

IgG anti-IgE in circulating immune complexes in the hyper-IgE syndrome

R. PAGANELLI*, I. QUINTI, M. CARBONARI, O. PONTESILLI,
G. P. D'OFFIZI, T. LETTA *and* F. AIUTI

Department of Allergy & Clinical Immunology, University 'La Sapienza', Rome, Italy

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Summary

We fractionated, by gel chromatography, sera with high IgE content from atopic subjects and five cases with the hyper-IgE syndrome, and measured the presence of IgE in high molecular weight (HMW) fractions. Two out of four asthmatics and four out of five hyper-IgE had HMW IgE. The same serum fractions gave positive results for conglutinin binding IgG (all six) and IgA (three cases) as well as C1q binding complexes (five cases). IgG auto-antibodies to IgE were also detected together with IgE in HMW fractions. Anti-F(ab)₂ activity was present in five cases (one of them negative for IgG anti-IgE). Our data indicate that complexes made of IgE and IgG anti-IgE are present mainly in patients with chronic allergic symptoms and most frequent in cases of hyper-IgE syndrome.

Introduction

Antibodies directed against the Fc portion of immunoglobulin isotypes gamma, alpha and mu have been detected in rheumatoid arthritis (RA) and other autoimmune conditions [1], patients with IgA deficiency [2], and even in a low proportion of normal subjects, with increasing incidence in ageing. In some diseases, particularly RA, these antiglobulins have been extensively characterized, and their participation in immune complex formation with serum immunoglobulins of the corresponding specificity has been confirmed [3]. However, their role in the pathogenesis of both lesions and symptoms is still controversial, as well as the nature of the immunological aberration causing their production [4].

Naturally occurring auto-antibodies directed against IgE have been described by Williams *et al.* [5] and successively confirmed by Inganas, Johansson & Bennich [6] and other groups [7,8] using different techniques. These antiglobulins have been found mainly in sera from patients with high IgE levels, and it is conceivable that they may arise, as in RA, either as a response to molecular changes of self immunoglobulins, or

Correspondence: Dr R. Paganelli, Department of Allergy & Clinical Immunology, University 'La Sapienza', Viale Università 37, 00185 Rome, Italy.

to antigenic sites of IgE exposed after combination with foreign antigens. An alternative hypothesis for anti-IgE antibodies would be to postulate their immunoregulatory role on IgE function or production [5,8].

Additional problems to be studied are the sub-class distribution of IgG anti-IgE, and their specificity, since other antiglobulins reacting with F(ab)₂ fragments [9] and idiotopes within the antigen-binding variable region (anti-idiotypic antibodies) have been detected [10] and may react positively in tests for anti-IgE antibodies. We studied the presence of such anti-IgE antibodies in atopic patients and patients with the hyper-IgE syndrome, and their participation in IgE-containing immune complexes formation, together with other antibodies and anti-F(ab)₂.

Subjects and Methods

Subjects

We examined sera from eighteen subjects: five of them were healthy laboratory workers used as normal controls; four suffered from allergic rhinitis, caused by grass pollens or house dust mite hypersensitivity; four were asthmatics, with proven sensitivity to common inhalant allergens, and in one case with allergy to cow's milk; five were affected by the hyper-IgE syndrome, as defined by recurrent infections (viral, fungal or staphylococcal), defective chemotaxis of polymorphonuclear leukocytes, cell-mediated immunodeficiency, eczema with or without asthma, polyclonal hyper-immunoglobulinemia E and eosinophilia [11,12]. Blood samples were obtained after an overnight fast, clotted at room temperature and stored in aliquots at -70°C until analysis.

Total and specific IgE

The Phadebas paper radio-immuno-sorbent test (PRIST) and radio-allergo-sorbent test (RAST) methods (Pharmacia diagnostics, Uppsala, Sweden), using commercially available reagents, were used to measure total and specific IgE in sera. A modified PRIST was adopted for testing the IgE content of chromatographic fractions obtained from selected sera, and the international standard 69/204 from the WHO, reconstituted at the concentration of 10 948 IU of IgE/ml. IgE antibodies to isolated food proteins were measured by solid phase RIA.

Antibodies to food proteins

These were detected by solid phase RIA (SP-RIA) as previously described [13]. Briefly, sera or their fractions were incubated in PVC flexible microplates (Dynatech, Cooke Alexandria, VA., U.S.A.) coated with bovine β -lactoglobulin (BLG), purchased from Sigma Chem. Co., St Louis, MO. The binding of the relevant antibody isotype was detected by adding radiolabelled purified anti-human IgG, IgM (Tago, Burlingame, U.S.A.) or IgE (anti-E2 from RAST kit). Results were expressed as net counts/min (c.p.m.) bound, after background subtraction.

Chromatographic separation of sera

Gel filtration on Sephacryl S-200 and S-300 calibrated columns (Pharmacia Fine Chem. Uppsala) was performed by elution with Tris HCl buffer 0.1M, pH=8, containing 0.5M NaCl. Columns were run at 2.5 ml/cm²/hr at room temperature, and fractions analysed immediately or frozen at -70°C .

Circulating immune complexes

They were detected in high molecular weight (MW) fractions by radiolabelled C1q precipitation (C1q binding assay-C1q BA) as described by Zubler *et al.* [14], and by modified conglutinin binding (KgB) test for IgG and IgA as described by Barnet, Carpentier & Lambert [15].

Antibodies to F(ab')₂

These were detected in high MW fractions by solid phase RIA, following a modification of the method described by Davey & Korngold [16]. Antibodies were identified by the specific binding of radiolabelled protein A (PA) and results expressed as ng of PA bound.

IgG anti-IgE

The method was an ELISA, used for detection of IgG anti-IgE in high MW fractions from selected sera, as described by Quinti *et al.* [17]. Briefly, dilutions of fractions were incubated overnight at 37 °C in microtiter plates coated with 10 µg ml⁻¹ of an IgE myeloma (ADZ) and blocked with 0.1% gelatin. Immunoabsorbent purified anti-human IgG conjugated with alkaline phosphatase (Tago) was then added for 1 hr and the antiserum bound revealed by the addition of the enzyme substrate for 30 min. The reaction was read by a Titertek multiscan apparatus and results expressed as optical density (OD) units after blank and background subtraction.

Results*IgE and antibodies to specific allergens*

Total serum IgE was markedly elevated in patients with the hyper-IgE syndrome, with levels ranging from 1700 to 20 000 IU/ml. High amounts of IgE were obviously present in patients with atopic diseases, some with levels exceeding 1000 IU/ml. RAST for common inhalant allergens was positive in cases with asthma and rhinitis. House dust mite-specific IgE were detected in four cases of hyper-IgE tested, and egg white or cow's milk-specific IgE in all five. RAST for cow's milk was also positive in the asthmatic case with cow's milk hypersensitivity.

The RAST results for foods were further analysed at different serum dilutions in patients who had very high IgE levels, and confirmed by inhibition with isolated food proteins (not shown). Most patients with IgE antibodies to foods also had specific IgG. Four out of five hyper-IgE had high levels of IgG anti-BLG, but only one also had elevated specific IgM to the same antigen. The patient with asthma and cow's milk allergy had low amounts of IgG and IgM to BLG, despite the presence of a strongly positive RAST.

Antibodies and immune complexes in serum fractions

The WHO standard 69/204, containing 10 948 IU of IgE/ml, was run on the calibrated separation columns, and fractions were assayed for IgE content to establish the elution position of monomer IgE. We then analysed the fractions obtained by gel chromatography from the sera, with estimated MW above the peak of monomer IgE. The results are summarized in Table 1.

No IgEs were detected in high MW (HMW) fractions from controls, patients with rhinitis, and two of the four asthmatic subjects. The other two cases had a well defined two-peak profile, with IgE in HMW fractions. One of them was the patient with cow's milk hypersensitivity.

Table 1. Summary of the results obtained in patients' and control sera and their chromatographical fractions

	Controls <i>n</i> = 5	Allergic rhinitis <i>n</i> = 4	Asthmatics <i>n</i> = 4	Hyper-IgE <i>n</i> = 5
Number with raised total and specific IgE	0	4	4	5
Number with HMW IgE	0	0	2	4
Number with IgG anti-IgE	0	1	2	4
Number with anti F(ab') ₂	0	1	3	4
Number with IgG or IgA immune complexes	0	0	1	5

Four out of five hyper-IgE cases had HMW IgE (i.e., > 200 Kd). The elution profile of a hyper-IgE serum on Sephacryl S-200 is shown in Fig. 1. Two distinct HMW IgE peaks can be observed, one of about 10^6 daltons, the other extending from 4.2 to 6.5×10^5 daltons (Fig. 2). HMW fractions were also analysed for presence of F(ab')₂ antibodies, and reactivity with conglutinin and C1q in the immune complexes assays. In one case of allergic rhinitis we found antibodies to F(ab')₂ and IgG anti-IgE in fractions slightly ahead of the IgE peak, which was probably monomer. Tests for C1q BA, K_gB-IgG and IgA were all negative in this as well as other cases of rhinitis.

Three out of four patients with asthma had antibodies to F(ab')₂, but only one of these had IgG anti-IgE. This patient (M.G.) was the cow's milk allergic, who also presented HMW IgE and positivity of the K_gB- IgG test in HMW fractions (Table 2). The fourth asthmatic also had HMW IgE, IgG anti-IgE of HMW, but no detectable antibodies to F(ab')₂. Immune complexes were found with K_gB- IgG and IgA methods, but not with C1q BA in this patient, whereas they were all negative in the other asthmatics.

All patients with the hyper-IgE syndrome had complexes in unfractionated sera. K_gB- IgG of > 400 Kd were present in four out of five, IgA in two, and C1q BA in four out of four tested. Antibodies to F(ab')₂ were detected in four out of five patients in HMW fractions, and IgG anti-IgE also in four out of five, but only one case had both in the same fractions (Table 2). Our findings of mutually exclusive antibodies directed against the F(ab')₂ and IgE, since they were present in different fractions, seem to rule out an interference in their respective assays. Direct evidence of this has been reported by one of us [17] by absorbing out anti-F(ab')₂ activity on immunosorbent columns and retesting the fractions for IgG anti-IgE.

Specific IgG to BLG were found in HMW fractions from hyper-IgE, with values increasing together with specific IgE, in fractions of lower MW, closer to that reported for monomer IgE and IgG (Table 3).

Discussion

We described the simultaneous presence of IgE and IgG anti-IgE in HMW fractions of sera from patients with hyper IgE syndrome and some cases of allergic asthma. The occurrence of a distinctive antiglobulin, IgG anti-IgE, in atopic disorders has been previously described by others [5, 6, 8] but its relevance in the hyper-IgE syndrome and its involvement in immune complexes formation has not been established. Our data

suggest that this antiglobulin may be responsible for mixed IgG/IgE complexes which have been described in atopy [18] and particularly in food allergic subjects [19,20]. Free as well as complexed IgG anti-IgE may in fact be present even in absence of antigen or allergen. Moreover, the combination of anti-IgE to IgE-antigen complexes may lead to the formation of larger aggregates containing different antibody classes with several specificities. The large complexes (up to 10^6 daltons in our hyper-IgE sera) may be more easily detected by relatively insensitive methods, such as C1q BA and KgB tests [21].

Nawata and co-workers described the presence of IgG anti-IgE in sera of patients with bronchial asthma [8] and atopic dermatitis [22]. They also performed gel fractionation studies and detected a small portion of complexed IgE together with anti-IgE auto-antibody in highly positive sera. The MW of complexes was around 600 Kd, and very little anti IgE was present in monomeric form. These authors found no

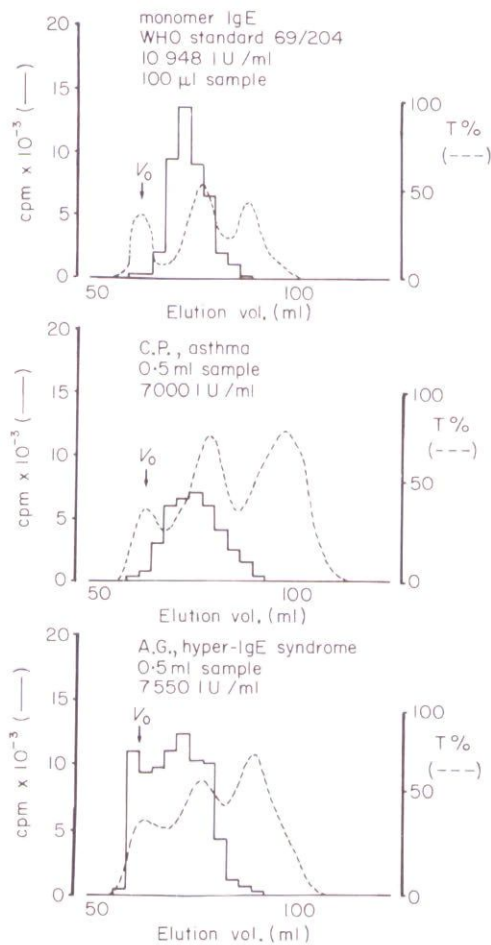


Fig. 1. Elution profiles of the WHO standard (upper panel), serum of an asthmatic patient without complexes (middle panel) and a patient with hyper-IgE syndrome. The IgE content is represented by the histograms as net c.p.m. measured by paper radio-immuno-sorbent test (PRIST). Dotted lines represent % transmitted light as read by Uvicord II unit at 280 nm wavelength. V_0 indicates the exclusion volume and corresponds to MW 250 Kd. Total IgE content of standard and sera are reported.

evidence of IgM anti-IgE, described by Williams *et al.* [5] and Quinti *et al.* [17]. We found high levels of IgG anti-IgE antibody in sera from atopic patients, including allergic rhinitis and asthma, atopic dermatitis and hyper-IgE (Carini C., Quinti I., Paganelli R., unpublished 1986). A small part of IgG anti-IgE activity was due to IgG anti-F(ab)₂. Complexes of IgE with autologous anti-IgE were found in all patients groups; however large MW complexes (> 900 Kd) were not detected in patients with asthma or rhinitis [17]. Swainson and co-workers [23] have described the occurrence of intermediate size IgE complexes in atopic dermatitis (MW about 470 Kd), also containing anti-IgE. We postulate that fractionation methods and variations of assay specificity and sensitivity for anti-IgE are likely to account for the differences in size of complexes detected.

The occurrence of antibodies directed against the F(ab)₂ portion of the immunoglobulin molecules of different classes has been so far described in autoimmune

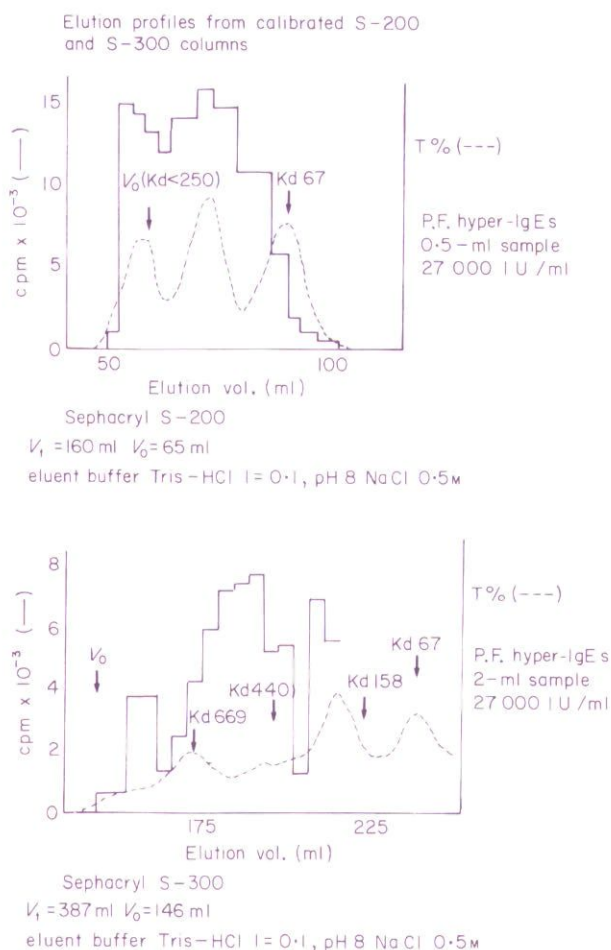


Fig. 2. Heterogeneity of HMW IgE demonstrated by chromatography with Sephacryl S-200 (upper profile) and Sephacryl S-300 (lower profile). Void volume (V_0) represents excluded MW for each gel bed: 250 Kd for S-200 and 10⁶d for S-300. V_t : total bed volume in ml. Resolution at HMW is more detailed for Sephacryl S-300 as shown in the comparison. MW of calibrators indicated are: dextran blue (V_0), thyroglobulin (699 Kd), ferritin (440 Kd), aldolase (158 Kd) and bovine albumin (67 Kd).

Table 2. Results of tests for IgE, auto-antibodies to IgE and F(ab)₂ in serum fractions containing immune complexes detected by C1q and conglutinin (Kg) binding methods. Fraction I contains material of approx 1000 Kd, fraction II of approx 500–300 Kd and fraction III of approx 200 Kd (monomer immunoglobulins)

Patient	Fraction	IgE (net c.p.m.)	IgG anti-IgE (OD)	Anti-F(ab) ₂ (OD)	Kgb IgG (ng PA*)	C1qBA (net c.p.m.)
MG. Asthma	I	11836	0.255	0.24	1.51	0
	II	10042	0.084	0.08	3.15	200
	III	10202	0.457	0.45	0.24	n.d.
FP. Hyper-E	I	15821	0	0.44	3.39	1764
	II	13156	0	0	4.63	1409
	III	16711	0.07	0.39	1.34	0
AG. Hyper-E	I	10297	0.081	0.90	0	1377
	II	11211	0.143	1.46	0.55	2002
	III	11938	0	0.19	0.35	621
DF. Hyper-E	I	2545	0	0.31	0.36	n.d.
	II	5799	0.330	0	0.72	n.d.
	III	5198	0.177	1.83	0	n.d.
IG. Hyper-E	I	4641	0	1.10	0.28	0
	II	8465	0.098	4.69	0.77	1703
	III	10249	0.202	0	0.94	n.d.
AM. Hyper-E	I	2065	0.126	0	0	2222
	II	10301	0.038	0	0.56	0
	III	9880	0	0	0	0

*ng of ¹²⁵I-labelled protein A from *Staphylococcus aureus* bound.

n.d. = Not done.

disorders, relatives of affected patients, and very recently in atopic diseases and at very low levels also in normal subjects [9,17]. Our data and those of Quinti *et al.* (1986) seem to rule out the relative interference of anti-F(ab)₂ and anti-IgE in their respective assays. However both of them might interfere on tests for measurement of total and specific circulating IgE. RAST for foods, which can be inhibited by the addition of antigens, seems not to be blocked by anti-IgE, but they might give false positive results for IgG antibodies in presence of specific IgE.

Several hypotheses on the biological significance of IgG anti-IgE have been discussed, as they do not seem to have a specific pathogenic role [5,6]. Human sera containing anti-IgE cannot release histamine from sensitized mast cells [6], although IgE containing complexes can induce weal reactions *in vivo*, [24] and also arachidonate release *in vitro* [25]. Mixed IgE/IgG complexes are associated with the development of symptoms in food allergic subjects after oral challenge [19] and in patients with asthma and rhinitis [26] and their chronic presence in patients with hyper-IgE may account for impaired chemotaxis [12,27], abnormal antibody responses and possibly defective T-cell function.

Despite the presence of anti-IgE in most atopic conditions, we found their occurrence in HMW fractions, together with IgE, only in patients with the hyper-IgE syndrome and in two with asthma, one of them being food allergic. In particular, none of the patients suffering from allergic rhinitis had HMW IgE and/or IgG anti-IgE in

Table 3. IgE and IgG antibodies to BLG in serum fractions from hyper-IgE sera. Fractions are numbered as in Table 1.

	Fraction	IgE net c.p.m.	IgG net c.p.m.
FP	I	0	1553
	II	150	2559
	III	214	12063
AG	I	0	0
	II	0	0
	III	0	331
DF	I	0	0
	II	560	2396
	II	1464	10009
IG	I	0	0
	II	0	0
	III	0	700
MA	I	0	0
	II	0	2130
	III	0	n.d.

n.d. = Not done.

HMW fractions. The most striking result of our study is the heterogeneity of the IgE-containing complexes found in patients with hyper-IgE. This may reflect the different composition of such complexes, e.g., the presence of anti-IgE, anti-F(ab)₂ and specific antibodies of other isotypes. The assessment of the biological action of this auto-antibody, whether in complexed or free form, is the goal of further investigations.

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