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Serum IgG subclass concentrations in healthy adults: a study using monoclonal antisera

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SUMMARY

IgG subclass concentrations have been measured in the sera of 172 healthy adults using monoclonal antisera. 'Normal ranges' are given. The findings were similar to those of previous studies of European populations but differed from the findings of the only other study of a large British population. In particular, low proportions of IgG4 in a British population have not been confirmed. IgG4 concentrations were higher in males than females ($P = < 0{\cdot}0\bar{0}003$) and IgG3 concentrations were higher in females than males ($P = 0{\cdot}034$).

Keywords IgG subclasses monoclonal antisera

INTRODUCTION

The measurement of serum IgG subclass concentrations has found application in the study of immunoglobulin genetics and also increasingly in the diagnosis and management of some immunodeficiency diseases (Oxelius, 1979; Oxelius *et al.*, 1981). However, it is recognized that there have been some difficulties in measuring IgG subclass concentrations because of difficulties in producing monospecific antisera (Shakib & Stanworth, 1980). This might partly explain differences in 'normal' IgG subclass concentrations in different populations (Shakib & Stanworth, 1980). A potential improvement of this situation is the use of monoclonal antisera. Using such antisera we have measured IgG subclass concentrations in a large population of healthy British adults and compared our findings with previous similar studies.

MATERIALS AND METHODS

Sera. Sera from 88 male (mean age 37·8, range 19–78) and 84 female (mean age 32·8, range 19–85) healthy blood donors and hospital personnel were collected and stored at −20°C until used.

Serum IgG subclass concentrations. These were measured by radial immunodiffusion in 1% agarose in 0·1 M barbitone buffer, pH 8·6, containing 7% polyethylene glycol (PEG) 4000 (BDH Chemicals Ltd., Poole, UK). Monoclonal antisera (Seward Laboratory, London, UK) to IgG1 (JL 512), IgG2 (GOM 1), IgG3 (ZG4) and IgG4 (RJ4) were diluted appropriately in the agarose. We have found the optimal dilutions to be 0·7% for IgG1, 1·0% for IgG2, 0·1% for IgG3 and 0·6% for IgG4. Test sera were diluted 1/4 in barbitone buffer except for IgG4 when a 1/2 dilution was used. Plates were left at 4°C for 72 h and stained or incubated in 1% tannic acid prior to reading. Results

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were calculated from a standard curve constructed using WHO serum 67/97 and values for IgG subclasses described by Morrell & Skvaril (1971).

Statistical analysis. Statistical comparisons between groups were done using the Mann–Whitney U-test.

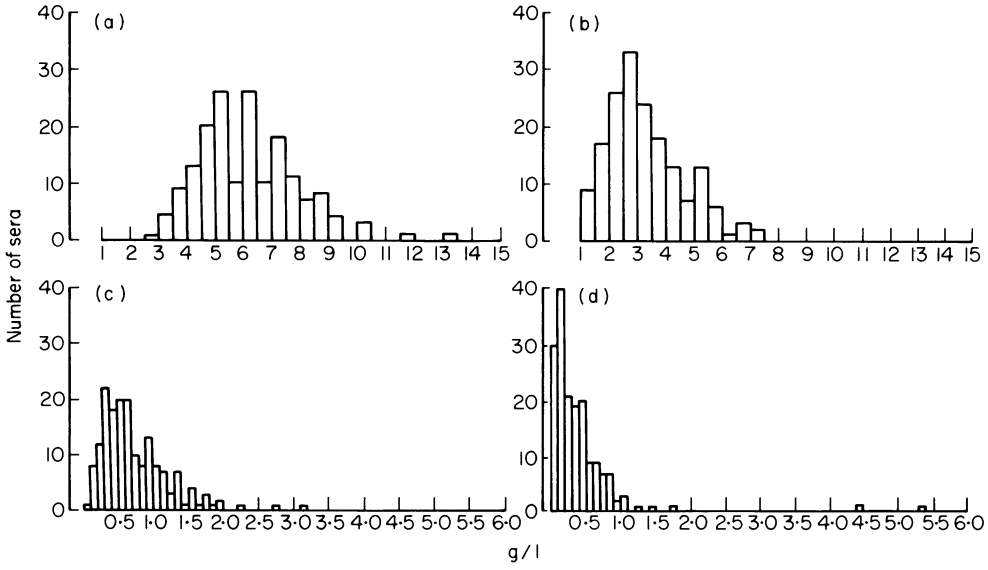

Fig. 1. Frequency distribution of (a) IgG1 (b) IgG2 (c) IgG3 and (d) IgG4 in the sera of 172 healthy adults.

RESULTS

Serum IgG subclass concentrations

As can be seen in Fig. 1, the frequency distribution of all subclasses except for IgG1 is skewed, particularly for IgG4. Log transformation of data made the frequency distribution more 'normal' except in the case of IgG4. Mean values, 95th centile ranges, and mean concentration of each subclass as a proportion of total IgG are shown in Table 1.

Effect of sex and age on IgG subclass concentrations

For IgG1 and IgG2 there was not a statistically significant difference between IgG subclass concentrations in males and females. However, IgG3 concentrations were higher in females ($0{\cdot}83 \pm 0{\cdot}51$, mean $\pm$ s.d.) than males ($0{\cdot}67 \pm 0{\cdot}45$) ($P < 0{\cdot}034$) and IgG4 concentrations were very significantly higher in males ($0{\cdot}44 \pm 0{\cdot}51$) than in females ($0{\cdot}35 \pm 0{\cdot}63$) ($P = < 0{\cdot}00003$). IgG4 was

Table 1. Serum IgG subclass concentrations in the sera of 172 healthy adults

	Mean $\pm$ 2 s.d. (Log transformed) (g/l)	95th centile ranges (g/l)	Range (g/l)	% Total
IgG1	$5{\cdot}91 \pm 2{\cdot}64$	3·19–10·2	2·86–13·46	60·3
IgG2	$3{\cdot}04 \pm 2{\cdot}52$	1·23– 6·63	1·00– 7·25	31·0
IgG3	$0{\cdot}61 \pm 0{\cdot}56$	0·16– 1·94	0·09– 3·16	6·2
IgG4	$0{\cdot}24 \pm 0{\cdot}90$	<0·03– 1·33	<0·03– 5·39	2·5

undetectable ($<0{\cdot}03$ g/l) in only two (2%) males compared with 15 (18%) females. When sera with undetectable IgG4 were excluded there remained a significant difference between males and females ($P=0{\cdot}0045$).

There appeared to be a decline in IgG1 concentrations with age until the sixth decade. However, statistical analysis of the data could not be performed because of unequal numbers amongst age groups. There was no apparent differences in the other subclasses amongst different age groups.

DISCUSSION

Our findings for IgG subclass concentrations in the sera of healthy adults are in general agreement with those of Morrell & Skvaril (1971) and Van der Giessen *et al.* (1975). However, there are significant differences from the findings of the only other study of a large British population reported by Shakib *et al.* (1975). In particular, we have not confirmed the very low IgG4 concentrations found in that study. Indeed, our finding that IgG4 as a proportion of total IgG is lower than that found by Morrell & Skvaril (1971) and by Van der Giessen *et al.* (1975) is explained by our use of log transformed means rather than arithmetic means as used by the others. The differences between our study and that of Shakib *et al.* (1975) might be explained by our use of monoclonal antisera rather than polyclonal antisera. It is possible that the extensive absorption of the polyclonal antisera resulted in an IgG4 antiserum which did not detect a subpopulation of IgG4, such as the 4a or 4b subtype, resulting in lower total IgG4 concentrations.

Significant sex related differences in IgG subclass concentrations have not previously been described. This was possible in only two (Shakib *et al.*, 1975; Van der Giessen *et al.*, 1975) of the previous studies on large populations. There were no statistically significant differences between males and females for any of the subclasses in the population studied by Shakib *et al.* (1975) but this could be explained by the use of inappropriate statistical analysis. In the population studied by Van der Giessen *et al.* (1975) females had a higher mean concentration of IgG2 whilst males had a higher mean concentration of IgG4. However, using appropriate statistical analysis the differences were not significant. We have confirmed the higher concentrations of IgG4 in males and found this to be statistically significant. In addition, we have found the mean IgG3 concentration to be higher in females than males.

In agreement with Oxelius (1979), we have found some healthy adults to be deficient in IgG4, although not to the same extent. Approximately 10% of the population reported here had undetectable IgG4 whereas Oxelius found one in four to be deficient. Whatever the frequency, caution must be exercised in attributing immunodeficiency to isolated IgG4 deficiency (Beck & Heiner, 1981).

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