

Binding affinities of allergens from pollen, mites, and house dust for specific IgG subclass antibodies

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The distribution and affinity of IgG subclasses against various aeroallergens were assessed by inhibition of specific antibody binding. Two parameters from the dose-response curves were taken as indicative of antibody affinity: the point of 50% inhibition and the value of the slopes on double-log plots. It was found that IgG4 antibody specific for aeroallergens (i.e., from pollens of several species of Gramineae, *Olea europaea*, and *Parietaria judaica* and from house dust) usually exhibits high affinity, except for *Dermatophagoides pteronyssinus*. High binding affinity was also displayed by IgG1 subclass antibodies against the allergens of *O. europaea* and *P. judaica*. Distinct IgG subclass affinity profiles were observed for the allergens of grass pollen (i.e., *Holcus lanatus*) and dust mites (i.e., *D. pteronyssinus*). These results demonstrate that IgG subclass distribution, as well as antibody affinity, depends on the nature of the sensitizing allergen.

Key words: aeroallergens; affinity; IgG subclasses.

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Immediate-type allergic disease is usually associated with an antibody response to the common allergens, mainly due to immunoglobulins of the IgE and IgG isotypes. Allergen-specific IgE antibodies are related to the clinical hypersensitivity reactions, whereas the IgG class antibodies have been considered to “block” the allergic response (1–6). IgG antibodies habitually appear during immunotherapy and are therefore often held responsible for the beneficial effect of the treatment (7).

In most investigations, IgG subclass antibodies are determined by direct immunoassay during which serum samples are made to react with an immobilized allergen. This system cannot distinguish IgG antibodies of high or low affinity. Nevertheless, the affinity of allergen binding is an important factor because it may influence the biologic effects of the antibodies (8, 9), as well as their presumed “blocking” potential (10). The relevance of antibody affinity has been demonstrated in patients immunized with tetanus toxoid, where the immune response mainly involved IgG1 and

IgG4 antibodies, although only the IgG1 isotype was of high affinity (11). Similar results were obtained in patients immunized with hepatitis B virus (12).

Only a few studies have investigated the affinity of IgG subclass antibodies specific to the common inhalant allergens. As regards allergies to dietary proteins, high-affinity IgG4 responses to both casein and ovalbumin have been described in mothers, whereas IgG1 antibodies to ovalbumin were of low affinity (13). It was suggested by these investigators that antigen exposure is an important factor in driving the affinity maturation process. However, high-affinity IgG4 and IgG2 antibodies to casein have also been described (14, 15). High-affinity IgG1 subclass antibodies to *Dermatophagoides pteronyssinus* were observed in allergic children and adults, whereas IgG1 antibodies to *Lolium perenne* were of low affinity in children, but not in adults (16). The present work examines the affinity distribution of IgG subclasses to different kinds of aeroallergens by an inhibition format of enzyme immunoassay.

Material and methods

Blood sera

Serum samples were collected from patients with symptoms indicative of type I allergy on the basis of the clinical history in association with positive immediate skin reactions to allergenic extracts. Sera were used solely from patients never treated by immunotherapy. Some of these sera were examined as individual samples; i.e., S1 from patients allergic to the pollen of the Gramineae; S2, S3, and S4 allergic to the pollen of *Olea europaea*; S5, S6, S7, S8, and S9 allergic to the pollen of *Parietaria judaica*; and S10, S11, S12, and S13 allergic to mite allergens of *Dermatophagoides* spp. Other sera were organized into four groups and pooled; i.e., the sera of patients allergic to the Gramineae (pools P1 and P2), to *O. europaea* (P4 and P5), and to *Dermatophagoides* spp. (P6, P7, and P8) and a pool of sera from adult patients with widespread atopic dermatitis (P-AD) having IgE and IgG antibodies to the allergens of both Gramineae pollen and house-dust mites. All sera were stored in aliquots at -20°C until use.

Allergens

Extracts were prepared from dried and defatted pollen grains (BioPol, Spokane, WA, USA). The allergenic pollen preparations for investigation comprised the unfractionated nondialyzable portions (nominal cutoff 10 kDa) of aqueous extracts manufactured by Laboratorios C.B.F. LETI SA (Madrid, Spain) and retained in the lyophilized form as in-house reference standards. The investigated species were *Cynodon dactylon*, *Dactylis glomerata*, *Holcus lanatus*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *O. europaea*, and *P. judaica*. The freeze-dried allergen preparations were dissolved in phosphate-buffered saline (PBS), pH 7.4, just before use. A second group of allergens consisted of whole-culture extract of *D. pteronyssinus* (Laboratorios C.B.F. LETI SA, Madrid, Spain) and an enriched protease fraction mainly comprising *Der p* I and *Der p* III, concentrated by molecular sieving through columns of Sephacryl S300. Finally, two house-dust extracts were investigated, i.e., an in-house standard product and a previously described highly purified house-dust preparation R1 (17).

Inhibition of IgG subclass antibody binding

IgG-antibody binding was evaluated by enzyme immunoassay using allergens coated to the wells of flat-bottom microtiter plates (Nunc-Immuno PolySorp, LabClinics, Barcelona, Spain) as a modifi-

cation of the ELAST system marketed by C.B.F. LETI SA (Madrid, Spain). Allergen coating was done by dissolving allergenic preparations at $10\text{ }\mu\text{g/ml}$ in sodium bicarbonate buffer (100 mM, pH 9.4) and incubating $150\text{-}\mu\text{l}$ aliquots per well overnight at 37°C . After a series of washings with PBS containing 0.4% Triton-X-405, the plates were stored at 4°C until use.

Before the inhibition assay, the optimal serum dilution giving a suitable optical density range of 0.5–1.5 was established by titration (usual range 1 : 20–1 : 100). Sequential allergen concentrations were then preincubated for 3 h at room temperature with the optimally diluted serum sample in a blocking solution (A) composed of Triton-X-405 (0.5%), thimerosal (1 mM), and PBS (0.01 M). This period of incubation was found to be sufficient to reach equilibrium. The absolute amount of IgG subclass antibodies was not taken into account because different dilutions of serum samples did not affect the inhibition assay (data not shown). In the following step, $100\text{ }\mu\text{l}$ of allergen-serum mixtures was pipetted into the wells of the allergen-coated plates to capture residual free IgG-antibody for 30 min at ambient temperature. After a series of washing cycles with Triton-X-405 solution, $100\text{ }\mu\text{l}$ of horseradish peroxidase (HRP)-labeled monoclonal antitotal IgG, IgG1, IgG2, IgG3, or IgG4 (Janssen Biochimica, Beerse, Belgium) in the blocking solution A was introduced to react with bound IgG and IgG subclasses for 30 min at room temperature. The plates were finally washed again with Triton-X-405, and bound peroxidase was evaluated at room temperature by adding $100\text{ }\mu\text{l}$ of 0.16 M sodium acetate buffer, pH 6.0, containing $2\text{ }\mu\text{l}$ tetramethylbenzidine in DMSO and $0.2\text{ }\mu\text{l}$ 1 M hydrogen peroxide. After 30 min, the reaction was stopped with $100\text{ }\mu\text{l}$ 2 M sulfuric acid, and the color was measured at 450 nm in an ELISA plate reader (Titertek Multiskan) and corrected for appropriate blanks.

Expression of the results

Binding affinities were evaluated by establishing the point of 50% inhibition and the slope of the regression lines computed from double-log plots of percent inhibition against allergen concentration. In this mode of data presentation, the equation for the linear regression line approaches the log transformation of the law of mass action for allergen-IgG subclass binding:

$$\log(\text{fractional inhibition}) = k_1 \log(\text{allergen concentration}) + k_2$$

in which k_1 represents the affinity-related slope of the linear portion of the dose-response curve (18, 19).

Results

Inhibition experiments with pollen extracts

In the inhibition experiments, we used six extracts from different pollen species of the family of the Gramineae. As respective representatives of allergenic trees and weeds, we employed extracts of the pollen of *O. europaea* and *P. judaica*. Serial dilutions of these allergenic preparations were incubated with their corresponding serum pools to examine the interaction with specific IgG subclass antibodies. From the regression lines of the inhibition curves thus established, the point of 50% inhibition was computed for total IgG (as control) and each of the subclasses, together with the slope of the linear portion of the dose-response curve.

The results for 50% inhibition with the pollen extracts are listed in Table 1. The data show the high capacity of the Gramineae pollen extracts to inhibit the binding of IgG4 to the immobilized allergens, together with a low capacity for inhibiting IgG1 and IgG3 subclass binding. Specific IgG2 levels generally were quite low; indeed, undetectable in several serum samples. In those cases where IgG2 attained detectable levels, the antibodies appeared to be of high binding capacity.

In contrast, the allergenic pollen proteins of *O. europaea* and *P. judaica* presented a high binding capacity for both IgG1 and IgG4 antibodies, though exhibiting low affinity for the IgG3 subclass. IgG2 antibodies against these allergens were not detected in any case (Table 1).

As shown in Table 2, the slopes of the double-log inhibition lines, representing binding affinity, follow a numeric pattern similar to the values for 50% inhibition values given in Table 1. For the Gramineae, *O. europaea*, and *P. judaica*, the affinity for IgG4 binding was considerably higher than observed with the antibodies of the IgG1, IgG2, and IgG3 isotypes. However, in the case of the *Parietaria* allergens, the binding potential for IgG1 subclass antibodies was of the same order of magnitude as observed with IgG4 (Table 2).

Inhibition experiments with mite and house-dust extracts

For these experiments, two batches of *D. pteronysinus* (1 and 2) were employed and two different preparations from house dust (see Material and methods). Inhibition curves were established by preincubation with the corresponding serum pools.

The potencies found for 50% inhibition are shown in Table 3, which demonstrates absence of a clear-cut pattern for the mite extracts, although, with some sera, appreciable inhibition was observed for IgG3 subclass antibodies. In contrast,

Table 1. Quantity of allergenic extract (ng) of pollen of various species of Gramineae, and of *O. europaea* and *P. judaica*, required for 50% inhibition of specific pan-IgG and IgG-subclass antibodies

Extracts	Sera	IgG	IgG1	IgG2	IgG3	IgG4
<i>P. pratense</i>	P1	71	no inh.	n.d.	no inh.	71.5
<i>L. perenne</i>	P1	8.2	70.5	72.5	no inh.	9.1
<i>L. perenne</i>	P3	14.6	1083.0	n.d.	311.0	45.9
<i>L. perenne</i>	S1	603.0	9051.0	n.d.	2881.0	9.7
<i>C. dactylon</i>	P2	12.1	no inh.	5.6	no inh.	2.9
<i>P. pratensis</i>	P2	6.1	256.0	22.3	222.0	3.2
<i>D. glomerata</i>	P2	9.9	83.3	18.3	149.0	2.3
<i>H. lanatus</i>	P1	4.5	20.8	5.3	401.0	2.8
<i>H. lanatus</i>	P2	1.8	no inh.	1.9	no inh.	0.4
<i>H. lanatus</i>	P-AD	1.5	98.8	n.d.	no inh.	0.9
<i>O. europaea</i>	P4	46.2	859.0	n.d.	289.0	571.0
<i>O. europaea</i>	P5	28.8	49.3	n.d.	1267.0	78.4
<i>O. europaea</i>	S2	1670.0	414.0	n.d.	1926.0	1803.0
<i>O. europaea</i>	S3	522.0	433.0	n.d.	1541.0	571.0
<i>O. europaea</i>	S4	347.0	1357.0	n.d.	n.d.	2628.0
<i>P. judaica</i>	S5	2.8	4.1	n.d.	164	2.8
<i>P. judaica</i>	S6	38.4	61.9	n.d.	65	6.0
<i>P. judaica</i>	S7	9.8	9.5	n.d.	394	14.8
<i>P. judaica</i>	S8	2.7	5.1	n.d.	n.d.	5.4
<i>P. judaica</i>	S9	—	11.9	n.d.	84.5	0.8

P=serum pools; S=individual serum samples; n.d.= not detectable; no inh.= no inhibition within the chosen range of allergen concentrations; figures in **bold**=low values for 50% inhibition representing high binding potencies.

Table 2. Values of k_1 for slopes derived from pan-IgG- and specific IgG-subclass antibody inhibition curves with pollen extracts of various species of Gramineae, and for *O. europaea* and *P. judaica*

Extracts	Sera	IgG	IgG1	IgG2	IgG3	IgG4
<i>P. pratense</i>	P1	0.41	no inh.	n.d.	no inh.	0.42
<i>L. perenne</i>	P1	0.37	0.27	0.51	no inh.	0.56
<i>L. perenne</i>	P3	0.48	0.20	n.d.	0.17	0.30
<i>L. perenne</i>	S1	0.38	0.27	n.d.	0.18	0.81
<i>C. dactylon</i>	P2	0.23	no inh.	0.28	no inh.	0.48
<i>P. pratensis</i>	P2	0.33	0.12	0.40	0.17	0.78
<i>D. glomerata</i>	P2	0.50	0.39	0.57	0.11	0.70
<i>H. lanatus</i>	P1	0.43	0.41	0.47	0.23	1.26
<i>H. lanatus</i>	P2	0.27	no inh.	0.47	no inh.	0.35
<i>H. lanatus</i>	P-AD	0.60	0.21	n.d.	no inh.	0.68
<i>O. europaea</i>	P4	0.39	0.17	n.d.	0.11	0.22
<i>O. europaea</i>	P5	0.60	0.24	n.d.	0.20	0.58
<i>O. europaea</i>	S2	1.38	0.20	n.d.	0.45	1.01
<i>O. europaea</i>	S3	0.45	0.60	n.d.	0.75	1.20
<i>O. europaea</i>	S4	0.55	0.50	n.d.	n.d.	0.57
<i>P. judaica</i>	S5	0.59	0.86	n.d.	0.55	0.68
<i>P. judaica</i>	S6	0.39	0.70	n.d.	0.62	0.82
<i>P. judaica</i>	S7	0.82	0.83	n.d.	0.26	0.49
<i>P. judaica</i>	S8	0.63	0.73	n.d.	n.d.	1.20
<i>P. judaica</i>	S9	—	0.36	n.d.	0.25	0.27

Abbreviations as for Table 1. Figures in **bold**=high values of k_1 for slopes corresponding to high affinities.

Table 3. Quantity of *D. pteronyssinus* extract (in ng) and house-dust extract (in µg) for 50% inhibition of specific pan-IgG and IgG-subclass antibodies

Extracts		Serum	IgG	IgG1	IgG2	IgG3	IgG4
<i>D. pteronyssinus</i>	(1)	P6	27.4	108.0	85.3	19.0	n.d.
<i>D. pteronyssinus</i>	(1)	P-AD	32.9	149.0	144.0	42.5	n.d.
<i>D. pteronyssinus</i>	(1)	S10	23.4	1182.0	n.d.	6.0	n.d.
<i>D. pteronyssinus</i>	(2)	S11	447.0	1885.0	n.d.	235.0	179.0
<i>D. pteronyssinus</i>	(2)*	P7	1140.0	26.0	114.0	368.0	n.d.
<i>D. pteronyssinus</i>	(2)	S12	324.0	2522.0	1240.0	1691.0	1103.0
<i>D. pteronyssinus</i>	(2)	S13	157.0	8669.0	n.d.	42.0	79.0
House dust	(1)	P8	—	155.0	n.d.	233.0	0.2
House dust	(1)	S10	4.2	no inh.	n.d.	33.9	8.0
House dust	(1)	S11	0.7	112.0	n.d.	161.0	15.1
House dust	(2)	P6	0.6	8.77	n.d.	n.d.	0.2
House dust	(2)	P7	0.4	no inh.	n.d.	n.d.	0.2
House dust	(2)	S10	0.2	no inh.	n.d.	no inh.	0.2
House dust	(2)	S11	—	no inh.	n.d.	no inh.	1.0

Abbreviations as for Table 1.

*Protease fraction (*Der p* I + *Der p* III).Figures in **bold** = low values (i.e., high potency) for 50% inhibition.

house-dust extracts displayed a strong capacity for inhibition of IgG4 antibody binding, as against a weak potential for reacting with IgG1 and IgG3.

As regards the antibody affinities represented by the slopes listed in Table 4, again no fixed pattern was evident for the mite allergens, not even with respect to the IgG3 subclass. On the other hand, slope values for the house-dust extracts with IgG4 were larger than with IgG1 and IgG3, in agreement with the data for 50% inhibition in Table 3.

Inhibition experiments with pollen and mite allergens using a pool of sera from multisensitized patients

For investigation of the effect of the allergen nature on IgG subclass binding, a pool of sera was prepared from atopic dermatitis patients sensitized, among others, to both grass-pollen and mite allergens (serum pool P-AD, Tables 1–4). As inhibitors in the immuno-assay, two allergen preparations were used, *H. lanatus* and *D. pteronyssinus*. The dose-response graphs observed for the IgG subclass antibodies against *H. lanatus* are shown in Fig. 1, which illustrates the great similarity in inhibition characteristics for the IgG4 and total (pan-) IgG antibodies, both in terms of 50% binding and with respect to affinity, i.e., the slope of the regression lines. Both parameters were quite distinct for the pollen-specific IgG1 and IgG3 antibodies. Binding of IgG2 antibodies was not detectable with this serum pool (Table 1).

The IgG subclass distribution of Fig. 1 is entirely different from that observed with the *D. pteronyssinus*

Table 4. Values for k_i for slopes derived from pan-IgG- and specific IgG-subclass antibody inhibition curves with *D. pteronyssinus* extract and house-dust extract

Extracts		Serum	IgG	IgG1	IgG2	IgG3	IgG4
<i>D. pteronyssinus</i>	(1)	P6	0.19	0.28	0.45	0.40	n.d.
<i>D. pteronyssinus</i>	(1)	P-AD	0.55	0.51	0.50	0.38	n.d.
<i>D. pteronyssinus</i>	(1)	S10	0.29	0.51	n.d.	0.83	n.d.
<i>D. pteronyssinus</i>	(2)	S11	0.37	0.68	n.d.	0.28	0.76
<i>D. pteronyssinus</i>	(2)*	P7	0.34	0.31	0.50	0.35	n.d.
<i>D. pteronyssinus</i>	(2)	S12	0.37	0.34	0.63	0.73	0.73
<i>D. pteronyssinus</i>	(2)	S13	0.44	0.71	n.d.	0.47	0.33
House dust	(1)	P8	—	0.16	n.d.	0.14	0.81
House dust	(1)	S10	0.39	no inh.	n.d.	0.41	0.42
House dust	(1)	S11	0.65	0.39	n.d.	0.46	0.74
House dust	(2)	P6	0.22	0.18	n.d.	n.d.	0.27
House dust	(2)	P7	0.29	no inh.	n.d.	n.d.	0.22
House dust	(2)	S10	0.27	no inh.	n.d.	no inh.	0.36
House dust	(2)	S11	—	no inh.	n.d.	no inh.	0.45

Abbreviations as for Table 1.

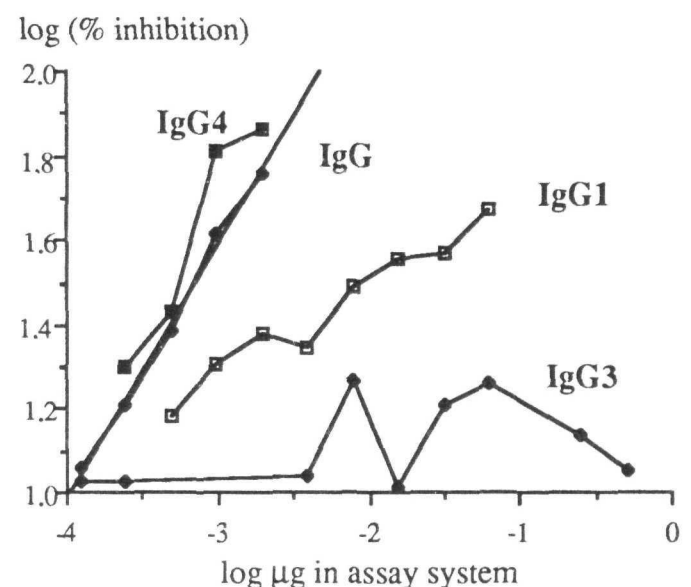
*Protease fraction (*Der p* I + III).Figures for **bold** = high-affinity values.

Fig. 1. Double logarithmic plot of dose-dependent binding of dialyzed allergenic extract of dried and defatted pollen of *Holcus lanatus* to specific pan-IgG and IgG-subclass antibodies in pool of sera from adult patients with atopic dermatitis (P-AD). Equation for linear regression with pan-IgG was $y = 0.60x + 3.39$.

allergens, despite the use of the same serum source. In Fig. 2, the slopes for all regression lines are virtually identical, but the potency for 50% IgG binding was greater for total IgG and the IgG3 subclass than that established for the IgG1 and IgG2 antibodies. In this serum pool, P-AD, antimite IgG4 antibodies were undetectable (Tables 1–4).

Discussion

This work investigated the affinity and distribution of allergen-specific IgG subclass antibodies to

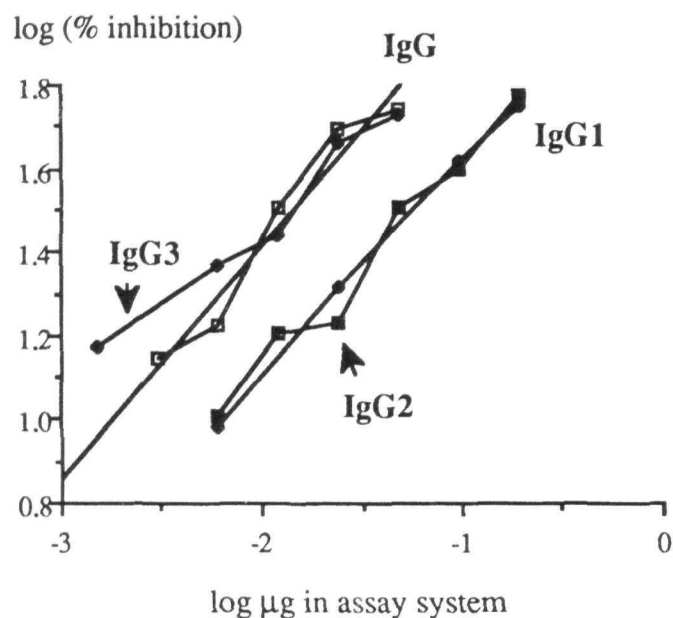


Fig. 2. Double logarithmic plot of dose-dependent binding of dialyzed allergenic extract of whole cultures of *Dermatophagoides pteronyssinus* to specific pan-IgG and IgG-subclass antibodies in pool of sera from adult patients with atopic dermatitis (P-AD). Equation for linear regression with pan-IgG was $y = 0.55x + 2.52$.

different kinds of allergens. Antibody affinity was assessed by establishing two parameters: 1) the point of 50% inhibition; 2) the slopes of the regression lines from double-log plots of the inhibition curves in immunoassay. The first parameter is usually considered to relate to allergenic potency, i.e., to the binding affinity and chemical concentration of the allergen, which, among other factors, depends on the nature and density of the epitopes binding to the IgG antibodies. The second parameter is related to the equilibrium constant for the antigen-antibody interaction and therefore to antibody affinity or avidity. It is conceivable that an accurate estimate of the *in vitro* affinity (50% and k_1) for an isotype may be influenced by antibodies of other isotypes. However, all isotypes present in the blood serum can compete with each other for antigen binding. Despite all this, differences in affinity are indeed being observed with different allergenic extracts.

From our data we conclude that IgG4 is the most pronounced subclass for pollen allergens, but not for *D. pteronyssinus*. In the case of the allergens of *O. europaea* and *P. judaica*, the IgG1 subclass seems to be important as well. These results are in partial agreement with reports on IgG1 and IgG4 subclass antibodies induced by natural exposure to the pollen of the grasses and of *P. judaica*, as well as to dust mites (4, 20, 21). However, a study performed in neonates found that the IgG antibodies for *D. pteronyssinus* were predominantly of the IgG1 isotype (22), which exhibited high binding affinity (16).

The results lend some support to the hypothesis that antiallergen IgG4 may act as a "blocking"

antibody. High-affinity IgG4 antibodies may facilitate the clearing of allergens by forming IgG4-allergen complexes which are incapable of activating complement and may therefore restrain the inflammatory response. Because of the possible relevance of affinity for the presumed blocking activity of IgG (IgG4) antibodies, it may be of interest to monitor IgG antibody affinity during immunotherapy with aeroallergens. van Ree & Aalberse (10) have recently reported that the blocking effect of IgG antibodies depends on their avidity of binding to a synthetic C-terminal peptide of the "major" allergen *Lol p I*. The application of an affinity parameter for the follow-up of immunotherapy has also been advocated for bee-venom immunotherapy by Devey et al. (23), who found high-IgG4- and low-IgG1-affinity antibodies to phospholipase A_2 .

The distinct IgG subclass distribution observed with various types of allergens from sources such as grass pollen, tree pollen, whole-mite culture, and house dust underscores the importance of the chemical nature of the sensitizing allergens in determining the antibody response. Allergen dependency of the IgG subclass pattern was observed not only in individual several serum samples (Tables 1-4), but also in a single pool of sera from patients with atopic dermatitis (Figs. 1 and 2). The immunologic basis for these distinctive subclass profiles may be that each type of allergen is processed by antigen-presenting cells in such a way that the T-helper cells develop a special allergen-driven pattern of cytokines controlling IgG subclass expression as well as antibody affinity (24). The present work suggests that inhibition assays may be valuable to assess the specificity of the IgG subclass antibodies induced by allergens. Besides, this property may help us understand the role of IgG subclass affinity after allergen exposure either by way of the respiratory tract or during immunotherapy.

The data shown in Tables 1-4 clearly demonstrate that the pan-IgG affinities correlate quite well with the main allergen-binding IgG-subclasses, independently of the nature of the allergen. For practical diagnostic purposes and for longitudinal studies in immunotherapy, it may therefore suffice to establish total IgG allergen-binding curves. In this context, it has already been disclosed that the allergen dosages for 50% IgG binding are closely correlated with those for 50% binding of specific IgE antibodies (18).

References

1. Devey ME, Wilson AV, Wheeler AW. The IgG subclasses of antibody to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 1976;6:227-36.
2. Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK,

- Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973;52:472-82.
3. McHugh SM, Lavelle B, Kemeny DM, Patel S, Ewan PW. A placebo-controlled trial of immunotherapy with two extracts of *Dermatophagoides pteronyssinus* in allergic rhinitis, comparing clinical outcome with changes in antigen-specific IgE, IgG, and IgG subclass. *J Allergy Clin Immunol* 1990;86:521-32.
 4. Moss RB, Hsu Y-P, Kwashicki JM, Sullivan MM, Reid MJ. Isotypic and antigenic restriction of the blocking antibody response to ryegrass pollen: correlation of the rye group I antigen-specific IgG1 with clinical response. *J Allergy Clin Immunol* 1987;79:387-98.
 5. Nakagawa T, Kozeki H, Katagiri J, et al. Changes of house dust mite-specific IgE, IgG and IgG subclass antibodies during immunotherapy in patients with perennial rhinitis. *Int Arch Allergy Appl Immunol* 1987;82:95-9.
 6. Pastorello EA, Pravettoni V, Incorvaia C, et al. Clinical and immunological effects of immunotherapy with alum-absorbed grass allergoid in grass-pollen-induced hay fever. *Allergy* 1992;47:281-90.
 7. Aalberse RC. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;130:722-6.
 8. Steward MW. The biological significance of antibody affinity. *Immunol Today* 1981;2:134-40.
 9. Steward MW, Lew AM. The importance of antibody affinity in the performance of immunoassays for antibody. *J Immunol Methods* 1985;78:173-90.
 10. van Ree R, Aalberse RC. Demonstration of carbohydrate specific immunoglobulin G4 antibodies in sera of patients receiving grass pollen immunotherapy. *Int Arch Allergy Immunol* 1995;106:146-8.
 11. Devey ME, Bleasdale K, Lee S, Rath S. Determination of the functional affinity of IgG1 and IgG4 antibodies to tetanus toxoid by isotype-specific solid-phase assays. *J Immunol Methods* 1988;106:119-25.
 12. Persson MAA, Brown SE, Steward MW, et al. IgG subclass associated affinity differences of specific antibodies in humans. *J Immunol* 1988;140:3875-9.
 13. Devey ME, Beckman S, Kemeny DM. The functional affinities of antibodies of different IgG subclasses to dietary antigens in mothers and their babies. *Clin Exp Immunol* 1993;94:117-21.
 14. Barnes RMR, Johnson PM, Blears J, Harvey MM, Finn R. Human serum antibodies reactive with dietary proteins. Antigen specificity. *Int Arch Allergy Appl Immunol* 1988;87:189-93.
 15. Barnes RMR, Johnson PM, Harvey MM, Finn R. Human serum antibodies reactive with dietary proteins. IgG subclass distribution. *Int Arch Allergy Appl Immunol* 1988;87:184-8.
 16. Ruiz RGG, Price JF, Kemeny DM. Specificity of ELISA for IgG subclass antibodies against inhalant antigens in early childhood. *Allergy* 1994;49:719-23.
 17. Berrens L, Bruijnzeel PLB, Fradkin VA, et al. Purification and standardization of house dust allergens based on cutaneous test, RAST-inhibition and complement assay. *Allergy* 1980;35:222-4.
 18. Boluda L, Gallego MT, Glez Romano ML, Cambra O, Berrens L. Evaluation of the potency of allergenic extracts by inhibition of IgG-antibody binding. *Biologicals* 1994;22:171-7.
 19. Okazaki T, Ilea VS, Wypych JI, Reisman RE, Arbesman CE. Detoxified Hymenoptera venoms: preliminary studies of *in vitro* cytotoxicity and antigenicity of *Apis mellifera* "venomoid". *J Allergy Clin Immunol* 1977;53:261-8.
 20. Kemeny DM, Urbanek R, Ewan P, et al. The subclass of IgG antibody in allergic disease. II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals. *Clin Exp Allergy* 1989;19:545-9.
 21. Palumbo S, Di Felice G, Mari A, et al. IgG subclass antibodies against *Parietaria judaica* in normal and allergic subjects. *Allergy* 1994;49:222-9.
 22. Mariani F, Price JR, Kemeny DM. The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of the immune system following natural exposure. *Clin Exp Allergy* 1992;22:29-33.
 23. Devey ME, Lee SR, Richards D, Kemeny DM. Serial studies on the functional affinity and heterogeneity of antibodies of different IgG subclasses to phospholipase A₂ produced in response to bee-venom immunotherapy. *J Allergy Clin Immunol* 1989;84:326-30.
 24. Martinez Alonso C, Coutinho A, Agustin AA. Immunoglobulin G-gene expression. I. The commitment to IgG subclass of secretory cells is determined by the quality of the non-specific stimuli. *Eur J Immunol* 1980;10:698-702.

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