Age related IgG subclass concentrations in asthma

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Abstract
The prevalence of IgG subclass deficiency in asthma is still controversial. Earlier studies often included patients receiving treatment with systemic steroids which can induce hypogammaglobulinaemia. Concentrations of IgG subclasses were studied in 200 children (aged 2–17 years) with asthma (mean asthma severity score (ASS) 2, range 1–4) who had not received systemic steroids for at least six weeks before investigation, and in 226 healthy age matched controls. The mean concentrations of IgG subclasses in children with asthma were within the ISD range of those of the control group. In the group with asthma there was a trend towards higher levels of IgG1 and IgG2, whereas the number of children with low concentrations of IgG1 (<2 SD of control serum samples; absolute concentrations 0.08–1.25 g/l) was slightly greater than in the group who did not have asthma (4.5% v 2.9%). Patients with subnormal concentrations of IgG2 could not be distinguished clinically or on the basis of case history and additional immunological studies did not show further abnormalities. Patients with severe asthma (ASS 3–4) had significantly higher concentrations of IgG4 (mean (SE) 0.53 (0.09) v 0.26 (0.04) g/l) than patients with mild asthma (ASS 1). No significant difference in subclass concentration was found between patients with atopic and those with non-atopic asthma.

It is concluded that in an unselected group of children with asthma the mean IgG subclass concentrations do not differ significantly from a group of healthy age matched controls.

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Asthma is characterised by acute episodes of airway obstruction precipitated by respiratory infections and the release of IgE dependent mediators. Airway inflammation resulting from an inappropriate response to either infectious or allergic antigens is a finding common to the different manifestations of asthma. Activation of CD4+ T lymphocytes and alterations in the pattern of lymphokines released contribute to its pathogenesis. In patients with atopic asthma enhanced production of interleukin 4 (IL-4) and decreased production of interferon γ are associated with increased serum concentrations of IgE, IgG1, and IgG4. Various immunological abnormalities have been reported in patients with asthma, including hypogammaglobulinaemia (IgG, IgA) and a deficiency of IgG subclasses. Some studies have indicated a therapeutic role for intravenous immunoglobulins in patients with severe asthma. These studies were mainly of adults, however. Reports about concentrations of IgG subclasses in children are rare and mostly focus on children with severe forms of asthma. Furthermore, many studies are hampered by concomitant treatment with systemic steroids, which can induce hypogammaglobulinaemia and IgG subclass deficiency.

The prevalence of IgG subclass deficiency in children with asthma still remains to be elucidated. We therefore studied 200 children with asthma of different grades of severity. To control for the effects of systemic corticosteroids on immunoglobulin concentrations, none of the patients studied had received systemic steroids for at least six weeks before the investigation. A group of 226 age matched children unaffected by asthma or atopy were studied as a control group. We tried to correlate IgG subclass concentrations with age, disease severity, and concentrations of IgE.

Patients and methods
PATIENTS AND CONTROLS
Two hundred children aged 2–17 years (139 boys) with a history of recurrent airway obstruction of at least six months’ duration were studied. The age distribution was as follows: 2–3 years, 25; 4–5 years, 30; 6–7 years, 35; 8–9 years, 31; 10–11 years, 22; 12–13 years, 25; 14–15 years, 22; and 16–17 years, 10 children. The diagnosis of bronchial asthma was established according to the criteria of the American Thoracic Society. All patients were assigned an asthma severity score (ASS) based on clinical data (frequency and severity of symptoms) and lung function parameters.

The median score was 2.0 (range 1–4; grade 1, 28%; grade 2, 53%; grade 3, 17%; grade 4, 14%). Individual or family history, or both, was positive for atopic diseases in 183 (92%) children. The mean (range) serum IgE level was 483 (1–4120) kU/l. Seventeen (9%) children had non-atopic asthma triggered by infections or exertion, or both, and characterised by the absence of allergen related symptoms, normal IgE (mean (range) serum IgE 22 (3–59) kU/l) and a negative radio allergosorbent test (RAST) to seven common inhalative allergens. Treatment for asthma consisted of inhaled sodium cromoglycate and bronchodilators, inhaled budesonide or theophylline by mouth, or both, according to disease activity. None of the patients with asthma had received systemic steroids for at least six weeks before investigation.
Occurrence of high (>2 SD) and low (<2 SD, IgG < 0.05 g/l) concentrations of IgG subclasses in children with asthma and in healthy controls. Values are number (%)

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Patients (n=200)</th>
<th>Controls (n=226)</th>
<th>Patients (n=200)</th>
<th>Controls (n=226)</th>
<th>Patients (n=200)</th>
<th>Controls (n=226)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 SD</td>
<td>9 (4.5)</td>
<td>17 (7.5)</td>
<td>16 (8.0)</td>
<td>15 (6.6)</td>
<td>14 (7.0)</td>
<td>18 (8.0)</td>
</tr>
<tr>
<td>&lt;2 SD</td>
<td>5 (2.5)</td>
<td>4 (1.8)</td>
<td>9 (4.5)</td>
<td>5 (2.2)</td>
<td>5 (2.5)</td>
<td>3 (1.3)</td>
</tr>
</tbody>
</table>

The ranges of low subclass concentrations in the different age groups were (g/l): IgG1 2.5–4.1; IgG2 0.08–1.25; IgG3 0.08–0.14; IgG4 0.0–0.04 g/l. Differences in the frequency of low or high subclass concentrations between patient and control group were not significant (p>0.05; χ² test).

Two hundred and twenty six children presenting to the outpatient clinic of the Children’s Hospital, University of Hamburg, for evaluation before an operation (elective surgery for cleft palate, hernia, dental procedures) or for evaluation of presumed growth disorders served as age matched controls. Children with an active or chronic inflammatory process, or with a known history of allergies or increased IgE (>100 kU/l) were excluded. Serum samples were collected, with informed parental consent, during routine venepuncture.

IMMUNOGLOBULIN ASSAYS
All serum samples were analysed for total IgG, IgM, IgA, and IgE. IgG1, IgG2, and IgG3 were determined by nephelometry (Behring Nephelometer 100, Behring, Marburg, Germany) and IgE by enzyme linked immunosorbent assay (ELISA) (Photometric Analyzer PA2, Pharmacia, Freiburg, Germany).

DETERMINATION OF IGG SUBCLASSES
IgG subclasses 1–4 were measured by radial immunodiffusion (Binding Site Ltd, Birmingham, UK) using polyclonal sheep anti-human antibodies. Polyclonal antisera were chosen because of concerns about monoclonal antibodies being too restricted in their specificity.19 20 The lower detection limits for IgG1, IgG2, IgG3, and IgG4 were 14, 8, 12, and 5 mg/l respectively. The interassay coefficient of correlation for all subclasses was less than 10%. Serum samples with known IgG subclass concentrations (low, intermediate, and high concentrations) served as internal calibrators. World Health Organisation reference serum 6797 was used as a standard. The concentrations of total IgG correlated well with the sum of all subclasses (linear regression analysis r>0.9; p<0.001).

STATISTICAL ANALYSIS
Student’s t test, Wilcoxon rank sum tests, the χ² test, and regression analysis according to Pearson and Bravais were used as indicated.

**Results**
SERUM IMMUNOGLOBULINS IgG, IgA, IgM, and IgE.
There was no significant difference in mean serum concentrations of total IgG, IgA, and IgM between patients and controls (data not shown). Concentrations of IgE, however, were significantly higher in the group with asthma than in the group without asthma (mean (SE) 483 (48) v 25 (2) kU/l).

AGE RELATED IGG SUBCLASS CONCENTRATIONS
Mean concentrations of all IgG subclasses in children with asthma were within the 1SD range of those of the control group (data not shown). There was a weak trend towards higher concentrations of IgG3 in all children with asthma and of IgG1 in patients who were older than 13 years. The difference for IgG2 was significant (p<0.05) in the 4–5 year old age group.

The prevalence of very low (IgG1, IgG2, IgG3 <2SD; IgG4 <0.05 g/l) and very high (>2 SD beyond the mean of the pertinent control group) subclass concentrations did not differ significantly (χ² test) between the two groups, though the number of children with low IgG2 concentrations was slightly higher in the group with asthma (table). The subgroup of children with asthma with low IgG2 consisted of nine children (mean (range) age 11–7 (7–14–7) years). All had atopic asthma (mean (range) ASS 1.8 (1–3)) and sensitisation to aerosol allergens was found in all nine (mean (range) total IgE 728 (73–1619) kU/l; RAST class 3–4). No further clinical nor laboratory abnormality was noted.

ASTHMA SEVERITY AND IGG SUBCLASS CONCENTRATIONS
Total IgG and IgG subclass concentrations were compared between children with mild asthma (ASS 1) and children with severe asthma (ASS 3–4). Children with severe asthma had significantly (p<0.02, Wilcoxon test) higher concentrations of IgG4 (mean (SE) 0.53 (0.09) g/l) than children with mild asthma (0.26 (0.04) g/l). This difference was independent of serum IgE concentrations (severe asthma 367 (81) kU/l; mild asthma 515 (142); p=NS).

ATOPIC v NON-ATOPIC ASTHMA
Subclass concentrations of 17 children with non-atopic asthma were compared with subclass concentrations of 17 patients with atopic asthma matched for age and ASS. The following mean (SE) concentrations (g/l) were found in patients with atopic (non-atopic) asthma: IgG1 6.24 (0.40) (6.50 (0.39)); IgG2 1.77 (0.20) (1.82 (0.20)); IgG3 0.47 (0.04) (0.50...
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(0.06)); IgG2 0.46 (0.12); IgG3 0.32 (0.05)). Thus atopic asthma patients had higher concentrations of IgG4, but the differences between the two groups were not significant.

CORRELATION WITH IgE

IgG subclass concentrations were plotted against the pertinent IgE concentrations. The correlation coefficients were as follows (all age groups summarised): IgG1 r=0.19, p<0.05; IgG2 r=0.15, p<0.05; IgG3 r=0.29, p<0.05; IgG4 r=0.10, p<0.15.

Discussion

In this study of 200 children with asthma their mean IgG subclass concentrations were within the 1SD range of those of healthy age matched controls. There was a trend towards higher concentrations of IgG1 and IgG4 and the prevalence of low concentrations of IgG2 was slightly increased among patients with asthma. IgG4 correlated with disease severity.

The role of IgG subclass deficiency in asthma is still controversial. The prevalence of subclass deficiency in a previous report ranged from 12%10 to 42%7 and 50%9 in adults, and from 7%11 to 34%14 in children. The patient groups in several of these studies were selected, however, including patients prone to infection,7 11 children with concomitant IgA deficiency (5/28 patients in Loftus et al15), or children less than 2 years of age,14 when considerable variations in normal values exist.21 22

Most importantly, many previous studies of the occurrence of subclass deficiency in asthma were not controlled for the confounding effects of treatment with systemic steroids.5 9 11 12 Concentrations of all immunoglobulins are known to decrease as early as two weeks after the onset of steroid therapy.23 All IgG subclasses are equally affected.10 15 24 Unlike systemic steroids, inhaled steroids rarely cause side effects;18 in particular, alterations of immune function have not been reported in association with these drugs.

In addition to drug induced subclass deficiency, the occurrence of healthy, asymptomatic subjects with subclass deficiency21 has to be taken into consideration. We were unable to distinguish clinically or on the basis of case history those patients with subnormal concentrations of IgG2 (table). Given the absence of further immunoglobulin alterations, these patients apparently belong to the group of asymptomatic children with IgG2 deficiency described by Shackelford et al.21 To identify patients with asthma with inherent humoral immunodeficiency, additional tests are required such as the assessment of functional – for example, postvaccination – antibodies or immunoglobulin synthesis in vitro.10 20 25

Taking the results of these assays into consideration, Hamilos et al concluded that true hypogammaglobulinaemia was uncommon in their group of patients with asthma.10 Moreover, patients with atopic asthma not receiving treatment with systemic steroids show a trend towards increased concentrations of IgG2 and IgG4 in addition to increased IgE.5 In accordance with this study, we observed higher concentrations of IgG4 and IgG2 in our patients with asthma than in the control group, and higher concentration of IgG4 in the patients with atopic than in those with non-atopic asthma. Concentrations of IgG4 correlated weakly with IgE (r=0.10; p<0.15). The increase in IgG4 in patients with asthma has been attributed either to long term antigen stimulation24 or to the existence of allergen specific 'blocking antibodies'.25 26 The concomitant increase of IgE, IgG4, and IgG3 in atopic subjects may at least partly be explained by the action of IL-4, which induces the isotype switch to IgE, IgG4,3 and IgG4.4 Dysregulation of lymphokine production with increased synthesis of IL-4 and decreased synthesis of interferon γ is a characteristic feature of atopic disorders.2

Interestingly, however, IgG4 was significantly (p<0.02) higher in patients with severe asthma than in those with mild asthma, whereas IgE concentrations were similar in the two groups. Gwynn et al reported increased concentrations of IgG4 in patients with severe asthma and speculated about a role for IgG4 in late asthmatic reactions.28 This is, however, controversial.27 So far, the reasons for the increase in IgG4 concentrations in patients with severe asthma remain obscure. It is suggestive of an underlying process which is not related to IgE, but further studies are needed to clarify this point.

Apart from differences in treatment modalities and patient characteristics, reported IgG subclass concentrations are considerably influenced by the method applied for their assessment. A variety of methods and reagents are presently used for the determination of IgG subclasses; these include radial immunodiffusion, ELISA, radioimmunoassay, and electroimmunoassay, and different polyclonal or monoclonal antisera.20 22 This renders direct comparisons between different studies difficult. Furthermore, concentrations of IgG subclasses are age dependent. It is therefore essential to establish age related reference values using the same method for controls and patients.20 22 Given the high prevalence of asymptomatic subjects with subnormal concentrations of IgG2, additional tests are required before establishing the diagnosis of (and eventually treating) 'subclass deficiency'.

In summary, our findings suggest that childhood asthma is associated with IgG2 subclass deficiency. Low concentrations of IgG2, though slightly more common in patients with asthma, are not necessarily clinically relevant. We suggest that the effectiveness of high dose intravenous gammaglobulin treatment in patients with severe asthma11 12 is either due to the correction of steroid induced hypogammaglobulinaemia or to immunomodulatory effects unrelated to alterations in the concentration of IgG subclasses.

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