

Immunoglobulin subclass distribution of specific antibodies in allergic patients

Prediction of the IgE inducing capacity of potential allergens

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The IgG and IgA subclass distribution of specific antibodies against a variety of protein and polysaccharide antigens was determined in sera from individuals with high levels of IgE. No shift of the antibody pattern could be observed, suggesting that the aberrant regulation of responses against allergens noted in these patients is limited, encompassing selected antigens only. Antibodies against protein antigens are mainly of the IgG1 subclass. In addition, low levels of specific IgG3 or IgG4 antibodies may be formed. Our data suggest that a given antigen induces *either* IgG3 *or* IgG4 and that potential allergens, in addition to IgG1 and IgE, elicit a response restricted to IgG4.

Key words: immunoglobulin subclass; IgG subclass; IgE.

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Specific IgE antibodies have been incriminated in various forms of allergy, and short-term sensitizing antibodies of the IgG4 subclass have also been suggested previously in allergic patients.

Allergens are mainly protein antigens, a type of antigen which gives rise to IgG1 antibodies in normal individuals (for review see 9). Immunization may result in the formation of specific IgG3 and IgG4 antibodies particularly after repeated or prolonged exposure (9). Immunotherapy usually induces quite marked levels of specific IgG4 antibodies (for review see 4). Antibodies against carbohydrate antigens, on the other hand, are usually restricted to the IgG2 subclass.

Levels of immunoglobulin classes and subclasses are usually markedly correlated in man. Thus, serum concentration of IgG3 and IgG1, IgG2 and IgG4 and IgA and IgE have all been shown to be interrelated both in normal and immunodeficient individuals (for review see 10). In IgA deficient patients, levels of IgG4

and IgE have also recently been shown to be co-variable (7). These observations are suggestive of a regulated expression of closely linked immunoglobulin genes; the gene order being $\gamma 3-\gamma 1-\alpha 1-\gamma 2-\gamma 4-\epsilon-\alpha 2$ (6).

Allergy appears to be a genetically determined disorder characterized by a tendency to form IgE antibodies in response to challenge with protein antigens. The basic defect may be a dysregulation of immune responsiveness, affecting also the antibody pattern normally induced. Thus, specific responses against *any* protein or carbohydrate antigen could theoretically be anticipated to display a marked preference for immunoglobulin subclasses located adjacent to the gene, i.e. the heavy chain constant region of IgG4 and IgA2, a shift which would not be detected by routine analysis of the class distribution of specific antibodies. Total serum levels of IgG4 have indeed previously been shown to be elevated in allergic patients, thus supporting this hypothesis (4) and we therefore determined the IgG and IgA sub-

Table 1
The immunoglobulin subclass pattern of specific antibodies in allergic patients^a

Do- nor	Age	Diag- nosis	Offending ^b allergen		Total serum	Tetanus toxoid						α-toxin					
						IgE ^c	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgG1	IgG2	IgG3	IgG4	IgA1
1	26	Atopic dermatitis	— ^d	—	17000	1.46	0.07	0.07	0.48	0.28	0.02	1.54	0.13	0.05	1.07	0.69	0.04
2	38	Atopic dermatitis	— ^e	—	15000	0.19	0.11	0.16	0.03	0.13	0.00	1.14	0.05	0.01	0.87	0.80	0.00
3	19	Eczema	— ^f	—	11000	0.82	0.01	0.01	0.04	0.27	0.00	1.22	0.82	0.07	1.20	0.46	0.06
4	77	Eczema	— ^g	—	14000	1.29	0.02	0.07	0.03	0.09	0.00	1.30	0.14	0.05	0.86	0.95	0.04
5	20	Atopic dermatitis	— ^h	—	18000	0.04	0.00	0.00	0.89	0.13	0.00	1.26	0.22	0.05	0.44	0.34	0.01
6	23	Asthma	Cat epithe- lium	55	540	0.17	0.01	0.03	0.00	0.21	0.00	1.22	0.05	0.03	1.04	0.98	0.00
7	21	Eczema	—	—	1100	0.97	0.00	0.04	0.00	0.17	0.00	1.36	0.31	0.03	0.29	0.24	0.00
8	38	Rhinitis	Timothy	86	390	1.27	0.17	0.77	0.59	0.34	0.02	1.30	0.26	0.10	0.76	0.62	0.00
9	53	Atopic dermatitis	—	—	710	0.11	0.07	0.08	0.12	0.12	0.00	1.30	0.08	0.06	0.92	0.76	0.00
10	24	Rhinitis	—	—	660	1.38	0.20	0.13	0.56	0.56	0.09	1.28	0.16	0.01	0.87	0.66	0.00
11	41	Rhinitis	Timothy	91	380	1.11	0.01	0.09	0.36	0.11	0.01	1.07	0.12	0.00	0.46	0.31	0.00
12	23	Rhinitis	Timothy	67	340	0.65	0.02	0.03	0.12	0.21	0.00	1.00	0.04	0.01	0.13	0.27	0.00
13	7	Rhinitis	Birch	24	490	1.17	0.06	0.13	0.26	0.17	0.00	0.96	0.06	0.02	0.36	0.44	0.00
14	23	Rhinitis	Timothy	67	770	0.43	0.03	0.06	0.04	0.07	0.02	0.85	0.27	0.09	0.12	0.17	0.00
15	13	Rhinitis	Timothy	58	430	0.99	0.08	0.02	0.44	0.25	0.00	1.23	0.14	0.06	0.73	0.45	0.00
16	23	Control	—	—	32	0.88	0.06	0.09	0.29	0.15	0.00	1.23	0.09	0.00	0.75	0.53	0.03
17	26	Control	—	—	5	1.22	0.05	0.14	0.52	0.29	0.02	0.97	0.21	0.00	0.64	0.48	0.01
18	34	Control	—	—	< 2.5	0.96	0.01	0.05	0.03	0.15	0.01	1.05	0.06	0.01	0.96	0.61	0.01
19	27	Control	—	—	12	0.30	0.04	0.01	0.09	0.09	0.00	0.97	0.01	0.01	0.05	0.31	0.02
20	18	Control	—	—	23	1.05	0.07	0.00	0.69	0.30	0.00	1.31	0.15	0.06	0.87	0.48	0.05

^a Results are given as net absorbance values in ELISA after 10 (tetanus toxoid, α-toxin, *H. influenzae* and teichoic acid) or 20 minutes (pneumococcal polysaccharide).
^b Given as offending allergen and PRU units.
^c Given as kU/l. Normal reference value for adults 2.5–122. kU/l.
^d Positive against a number of tested antigens including cat epithelium (8.3 PRU), dog epithelium (0.6 PRU), cow epithelium (0.9 PRU) and feathers (1.1 PRU).
^e Positive against peanut antigen (2.4 PRU).
^f Positive against a number of tested antigens including birch pollen (134 PRU) and timothy pollen (330 PRU).
^g Positive against a number of tested antigens including oats (47 PRU), wheat (3.5 PRU), rye (4.1 PRU) and cow epithelium (4.3 PRU).
^h Positive against a number of tested antigens including cheddar cheese (65 PRU) and mold (64 PRU).

class pattern of specific antibodies against a variety of antigens in patients with raised levels of serum IgE.

MATERIAL AND METHODS

Sera

Sera from 23 patients with suspected or confirmed IgE-mediated allergic disorders were col-

lected and stored at -20°C until used. IgE levels were measured in a radioimmunoassay (lower limit 2.5 µl). Sera from normal blood donors served as controls.

ELISA

The immunoglobulin subclass distribution of antibodies against a variety of antigens was tested as previously described in detail (15). Briefly, antigens (pneumococccal capsular

Table 1 (continued)

<i>H. influenzae</i> membrane protein						Teichoic acid						Pneumococcal polysaccharide type 19 F					
IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2
0.97	0.00	0.00	0.01	0.33	0.00	0.26	0.65	0.00	0.04	0.06	0.00	0.20	0.55	0.04	0.06	0.49	0.11
0.96	0.07	0.07	0.00	0.46	0.00	0.01	0.67	0.00	0.01	0.05	0.00	0.08	0.71	0.03	0.04	0.42	0.05
1.07	0.04	0.08	0.04	0.15	0.00	1.24	1.28	0.05	0.24	0.06	0.00	0.32	0.84	0.02	0.08	0.15	0.02
0.87	0.05	0.31	0.01	0.10	0.01	0.08	0.44	0.01	0.01	0.08	0.00	0.97	0.66	0.02	0.08	0.28	0.03
0.73	0.02	0.13	0.01	0.21	0.00	0.76	0.17	0.02	0.07	0.03	0.00	0.23	0.65	0.05	0.07	0.13	0.01
1.23	0.07	0.71	0.01	0.62	0.00	0.77	0.84	0.05	0.07	0.69	0.00	0.48	0.81	0.03	0.06	0.22	0.03
0.49	0.00	0.01	0.00	0.03	0.00	1.52	1.07	0.02	0.11	0.06	0.00	0.23	0.92	0.00	0.02	0.21	0.01
1.03	0.70	0.24	0.02	0.80	0.00	0.00	0.98	0.02	0.09	0.04	0.00	0.11	1.17	0.02	0.02	0.20	0.00
1.17	0.04	0.19	0.01	0.21	0.00	0.31	0.03	0.00	0.00	0.30	0.00	0.19	0.64	0.03	0.01	0.35	0.11
1.42	0.25	0.61	0.05	0.67	0.01	0.07	1.00	0.01	0.05	0.09	0.00	1.38	1.03	0.02	0.10	0.44	0.00
0.70	0.00	0.05	0.03	0.26	0.00	0.23	0.87	0.02	0.01	0.12	0.00	0.12	0.56	0.01	0.00	0.22	0.00
1.45	0.30	0.44	0.10	0.22	0.00	0.10	0.76	0.01	0.03	0.04	0.00	0.17	0.63	0.02	0.03	0.14	0.00
1.03	0.04	0.08	0.03	0.28	0.00	0.43	1.03	0.03	0.02	0.09	0.00	0.25	0.45	0.09	0.01	0.23	0.02
1.70	0.15	0.18	0.03	0.19	0.00	0.09	0.64	0.00	0.01	0.03	0.00	0.09	0.79	0.03	0.06	0.11	0.01
0.78	0.06	0.09	0.02	0.21	0.00	0.17	0.79	0.04	0.08	0.02	0.00	0.12	0.91	0.04	0.02	0.16	0.04
1.27	0.05	0.23	0.00	0.24	0.00	0.46	1.14	0.04	0.16	0.14	0.01	0.19	0.88	0.04	0.08	0.26	0.03
1.03	0.09	0.16	0.08	0.36	0.01	0.27	0.89	0.02	0.09	0.05	0.00	0.12	1.02	0.05	0.07	0.17	0.02
1.26	0.00	0.29	0.00	0.35	0.00	0.19	0.76	0.00	0.04	0.05	0.01	0.11	0.61	0.03	0.05	0.27	0.09
0.54	0.02	0.01	0.00	0.12	0.00	0.10	0.52	0.00	0.01	0.06	0.00	0.09	0.58	0.04	0.01	0.13	0.04
1.02	0.10	0.23	0.06	0.24	0.01	0.61	1.13	0.05	0.12	0.02	0.01	0.28	1.07	0.06	0.09	0.46	0.01

polysaccharide type 19F) (a gift from MSD, Mechelem, Belgium), teichoic acid (batch 8a) and α -toxin from *S. aureus* (gifts from Dr. R. Möllby, National Bacteriological Laboratory, Stockholm, Sweden), tetanus toxoid (a gift from Dr. M. Fall-Persson, National Bacteriological Laboratory, Stockholm, Sweden) and outer membrane protein from *H. influenzae* (a gift from Dr. I. Allan, Oxford, England) were coated onto microtiter plates. After incubation with patient serum samples (diluted 1:100), commercially available monoclonal antibodies against the various IgG (Unipath, Bedford, England) or IgA subclasses (Nordic, Tilburg, The Netherlands) were added followed by a rabbit anti-mouse (Dakopatt, Glostrup, Denmark) and alkaline phosphatase conjugated goat anti-rabbit immunoglobulin antibodies (Sigma Chemical Company, St.

Louis, MO, USA) following addition of substrate, absorbance at 405 nm were measured. Statistical analysis was performed using Wilcoxon's non-parametric ranking test.

RESULTS

Response to protein antigens

The IgG and IgA subclass distribution against protein antigens such as tetanus toxoid, α -toxin from *S. aureus* and outer membrane proteins from *H. influenzae* showed a normal pattern (i.e. mainly IgG1 and IgA1, both in patients with high or moderate serum IgE levels and in normal donors (Table 1). IgG3 and IgG4 antibodies were also detected in levels comparable to those seen in normal donors and no

statistically significant shift of specific antibodies to the IgG4 subclass was noted. The response to certain antigens, such as outer membrane protein from *H. influenzae*, was almost completely restricted to the IgG3 subclass (in addition to the dominant IgG1 subclass), whereas other antigens (tetanus toxoid and α -toxin from *S. aureus*) appear to induce mainly IgG4 antibodies as the second most dominant antibody subclass.

Response to polysaccharide antigens

Antibodies against carbohydrate antigens, such as pneumococcal capsular polysaccharide and teichoic acid from *S. aureus*, were restricted mainly to the IgG2 subclass in all donors and no increment in levels of specific IgG4 or IgA2 antibodies was observed in patients with raised levels of serum IgE (Table 1).

DISCUSSION

Chronic stimulation with a protein antigen has previously been suggested to result in an IgG4 dominated immune response (1) and this hypothesis appears to be supported by a number of different reports. Allergens are known to induce IgE antibodies, but IgG1 and IgG4 antibodies are also formed during the immunization process, and prolonged antigenic exposure, as seen during immunotherapy, will ultimately result in the induction of vast amounts of IgG4 (4). Chronic parasitic infections may also result in the formation of specific IgE and IgG4 antibodies. In addition, some autoantigens also appear to induce IgE and IgG4 antibodies. In view of the genomic localization of the $\gamma 4$ and ϵ genes, the genetic factor underlying the allergic state may be a T helper cell-mediated switch preference resulting in an antibody response skewed towards utilization of the 3' heavy chain constant region genes. As a consequence, the normal subclass pattern of antibodies against all antigens would be expected to be disturbed if this was a general phenomenon, and that the heavy chain genes most distal to the variable genes, i.e. $\gamma 4$, and $\alpha 2$, would be employed at a

higher rate than expected. However, our data clearly suggest that the normal antibody pattern is retained in "allergic" individuals, supporting the notion that only selected antigens are able to induce the disproportionately high levels of IgG4 and IgE seen in these patients.

Protein antigens are known to induce IgG3 and IgG4 antibodies (in addition to the normally dominant IgG1 antibodies). However, it is becoming increasingly clear that a given antigen will induce *either* IgG3 *or* IgG4 (Table 1). In view of the close association between levels of IgG4 and IgE antibodies, this finding may also have bearing on the potential IgE inducing, and thus the possible "allergenic" capacity, of protein antigens. Parasites are generally thought to induce vast amounts of IgE antibodies. However, not all parasites give rise to IgE and we predict that only those capable of inducing IgG4 display this ability. Unfortunately, the IgG subclass pattern of specific antibodies has as yet only been determined in a few chronic infections. However, potent inducers of IgE, such as *Schistosoma* and *Filaria* both give rise to IgG4 antibodies (11, 12). In contrast, diseases such as trypanosomiasis (19), leishmaniasis (5), spirochetosis (lyme disease) (13) and malaria (20) which to our knowledge are not known to induce large amounts of IgE, all give rise to a pattern of mainly, although not exclusively, IgG1 and IgG3 antibodies. In the latter case it is noteworthy that total levels of IgG3 may remain normal in spite of infection (18). A similar argument appears to be valid for responses both against autologous protein antigens (such as blood group antigens (A, D and P), which induce IgG1 and IgG3 (9) but not IgE (16) and immunoglobulins which induce IgG1, IgG4 and IgE (2, 8, 14, 17)) and heterologous antigens (such as tetanus toxoid (IgG1, IgG4 and IgE) (21)). Although these two patterns appear rather consistent, exceptions may exist and trace amounts of the non-preferred subclasses (such as IgG2 and IgG3 or IgG4) may be found in the sera of hyperimmunized individuals.

The strong preference for either IgG3 *or* IgG4 may suggest that the antibody repertoire that can be expressed in a given subclass is

limited and that certain V_H -genes may only, or at least preferentially, combine with a limited number of immunoglobulin constant region genes. The alternative hypothesis is that the induction of the antibodies (via T cell help) differ and that particular sets of T helper cells will produce factors that switch B cells to either IgG3 or IgG4 production. This could either be because T cells distinguish between various types of protein antigens and subsequently induce factors initiating this switch (to IgG3 or IgG4) or two distinct subsets of T helper cells exist, predestined to produce either an IgG3 switch factor or an IgG4 switch factor. In the latter case we would predict that the T cells would also be able to effectively induce IgE production, possibly by the same lymphokine, and the antigen receptor repertoire in the two subsets of T cells must then be anticipated to differ markedly. In this context it is of considerable interest to note that in the mouse a factor derived from T helper cells that will help B cells to switch to the production of multiple IgG subclasses and IgE has recently been described (3).

Our hypothesis on switch patterns may predict the IgE-inducing potential of a given antigen and suggest that the helper factor producing T cells may be an attractive target for pharmacological intervention in "allergic" patients.

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