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IgG subclass levels in chronic rhinosinusitis*

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SUMMARY

Sera from seventy-four adult patients with chronic or recurrent rhinosinusitis (mean duration 10.3 years) were tested for levels of total immunoglobulin and IgG subclasses. Fourteen (19%) had low levels of one of the major immunoglobulin classes and 23 (31%) had one or more IgG-subclass deficiencies, i.e. values less than the mean minus 2 standard deviations of a control population sample. Nineteen patients had low IgG3 levels. The group as a whole showed significantly low mean levels of IgG3, 46.9±19.5 mg/dl compared to 76±21 mg/dl for the controls, $p < 0.0005$ (Student's t-test). Since there was no clinical difference between those with and without IgG3 deficiency, there exists the possibility of an underlying immune defect, possibly involving heavy chain switching, in all these patients.

Key words: immunoglobulins, rhinosinusitis, immunodeficiency

INTRODUCTION

Human IgG consists of four subclasses based on antigenic differences in their heavy polypeptide chains. The IgG subclasses are found in normal serum in the relative proportions: IgG1: 60-70%; IgG2: 14-20%; IgG3: 4-8%; and IgG4: 2-6%. In adults IgG1 and IgG3 are thought to be the subclasses preferentially produced in response to protein antigens, while IgG2 and IgG1 have predominantly been associated with responses to carbohydrate antigens. The effects of subclass deficiencies are unclear since although profound or partial deficiencies may be associated with an increased susceptibility to particular infections, rare instances of complete absence of one subclass without any apparent clinical abnormality do occur.

Chronic or recurrent chest infections may be associated with IgG2 deficiency, with or without absent IgG4 and/or low IgA levels (Stanley et al., 1984). Antibodies to polysaccharide antigens are particularly associated with the IgG2 subclass and patients with this deficiency suffer repeated infections with bacteria having a polysaccharide capsule, such as *Haemophilus influenzae* and *Pneumococci.* Since such individuals frequently give a history of upper respiratory tract infections preceding any lower respiratory tract symptoms it seemed appropriate to investigate our patients for IgG subclass deficiencies.

MATERIALS AND METHODS

Patients

Seventy-four patients (30 males, 44 females) attending the Rhinology Clinic at the Royal National Throat, Nose and Ear Hospital for chronic or recurring infection of nose and sinuses with over three episodes of purulent infection per year were tested. Approximately two-thirds were non-smokers and only two drank alcohol in excess of the maximum recommended number of units per week. Their ages ranged from 15 to 60 years, with a mean of 38.4 years. The length of history was from 1.5 to over 30 years, with a mean of 10.3±8.3 years. Forty-seven patients had undergone operations, 25 of them on more than one occasion. Twenty-seven had lower respiratory tract involvement, 11 of these were asthmatic. All patients had ciliary function which was within normal limits as assessed by nasal mucociliary clearance and/or ciliary beat frequency (Greenstone et al., 1984). Patients with known secondary causes of immunoglobulin deficiency were excluded from the study.

Methods

Venous blood was taken and the serum removed and stored at -20°C until IgG-subclass determinations were made by single radial immunodiffusion using monoclonal antibodies (Goldblatt et al., 1989) in the Immunology Laboratory,

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Institute of Child Health. A pool of serum from healthy laboratory staff members was calibrated for IgG1, IgG2 and IgG4 levels against commercially available IgG-subclass standard preparations obtained from Serotec (Kidlington, UK; code Nos. HPRO11-HPRO14). A departmental normal range was derived for each subclass from elective surgical cases and healthy schoolchildren. A cohort of individuals aged more than 12 years was regarded as equivalent to the adult population.

Serum immunoglobulins were measured in the routine chemical pathology laboratory (Middlesex and University College Hospital) by nephelometry and were compared to the normal range of values established by testing several hundreds of normal adults.

Skin prick testing was performed to 10 common allergens (house dust mite, grass pollen, tree pollen, house dust, *Cladosporium*, *Aspergillus*, feathers, cat, milk, egg; supplied by Bencard Ltd, UK), with saline as a negative control and histamine as a positive control. Patients with one or more reactions to allergens, 3 mm greater in diameter than the negative control, were classified as allergic.

Microbiology: The results of cultures of sinus material taken at operation were obtained from the patients' notes.

Statistics: Student's t-test was used to compare mean values of IgG and IgG subclasses; associations between clinical symptoms and subclass deficiency were examined using the Chi-square test.

RESULTS

Skin prick tests

Forty-two individuals (57%) gave at least one positive skin prick test result, the commonest being the house dust mite (82%), 40 individuals showed multiple sensitivities.

Serum immunoglobulins

The total IgG, IgA and IgM levels of most patients fell within the expected normal ranges. Four individuals had low levels of IgG, five others showed low IgA levels, and a further five had reduced IgM levels. Two patients had IgM values above the normal range and one had a raised IgA.

The mean value for IgG (as derived by nephelometry in the Chemical Pathology Department of Middlesex and University College Hospitals) in the patient group as a whole was 10.8±2.5 mg/ml, significantly lower than the mean for the age-matched control group, 13±2.5 mg/ml ($p<0.001$; Student's t-test; Figure 1).

The mean values for IgA and IgM were not different from those of the normal population (Table 1).

IgG subclasses

For all four subclass assays the maximum intra-assay coefficient of variation was 2.5%. For all four subclass assay the maximum inter-assay coefficient of variation was 6%.

The lower limits of detection were: IgG1: 1.5 mg/dl; IgG2: 1.0 mg/dl; IgG3: 1.0 mg/dl; IgG4: 0.6 mg/dl.

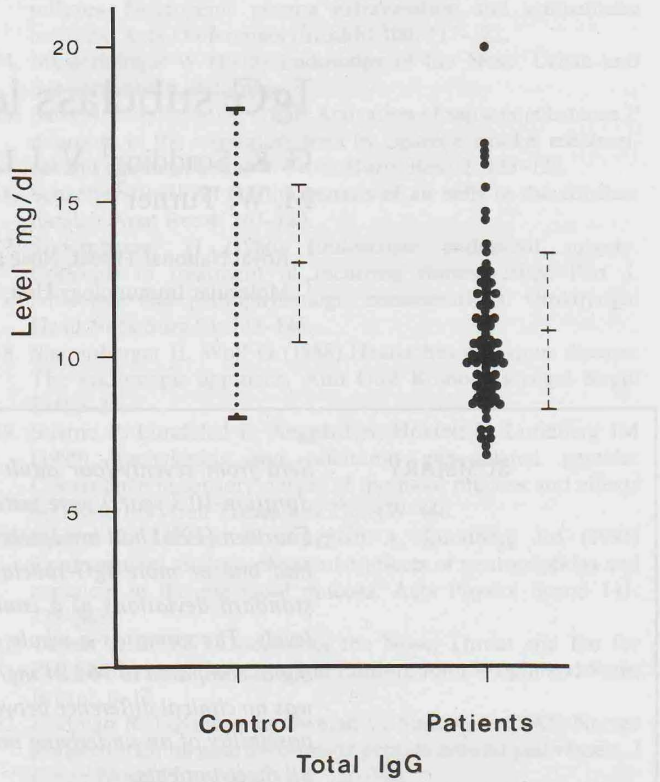

Figure 1. Total serum immunoglobulin G (IgG) levels in an apparently healthy control population compared to values of patients with chronic or recurrent rhinosinusitis. The mean and standard deviations for each group are indicated by a dashed line. The control range is shown by a dotted line representing 0-95% confidence intervals. Individual values for the patients are shown as black dots. The patient and control means were significantly different ($p<0.001$; Student's t-test). Measurements were made at the Biochemistry Laboratory, University College and Middlesex Hospitals.

Table 1. Serum levels in mg/ml of immunoglobulins G, A, and M in chronic rhinosinusitis patients compared to control ranges. All measurements were derived from the Chemical Pathology Laboratory, Middlesex and University College Hospitals.

	serum levels (mg/ml)		
	patients	controls	
IgG	10.8±2.5	13±2.5	$p<0.001$
IgA	2.1±0.9	2.7±0.9	p=ns
IgM	1.4±0.7	1.7±0.55	p=ns

IgG1

There was no difference between the patient (778±283 mg/dl) and control (783±203 mg/dl) mean values of IgG1 (Figure 2). Five individuals had values greater than 2 standard deviations below the control mean value and five others had raised levels.

IgG2

Raised IgG2 values were present in thirty-one patients whereas three individuals had low values (Figure 3). The

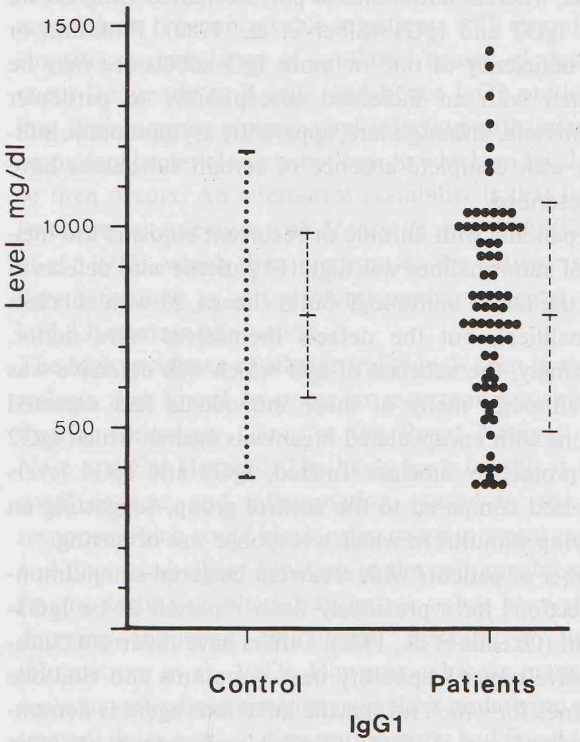

Figure 2. Serum IgG1 values in the control population compared to levels in chronic rhinosinusitis patients. The dashed lines indicate mean and standard deviations, the dotted line the range of values in the control population. The mean levels of the patient and control groups were not significantly different (Student's t-test).

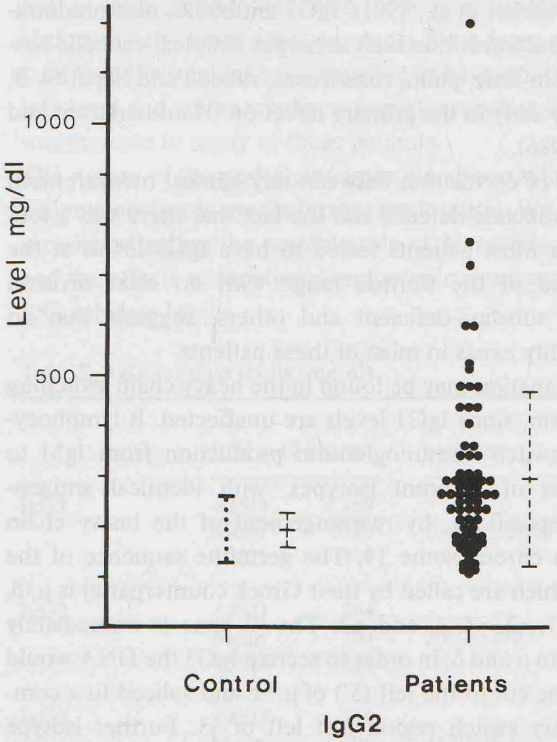

Figure 3. Serum IgG2 values in the control population compared to levels seen in chronic rhinosinusitis patients. The dashed lines indicate mean and standard deviations, the dotted line the range of values in the control population. The mean levels of patient and control groups were significantly different ($p < 0.005$; Student's t-test).

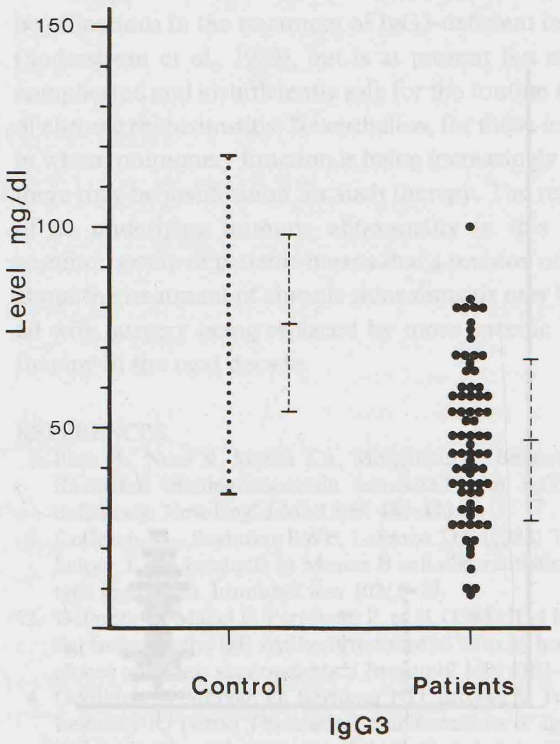

Figure 4. Serum IgG3 values in the control population compared to levels in chronic rhinosinusitis patients. The dashed lines indicate mean and standard deviations, the dotted line the range of values in the control population. The patient and control means were significantly different ($p < 0.0005$; Student's t-test).

mean for the group as a whole was raised at 296.2±173 mg/dl, compared to 194±35 mg/dl for the control group ($p < 0.005$; Student's t-test).

IgG3

No individual had a complete lack of detectable IgG3. Nineteen patients had IgG3 levels at or below the lower limits of the normal range (Figure 4). The mean value was 46.9±19.5 mg/dl for the patient group, compared to 76±21 mg/dl for the controls ($p < 0.0005$; Student's t-test). No raised levels were found.

IgG4

The IgG4 mean value of 39.7±22.1 mg/dl was significantly higher than that of the control group, 32±12.5 mg/dl ($p < 0.025$; Student's t-test). Twenty individuals were above, and six below the normal range (Figure 5).

Combined deficiencies

Seven individuals showed deficiencies in more than one subclass: three involved IgG3 and IgG4; one IgG1 and 3, one IgG1, 2 and 3, and one all IgG subclasses. There was no correlation between the total IgG levels and the levels of any individual subclass, nor between any of the subclasses. Length of history did not correlate with any immunoglobulin or subclass level, nor did age.

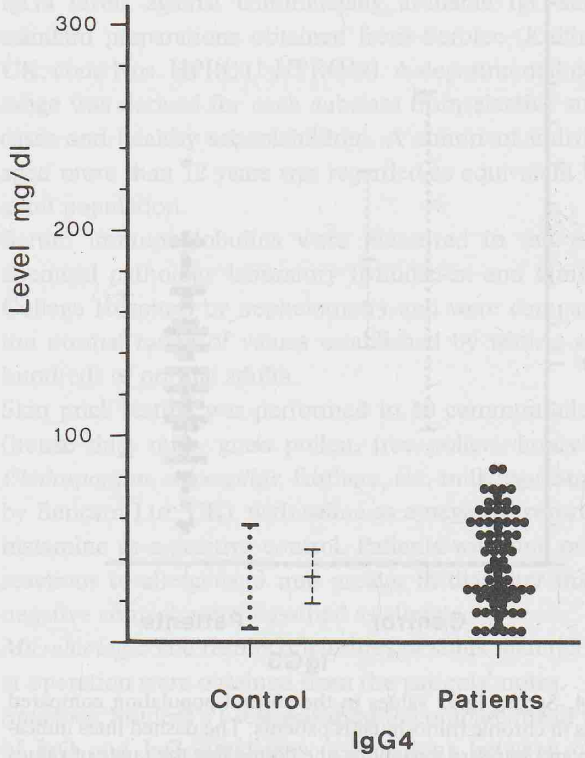

Figure 5. Serum IgG4 values in the control population compared to levels in chronic rhinosinusitis patients. The dashed lines indicate mean and standard deviations, the dotted line the range of values in the control population. The mean levels of the patient and control were significantly different ($p < 0.025$; Student's t-test).

There was no significant difference in the incidence of lower respiratory tract involvement (even with asthma excluded), operative history or allergy when subclass-deficient patients were compared to the non-deficient group (chi-square test). There was a tendency for the subclass-deficient patients to have more frequent infective episodes, but this failed to reach significance. Of the two most severely affected individuals in the group, one had a low IgA and the other was deficient in IgG1, IgG2 and IgG3.

Microbiology

Fifty-one bacteriological reports on material removed from sinuses at operation were available on twenty-three patients. Eleven (22%) showed no bacterial growth. Seventeen samples (33%) grew *Staphylococcus aureus*, 10 samples (20%) *Haemophilus influenzae* and four samples (8%) *Pneumococci.* The remainder grew *Pseudomonas aeruginosa* (3), *Streptococci* (2), mixed growth (2), *Bacteroides* (1) and *Enterobacteria* (1).

DISCUSSION

IgG subclasses were first recognized more than 25 years ago, but the effects of deficiencies in one or more subclasses are still unclear. In general terms IgG1 and IgG3 are the predominant IgG isotypes produced in response to protein antigens, whereas antibodies to polysaccharide antigens are mainly IgG2 and IgG1 (Siber et al., 1980). Profound or partial deficiency of one or more IgG subclasses may be associated with an increased susceptibility to particular infections and, although rare, apparently asymptomatic individuals with complete absence of certain subclasses have been described.

In our patients with chronic or recurrent sinusitis the incidence of abnormalities was high (14 patients with defects in one of the major immunoglobulin classes, 23 with subclass abnormalities), but the defects themselves were minor. Interestingly, the subclass of IgG which was defective was IgG3, although many of these individuals had repeated infections with encapsulated organisms against which IgG2 is the protective subclass. Indeed, IgG2 and IgG4 levels were raised compared to the control group, suggesting an underlying stimulus to which a response was occurring.

A number of patients with recurrent bacterial sinopulmonary infections have previously been reported to be IgG3-deficient (Oxelius et al., 1986). Others have recurrent culture-negative upper respiratory tract symptoms and viral-like syndromes for which no specific infectious agent is demonstrable (Oxelius et al., 1986). Clinical improvement has been noted in some of these following the use of intravenous gammaglobulin (Soderstrom et al., 1989). IgG3-subclass responses have been measured in response to various environmental antigens and have been found to be disproportionately increased in response to *Moraxella catarrhalis* and *Streptococcus pyogenes* (Walker et al., 1983; Skarvil, 1986; Goldblatt et al., 1991). IgG3 antibodies also predominate in viral infections such as herpes simplex, varicella zoster, Epstein-Barr, polio, rotaviruses, rubella and hepatitis B, especially early in the primary infection (Hammarstrom and Smith, 1986).

The lack of correlation between any clinical measurement and the subclass defects, and the fact that there was a tendency for most patients tested to have IgG3 levels at the lower end of the normal range with no clear division between subclass-deficient and others, suggests that an abnormality exists in most of these patients.

One explanation may be found in the heavy chain switching mechanism, since IgG1 levels are unaffected. B lymphocytes can switch immunoglobulin production from IgM to antibodies of different isotypes, with identical antigen-binding specificity, by rearrangement of the heavy chain genes on chromosome 14. The germline sequence of the genes (which are called by their Greek counterparts) is μ, δ, γ3, γ1, α1, γ2, γ4, ε, and α2. The γ3 gene is immediately adjacent to μ and δ; in order to secrete IgG3 the DNA would need to be cut to the left (5′) of μ + ε and spliced to a complementary switch region just left of γ3. Further isotype switching would result in deletion of γ3 with preservation of the other γ-genes.

The regulation of isotype switching is an area of intense study. T cells and their cytokines appear to be important regulators of specific isotype production (Coffman et al.,

1988; Del Prete et al., 1988), but the mechanisms involved are unclear. It is conceivable in patients with chronic rhinosinusitis an imbalance of cytokines causes γ3 deletion to occur frequently in B cells, and hence IgG3 production is low. This permits chronic viral infection with intermittent secondary bacterial exacerbations, to which an IgG2 response then occurs. An alternative possibility is that low IgG3 levels permit infection with *Moraxella catarrhalis* (Goldblatt et al., 1991), which then predisposes the patient to secondary infections by virtue of microenvironmental changes and β-lactamase production.

The high incidence of allergic individuals seen in this study reflects that found to occur in recurrent sinusitis in the Special Rhinology Clinic at the Royal National Throat, Nose and Ear Hospital. The likely basis for this is the mucosal oedema and inflammation caused by the allergic response which could block ostia, cause mucosal apposition and decreased ciliary function in the ostiomeatal complex. *In vitro* testing of ciliary beating *per se* has been shown to decrease following an allergic reaction in the nose (Holmstrom et al., 1992). However, whereas many atopics cease to suffer from sinusitis once their underlying allergy is treated, those participating in this study had failed to do so, suggesting a further predisposing factor. The role of local immunity in this group of patients is also under investigation and will be the subject of a separate paper.

Otorhinolaryngologists are frequently exhorted to undertake measurement of serum immunoglobulin levels in patients with recurrent nose and sinus infections in order not to miss individuals with hypogammaglobulinaemia. Unfortunately, since IgG1 accounts for a large proportion (>60%) of the total IgG, it is possible to have a normal serum IgG level and yet be wholly or partially subclass deficient as was the case in many of these patients.

The nature of the underlying immune abnormality in these patients obviously merits further exploration. We are at present investigating the possible role of *Moraxella catarrhalis* and the effects of functional endoscopic sinus surgery upon IgG subclass levels.

Table 2. IgG subclass levels (mg/dl)

	ICH (>12 years)		patients	normal range
IgG1	+2SD	1,189		
	Mean	783	778.13±282.9	400–1,300
	-2SD	377		
IgG2	+2SD	264		
	Mean	194	296.2±172.6	100–750
	-2SD	124		
IgG3	+2SD	118		
	Mean	76	46.9±19.5	34-118
	-2SD	34		
IgG4	+2SD	57		
	Mean	32	39.7±22.1	<1-300
	-2SD	7		

Immunoglobulin replacement therapy has been shown to be efficacious in the treatment of IgG3-deficient individuals (Soderstrom et al., 1989), but is at present too expensive, complicated and insufficiently safe for the routine treatment of chronic rhinosinusitis. Nevertheless, for those individuals in whom pulmonary function is being increasingly damaged there may be justification for such therapy. The recognition of an underlying immune abnormality in this relatively common group of patients means that a revision of attitudes about the treatment of chronic rhinosinusitis may be required with surgery being replaced by more specific immunotherapy in the next decade.

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