ (four of 41), IgG₁-IgG₃ (two of 41), and IgG₁-IgG₃-IgG₄ (one of 41). There were a higher proportion of children with low IgG₄ levels in group I than in group II (p = 0.01). To conclude, IgA and IgG subclass deficiencies were detected in both severely asthmatic groups, with a predominance of IgG₃ subclass deficiency.

However, low IgG subclass levels appear not to be a suitable predictor of the development of infections in asthmatic children.

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Upper respiratory infections are reported to be trigger factors of acute attacks of asthma in children, with viruses appearing to be the primary causative agents. There are few data available that correlate bacterial respiratory infections (except sinusitis) with the acute symptoms of asthma. Although bacterial infections do not represent an important risk factor for asthma, they have been associated with secondary complications of viral infections (1). Severely asthmatic patients present several conditions that predispose to an increased susceptibility to infections of the respiratory tract, which are either directly associated with the pathophysiol-

ogy of asthma or occur as a result of medication influencing the immunologic response. Decreased levels of immunoglobulins or immunoglobulin G (IgG) subclasses may then further increase the risk for infection.

The principal immune response against different infectious agents invading the respiratory tract is humoral. Different immunoglobulin isotypes, and even the four IgG subclasses (serum distribution: 58–71.5% IgG₁, 19–31% IgG₂, 5–8.4% IgG₃, and 0.7–5% IgG₄), exert distinct biological activities. The humoral immune response to a certain antigen may be restricted to one subclass. Isolated or combined

immunoglobulin subclass deficiencies, even without a decrease in total IgG serum concentrations, have been described (2,3).

The absence or deficiency of one or more IgG subclasses has been associated with an increased risk of infections (4). The severity of clinical manifestations is unrelated to the degree of IgG subclass deficiency (3). Asymptomatic patients with a complete IgG subclass deficiency caused by γ heavy chain deletions, have been described (5). Antibodies against protein antigens, including bacterial, viral and autologous proteins, are usually of IgG₁ and IgG₃ subclasses. The IgG₂ subclass predominates in the response to polysaccharide antigens, although specific antibodies of other immunoglobulin subclasses have also been observed. An increased susceptibility to pathogens of the respiratory tract has been described for IgG₂ deficiencies, which is associated with the inability to mount a specific antibody response against polysaccharides (4).

The molecular background of IgG subclass deficiencies is currently not well understood. The defect is probably related to an impaired regulation of IgG gene expression in B cells during isotype switching. In some cases it occurs as a consequence of abnormal T-cell control or altered B-cell function (6). The reduction of T-lymphocyte help directed to specific subclass induction or excessive T-cell suppression may result in subclass deficiency (7).

Among clinical manifestations, severe or recurrent respiratory infections are those most frequently observed, mainly if IgG₂ is absent (4). In addition, a high frequency of atopic diseases has been described in IgG subclass-deficient individuals (6).

Immunoglobulin or IgG subclass deficiencies in asthmatic patients have been described (8–10). The patterns of humoral immunity dysfunction observed in asthmatic patients are variable. Increased IgG₄ levels (11–13), as well as selective or associated deficiency of IgG subclasses, were found (14); high IgE concentrations and IgA deficiency often occurs (8,9). Hoeger et al. described IgG subclass levels in 200 asthmatic children with variable severity of the disease, and without previous systemic steroid treatment, to be within the normal range and comparable to those in healthy children of the same age (15). There was, however, a tendency to find higher concentrations of IgG₁ and IgG₄ and decreased levels of IgG₂ in those patients.

Patients with recurrent infections have been described to respond favorably to intravenous gamma globulin. Page et al. observed fewer sino-pulmonary infections, an improvement of asthma

severity, and a reduction in the necessity for medication in asthmatics with IgG subclass deficiency upon intravenous immunoglobulin treatment (9). In contrast, Niggemann et al. did not achieve a significant reduction in the incidence of upper respiratory tract infections in a prospective, double-blind, placebo-controlled study (16).

With respect to the possible complications caused by recurrent respiratory infections in severely asthmatic patients, the aim of this study was to analyze parameters of humoral immunity in these children and adolescents.

Materials and methods

Forty-one patients (19 male, 22 female; 7–15 years of age) with severe asthma were included in the study. They were followed-up during a 15-month time-period in the Allergy and Immunology Unit at the Department of Pediatrics, University of São Paulo (São Paulo, Brazil).

A diagnosis of asthma and its severity were determined according to the Third International Pediatric Consensus Statement on the Management of Childhood Asthma (17) and included the following criteria:

- persistent symptoms;
- school absence or symptoms after exercise;
- frequent hospitalizations or steroid dependency;
- nocturnal symptoms;
- use of bronchodilators >2 times/day;
- continuous use of steroids;
- peak flow below 75% of the expected value and pulmonary functions.

Patients were classified into two groups according to the presence or absence of recurrent respiratory infections:

- Group I comprised 12 (four male and eight female) patients with respiratory infections (three or more episodes of pulmonary infections per year and/or six or more infections of the upper respiratory tract as sinusitis or otitis per year.
- Group II comprised 29 (15 male and 14 female) patients without recurrent infections.

During routine diagnosis, the following tests were performed on all patients: blood smear; stool parasitologic tests; delayed cutaneous hypersensitivity test for tuberculosis (purified protein derivative [PPD]); pulmonary function tests; thoracic, sinusal and adenoid X-rays; and immediate cutaneous prick tests with *Dermatophagoides pteronyssinus*, cat and dog dander, house dust, a mixture of fungal antigens, and wool. Blood samples were collected during remission

when the patients were clinically well and showed no signs of infection.

Immunoglobulin levels were analyzed by nephelometry, using a Behring Nephelometer 100 (Software version N 1,1 A (Behring, Marburg, Germany) with normal standards (Behring). Normal age-related values, as a Brazilian reference, were as reported by Fujimura et al. (18). IgA deficiency was defined for plasma concentrations ≤ 7 mg/dl (3). IgE levels were obtained by radioimmunoassay (RIA), using lyophilized particles of polystyrene coated with rabbit anti-human IgE.

IgG subclass analysis was performed by enzyme-linked immunosorbent assay (ELISA). In brief, each well of a microtiter plate (Nunc, Wiesbaden, Germany) was coated with 100 μ l of subclass-specific monoclonal antibodies (mAbs) to IgG₁ (1 μ g/ml), IgG₂ (1 μ g/ml), IgG₃ (10 μ g/ml), and IgG₄ (10 μ g/ml) (Calbiochem, Bad Soden, Germany) and incubated for 18 h at -4°C . Blocking solution (a 10% solution of dried milk powder in phosphate-buffered saline [PBS]) (200 μ l) was added and incubated for 90 min at room temperature. After washing six times with PBS–Tween-20, 0.1 μ l of diluted standard serum (1:500, 1:1000, 1:2000, 1:4000 and 1:8000; 'Binding site-code KC1, Batch CS253') or diluted patients' sera (1:1000 and/or 1:4000) were added

and incubated for 90 min at room temperature. After washing, 100 μ l of peroxidase-conjugated goat anti-human IgG (Sigma, Deisenhofen, Germany), diluted 1:1000 for IgG₁ and IgG₂ and 1:500 for IgG₃ and IgG₄, was added and incubation continued for 90 min at room temperature. Orthophenylenediamine (OPD)/H₂O₂ was added as the reaction was terminated with 100 μ l of sulfuric acid (12%) and the absorbance was measured in an ELISA reader at $\lambda=492$. Subclass deficiency was defined for values <2 SD of normal concentrations for the respective age-group, as established by Ribeiro (19).

Upon receiving informed consent of the parents/guardians of the patients, blood samples were collected at the same time that routine tests were performed. The protocol was approved by the Ethical Committee of the Department of Pediatrics and Hospital das Clínicas, University of São Paulo.

Descriptive data are presented in tables. Differences in concentrations of immunoglobulin classes and IgG subclasses were calculated for statistical significance by using analysis of variance. For continuous variables, variance analysis was performed by the Fischer and Student's *t*-tests. Clinical symptoms were compared between both groups by using the chi-

Table 1. Clinical and laboratory data and diagnostic criteria for severe asthma, according to the Second Brazilian Consensus on Asthma and International Consensus of Asthma (19), as applied in group I (GI) and group II (GII)

	Group		p-value
	I	II	
Total number of patients	12	29	
Male : female	4 : 8	15 : 14	NS
Age ≤ 10 years (%)	4 (33.3)	12 (41.4)	NS
Age of first symptoms ≤ 12 months, n (%)	9 (75)	14 (48.3)	p=0.0094
Other atopic diseases, n (%)	10 (83.3)	28 (96.5)	NS
Family history of atopy, n (%)	12 (100)	27 (93.1)	NS
Thoracic deformity, n (%)	7 (58.3)	6 (20.7)	p=0.0239
Positive stool parastitologic tests, n (%)	1/12	2/27	NS
Continuous symptoms, n (%)	11 (91.7)	20 (69)	NS
Hospital assistance/year, n (%)			
In-patient ≥ 4	4 (33.3)	2 (6.9)	0.0293
ER ≥ 10	9 (75)	7 (24)	p=0.02
Use of bronchodilator, n (%)	10 (83.3)	18 (62)	NS
Steroid use, n (%)			
Oral	6 (50)*	10 (34.5)*	GI>GII, p<0.0028
Inhalatory	6 (20.7)	20 (69)	NS
Infections/year, n (%)			
BCP ≥ 3	9 (75)	0	p<0.0001
URI ≥ 6	3 (25)	0	p=0.005
Positive allergy tests, n (%)	9/11	24/25	NS
Lung function, n (%)			
(FEV ₁ < 65%)	9/11	16/25	NS

*Corticoid dependent.

BCP, bronchopneumonia; ER, emergency room; FEV₁, forced expiratory volume; NS, not significant; URI, upper respiratory infection.

Thoracic deformity: increased antero-posterior diameter, kyphosis, sternum retraction.

Group I, asthmatics with recurrent infections; group II, asthmatics without recurrent infections.

Table 2. Immunoglobulin A (IgA) and immunoglobulin G (IgG) subclass deficiencies

Deficiencies*	Group I (n=12)	Group II (n=29)	Total (n=41)	p-value
IgA	1	0	1	p=0.2927
IgG ₁	0	1	1	p=1.0
IgG ₂	1	1	2	p=0.5049
IgG ₃	1	9	10	p=0.2313
IgG ₄	1	2	3	p=1.0
IgG ₁ -IgG ₃	0	2	2	p=1.0
IgG ₃ -IgG ₄	3	1	4	p=0.0679
IgG ₁ -IgG ₃ -IgG ₄	1	0	1	p=0.2927
IgA/IgG ₃ /IgG ₄	1	0	1	p=0.2927
Total†	9	16	25	p=0.3054

Group I, asthmatics with recurrent infections; group II, asthmatics without recurrent infections.

*Group I vs. group II, not significant.

†Isolated and combined IgG₄ subclass deficiency: group I (six of 12) > group II (three of 29); p=0.0106. IgA deficiency was defined as IgA <7mg/dl and IgG subclass deficiencies were defined as values <2.5%.

square test. Values were considered significant at a p-value of <0.05.

Results

Clinical and laboratory data, as well as the main characteristics for asthma classification, are summarized in Table 1. A statistical difference between both groups was detected for the frequency of emergency visits (p=0.02). Group I comprised eight (67%) female and four (33%) male patients (mean age 10.7 years: 7–10 years, n=4; 10–13 years, n=4; 13–15 years, n=4). Group II comprised 14 (48%) female and 15 (52%) male patients (mean age 11 years: 7–10 years, n=12; 10–13 years, n=9; 13–15 years, n=8). Nine patients (75%) of group I presented three or more pulmonary infections/year and three children (25%) had six or more episodes of sinusitis/year. The mean age at the first asthmatic symptoms was lower in group I than in group II (p = 0.0094). Most of the children of groups I and II (10/12 and 28/29, respectively) were affected by other atopic conditions besides asthma, with rhinitis being the most common (10/12 and 27/29 in groups I and II, respectively). All the patients from group I (n=12) and 27/29 of group II presented a family history of atopic diseases. There were a significantly greater number of corticoid-dependent children in group I than in group II (p= 0.0028).

Comparative mean IgG (p= 0.1773) and IgA (p = 0.7568) levels were found in both groups. Two children of group I were IgA deficient. Mean IgM levels of group I patients exceeded the upper limit for age (p = 0.0036), whereas IgM concentrations in group II were within the normal range. There was no statistical difference between both groups for IgM levels (p = 0.2661).

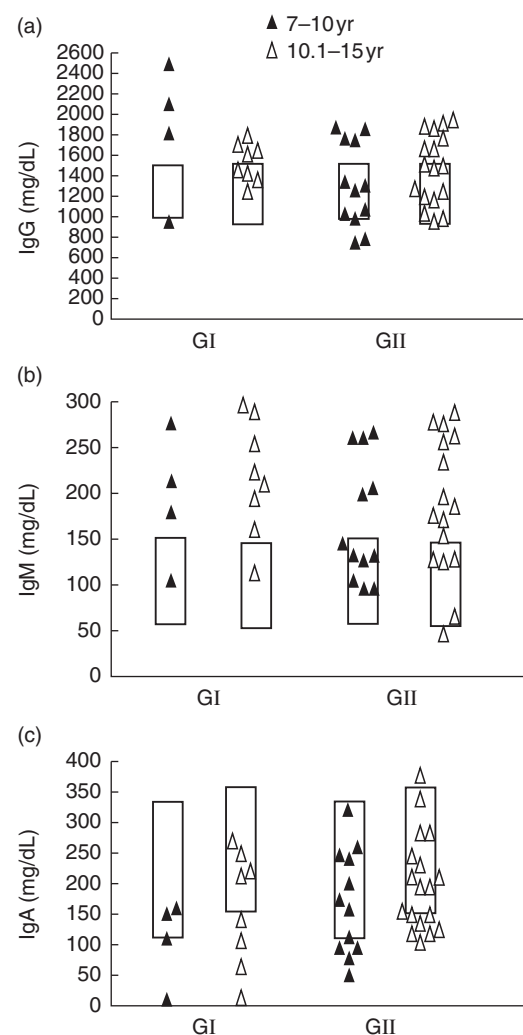
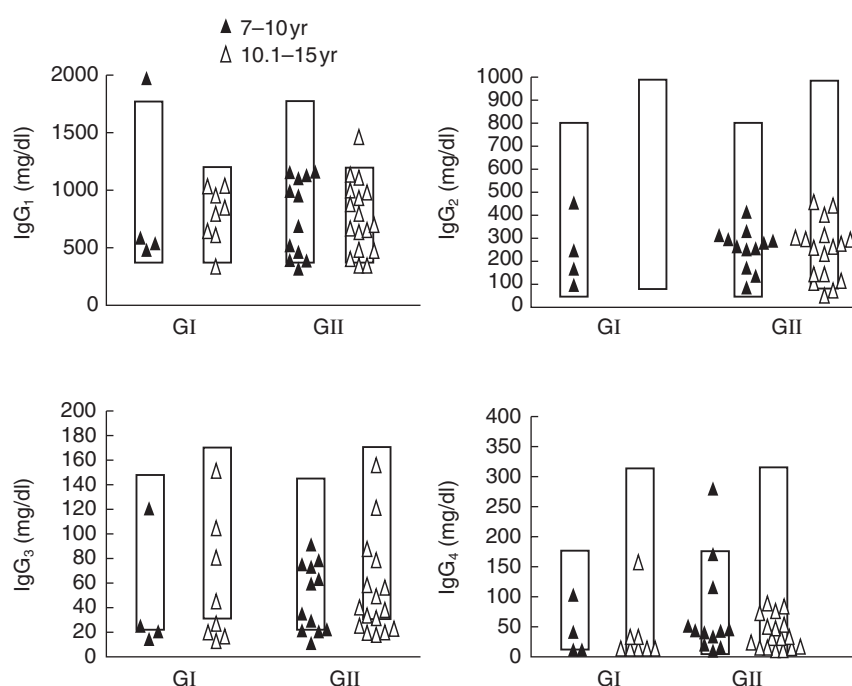


Fig. 1. Immunoglobulin levels in severe asthmatics with recurrent infections (group I [GI]) or without infections (group II [GII]). (a) immunoglobulin (Ig)G levels; (b) IgM levels; (c) IgA levels. Boxes represent variability of concentrations from percentile 2.5 to 97.5 in healthy individuals.

Fig. 2. Distribution of immunoglobulin G (IgG) subclasses in asthmatics with recurrent infections (group I [GI]) or without infections (group II [GII]). Boxes represent variability of concentrations from percentile 2.5 to 97.5 in healthy individuals.



IgG subclass deficiencies are summarized in Table 2. IgA or IgG subclass deficiency was found in nine of 12 patients from group I (75%) and in 16/29 from group II (55%). Isolated IgG₃ deficiency was the most frequent (10/41; 24.3%) subclass deficiency, followed by IgG₄ (three of 41; 7.3%), IgG₂ (two of 41; 4.8%), IgG₁ (one of 41; 2.4%), and IgA (one of 41; 2.4%). Combined subclass deficiencies were: IgG₃-IgG₄ (four of 41; 9.7%), IgG₁-IgG₃ (two of 41; 4.8%), IgG₁-IgG₃-IgG₄ (one of 41; 2.4%), and IgA-IgG₃-IgG₄ (one of 41-2.4%). The distribution of IgG, IgM and IgA levels, and of IgG subclasses, are presented in Fig. 1 and Fig. 2, respectively. IgE levels are presented in Fig. 3 (group I vs. group II; $p=0.3856$). No correlation was found between IgE and IgG₄

concentrations (group I: $p=0.2567$; group II: $p=0.0650$).

A higher number of single and combined IgG₄-deficient patients was detected in the group of children with recurrent infections ($p=0.0312$) (Table 2). Increased numbers of immunoglobulin deficiencies were not necessarily associated with the occurrence of infections: IgG₁, $p=0.8293$; IgG₂, $p=0.961$; IgG₃, $p=0.2503$; IgG₄, $p=0.6883$ (the p -value refers to IgG subclass vs. occurrence of infections).

Discussion

Asthma is considered to be one of the most important chronic diseases in childhood with respect to its high incidence and the increasing morbidity/mortality observed in the last years. Considering the fact that a subgroup of severe asthmatics present upper respiratory infections, we were interested in studying whether certain immunologic parameters reflected a higher risk of infections in these children. In particular, this study was performed to identify possible deficiencies of immunoglobulins and IgG subclass levels in severe asthmatics, as a possible explanation of an increased susceptibility to infections in these children.

The asthmatic patients were diagnosed and classified according to a previous publication (17), and additional criteria (functional pulmonary tests, thoracic deformation and psychological problems) were included. Patients of group I

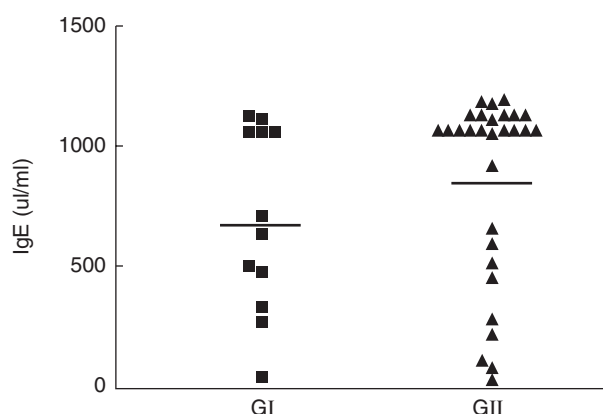


Fig. 3. Immunoglobulin E (IgE) concentrations in asthmatics with recurrent infections (group I [GI]) or without infections (group II [GII]). GI×GII=not significant.

(those with recurrent infections) had an increased number of hospitalizations and visits to the emergency room, a more pronounced thoracic deformation, worse pulmonary function, and a stronger need for steroids and bronchodilators than patients of group II.

Classification of the patients into two groups also took into account the frequency of infections and the age, being limited to 7–18 yr. Seven years, as the lower age limit for study entry, was chosen to exclude children with immunologic immaturity, together with lower levels of immunoglobulins and/or an inappropriate response to polysaccharide antigens.

Mean IgA levels were comparable in both groups. However, two IgA-deficient patients were identified in group I, one also presenting with a combined IgG₃-IgG₄ deficiency. It is estimated that $\approx 50\%$ of IgA-deficient patients are asymptomatic but may develop severe allergy at a frequency three times higher than in those who are not IgA deficient (20). In $\approx 50\%$ of 60 symptomatic IgA-deficient patients followed up in our Unit we have observed symptoms of allergy, mainly asthma (21). Ayres & Thompson evaluated asthmatic patients with mild, persistent asthma, or affected by infections, and detected a reduction of mean IgA and IgG₃ levels, but observed no cases of IgA deficiency (22).

The association of IgA and IgG subclass deficiencies (mainly of IgG₂ or IgG₄) with respiratory infections has been described previously (23,24). These patients present with a greater number of infections compared to those with isolated IgA deficiency. Impaired pulmonary function, bronchiectasia, and poor prognosis were described in these patients (24). Both of the IgA-deficient patients described in the present study were detected in the group with recurrent infections and one of them had associated IgG₃-IgG₄ subclass deficiency.

Patients of group I had higher levels of IgM than those of group II, confirming previous findings (25). Stanley et al. (26) detected high concentrations of IgG, IgA, IgM, IgG₁ or IgG₂ in 27% of 47 patients with chronic or recurrent respiratory infections. Ribeiro (19) also observed similar elevated mean IgM levels in asthmatics, independently of the existence of any pulmonary infections.

Although mean IgG concentrations were almost never affected, we observed an increased frequency of IgG subclass deficiency. The four IgG subclasses have different biological activities, and the humoral immune response to a certain antigen may be restricted to one subclass. A deficiency may result in a defective response to a

pathogen of the respiratory tract, as described for IgG₂ deficiency with a lack of antibodies against polysaccharides (4).

Since Schur et al. (27) described IgG subclass deficiencies in patients with recurrent bacterial infections, many studies have been performed aiming to better define the associated clinical syndrome. Alterations in serum concentrations of IgG subclasses have been reported in recurrent or chronic respiratory infections (4,10,19,25,26,28) and also in asthmatic patients (8,10). Establishing normal ranges of plasma levels for each IgG subclass in a given population is hampered by a lack of comparability, as a result of methodological variations (29). In the present study, we compared our data with those of a Brazilian study (19), applying the same methodology (ELISA).

IgG₁ deficiency was detected in one of 12 group I patients and in three of 29 group II patients (one of whom had additional isolated IgG₃ deficiency). IgG₁ constitutes the dominant isotype against viral proteic antigens. Smith et al. described the occurrence of an isolated IgG₁ deficiency in a family where a 5-year-old girl suffered from asthma and recurrent respiratory infections. Her father and maternal grandfather were also IgG₁ deficient and had asthma, but no infections. Her aunt and maternal grandfather with the same deficiency were asymptomatic (30). Increased IgG₃ values in the patient and her father were interpreted as a compensatory mechanism.

Isolated IgG₂ deficiency was detected in one patient in each of group I and group II, a frequency lower than reported previously (23,26,28). Isolated IgG₂ deficiency has been described in asthmatics with recurrent respiratory infections (10).

IgG₃ deficiency was the most common deficiency in this study, affecting six (50%) patients of group I and 12 (41%) of group II. IgG₃ is the predominant subclass involved in the primary immune response to proteic viral antigens. In addition, IgG₃ is responsible for the primary immune response to *Moraxella catarrhalis* and to the M component of *Streptococcus pyogenes*. As IgG₃ shares functional properties with the IgG₁ subclass, complete IgG₃-deficient individuals may remain asymptomatic (31).

In a previous study evaluating IgG subclasses in IgA-deficient patients, radial immunodiffusion demonstrated IgG₄ deficiency in $\approx 50\%$ (32). With respect to the limited sensitivity of this method, a partial deficiency cannot be distinguished. To improve sensitivity, enabling us to distinguish complete from partial immunoglobulin

lin deficiency, all measurements of immunoglobulins were performed by ELISA (19,29).

In our study, IgG₄ deficiency was detected in six (50%) patients of group I and only in three (10%) of group II, with a significant difference between both groups. Other studies, using different diagnostic methods, have frequently demonstrated IgG₄ deficiency in patients affected by recurrent pulmonary infections. Beck & Heiner studied IgG subclasses (using radioimmunoassay) in 422 individuals; four of 29 IgG-deficient patients presented recurrent infections (25). Moss et al. detected an IgG₄ deficiency in 21/123 children with recurrent respiratory infections (17.1%) (33).

Treatment of severely asthmatic patients with steroids may influence immunoglobulin or IgG subclass levels (34). Klaustermeyer et al. (35) detected low levels of IgG subclasses in six of 12 corticosteroid-dependent asthmatics with sino-pulmonary infections, whereas none of 12 asthmatics without steroid treatment had decreased IgG subclass levels. The underlying mechanism of the impact of steroids on immunoglobulin synthesis is still obscure. Multiple effects have been described, including inhibition of antigen uptake by antigen-presenting cells and a decreased number and function of CD4⁺ T cells, leading to impaired immunoglobulin production (35). However, we could not detect a significant correlation between steroid doses received and immunoglobulin or IgG subclass concentrations in both groups of the present study. Continuous steroid use was significantly higher in group I and may have influenced immunoglobulin levels and/or the occurrence of recurrent infections.

The molecular mechanisms responsible for IgG subclass deficiency are not entirely clear. The defect is probably related to an impaired regulation of IgG gene expression in B cells during isotype switching. In some cases, it also may occur as a consequence of abnormal T-cell control or altered B-cell function (6). Diminished T-lymphocyte function directed to specific subclass induction or excessive T-cell suppression may result in subclass deficiency (7).

Severe asthma may become associated with an increased susceptibility to infections if immunoglobulin or IgG subclass levels are suppressed as a result of various diseases or treatment-associated conditions. Considering that many healthy individuals present with IgG subclass deficiencies (7,31), a direct relationship of these immunodeficiencies to infections should be considered with special care, especially with respect to the need for gamma globulin treatment. Some studies show an improvement of clinical conditions in patients

with established immunodeficiencies upon treatment with gamma globulin (36). However, this effect was not observed in asthmatic patients with low immunoglobulin subclass levels (16). In our study, the detection of a relative humoral immunodeficiency was not related to the occurrence of pulmonary infections. However, a higher frequency of partial immunoglobulin deficiency could be demonstrated in severe asthmatics.

References

1. CYPGAR D, STARK J, LEMANSKE RF. The impact of respiratory infections on asthma. *Pediatr Clin N Am* 1992; 39: 1259–75.
2. FADAL RG. Chronic sinusitis, steroid dependent asthma, and IgG subclass and selective antibody deficiencies. *Otolaryngol Head Neck Surg* 1993; 109: 606–10.
3. SORESENSEN RU, MOORE C. Antibody deficiency syndromes. *Pediatr Clin N Am* 2000; 47: 1225–52.
4. GROSS S, BLAISS MS, HERROD HG. Role of immunoglobulin subclasses and specific antibody determinations in the evaluation of recurrent infection in children. *J Pediatr* 1992; 121: 516–22.
5. LEFRANC MP, HAMMARSTRÖM L, SMITH CI, et al. Gene deletions in the human immunoglobulin heavy chain constant region locus: molecular and immunological analysis. *Immunol Rev* 1991; 2: 265–81.
6. MORGAN G, LEVINSKY RJ. Clinical significance of IgG subclass deficiency. *Arch Dis Child* 1988; 63: 771–3.
7. HAMMARSTRÖM L, HOLM G, PALBLAD J, PERSSON MAA, SMITH CIE. Lack of IgG in a healthy adult: a rare case of dysgammaglobulinemia with undetectable serum IgG, IgA2, and IgE. *Clin Immunol Immunopathol* 1984; 30: 1–10.
8. LOFTUS BG, PRICE JF, LOBO-YEO A, VEGANI D. IgG subclass deficiency in asthma. *Arch Dis Child* 1988; 63: 1434–7.
9. PAGE R, FRIDAY G, STILIWAGON P, SKONER D, CALIGURI L, FIREMAN P. Clinical and laboratory observations. Asthma and selective immunoglobulin subclass deficiency: improvement of asthma after immunoglobulin replacement therapy. *J Pediatr* 1988; 112: 127–31.
10. SILK H, GEHA RS. Asthma, recurrent infections and IgG2 deficiency. *Ann Allergy* 1988; 60: 134–6.
11. GWYNN CM, SMITH JM, LEON GL, STANWORTH DR. Role of IgG4 subclass in childhood allergy. *Lancet* 1978; 29: 910–1.
12. SCHUR PH. IgG subclasses – a review. *Ann Allergy* 1987; 58: 89–101.
13. HEINER DC. Significance of immunoglobulin G subclasses. *Am J Med* 1984; 76: 1–6.
14. DE JOSE GOMEZ MI, GONZALEZ DE DIOS J, DE HERNANDO LARRAMENDI C, JIMENEZ GARCIA A, VIDAL LOPEZ ML, GARCIA HORTIELANO J. IgG2 deficiency associated with recurrent pneumonia and asthma. *An Esp Pediatr* 1990; 33: 258–64.
15. HOEGER PH, NIGGEMANN B, HAEUSER G. Age related IgG subclass concentration in asthma. *Arch Dis Child* 1994; 70: 179–82.
16. NIGGEMANN B, LEUPOLD W, SCHUSTER A, et al. A prospective, double blind, placebo controlled, multicentre study on the effect of high dose, intravenous immunoglobulin in children and adolescents with severe bronchial asthma. *Clin Exp Allergy* 1998; 28: 205–10.
17. WARNER JO, NASPITZ CK, CROPP GJA, et al. Third International Pediatric Consensus Statement on the Management of Childhood Asthma. *Pediatr Pulmonol* 1998; 25: 1–17.
18. FUJIMURA MD. Níveis séricos das subclasses de imunoglobulina g em crianças normais e nefróticas. Doctoral Thesis. São Paulo: Faculty of Medicine, University of São Paulo, 1990.

19. RIBEIRO JD. Níveis séricos das subclasses de IgG em crianças asmáticas graves com e sem pneumonias de repetição, Doctoral Thesis. São Paulo: Faculty of Medical Science, University of Campinas, 1994.
20. BUCKLEY RH. Clinical and immunologic features of selective IgA deficiency. *Birth Defects. Original Article Series* 1975; XI: 134–141.
21. GRUMACH AS, JACOB CMA, PASTORINO AC. Deficiência de IgA: Avaliação clínico-laboratorial de 60 pacientes do Instituto da Criança. *Rev Ass Med Bras* 1998; 44: 277–82.
22. AYRES JG, THOMPSON RA. Low IgG subclass levels in brittle asthma and in patients with exacerbations of asthma associated with respiratory infection. *Respir Med* 1997; 91: 464–9.
23. UMETSU DT, AMBROSINO DM, QUINTI I, SIBER GR, GEHA RS. Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG subclass deficiency. *N Engl J Med* 1985; 313: 1247–51.
24. OXELIUS VA, LAURELL AB, KINDQUIST B, et al. IgG subclasses in selective IgA deficiency. Importance of IgG2-IgA deficiency. *N Engl J Med* 1981; 304: 1476–7.
25. BECK CS, HEINER DC. Selective immunoglobulin G deficiency and recurrent infections of the respiratory tract. *Am Rev Respir Dis* 1981; 124: 94–6.
26. STANLEY PJ, CORBO G, COLE PJ. Serum IgG subclasses in chronic and recurrent respiratory infections. *Clin Exp Immunol* 1984; 58: 703–8.
27. SCHUR PH, BOREL H, GELFAND EW, ALPER CA, ROSEN FS. Selective gamma G globulin deficiencies in patients with recurrent pyogenic infections. *N Engl J Med* 1970; 283: 631–4.
28. DE BAETS F, KINT J, PAUWELS R, LEROY J. IgG subclass deficiency in children with recurrent bronchitis. *Eur J Pediatr* 1992; 151: 274–8.
29. MELSSNER C, REIMER CG, BLACK C, et al. Interpretation of IgG subclass values: a comparison of two assays. *J Pediatr* 1990; 117: 726–31.
30. SMITH CI, HAMMARSTRÖM L, DE HENTER JJ, LANGE GG. Molecular and serologic analysis of IgG1 deficiency caused by new forms of the constant region of the Ig H-chain gene deletions. *J Immunol* 1989; 142: 4514–9.
31. LEFRANC G, DUMITRESCO SM, SALIER JP, et al. Familial lack of the IgG3 subclass gene elimination or turning off expression and neutral evolution in the immune system. *J Immunogenet* 1979; 6: 215–21.
32. CASTRIGNANO SB, CARLSSON B, CARNEIRO-SAMPAIO MS, SODERSTROM T, HANSON L. IgA and IgG subclass deficiency in a poor population in a developing country. *Scand J Immunol* 1993; 37: 509–14.
33. MOSS RB, CARMACI MA, ESRIG S. Deficiency of IgG4 in children: Association of isolated IgG4 deficiency with recurrent respiratory tract infection. *J Pediatr* 1992; 120: 16–21.
34. SETTIPANE GA, PUDUPAKKAM RK, MCGOWAN JH. Corticosteroid effect on immunoglobulins. *J Allergy Clin Immunol* 1978; 62: 162–6.
35. KLAUSTERMEYER WB, WONG SC, SCHOETTLER JJ, GIANOS ME, HEINER DC. Quantitative immunoglobulins and IgG subclasses in patients with corticosteroid dependent reversible airway obstruction. *Ann Allergy* 1989; 63: 327–30.
36. GRAY BM. Immune globulin administration as an approach to prevention of acute otitis media. *J Pediatr* 1993; 123: 739–41.