

Isotypic analysis of grass-pollen-specific antibodies in human plasma. III. Relationship to autoantibodies to IgE

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Since it has been shown that autoanti-IgE may be mistaken for antiallergen antibodies, thus appearing as pseudo-allergen-specific antibodies, it is crucial to separate true- from pseudo-allergen-specific antibodies and to determine to what extent autoanti-IgE appeared as pseudo-allergen-specific antibodies. For this purpose, human Ig pools were affinity-purified successively on a grass-pollen column and then on an antihuman-IgE column. IgG1–4, IgA, and IgM antibodies that were eluted from the grass-pollen column separated into pseudo- (~30–40%) and true-allergen-specific antibodies that were coretained and not coretained, respectively, with the IgE on the anti-IgE column. Levels of autoanti-IgE were determined in individual plasma samples by surface plasmon resonance and statistically compared to the concentrations of allergen-specific antibodies obtained previously in the same plasma samples. A positive correlation between IgM autoanti-IgE levels and grass-pollen-“specific” IgM concentrations ($P < 0.0002$), and negative correlations between IgA autoanti-IgE and both IgE anti-grass pollen and IgG2 autoanti-IgE levels ($P < 0.03$, in both cases) were observed for the first time. This supports the contentions that: (1) autoanti-IgE antibodies appeared as pseudo-grass-pollen-specific antibodies, (2) they hid IgE antibodies when the latter were measured, and (3) they compete with one another in binding IgE. Lastly, a model of large Ig complexes is discussed.

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Since their discovery by Williams et al. (1), anti-IgE autoantibodies (autoanti-IgE) of the IgM, IgG, or IgA class have been extensively described. Complexes may form, involving 50–100% of the autoanti-IgE and 1–100% of the total IgE (reviewed by Magnusson & Johansson [2]). The role of autoanti-IgE, however, is still unclear. They might be involved in the mechanisms of allergic diseases, as their levels have been reported to be, firstly, significantly increased in allergic patients compared to nonatopic subjects (3–10), and, secondly, correlated to total serum IgE concentrations (6–8, 11, 12), although this correlation is not invariably observed (13). They might also be a marker for both atopic and intrinsic asthma (7) and for atopic dermatitis (7, 10, 12, 14). Although serum concentrations of both free and complexed IgE have been shown to change during allergen

immunotherapy (7), the role of autoanti-IgE in allergen-induced IgE suppression during immunotherapy remains controversial (15).

In any case, autoanti-IgE in the serum may interfere with the determination of both total and specific IgE by masking part or all of the IgE to be measured (16–18). Alternatively, it has been demonstrated (19) that IgG autoanti-IgE may be mistaken for allergen-specific IgG when they bind to allergen-specific IgE. Therefore, these autoanti-IgE may be considered to be pseudo-allergen-specific antibodies.

Previously, we analyzed the isotype specificity and concentration of anti-grass-pollen antibodies in human plasma samples (20, 21). We demonstrated that not only IgG1 and IgG4 but also IgA2 were potential blocking antibodies due to their apparent specificity for grass-pollen antigens (20).

The techniques we used, however, did not allow us to distinguish between antibodies truly specific for grass-pollen allergens, that is, those binding directly to the allergens, and pseudo-allergen-specific autoanti-IgE. Because only true-allergen-specific antibodies should be considered potential blocking antibodies, it appeared paramount to us to separate true- from pseudo-allergen-specific antibodies and to determine to what extent autoanti-IgE appeared as pseudo-allergen-specific antibodies.

Material and methods

Ig sources

Twenty-six human plasma samples, referred to hereafter as "IgG4+ plasma", were selected on the basis of their having high levels of *Dactylis glomerata* (cocksfoot)-pollen-specific IgG4, as detected by ELISA. They were obtained mainly from desensitized grass-pollen-allergic patients (20, 22). Those plasma samples were pooled, defibrinylated, and filtered down to 0.2 μ m.

IgGAM (BioTransfusion, Villepinte, France) (23) is an Ig solution that had been prepared from a pool of over 10 000 plasma samples by caprylic acid fractionation of the subfractions 1 and 3, according to the procedure of Deutsch et al. (24) and Nitschmann and Kistler (25, 26), as modified by Steinbuch et al. (27). Most of the starting plasma samples were obtained from nonatopic subjects and therefore were equivalent to the previously described "normal plasma", which contained very little anti-*Dactylis*-pollen IgG4 (20). IgGAM contained over 95% Ig, consisting of approximately 65% IgG, 15% IgA, and 20% IgM (23).

Allergam (Gamma TS, France) (28), which was used as control, is an Ig solution with no detectable IgE that was obtained from a pool of over 10 000 plasma samples, mostly of nonatopic donors. It was obtained by caprylic acid fractionation of the subfractions 1, 2, and 3, according to the procedure described above.

Dactylis glomerata (cocksfoot)-pollen and anti-human IgE affinity columns

The grass-pollen column consisted of 25 ml of *Dactylis*-pollen allergosorbent packed in a 25-mm-diameter column (Sepracor, USA), as previously described (21). The anti-IgE column consisted of 5.5 ml of packed CNBr-Sepharose 4B gel (Pharmacia, Uppsala, Sweden) coupled to goat polyclonal antibodies to IgE Fc (Nordic Immunology, Norway) in an 11-mm-diameter column (Sepracor).

Successive chromatography on grass-pollen and anti-IgE affinity columns

A volume of 350 ml of pooled IgG4+ plasma and, in another experiment, 630 ml of IgGAM were passed through the grass-pollen column at 75 ml/h. The gel was then extensively washed with 0.1 M phosphate buffer, pH 7.5, and antibodies were eluted with 0.1 M citric acid/glycine buffer, pH 2.5 (21 g citric acid, 6 g glycine, qsp 1 l deionized water). As previously shown (21), this elution procedure leads to the complete removal of the antibody bound to the allergosorbent. Eluted antibodies were immediately neutralized with solid Tris(hydroxymethyl)-aminomethane (Merck, Germany), concentrated six times, and then dialyzed against 0.1 M phosphate buffer, pH 7.5, for 16 h at 4°C. They were >95% pure (data not shown). Eluted antibodies were then passed through the anti-IgE column at 14 ml/h. The immunosorbent was washed, and retained antibodies were eluted and neutralized as described above.

A control experiment was conducted as follows. A pool made with one of the IgG4+ plasma samples and with a plasma sample containing high levels of antibodies to bacterial lipopolysaccharide (LPS) was first passed through a precolumn packed with CNBr-Sepharose gel coupled to LPS. Antibodies contained in the flow-through were then passed through the grass-pollen column. The amount of antibody eluted from the grass-pollen column was <10% lower than the amount of antibody that was eluted from the same column when the pool of the two plasma samples was directly passed through this column, i.e., not passed first through the precolumn. This small decrease did not affect one Ig class more than another (data not shown).

Size-exclusion chromatography

Fractions (eluates and flow-throughs) of the anti-IgE chromatography underwent size-exclusion liquid chromatography. Before the experiments, each fraction was concentrated on a Centricon 10 filter and then passed through a 0.2- μ m filter (Dynagard, Microgon Inc., USA). A volume of 200 μ l of each concentrated fraction was injected into a Superose 12 column, the FPLC system being used (Pharmacia-LKB, Sweden).

Antibody analysis

IgG, IgA, and IgM concentrations were measured by nephelometry with an ISC II (Beckman, USA). IgE concentrations were determined with the IgE EIA 100 kit (Pharmacia Diagnostics AB, Sweden).

Concentrations of IgG1, IgG2, IgG3, and IgG4 were measured by a previously described "sandwich" ELISA (21), the WHO (67/97) standard being used with IgG subclass concentrations of 5.0 mg/ml IgG1, 2.6 mg/ml IgG2, 0.4 mg/ml IgG3, and 0.5 mg/ml IgG4, as reference (29).

The binding patterns of IgE, IgM class, and IgG subclass antibodies to *Dactylis*-pollen antigens were analyzed in the fractions of both the grass-pollen and the anti-IgE affinity chromatography. This was performed by the immunoprinting technique after isoelectric focusing of an aqueous extract of *D. glomerata* pollen, according to Peltre et al. (30), as described elsewhere (20). Samples were diluted so as to obtain 2–3 µg of Ig per strip.

Surface plasmon resonance assay

Semiquantitative determination of autoanti-IgE of the different isotypes was performed on individual plasma samples by surface plasmon resonance technology with the BIAcore system (Pharmacia Biosensor AB, Sweden) (31). Mouse monoclonal antihuman IgE (HP6061, produced by Hybridoma Reagents Laboratory and kindly provided by Dr R. G. Hamilton, Johns Hopkins University School of Medicine, Baltimore, MD, USA) was immobilized on the sensor chip as follows. The carboxylated dextran matrix was activated with *N*-ethyl-*N'*-(3-diethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide. Next, 30 µl of mouse monoclonal antihuman IgE at 33 µg/ml in 10 mM sodium acetate buffer, pH 5, was injected at a 5-µl/min flow rate. For deactivation of the unreacted groups, 35 µl of ethanolamine was injected. Then, a 2-µl/min flow rate was maintained during the whole experiment. A volume of 100 µl of an individual plasma sample was injected. To detect the different isotypes of autoantibodies that bound to autologous IgE (immobilized on the sensor chip through the anti-human IgE), 4-µl volumes of mouse monoclonal antibodies against human IgG1 Fc (HP6069), IgG2 Fc (HP6002), IgG3 Hinge (HP6047), IgG4 Fc (HP6025) (32), IgA (HP6123B), and IgM (HP6081B) (from Hybridoma Reagents Laboratory; see above) were injected in that sequence. The same sequence was repeated for every IgG4+ plasma sample after the sensor chip was regenerated with 15 µl of 100 mM HCl. Results were expressed in relative reflectance units (RU) for each isotype detected.

Statistics

The Spearman rank correlation coefficient and Mann-Whitney U tests were assessed with StatView II software (Abacus Concept, Berkeley, CA, USA).

Results

Successive chromatography on Dactylis-pollen and antihuman IgE affinity columns

According to the immunoprints and regarding all four IgG subclasses, *Dactylis*-pollen-specific antibodies were detected in the eluates of the grass-pollen column but not or hardly at all in the flow-throughs (Figs. 1 and 2). This indicated a nearly 100% absorptive capacity for the allergosorbent. Note that the Ig class composition of the antibodies isolated on the *Dactylis*-pollen column from the pooled IgG4+ plasma (Table 1) was consistent with the average composition of anti-*Dactylis*-pollen antibodies previously determined in the related individual plasma samples (21).

In order to control for the specificity of the anti-IgE immunosorbent, Allergam, with no detectable IgE or, as well, a sample that was not retained on the immunosorbent itself (flow-through), was injected into the column; no significant Ig was eluted (results not shown). In contrast, the anti-IgE column retained not only most of the IgE (from 70% to nearly 100%) but also a great part (~30–40%) of the other Ig classes and subclasses that eluted from the grass-pollen column when either the IgG4+ plasma pool or the IgGAM was applied to the latter (Tables 1 and 2). Over 50% of non-IgE antibodies that were eluted from the anti-IgE column consisted of IgG (IgG2 being the predominant subclass: >45% of the IgG), the rest being mainly IgM. Antibodies not retained on the anti-IgE column were also mainly of the IgG and IgM classes. The molar ratio of the non-IgE to the IgE antibodies that eluted from this column was calculated from data in Tables 1 and 2. It was 97 : 1 when antibodies were isolated from pooled IgG4+ plasma and 1073 : 1 when antibodies were isolated from IgGAM.

Like antibodies not retained on the anti-IgE column, those that were retained and eluted from this column acted as *Dactylis*-pollen-specific antibodies, as shown on the immunoprints (Figs. 1 and 2).

Size-exclusion chromatography

Whichever anti-IgE chromatography fraction was injected (flow-through or eluate), the chromatographic profile observed was composed essentially of two main peaks, one corresponding to molecular sizes over 300 kDa, and the other corresponding to a molecular size of about 150 kDa (see example, Fig. 3). As expected, IgM antibodies were detected in the high-molecular-size peak, but not in the 150-kDa peak. In contrast, IgG antibodies were detected only in the 150-kDa peak. Due to their

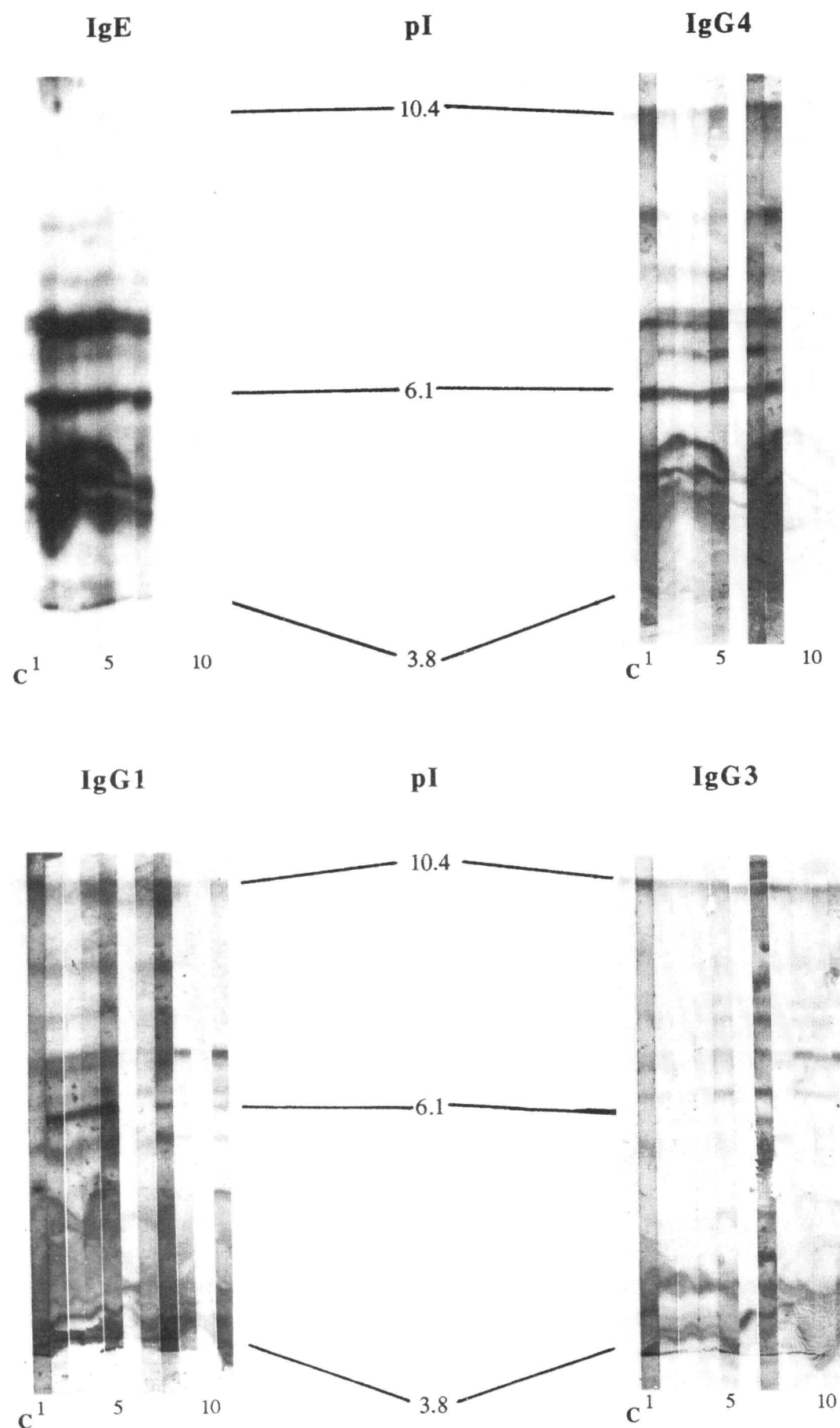


Fig. 1. Specificity analysis of IgE, IgG4, IgG1, and IgG3 in different chromatographic fractions, by technique of immunoprinting after isoelectric focusing of *Dactylis*-pollen aqueous extract. Lane C=controls (lanes incubated with PBS-Tween-BSA). Lane 1 = pooled IgG4+ plasma. Lanes 2 and 5 = antibodies of pooled IgG4+ plasma retained on and eluted from *Dactylis*-pollen column. Lane 3 = same eluate as on lanes 2 and 5 after dialysis against deionized water. Lane 4 = same sample as on lane 3 after heating for 2 h at 56°C. Lane 6 = antibodies of pooled IgG4+ plasma passed through and not retained on *Dactylis*-pollen column (flow-through). Lanes 7 and 8 = respectively, eluate and flow-through from anti-IgE column through which had been passed same sample as on lanes 2 and 5 (antibodies of pooled IgG4+ plasma eluted from *Dactylis*-pollen column). Lane 9 = antibodies of IgGAM (BioTransfusion, Villepinte, France) retained on and eluted from *Dactylis*-pollen column. Lanes 10 and 11 = respectively, eluate and flow-through from anti-IgE column through which had been passed same sample as on lane 9 (antibodies of IgGAM eluted from *Dactylis*-pollen column). Note two major allergens Dac g 1 and Dac g 4 at pI 6.1 and 10.4, respectively.

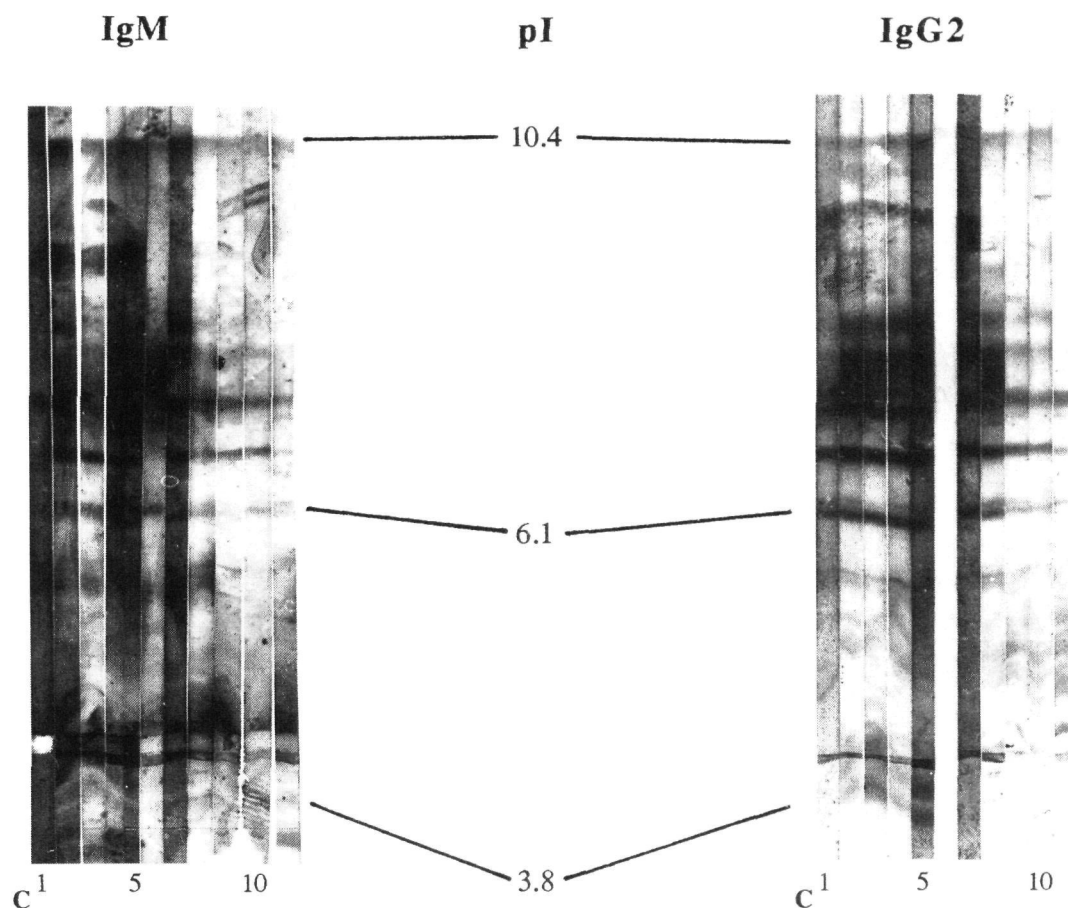


Fig. 2. Specificity analysis of IgM and IgG2 in different chromatographic fractions, by technique of immunoprinting after isoelectric focusing of *Dactylis*-pollen aqueous extract. See Fig. 1 legend.

Table 1. Distribution of Ig isotypes in antibody fractions isolated successively on *Dactylis*-pollen column and then on antihuman IgE column from pool of 26 plasma samples with high levels of IgG4 anti-*Dactylis* pollen, mainly from desensitized grass-pollen-allergic subjects (IgG4+ plasma)

μg of Ig corresponding to 1 ml of plasma pool					
<i>Dactylis</i> -pollen column			Anti-IgE column		
Sample	Eluate		Flow-through	Eluate	
IgG	9300 (74%)	33.14 (62%)	13.81 ^a (65%)	14.60 ^b (67%)	
IgA	1700 (13%)	4.14 (8%)	1.24 (6%)	1.66 (8%)	
IgM	1600 (13%)	15.74 (30%)	6.12 (29%)	5.25 (24%)	
IgE	1.6 (0.01%)	0.33 (0.6%)	1.8×10 ⁻⁴ (0.001%)	0.23 (1%)	

^a 40% IgG1, 31% IgG2, 3% IgG3, 26% IgG4.

^b 28% IgG1, 46% IgG2, 3% IgG3, 24% IgG4.

Table 2. Distribution of Ig isotypes in antibody fractions isolated successively on *Dactylis*-pollen column and then on antihuman IgE column from Ig pool with low levels of IgG4 anti-*Dactylis* pollen, mainly from nonatopic subjects (IgGAM)

μg of Ig corresponding to 1 ml of Ig pool					
<i>Dactylis</i> -pollen column			Anti-IgE column		
Sample	Eluate		Flow-through	Eluate	
IgG	42 800 (65%)	58.07 (46%)	38.11 ^a (44%)	19.96 ^b (51%)	
IgA	10 000 (15%)	14.52 (12%)	13.34 (15%)	1.18 (3%)	
IgM	13 200 (20%)	53.23 (42%)	35.49 (41%)	17.74 (46%)	
IgE	0.02 (0.000%)	0.02 (0.02%)	0.00 (0%)	0.02 (0.05%)	

^a 46% IgG1, 48% IgG2, 0.6% IgG3, 6% IgG4.

^b 17% IgG1, 81% IgG2, 0.1% IgG3, 2% IgG4.

lower concentrations (Tables 1 and 2), IgA antibodies were not detected. As expected, no IgE was detected when flow-throughs of anti-IgE chromatography were studied (results not shown). As regards the eluates, IgE antibodies were detected in both >300-kDa and 150-kDa peaks, being much more concentrated in the latter (Fig. 3).

Semiquantitative determination of autoanti-IgE levels by surface plasmon resonance assay

A signal was observed only when anti-IgG2, anti-IgA, and anti-IgM were injected (Table 3). The fact that monoclonal antibody reagents to IgG1, IgG3,

and IgG4 did not bind the sensor chip cannot be due to steric hindrance, since no reagent was injected before anti-IgG1, and reagents injected after anti-IgG3 and anti-IgG4, that is, anti-IgA and anti-IgM, were still able to bind. It is also important to stress that detected antibodies were not directed against the immobilized monoclonal mouse Ig anti-human IgE, since correlations were observed with *Dactylis*-pollen-specific antibodies (see below), and the mouse Ig antihuman isotype would compete with the mouse Ig antihuman IgE for binding those putative antimouse Ig antibodies.

A very significant positive correlation was observed between plasma IgM autoanti-IgE levels

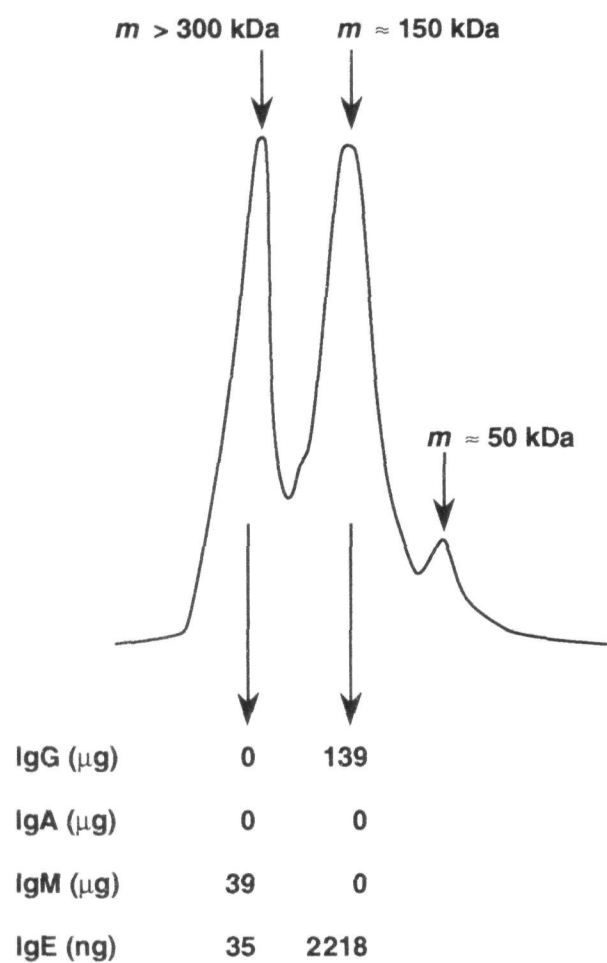


Fig. 3. Size-exclusion chromatographic profile of antibodies of pooled IgG4+ plasma that had been successively retained on and eluted from *Dactylis*-pollen and then anti-IgE columns.

and previously obtained (21) *Dactylis*-pollen-specific IgM concentrations ($r = 0.76$, $P < 0.0002$). Significant negative correlations were observed between plasma IgA autoanti-IgE levels and both plasma IgG2 autoanti-IgE levels and previously obtained (21) *Dactylis*-pollen-specific IgE concentrations ($r = -0.46$ and $P < 0.03$ in both cases).

Discussion

The present study indicates that when Igs were affinity purified successively on a *D. glomerata* (cocksfoot) grass-pollen column and then on an antihuman IgE column, not only IgE but also significant amounts of the other Ig classes and subclasses were successively retained on and eluted from both columns. Importantly, the non-IgE antibodies that were eluted from the anti-IgE column were not specific for the immobilized anti-IgE, as shown by control experiments performed with Igs from which IgE had been depleted. They were not directed against the polysaccharide matrix in those columns either, as adsorption of Igs on the same matrix coupled to a different antigen before passage through the grass-pollen column did not significantly decrease the amount of antibody that eluted from the grass pollen column (see details in Material and methods). Nor could eluted anti-

Table 3. Semiquantitative measurement (in relative reflectance units) of IgG2, IgA, and IgM autoanti-IgE in 26 plasma samples with high levels of *Dactylis*-pollen-specific IgG4 (IgG4+ plasma)

Plasma sample no.	IgG2	IgA	IgM
1	307	176	168
2	189	165	334
3	95	145	230
4	154	175	279
5	114	213	370
6	185	157	179
7	336	130	195
8	55	198	325
9	353	166	231
10	147	148	600
11	219	190	202
12	120	190	262
13	44	94	192
14	234	160	381
15	126	314	371
16	150	167	247
17	231	166	253
18	128	179	720
19	ND ^a	ND	ND
20	225	185	182
21	174	184	5126
22	163	191	263
23	81	152	365
24	133	163	248
25	76	139	424
26	359	132	228

^a Not determined.

bodies have formed immune complexes with allergens either removed from the *Dactylis*-pollen allergosorbent or present in the Ig sources. Indeed, the allergosorbent underwent tens of cycles with no loss of capacity (data not shown). In addition, *Dactylis*-pollen allergens are not large enough ($m < 70 \text{ kDa}$) (33) to bind at the same time one IgE molecule and about 100–1000 non-IgE antibody molecules (see Results). Lastly, all IgG4+ plasma was drawn 3–6 months after the *Dactylis*-pollen season, whereas IgGAM contained almost 100% Ig. Altogether, this implies that the non-IgE antibodies retained on and then eluted from the anti-IgE column were autoanti-IgE antibodies that bound to and coeluted with the *Dactylis*-pollen-specific IgE that had been retained on this column. The fact that autoanti-IgE bound to grass pollen-specific IgE explains why they were mistaken for grass-pollen-specific antibodies, as shown on the immunoprints. Therefore, detected autoanti-IgE were pseudo-allergen-specific antibodies.

Our results are in complete agreement with a previous report by Jensen-Jarolim et al. (19) indicating that successive passages of serum pools of allergic patients through a timothy-grass-pollen column and then on an anti-IgE column led to the recovery of IgG autoanti-IgE antibodies, which

also acted as allergen-specific antibodies. The present study is an extension of those findings, not only to the other Ig classes (IgA, IgM) and to IgG subclasses, but also to Igs from nonatopic subjects.

However, the molar ratios of autoanti-IgE to IgE in samples that were eluted from the anti-IgE column, that is, about 100 : 1 to 1000 : 1, were much higher than those published by Jensen-Jarolim et al. (19). Modelization studies indicated that, at most, 20 Ig Fabs can bind simultaneously to an IgE molecule (personal communication, R. J. Poljak et al., Institut Pasteur, Paris, France). It is possible that part of the IgE in the eluate of the anti-IgE column was hidden by autoanti-IgE. Therefore, its concentration would be underestimated when measured by the technique we used, leading to apparently high anti-IgE : IgE ratios (16, 18). In this respect, it has been demonstrated that a true anti-IgE : IgE ratio of 20 : 1 results in a maximum decrease of 40% of measured IgE concentration (16), thus leading to an apparent ratio of 20 : 0.6, that is, 33 : 1. Since we observed ratios much greater than 33 : 1, it would appear that large Ig complexes form on both grass-pollen and anti-IgE columns. If we extend the idea of Shakib (34), such large Ig complexes would involve not only autoanti-IgE but also autoantibodies to the autoanti-IgE, autoantibodies to these autoanti-(autoanti-IgE), and so forth. This hypothesis is supported by several reports describing IgM anti-IgG, IgM anti-IgA, and IgG anti-IgG autoantibodies in nonatopic subjects or/and allergic patients (19, 34–37). Large amounts of IgG could also be bound by the free sites of IgM autoanti-IgG that bind to IgG autoanti-IgE (Fig. 4).

Size-exclusion experiments were performed to determine whether large Ig complexes are stable or not. Since only IgM and IgE were detected in the multimeric Ig fraction, we assume that IgE in this fraction was complexed with IgM autoanti-IgE. Indeed, to our knowledge, aggregates made up of two or more IgE molecules have never been described. Interestingly, immunoprints indicated that significant amounts of grass-pollen-specific IgE, as well as apparently specific IgM, were not retained by the grass-pollen allergosorbent. Given that this was not due to a lack of absorptive capacity of the allergosorbent (see details in Results), those IgE and IgM were probably involved in IgE : IgM complexes in which the IgM was the autoanti-idiotypic antibody to grass-pollen-specific IgE. This would have prevented the IgE antibody from binding to the allergosorbent. To explain why the IgM and IgE bound on the immunoprints, we must point out that they were in contact with antigen much longer (overnight) than in the chromatography experiments (about 20 min).

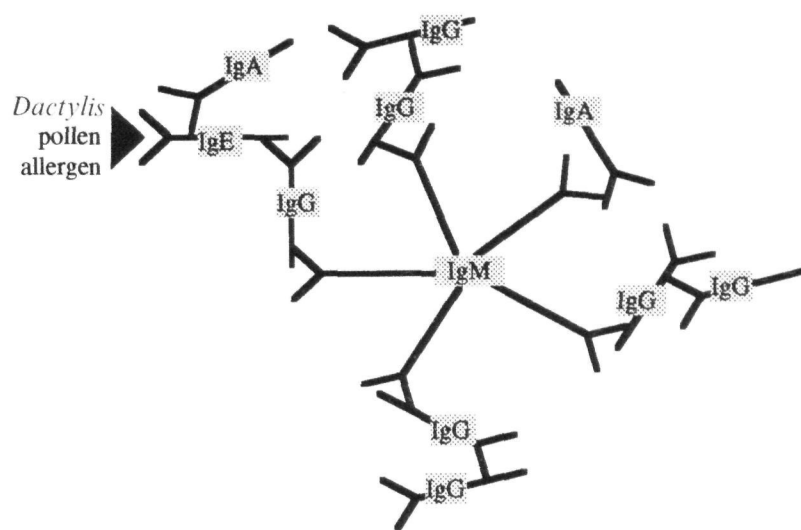


Fig. 4. Proposed simplified model of large Ig complexes forming on grass-pollen column and on anti-IgE column from Ig pools (see text).

Therefore, the only complexes that could be detected consisted of IgE and IgM autoanti-IgE. This indicates that large Ig complexes, involving IgE, IgA, IgM, and the four IgG subclasses, form preferentially when IgE is immobilized, as on grass-pollen and anti-IgE affinity columns. In contrast, when IgE is not immobilized, as in plasma samples and size-exclusion liquid chromatography, large Ig complexes are less likely to form, making it difficult to detect them directly. This is consistent with a recent report indicating that IgE-IgG autoanti-IgE complexes not occurring preformed in the blood circulation arose *de novo* on the anti-IgE-coated solid phase (38). To explain this, we propose that the likelihood that an antibody will encounter the relevant antigen is greater when the antigen is immobilized than when it is not. This is supported by a higher antibody affinity when the antigen is immobilized than when it is not (39). Therefore, large Ig complexes would probably form on basophil and mast-cell membranes which immobilize IgE antibodies through Fc ϵ RI receptors. This can have important clinical implications (40).

Experiments performed on individual plasma samples with surface plasmon resonance allowed us to determine to what extent autoanti-IgE appeared to be pseudo-grass-pollen-specific antibodies. First, those experiments seemed to confirm that IgG2 was the subclass most represented among IgG autoanti-IgE, it being the only one we detected, although the same IgG subclass reagents had been used for analyzing the chromatographic fractions. Note that anti-IgE coupled to the BIAcore sensor chip *immobilizes* the sample IgE, thus favoring the occurrence of large Ig complexes. Therefore, it is likely that IgG1 and IgG4 were not detected because they were not accessible. Indeed, as reported by Shakib (34), IgG4 autoanti-IgE and also, we assume, IgG1 autoanti-IgE can be sand-

wicked between IgE and IgM autoanti-IgG. The IgG4 and IgG1 autoanti-IgE then become inaccessible to antibody reagents, and the IgM autoanti-IgG could be considered to be pseudo-autoanti-IgE. The remaining peripheral binding sites of the bound IgM autoanti-IgG can bind free IgG2 antibodies, which then become very accessible to the IgG2 reagent. Those IgG2 antibodies could also be considered to be pseudo-autoanti-IgE.

The very significant correlation between the levels of IgM that appeared as autoanti-IgE and the concentrations of *Dactylis*-pollen-"specific" IgM suggests that the latter was in fact, at least in part, IgM autoantibody binding directly to *Dactylis*-pollen-specific IgE or indirectly through IgG autoanti-IgE. Indeed, *Dactylis*-pollen-specific antibody concentrations were previously obtained without differentiating between true- and pseudo-allergen-specific antibodies. The negative correlation between IgA autoanti-IgE levels and IgE anti-*Dactylis*-pollen concentrations also suggests a masking effect of specific IgE by IgA autoanti-IgE. Lastly, the negative correlation between IgA and IgG2 autoanti-IgE levels may suggest a masking effect or a competition between IgA and IgG2 for forming the large Ig complexes. To our knowledge, this is the first time that such fine relationships between pseudo-allergen-specific and autoanti-IgE antibodies have been described.

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