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IgG subclass deficiency in asthma

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SUMMARY Total immunoglobulin G (IgG) and subclasses were measured in serum samples from 82 children with chronic asthma, aged 1·5 to 6·3 years, and 76 controls. Concentrations of IgG_1, IgG_2, IgG_3, and total IgG were significantly lower in asthmatic children aged 1 to 5, and IgG_2 concentrations were also significantly lower in asthmatic children over 5 years of age. Twenty eight asthmatic children had at least one value in the deficient range, and 26 had IgG_2 deficiency alone or in combination. Five children had IgG_2 and IgA deficiency. These 28 children were significantly younger and fewer had raised IgE concentrations than the remainder.

IgG subclass deficiency, which may reflect delayed maturation of the immune system, is common in young asthmatic children, and may have a role in the pathogenesis of the disease.

The importance of respiratory infection in precipitating attacks of asthma is well known. Deficiencies of IgG limited to one or more subclasses have been recognised in children with recurrent infections[1] and one small study found evidence of IgG subclass deficiency in 'non-allergic children with chronic chest symptoms'.[2] Because other forms of immunodeficiency have been associated with atopic diseases, we decided to study IgG subclass concentrations in a large group of chronic asthmatic children.

Patients and methods

We studied 82 children with chronic asthma, all of whom were attending the paediatric respiratory clinic at King's College Hospital. Children were included if they had asthma severe enough to warrant regular bronchodilators or prophylactic medication—that is, at least one severe attack a month, or symptoms on most days. Fifty six boys and 26 girls aged 1·5 to 6·3 years were recruited. The first symptoms of asthma occurred under the age of 1 year in 36, and under the age of 2 in 69. Half the children had symptoms of rhinitis, and half had present or past symptoms of eczema. Skin testing to six common allergens was carried out on 69, and yielded one or more positive reactions in 55. Sixty six had raised serum IgE concentrations (more than two standard deviations above the age matched mean). Forty eight children required steroids by inhalation, five sodium cromoglycate, 10 theophylline, and 19 regular β agonists. Serum samples from 76 unselected healthy controls, 38 boys and 38 girls aged 1 to 6·8 years were also analysed. These children were recruited in conjunction with the Michael McGough Foundation; they were in good health at the time of the study and had no history of serious disease.

We compared values for male and female controls to determine whether there was any sex difference in IgG subclass concentrations. Because concentrations of IgG subclasses vary with age,[3] asthmatic and control children were divided in three groups 1 to 2·99 years, 3 to 4·99 years, 5 to 6·99 years, for the purposes of analysis so that age matched comparison could be carried out. In the 1–2·99 years age group there were 17 controls (eight of whom were boys) and 22 asthmatic children (15 of whom were boys); in the 3–4·99 years age group there were 22 controls (11 of whom were boys) and 42 asthmatic children (31 of whom were boys); and in the 5–6·99 years age group there were 37 controls (20 of whom were boys) and 18 asthmatic children (10 of whom were boys). In both asthmatic and control groups were calculated the geometric mean and ranges using logarithmic transformation of the data.[4] The significance of differences between values in control and asthmatic children were assessed using Student's *t* test and the Wilcoxon rank sum test, probabilities of less than 0·05 being accepted as significant.

Asthmatic children with abnormal concentrations of IgG subclasses were compared with the remainder for age, and clinical and atopic state. The χ^2 test was used to assess the significance of any differences.

IgG subclasses were measured using an enzyme linked immunosorbent assay (ELISA) based on the method described by Aucouturier *et al*.[5] The monoclonal antibodies used were: IgG_1, clone NL16 (WHO/IUIS HP 6012) diluted 1/100; IgG_2, a mixture of clone GOM2 (HP 6009) and HP 6014, diluted 1/100; IgG_3, clone ZG4 (HP 6010) diluted 1/1000; and IgG_4, clone RJ4 (HP 6011) diluted 1/100. All monoclonal antibodies were obtained from Oxoid (Bedford, UK).

Microtitre plates were coated with purified monoclonal antibody of the relevant subclass at optimal dilution, in 0·01 mol/l phosphate buffer, 0·15 mol/l in sodium chloride, pH 7·4 (phosphate buffered saline) overnight at 4°C. Assays were conducted in triplicate in a volume of 100 µl in wells of polystyrene microtitre plates (Dynatech Immulon M129B, Dynatech Laboratories Ltd, Sussex, UK). After discarding the coating solution the wells were incubated with 5% bovine serum albumin in phosphate buffered saline, for one hour at 37°C to block residual reactive sites on the plastic wells. The plates were then washed with casein trometamol (Tris) buffer (0·05% casein, 0·9% sodium chloride, 0·01% thimersol, 10 nmol/l Tris hydrochloric acid, pH 7·6). All washes were done with an automated plate washer (Dyantech Autowash 2000). Patient or control serum was added at a dilution of 1/40 in phosphate buffered saline containing 1% bovine serum albumin and incubated for two hours at 37°C. A standard curve was established for each subclass using a reference serum preparation SPS-01 (Royal Hallamshire Hospital, Sheffield). After a further wash with casein Tris buffer, a peroxidase conjugated goat antihuman IgG antibody (Tago Inc) diluted 1/1000 in phosphate buffered saline was added and incubated for 1·5 hours at 37°C. After a final wash with phosphate buffered saline, the peroxidase activity of each well was determined with the colorimetric substrate, O-Phenylenediamine (0·4 mg/ml) and the reaction stopped with 2 mol/l sulphuric acid. The absorbance of the wells was determined at 490 nm using a Dynatech MR700 Elisa reader.

Each serum sample was studied at least twice, and a sample from a normal serum pool was included in every plate. The intra-assay coefficient of variation was 8% and the interassay coefficient of variation was 14%. A good correlation between the concentrations of IgG obtained by summing subclasses and total IgG concentrations was found on measurement by laser nephelometry (linear regression analysis $r=0·89$).

Informed consent was obtained from all parents and the study was approved by the hospital ethical committee.

Table 1 *Mean (range) IgG concentrations (g/l) in the three age groupings*

	Control children	*Asthmatic children*	*t*	*p Value*
Age 1 to 2·99 years:				
Total IgG	10·04 (7·4–13·7)	7·8 (5·0–12·2)	4·16	<0·01
G_1	6·6 (4·0–10·8)	5·5 (3·4–8·7)	2·52	<0·05
G_2	2·1 (1·6–2·8)	1·3 (0·4–3·9)	3·53	<0·01
G_3	0·89 (0·4–1·9)	0·73 (0·4–1·3)		NS
G_4	0·3 (0·1–0·5)	0·2 (0·1–0·4)		NS
Age 3 to 4·99 years:				
Total IgG	9·9 (6·6–14·9)	8·1 (5·6–11·5)	4·26	<0·01
G_1	6·4 (4·2–9·9)	5·5 (3·7–8·2)	2·74	<0·01
G_2	2·3 (1·2–4·3)	1·5 (0·8–3·1)	3·91	<0·01
G_3	0·84 (0·42–1·7)	0·69 (0·42–1·13)	2·63	<0·05
G_4	0·2 (0·1–0·8)	0·2 (0·1–0·4)		NS
Age 5 to 6·99 years:				
Total IgG	9·7 (6·7–14·1)	9·05 (6·7–12·2)		NS
G_1	6·0 (3·8–9·6)	6·27 (4·5–8·8)		NS
G_2	2·5 (1·3–4·8)	1·72 (1·0–3·1)	3.87	<0·01
G_3	0·76 (0·33–1·74)	0·73 (0·4–1·35)		NS
G_4	0·2 (0·03–1·7)	0·2 (0·1–0·4)		NS

Results

Values for boys and girls in the control group were similar for all subclasses and for total IgG.

Concentrations of IgG_1, IgG_2, IgG_3 and total IgG were lower in asthmatic children aged 1 to 2·99 and 3 to 4·99 years when compared with age matched controls. IgG_2 values were also lower in asthmatics aged 5 to 6·99 years ($p<0·05$) (table 1, figure).

Twenty eight asthmatic children had one or more values more than two standard deviations below the mean for age matched controls. Twenty six had IgG_2 deficiency—alone in 13, and combined with total IgG deficiency in nine, IgG_1 and total in two, IgG_3 in one, and IgG_3 and total in one. One child had a low concentration of IgG with subclass concentrations within the normal ranges, and a further child had IgG and IgG_1 deficiency.

These 28 children with deficiencies, when compared with the remainder of the asthmatic group, were significantly younger and fewer had high IgE concentrations than the remainder. There were no significant differences in sex, age of onset, concomitant atopic disease, requirement for inhaled steroids, or skin test reactivity. Five children, all of whom had IgG_2 deficiency, also had IgA deficiency (table 2).

Discussion

Our results indicate that IgG subclass deficiency, predominantly IgG_2, is present in over one third of children under 7 with serious asthma. Five children

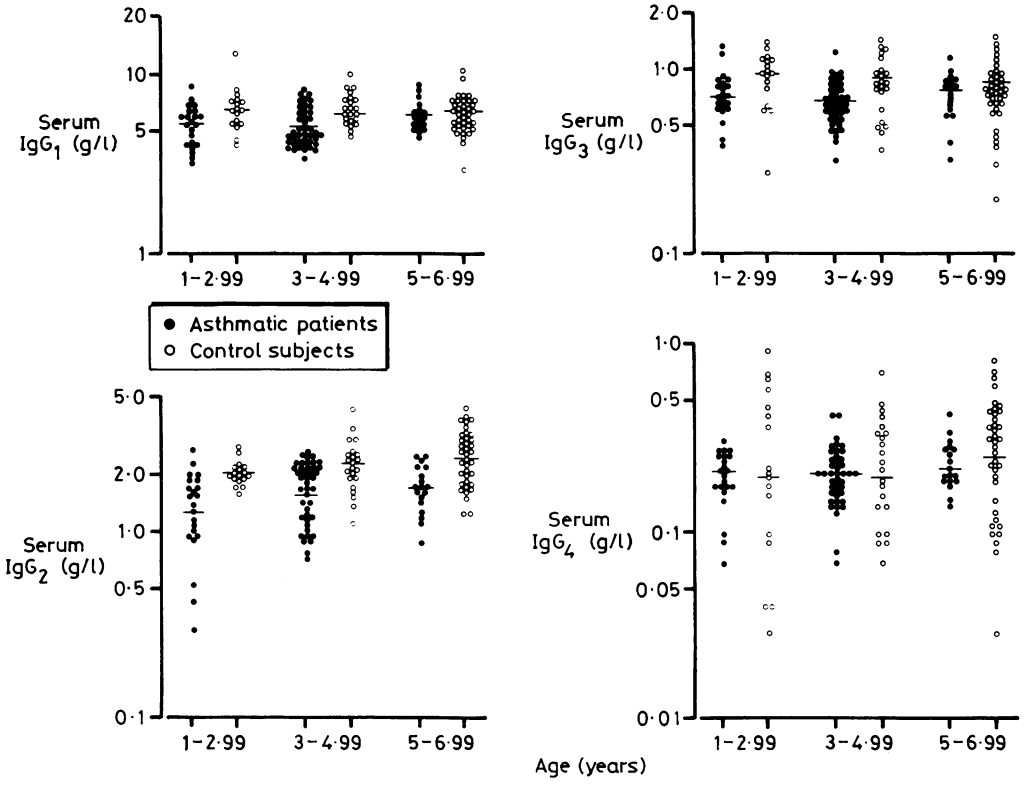

Figure *Individual values for IgG subclasses in asthmatic and control patients. The mean of each group is indicated.*

Table 2 *Comparison of asthmatics with at least one deficiency of IgG or subclass and the rest of the study group*

	Those with at least one deficiency (n=28)	*p Value*	*Those with no deficiencies (n=54)*
Mean (SD) age (months)	36·4 (16·4)	<0·05	47·8 (SD 14·9)
No (%) of boys	22 (79)	NS	34 (63)
Age at onset:			
No (%) under the age of 1 year	12 (43)	NS	24 (44)
No (%) between 1 and 2 years	10 (36)	NS	23 (43)
No (%) receiving treatment with inhaled steroids	16 (57)	NS	22 (40)
No (%) with rhinitis	15 (54)	NS	32 (59)
No (%) with eczema	13 (46)	NS	25 (46)
No (%) with positive skin tests	17/24 (71)	NS	38/45 (85)
No (%) with raised IgE concentration	17 (61)	<0·05	49 (91)
No (%) with IgA deficiency	5 (18)	NS	0

had abnormally low concentrations of IgA, and they all had concomitant IgG_2 deficiency. Total IgG deficiency has been reported in children taking oral steroids[6]; none of our chldren were taking oral steroids when studied, though many had received short courses previously. Multiple IgG subclass deficiency was present only in younger patients.

In the previous study of IgG subclasses in children with asthma, IgG_2 deficiency was noted in 10 of 37 'non-allergic children with chronic chest symptoms'; these children had negative skin tests and normal IgE concentrations.[2] In our study negative skin tests and normal concentrations of IgE were more common in children with deficiencies. These children were significantly younger, however, and because the incidence of positive skin tests increases with age,[7] the apparent association of G subclass deficiency and 'non-allergic' asthma may only be a reflection of a younger age group.

Atopic diseases including asthma have been linked with IgA deficiency,[8] defective yeast opsonising activity,[9] C2 deficiency,[10] and now IgG subclass deficiency. No specific immunodeficiency is accepted

as predisposing to childhood asthma but the association with a variety of abnormalities indicates that some form of dysregulation of immunity may be concerned in the pathogenesis. It has been suggested that defects of the immune system that affect exclusion or elimination of antigen could be relevant in early infancy at the time of first presentation of antigen to cells making IgE[11]; clearance of adsorbed antigen might be impaired in IgG subclass deficiency.

The IgG antibody response to bacterial polysaccharide antigen resides mainly within IgG_2 subclass[12] and the antibody response to some viruses may also be restricted to a particular subclass.[13] Inability to mount an adequate IgG response might provoke or exacerbate asthma in certain children. Respiratory infection, particularly when viral, results in an increase in bronchial reactivity.[14] Perhaps protracted infection and epithelial damage enhances this effect on airway function.

Our results show that IgG subclass concentrations are significantly reduced in young asthmatics. The hypothesis that IgG subclass deficiency predisposes to asthma depends on showing that the abnormalities predate the disease. We cannot be sure that the observed deficiencies were not a consequence of asthma or its treatment, but multiple deficiencies were present only in the younger children. This suggests some type of delayed maturation and tends to favour the concept that these deficiencies are primary.

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