

A community survey of IgG4 antibody levels

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Summary

Precise and specific radioimmunoassays have been developed to quantify IgG4 and IgG4 antibodies. A community survey has demonstrated that men have significantly higher total serum IgG4 levels than women: geometric mean values of 0.581 mg/ml versus 0.302 mg/ml. This sex difference was extended to total IgE values. Circulating levels of IgG4 antibodies against foods, especially egg and milk, were normally higher than those against the three most common U.K. inhalant allergens—grass pollen, house dust mite and cat epithelium.

Introduction

Atopic allergy affects about 20% of the U.K. population but is most common below the age of 30. The atopic person is stimulated to produce IgE antibodies after natural exposure to common environmental allergens (Pruzansky, 1972), and these can be quantified by the IgE RAST technique (Wide, Bennich & Johansson, 1967).

Discovery of short-term sensitizing (STS)-IgG antibody (Parish, 1970) and its association with immediate asthmatic reactions (Bryant, Lazarus & Burns, 1975) has led to speculation that it is in the sub-class IgG4 (Stanworth & Smith, 1973; Vijay & Perelmutter, 1977) and data has been presented to support the view that IgG antibodies mediate late asthmatic reactions (Gwynn *et al.*, 1982). However there is evidence against STS-IgG being in the sub-class IgG4: (1) although IgG4 antibodies can sensitize mast cells and challenge thereafter with anti-IgG4 will release histamine, this is non-specific because so will normal goat serum (Toorenenbergen & Aalberse, 1982), and (2) very high IgG4 titres to grass pollen have been reported in response to grass pollen hyposensitization (Giessen *et al.*, 1976).

Clearly, knowledge of the frequency with which levels of allergen-specific IgG4 antibodies are raised in the normal population would help to establish the relationship between IgG4 and STS-IgG antibody. We present the results of a community survey in

the U.K. which establishes the normal ranges of total serum IgG4 and compares the circulating levels of inhalant and food-specific IgG4 antibodies.

Materials and methods

Subjects

These were participants in a dietary survey of a small town in South Wales. Two random samples were selected from the electoral register, of seventy-five men and 121 women respectively. Blood was obtained from fifty-seven men (76%) and 99 women (82%), whose ages ranged from 18 to 86 years, the median being 41 years for men and 45 years for women.

IgG4 measurements

Radio-labelled anti-IgG4 was used to assay both total and specific IgG4 antibodies. Immunosorbent-purified anti-IgG4 (Dutch Red Cross, Amsterdam) was ^{125}I -labelled (NEN Ltd) by the chloramine-T method (Greenwood, Hunter and Glover, 1963) to a specific radioactivity of approximately $15\ \mu\text{Ci}/\mu\text{g}$; this tracer bound 90% to an excess of myeloma derived IgG4-cellulose particles. In the assay procedures radio-labelled anti-IgG4 was diluted so that $100\ \mu\text{l}$ added to each tube produced about 100 000 ct/min in an automatic γ -counter. Phadebas RIST buffer (Pharmacia Diagnostics) was used as a diluent for the tracer, cellulose particles and foetal calf serum. (1) Total serum IgG4 was measured by a 1 h radioimmunoassay (RIA) in which $50\ \mu\text{l}$ IgG4 sample competed with $100\ \mu\text{l}$ IgG4 covalently linked to CNBr-activated microcrystalline cellulose particles (Merrett & Merrett, 1980) for binding to $100\ \mu\text{l}$ of radiolabelled anti-IgG4. Test samples were diluted 200-fold with 0.9% saline and quantified by reference to a calibration curve varying in values from 0.06 to 7.36 mg IgG4/ml. The concentration of IgG4 particles was adjusted so that the maximum binding of labelled anti-IgG4 was 30% in the presence of a hypogammaglobulinaemic human serum. Particles were washed four times with 0.9% saline before measurement in a γ -counter. A reference serum calibrated as 0.46 mg/ml (Dutch Red Cross) was used to quality control the assays. (2) Circulating IgG4 antibodies were measured by the RAST technique against timothy grass pollen, *D. pteronyssinus*, cat epithelium, hen's egg-white, cow's milk, cod fish, wheat flour, peanut and almond nut. To a Phadebas RAST allergen disc (Pharmacia Diagnostics) was added $50\ \mu\text{l}$ of serum sample diluted ten-fold with 50% foetal calf serum then, after 1 hr incubation, the disc was washed free of excess serum and incubated overnight with $100\ \mu\text{l}$ of labelled anti-IgG4. The binding of test sera (Bi) were related to a milk-positive reference serum which bound 15% of labelled anti-IgG4 (Bref) in the assay procedure, and IgG4 levels expressed as $(\text{Bi}/\text{Bref}) \times 100$. Non-specific binding was estimated with a hypogammaglobulinaemic serum; it varied from 0.8–1.2% according to the allergen and was allowed for in the calculations. A result over 33% was regarded as positive.

Total serum IgE

Total serum IgE was measured by conventional RIA using a fast double antibody separation technique (Merrett & Merrett, 1978).

Statistical analyses

These were carried out using Student's *t*-test on log-transformed total IgG4 and IgE

data, and Whitney-Mann rank correlations for IgG4 RAST data. The null hypotheses were rejected when $P < 0.05$.

Results

Total serum IgG4

A myelomatous IgG4 serum diluted parallel to (1) the high-titre IgG4 serum pool used to calibrate the assay, and (2) the quality control reference serum (Fig. 1). These experiments supported the manufacturer's claim that the anti-IgG4 serum supplied was highly specific. There was some evidence for deterioration of specimens after storage for 1 year at -20° , but this was negligible at levels below 1 mg IgG4/ml of serum (Fig. 2). Sera were tested in five different assays and the mean value of the quality control serum was 0.464 mg/ml with a between assay coefficient of variation equal to 10.9%.

Frequency histograms and cumulative frequency distributions of log transformed data for men and women are shown (Fig. 3). The overall geometric mean value was 0.383 mg/ml but the men's geometric mean of 0.581 mg/ml (95% confidence interval:

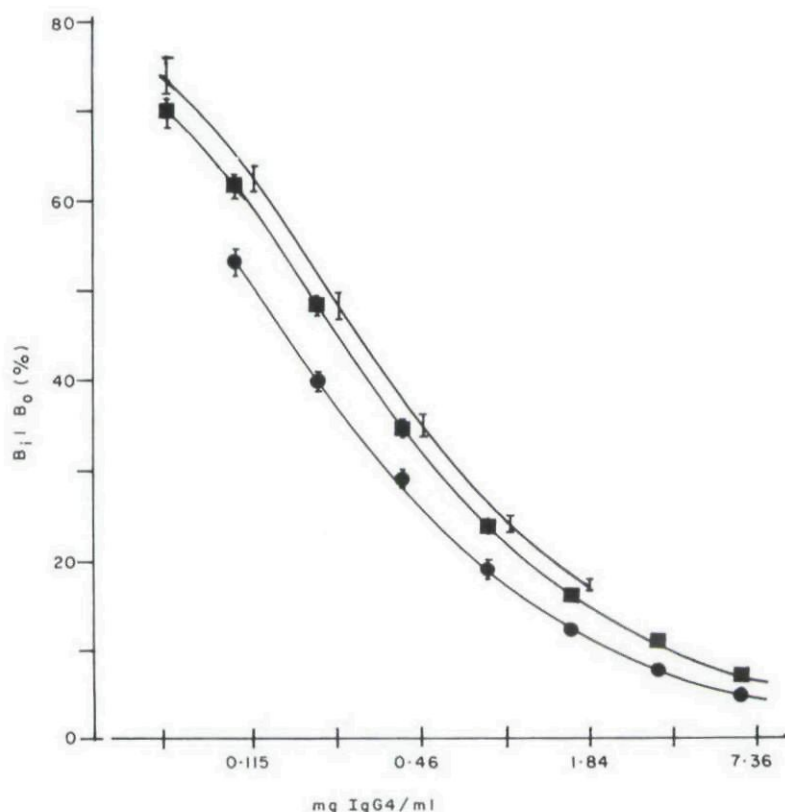


Fig. 1. Total IgG4 calibration curves demonstrating parallelism between a reference serum supplied by the Dutch Red Cross (—□—), a high titre patient serum pool (—■—) and an IgG4 myeloma serum (—●—). The maximum binding (B_0) of ^{125}I -anti-IgG4 to particle-linked IgG4 in the presence of an IgG4-free human serum was 30%.

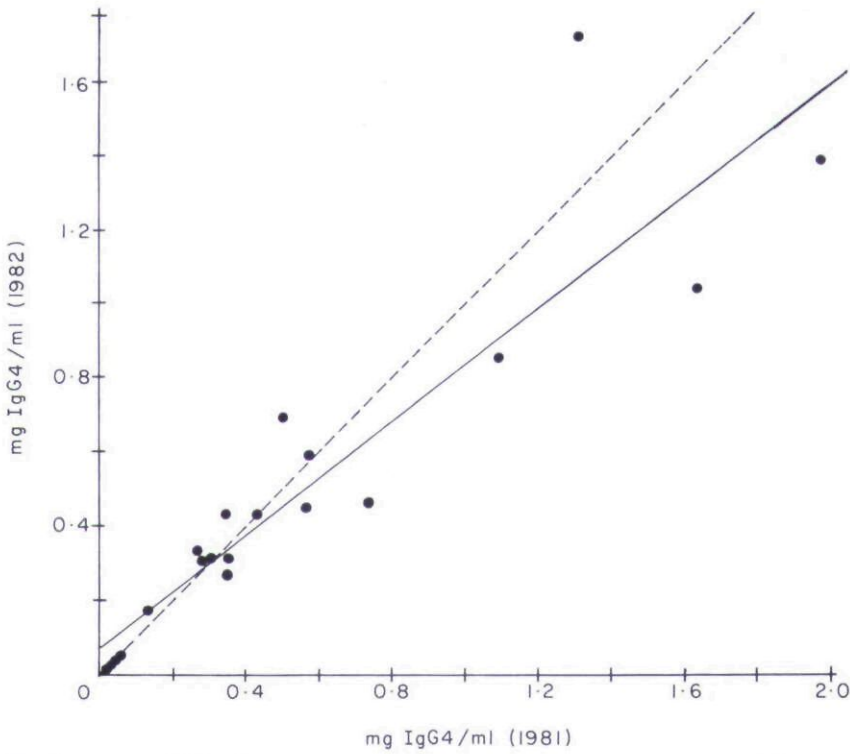


Fig. 2. Correlation of total serum IgG4 determinations performed 12 months apart. The regression line is: $y=0.76x+0.08$, and the correlation coefficient: 0.91.

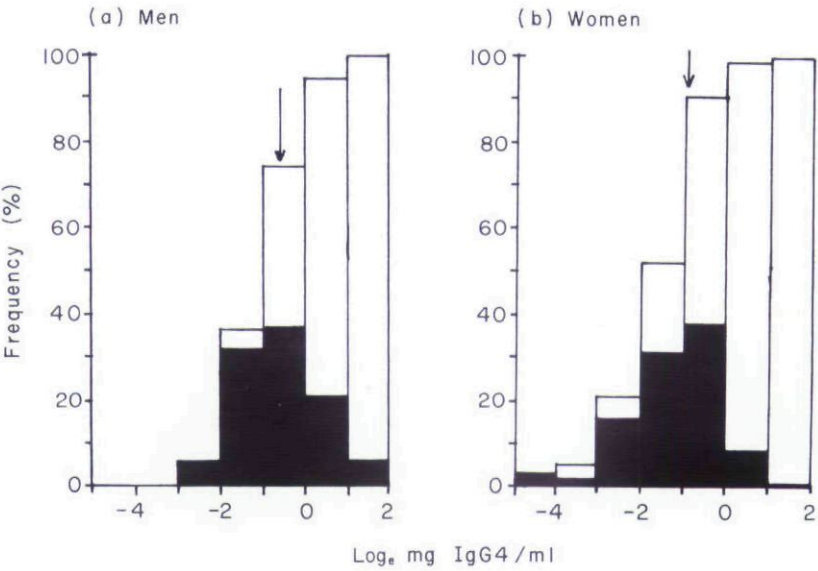


Fig. 3. Frequency histograms and cumulative frequency distributions of total IgG4 concentrations in (a) fifty-seven men and (b) ninety-nine women. The arrows indicate the geometric mean values of log_e transformed data.

0.446–0.759 mg/ml) was significantly greater than the women's value of 0.301 mg/ml (95% confidence interval: 0.242–0.375 mg/ml).

Total serum IgE

Sera were tested in three different assays and the between assay coefficient of variation for high, normal and low reference sera were 2.3, 2.9 and 5.0% respectively. Frequency histograms and cumulative frequency distributions of log-transformed data are illustrated for both sexes (Fig. 4). The geometric mean IgE value for men—42.5 kU/l (95% confidence interval: 30.1–60.0 kU/l) was also significantly higher than that for women—27.5 kU/l (95% confidence interval: 23.1–32.8 kU/l). The overall geometric mean value for men and women was 32.2 kU/l.

IgG4 antibodies

Hen's egg-white allergenic extract was pre-incubated with two sera previously shown to have detectable IgG4 antibodies to these allergens and then IgG4-RAST performed. The resulting inhibitions of the previously positive IgG4-RAST results (Table 1) provided evidence for the specificity of our IgG4-RAST technique.

Frequency distributions of IgG4 antibody levels to the three common inhalant and six food allergens tested were calculated and it can be seen (Table 2) that IgG4 antibody levels were generally higher for foods, especially egg and milk, than inhalants. Between assay coefficients of variation were less than 13% for all allergens except *D. pteronyssinus* (17.2%). Significant differences in IgG4 antibody levels between men and women were noted for grass, cat, wheat and peanut allergens (Table 3).

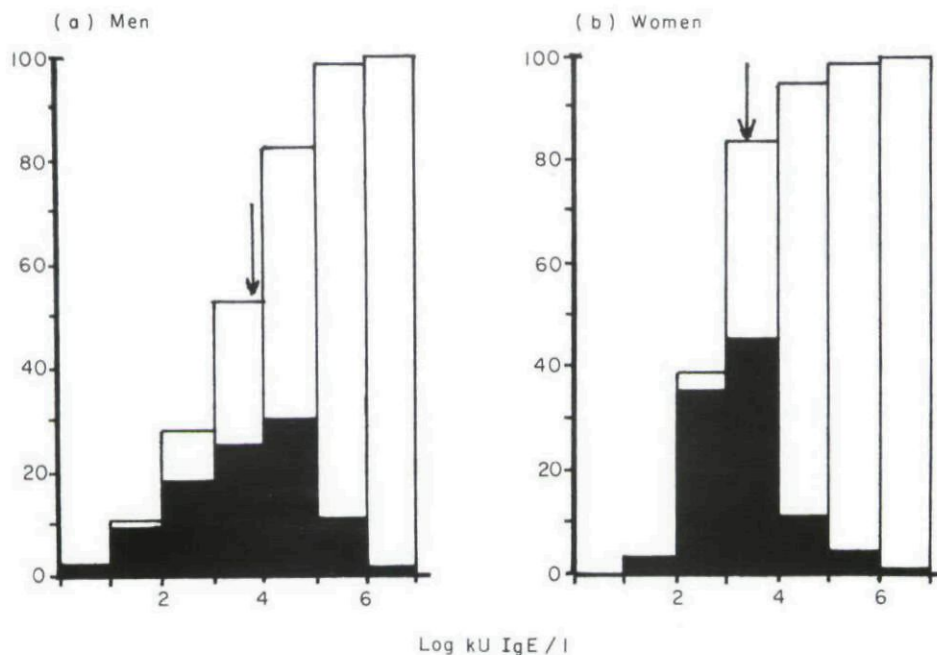


Fig. 4. Frequency histograms and cumulative frequency distributions of total IgE concentrations in (a) fifty-seven men and (b) ninety-nine women. The arrows indicate the geometric mean values of log_e transformed data.

Table 1. Inhibition of egg white IgG4-RAST by egg white

Egg white ($\mu\text{g}/\text{tube}$)	Inhibition (%)	
	Serum 'A'	Serum 'B'
250.0	85	82
25.0	69	59
2.5	37	34
0.25	6	2
0.025	0	0

Total radioactivity added per tube was 15000 ct/min, the count rate in the absence of egg allergen to inhibit the RAST was 3210 ct/min and the non-specific binding, as measured by allowing an IgG4 deficient human serum to react with egg allergen discs was 110 ct/min.

Comparisons of total IgG4 and IgG4 antibody levels

Despite having the higher total IgG4 levels, men generally had lower IgG4 antibody levels than women—the exceptions being grass pollen and wheat flour (Table 3). There was no correlation, in either sex, between total and specific IgG4 levels, e.g. between total IgG4 and egg-IgG4, the correlation coefficients were 0.24 and 0.23 for men and women respectively.

Table 2. Frequency distributions of IgG4 antibodies in fifty-seven men and ninety-nine women (log_e Transformed data)

Class	Lower limit	Frequency (%)								
		Grass	Mite	Cat	Egg	Milk	Cod	Wheat	Almond	Peanut
(a) Men										
1	1.5	0	38.6	2.0	3.5	19.3	49.1	5.3	40.3	68.4
2	2.0	50.9	42.1	61.4	21.0	28.1	29.8	36.8	36.8	15.8
3	2.5	42.1	19.3	29.8	24.6	21.0	15.8	35.1	10.5	12.3
4	3.0	7.0	0	1.8	12.3	8.8	5.3	7.0	8.8	1.8
5	3.5	0	0	0	12.3	14.0	0	14.0	1.8	1.7
6	4.0	0	0	0	21.0	8.8	0	1.8	0	0
7	4.5	0	0	0	5.3	0	0	0	0	0
(b) Women										
1	1.5	9.1	31.3	1.0	9.1	14.1	40.4	17.2	35.3	37.4
2	2.0	62.7	56.6	55.6	11.1	26.3	28.3	48.5	36.4	19.2
3	2.5	23.2	10.1	43.4	15.2	18.2	11.1	16.2	9.1	17.2
4	3.0	1.0	2.0	0	12.1	23.2	11.1	13.1	8.1	3.0
5	3.5	2.0	0	0	12.1	6.1	6.1	4.0	7.1	13.1
6	4.0	0	0	0	25.3	9.1	3.0	1.0	4.0	8.1
7	4.5	0	0	0	15.1	3.0	0	0	0	2.0

Table 3. Comparisons of IgG4 antibody levels in fifty-seven men and ninety-nine women

	Timothy grass*		<i>D. pteronyssinus</i>		Cat		Hen's egg		Cows' milk		Cod fish		Wheat flour		Almond		Peanut	
	M†	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Geometric mean (arbitrary units)	13.0	10.8	8.7	8.8	11.1	11.9	26.2	32.6	15.5	17.0	8.6	10.8	15.0	12.2	9.2	11.1	4.6	7.2
95% Confidence intervals of the geometric mean	12.2-13.8	10.0-11.6	7.9-9.5	8.3-9.4	10.3-12.0	11.3-12.4	20.9-32.9	27.0-39.4	12.6-19.0	14.5-19.9	7.6-9.6	9.3-12.4	12.9-17.4	11.0-13.6	8.2-10.4	9.7-12.7	3.6-5.8	5.8-9.0
Significant differences (P)	<0.002		NS		<0.05		NS		NS		NS		<0.007		NS		<0.006	

* Allergen.

† Sex.

Comparisons of total IgE and IgG4

There was no correlation between total IgE and total IgG4 levels: irrespective of whether the total IgE level was above or below 100 kU/l there were about twice as many subjects with IgG4 levels below 0.8 mg/ml as above it (Table 4). Similarly there was nothing to suggest that a high total IgE level was likely to be associated with a high IgG4 antibody level.

Table 4.

Total IgG4 (mg/ml)	Total IgE (kU/l)	
	< 100	≥ 100
< 0.8	92	16
≥ 0.8	43	8

Discussion

There are two major conclusions to be drawn from this community survey. Firstly IgG4 antibody concentrations were generally greater for food allergens, especially egg and milk, than inhalant allergens, and secondly, men usually had higher total IgG4 levels than women but, with the exceptions of grass pollen and wheat flour, this did not reflect higher specific IgG4 antibody results (Table 3). Before analysing the results it is necessary to consider the reliability of the IgG4 methods because the RAST technique is rarely applied to the determination of other immunoglobulin classes than IgE.

Assay methods

Allergen-specific IgG antibody is a much smaller percentage of total IgG than IgE antibody is of total IgE, e.g. the IgG:IgE ratio of circulating antibodies specific to ragweed pollen in untreated patients was 19:290 (Zeiss, Metzger & Levitz, 1977). This is the main reason why it is easier to measure IgE than IgG antibodies by the RAST method; even so falsely positive IgE-RAST results can be obtained when the total IgE exceeds 5000 kU/l and the specific IgE antibody to be determined is less than 1% of the total (Merrett, 1981). At these high IgE levels it is difficult to satisfactorily wash solid phase allergen-complex free from non-specific IgE prior to the addition of ^{125}I anti-IgE because even a 0.01% non-specific 'trapping' of IgE will be measured by the tracer as a Phadebas RAST score of '1'. Other possible causes of artefactually positive IgE-RAST results are: (1) the use of labelled sheep anti-IgE tracer—Foucard *et al.* (1975) found that 70% of human sera contained antibodies specific to bovine γ -globulin and these antibodies bound cows' milk allergen specifically and ^{125}I -sheep anti-IgE non-specifically; (2) the binding of non-antibody IgE to lectin present in some allergen extracts (Aalberse, 1981).

The possibility of similarly artefactual IgG4-RAST results must be considered. In practice non-specific binding was not a problem despite total IgG4 concentrations being 10 000 times greater than total IgE. The washing of insolubilized allergen free from non-antibody IgG4 prior to the addition of ^{125}I -anti IgG4 was obviously very efficient otherwise a correlation better than the 0.24 recorded would have been found

between egg IgG4-RAST and total IgG4 results; six sera which gave wholly negative RAST results had total IgG4 levels above 1.6 mg/ml. Interaction with human antibodies to bovine γ -globulin being responsible for elevated milk IgG4-RAST results can be discounted because foetal calf serum was used as diluent for the test specimens. Non-specific binding to lectins need only be considered for allergenic extracts which may contain lectins, that is wheat flour, peanut and almond, but since several test specimens with elevated total IgG4 levels produced low IgG4-RAST results to these allergens it seems to be of little or no significance.

The anti-sheep IgG4 had acceptable specificity and avidity for both IgG4-RAST (Table 1) and total IgG4 determinations (Fig. 1). Consequently the methods described are likely to present a true picture of IgG4 antibody and IgG4 immunoglobulin levels.

Some deterioration of specimens stored frozen for twelve months was noted (Fig. 2) but the comparison was excellent for IgG4 concentrations below 1 mg/ml. Therefore radioimmunoassay appears sufficiently robust for the batchwise assay in future studies of specimens sampled at different times.

Immunoglobulins: total IgE and total IgG4

The geometric mean IgE value of 32.2 kU/l was similar to other U.K. community surveys in which the value for people aged 20–44 years was 35.8 kU/l (Burr *et al.*, 1975) and for persons over 70 years it was 30.6 kU/l (Merrett *et al.*, 1980). None of these surveys excluded atopic persons and this accounts for a higher geometric mean than the 21 kU/l we previously recorded in a hospital population of definitely non-atopics (Nye *et al.*, 1975). In the current survey men had significantly higher IgE levels than women (Fig. 4) following the trend in the earlier surveys, although the sex difference was previously only significant among the over-70s. Therefore the participants in the current survey seem representative of the U.K. population, and consequently the IgG4 data recorded can probably be extended nationwide.

Normal IgG4 levels have usually been estimated by radial immunodiffusion techniques and might be expected to give less precise estimations than radioimmunoassay. Indeed a normal U.K. adult range of 0–0.187 mg/ml was quoted by Gwynn *et al.* (1978), but while demonstrating in the U.S.A. that IgG4 levels increased with age until puberty, a geometric mean adult level of 0.6 mg/ml was recorded (Schur, Rosen & Norman, 1979). Nevertheless a recent study, also using a radial immunodiffusion technique, quoted a geometric mean adult value of 0.4 mg/ml (Nieuwkoop, 1982) which is close to our overall value of 0.383 mg/ml. None of these earlier studies noted a sex difference, yet it can be seen (Fig. 3) that men had a significantly higher IgG4 concentration (0.581 mg/ml) than women (0.302 mg/ml).

We found no evidence for a positive correlation between IgE and IgG4 levels (Table 4) in agreement with one study involving thirty-eight patients (Gwynn *et al.*, 1978), but not another (Devey, Wilson & Wheeler, 1976).

IgG4 antibodies

Although men had higher total IgG4 levels than women this observation was not generally extended to antibody levels against the nine common allergens tested. Indeed women tended to have the higher geometric mean values—7/9 allergens (Table 3)—but only in the cases of cat and peanut was this significant. On the other hand in the remaining two tests—grass pollen and wheat flour—men did have significantly higher IgG4 antibody levels, and this is worth comment as follows: (1) hay fever seems to be

more common among men than women (Hagy & Settupane, 1969) and our results could argue either in favour of IgG4 being a monitor of allergy (Gwynn *et al.*, 1978; Vijay, Perelmutter & Bernstein, 1978)—or possibly that more of the men had been hyposensitized with grass pollen and IgG4 was acting as blocking antibody, as suggested by others (Giessen *et al.*, 1976; Iskander, Dars & Aalberse, 1981); (2) the concept that grass pollen and wheat flour share common antigens was used to explain why 20% of hay fever patients with no evidence of adverse reactions to wheat flour products gave positive IgE-RAST results to grass and wheat (Wraith *et al.*, 1979). However, they noted that grass IgE concentrations were usually higher, but careful examination of Table 2 reveals that both men and women had higher concentrations of IgG4 antibodies to wheat than grass. Thus even with these similar allergenic extracts the trend for ingestants to cause higher IgG4 antibody levels than inhalants was confirmed.

IgG4—Blocking antibody or STS-antibody?

The finding that it is normal to have high concentrations of egg- and milk-specific IgG4 antibodies does suggest that they do not play a role in food allergy—although of course levels among food allergic subjects could be even higher. However the situation with regard to inhalant allergy is different, because levels are normally low and therefore raised values may be of diagnostic value. These patient groups are currently being studied in order to provide more data on which a definite conclusion might be based.

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