

Natural anti-IgE auto-antibodies interfere with diagnostic IgE determination

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Summary

A simple method is described which enables measurement of anti-IgE antibodies in free form as well as in immune complexes of IgE and anti-IgE. Anti-IgE antibodies were purified from serum of one selected blood donor with highly elevated levels of such auto-antibodies. These purified anti-IgE auto-antibodies inhibited the measurement of myeloma IgE. Purification also revealed that 98% of the subject's serum IgE was masked by anti-IgE auto-antibodies. Our data suggest that IgE determinations in sera containing anti-IgE antibodies might be underestimated.

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Introduction

The existence of auto-antibodies directed against distinct immunoglobulin isotypes is well documented [1–10]. For example, anti-IgG auto-antibodies have been described as rheumatoid factors and have been associated with disease [1–4]. Anti-IgE auto-antibodies were first described in 1972 [5]. Later, such IgE specific auto-antibodies were reported in conjunction with different atopic disorders [8–14] as well as non-atopic diseases with elevated IgE serum levels [15]. However, high levels of anti-IgE antibodies were also encountered in controls [15, 16].

In vitro anti-IgE autoantibodies have been shown to trigger histamine release from human basophils, tissue mast cells and passively sensitized human bone marrow cells [13, 17, 18]. In other reports, anti-IgE antibodies failed to trigger histamine release from blood basophils [5, 18–20]. These findings suggested the existence of different types of anti-IgE antibodies being able to either block or to induce histamine release [18, 21]. Despite the fact that natural anti-IgE antibodies trigger histamine release *in vitro* and might thus also participate in allergic reactions *in vivo*, the pathophysiological role of anti-IgE auto-antibodies still remains unclear. In the present study we investigated why anti-IgE antibodies are not always correlated with elevated IgE serum levels. Furthermore, we investigated whether anti-IgE auto-antibodies are

intact antibodies or only an entity recognizable by immunological techniques.

To undertake the present study, we developed a simple assay for measuring IgG anti-IgE antibodies either occurring as 'free' antibodies (meaning antibodies which are capable of recognizing purified IgE) or in complexes with IgE, and we purified such anti-IgE antibodies from human serum.

Here, we show that natural anti-IgE antibodies indeed occur as intact antibodies. Furthermore, the purified anti-IgE antibodies inhibited IgE determinations. Thus, our results question the use of antibody-based immunological methods for IgE determinations in samples containing anti-IgE antibodies.

Materials and methods

Sera

Serum samples from 329 children and adults (ages between 1 month and 51 years) were collected. Among this group of blood donors 141 subjects had allergic disease.

Antibodies

Human IgE was purified from serum of a myeloma subject (PS IgE myeloma; a kind gift from Drs K. and T. Ishizaka, Baltimore, U.S.A.). Purification was carried out by HPLC gel-filtration using a 7.5 × 600 mm TSK-G-3000 SW column (LKB, Bromma, Sweden) and a sample load of 1 ml.

Two different types of murine monoclonal IgG₁ antibodies against human IgE were used, both of which were produced in our laboratory [22]. The anti-IgE antibody BSW17 recognizes the D_{e1} region of IgE, while the anti-IgE antibody Le27 binds to the D_{e2} region. These antibodies were purified from murine ascites by protein A affinity chromatography [23]. The monoclonal anti-human immunoglobulin antibody BS17 (a kind gift of Biotest AG, Frankfurt, FRG) recognizes all human IgG subclasses and to a lesser degree human IgA₂. However, no binding to human IgE was observed. This antibody was also purified by protein A chromatography [23].

Human myeloma-derived proteins of IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁ and IgA₂ isotypes were a kind gift from Dr F. Skvaril, Institute for Cancer Research, Bern, Switzerland. Monoclonal murine antibodies to human IgG₁, IgG₂, IgG₃, IgG₄, IgM and IgA respectively, labelled with horseradish peroxidase, were purchased from Nordic Immunological Laboratory (Tilburg, The Netherlands).

Dot immunobinding assay for detection of 'free' anti-IgE antibodies and IgE/anti-IgE complexes

Natural anti-IgE antibodies capable of directly recognizing purified solid-phase IgE were termed 'free' anti-IgE antibodies. In order to assess these 'free' anti-IgE antibodies in serum samples, we dotted 1 µl volumes of purified human IgE (PS; 50 µg/ml) onto nitrocellulose (Millipore, Bedford, U.S.A., 0.45 mm, HA 304 FO). IgE/anti-IgE complexes were measured in human serum by dotting the murine monoclonal anti-human IgE antibodies BSW17 or Le27 respectively, onto nitrocellulose (500 µg/ml). The nitrocellulose strips were then incubated in a solution of casein in Tris-buffered saline (TBS-casein, 1.5 mg/ml casein, 50 mM Tris-HCl in 200 mM NaCl, pH 7.4) in order to prevent unspecific protein adsorption. Subsequently, dotted nitrocellulose strips were incubated with diluted serum (dilutions 1/2 and 1/4 in TBS-casein) at room temperature on a shaker (Köttermann 4010, Schöenengrund, Switzerland) at 50 r.p.m. overnight. After three washings with TBS, bound human anti-IgE antibodies of IgG isotype were detected with a peroxidase-conjugated monoclonal anti-IgG antibody (BS17, diluted in TBS-casein). The strips were washed three times with TBS and developed with a 16.6% solution of 3 mg/ml 4-chloro-1-naphthol (Merck, Darmstadt, FRG) in methanol, and 0.042% hydrogen peroxide (stock solution 30% in water) in TBS for 30 min. After washing with distilled water, the strips were dried and the colour intensity of the dots measured using a hand-held reflection densitometer (Densitometer, Gretag, Regensdorf, Switzerland) at 600 nm to obtain relative densitometric units.

IgE determination in the presence of anti-IgE antibodies

IgE determination was carried out as described before [24]. In brief, the monoclonal anti-human IgE antibodies Le27 were diluted to 20 µg/ml in 0.05 M NaHCO₃/Na₂CO₃ buffer, pH 9.6, and added as 100 µl volumes to 96-well soft PVC microtitre plates (Dynatech, Plochingen, FRG). The plates were then kept in a humid atmosphere at 4°C overnight. Supernatants were discarded and the coated wells were filled with 200 µl of TBS-casein (as described above) for 2 hr in order to prevent unspecific protein adsorption. After three washings with 0.05% Tween-20 in phosphate-buffered saline (PBS) pH 7.4 (PBS-T), 100 µl of IgE standard (Pharmacia) or samples containing a mixture of standard IgE and purified natural anti-IgE antibodies in TBS-casein were added in triplicate. After incubation in a humid atmosphere at 4°C overnight, the plates were washed three times with PBS-T. Then, 50 µl of the ¹²⁵I-labelled monoclonal anti-IgE antibody BSW17 in 50% PBS-T/50% TBS-casein were added to each well. Afterwards, plates were incubated in a humid atmosphere at room temperature overnight, washed with PBS-T and cut into single wells. Subsequently single wells were counted in a gamma-counter (Gammamatic, Kontron Instruments AG, Zürich, Switzerland).

Purification of natural IgG anti-IgE antibodies

Anti-IgE antibodies were purified from serum of a drug-allergic patient (M.D) with 'normal' IgE levels (93 U/ml) and high levels of anti-IgE antibodies. In brief, purified human IgE (PS; 40 mg IgE/5 g carrier) was immobilized to a glutaraldehyde-activated silicate carrier matrix (Boehringer Mannheim, FRG) as described by the supplier. Then, by adding 2.66 ml of 1 N HCl to 20 ml of serum, pH of the serum was lowered to 3.0. After 5 min, the pH was raised again by adding 2.66 ml of 1 N NaOH. The serum was immediately diluted 1/2 with 0.9% NaCl. The diluted serum was loaded on the IgE affinity column (7.5 × 50 mm) with a constant flow rate of 8 ml/hr. The column was washed with 3% NaCl until absorbance at 280 nm was below 0.005 and then eluted with 3 M NaSCN. Fractions were collected (1 ml) at a constant flow rate of 20 ml/hr. Peak fractions were pooled and immediately dialysed against PBS pH 7.4. All isolation procedures were carried out at 4°C. IgG anti-IgE activity of the pooled fractions was assessed by the dot immunobinding assay.

Material, which passed the IgE affinity column without binding, was loaded on an affinity column based on a monoclonal antibody against IgE (40 mg BSW17/1 g carrier) at a constant flow rate of 10 ml/hr, in order to isolate IgE/anti-IgE immune complexes via IgE. This column was washed with 0.9% NaCl instead of 3% NaCl

until $A_{280} < 0.005$ and eluted with 3 M NaSCN. In order to isolate IgE/anti-IgE complexes which did not bind to the BSW17 anti-IgE column the effluent from the BSW17 column was additionally passed over another anti-IgE affinity column (35 mg Le27/ g carrier). This antibody recognizes the D_{e2} region instead of the D_{e1} region (BSW17). This anti-IgE column was also eluted as described above.

Further purification of IgG anti-IgE antibody was achieved by protein A chromatography. In brief, eluted material from the affinity columns containing anti-IgE activity was loaded on 2.5 ml of protein A agarose (7.5 × 50 mm column) (Affi-gel protein A MAPS II kit; Bio-Rad, Richmond, U.S.A.). Chromatography was performed as described by the supplier. As elution was carried out at pH 3.0, 500 µl fractions were eluted into tubes containing 150 µl 1 M Tris-HCl pH 9.5, in order to raise the pH immediately. Fractions were pooled according to IgE concentration, as measured in the dot immunobinding assay and then dialysed against 10 mM phosphate buffer pH 7.4.

Protein determination

Proteins were concentrated by ultrafiltration on a membrane of 10 000 molecular weight cut-off (Centricon-10 microconcentrator, Amicon, Danvers, U.S.A.). Centrifugation was carried out at 4200 × *g* using a Kontron A 8.24 fixed angle rotor (34°) (Kontron Instruments AG, Zürich, Switzerland). Protein concentrations were determined using a dye reagent from Bio-Rad, containing an acidic solution of Coomassie brilliant blue G-250 [25]. Purified human IgG (Nordic Immunological Lab., Tilburg, The Netherlands) was used for protein standard.

Gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and staining procedures were performed with the PhastSystem (Pharmacia, Uppsala, Sweden), consisting of a separation chamber, a development chamber and a programmable control unit with a microprocessor. Precast gradient gels were used with continuous 10–15% gradient gel zones with 2% crosslinking together with buffer strips of 0.20 M tricine, 0.20 M Tris and 0.55% SDS, pH 7.5 (Pharmacia). Samples containing 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, 2.5% SDS, 0.01% bromophenol blue and 5% β-mercaptoethanol, in case of reducing conditions, were heated to 95°C for 5 min, and applied to the gels in 4 µl volumes. The gel was run at 15°C under the following electric field conditions: 250 V, 10.0 mA, 3.0 W. After 60 accumulated volt-hours the run was stopped.

Coomassie staining was carried out with Coomassie brilliant blue R-250 (Fluka AG Buchs, Switzerland) as described in the PhastSystem manual. Silver staining was performed with a kit supplied by Bio-Rad, which was modified for application to the PhastSystem. In brief, the gel was fixed in 50% ethanol, 10% acetic acid in twice-distilled water for 2 min at 50°C, followed by two fixations in 10% ethanol, 5% acetic acid in twice-distilled water for 2 and 4 min respectively, at 50°C. Staining steps were carried out as described by the supplier of the staining kit. After development, the gel was exposed to 5% acetic acid in twice-distilled water for 2 min at 50°C, followed by treatment with 10% acetic acid, 5% glycerol (Biofinex, Praroman, Switzerland) in twice-distilled water for 3 min at 50°C.

Results

In order to find a suitable source of anti-IgE antibodies for purification, we first screened 329 randomly collected sera. 'Free' and complexed anti-IgE antibodies were measured with a dot immunobinding assay, which was developed for this purpose. Purified myeloma IgE and two different types of monoclonal anti-IgE antibodies were adsorbed onto nitrocellulose. Natural anti-IgE antibodies, which were able to bind the adsorbed solid phase IgE, were termed 'free' anti-IgE antibodies, whereas IgE/anti-IgE complexes were recognized by the solid-phase anti-IgE. Bound 'free' or complexed anti-IgE antibodies of different IgG isotypes were revealed with labelled monoclonal anti-IgG antibodies. Figure 1 shows that for most sera, more IgE-IgG immune complexes were detected than 'free' IgG anti-IgE antibodies ($r = 0.52$; $P < 0.001$). Using our assay we determined two types of immune complexes, namely by using either the monoclonal antibody Le27 or BSW17 on the solid phase. Figure 2a shows IgE-IgG immune complexes measured in 329 randomly collected sera in order to select a serum with high levels of IgG anti-IgE antibodies. This figure shows that the serum from subject M.D. contained very high levels of complexed anti-IgE antibodies of IgG isotype. Serum M.D., therefore, was chosen for the purification of IgG anti-IgE antibodies. Furthermore, this figure shows a high correlation for IgE-IgG complexes measured with either the monoclonal antibody Le27 or BSW17 on the solid phase ($r = 0.95$; $P < 0.001$). If serum M.D. was left out it becomes clear that in some subjects' sera fewer complexes were detected via the monoclonal antibody BSW17 then via Le27 ($r = 0.41$; $P < 0.001$); otherwise there seemed to be a good recognition by both types of monoclonal anti-IgE antibodies ($r = 0.96$; $P < 0.001$) (Fig. 2b).

A positive correlation between serum IgE and anti-IgE

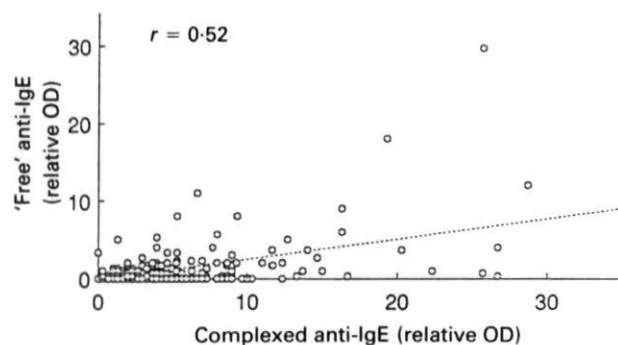


Fig. 1. 'Free' IgG anti-IgE antibodies and IgE-IgG immune complexes. 'Free' and complexed IgG anti-IgE antibodies were measured in 328 randomly collected sera. A dot immunobinding assay was used where either myeloma IgE or murine monoclonal anti-human IgE antibodies (Le27) were bound to the solid phase. Solid-phase antibodies were incubated with the serum samples. Bound 'free' IgG anti-IgE or IgE-IgG immune complexes were revealed with monoclonal anti-human IgG labelled with horseradish peroxidase.

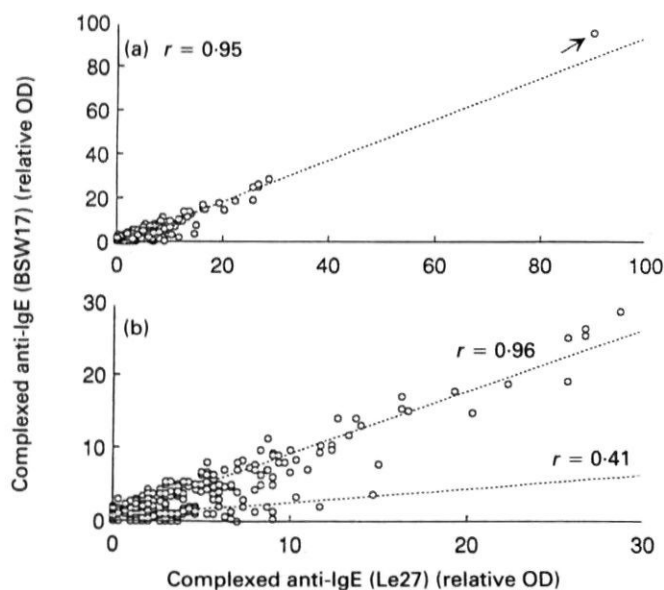


Fig. 2. IgE-anti-IgE immune complexes in human sera. Human sera ($n=329$) were screened for IgE-IgG complexes. Such complexes were measured as described in the legend to Fig. 1. (a) Results of 329 sera including the serum from subject M.D. (↑). (b) The same results excluding serum M.D. Each point represents the mean of triplicate measurements with both anti-IgE antibodies.

antibodies has been reported previously [5,6,8,12,14]. Interestingly, serum M.D. seemed to contain large amounts of anti-IgE antibodies, but only normal amounts of IgE (93 U/ml). We therefore selected 141 subjects with documented allergic disease and investi-

Table 1. IgE determination in serum M.D.*

	IgE (U/ml)					
	Serum dilution					
	1/2	1/5	1/10	1/20	1/50	1/100
PRIST†	4857	588	197	93	N.D.	48
Dot assay‡						
Le27	4900	2000	1000	n.d.	200	100
BSW17	4400	2000	1000	n.d.	200	100

* Units of IgE measured in serum M.D. corrected for the dilution factor.

† Commercial Phadebas IgE PRIST.

‡ Dot immunobinding assay, with either the murine monoclonal anti-human IgE antibody Le 27 or BSW17 dotted onto nitrocellulose as catching antibody and the labelled BSW17 or Le27 antibody as revealing antibodies.

gated the relationship between serum IgE levels and IgE-IgG immune complexes. IgE antibody levels were determined with the commercial Phadebas PRIST, while IgE-anti-IgE immune complexes were determined as described before. Figure 3 shows that in contrast with previous reports, we did not find a correlation between IgE levels and IgG-IgE complexes ($r = -0.01$; $P > 0.05$). Serum M.D. is marked with an arrow for illustration of the high levels of anti-IgE antibodies and the low levels of IgE detected in this serum.

This prompted us to investigate, whether the observed 'normal' IgE levels in serum M.D. might be due to an artefact caused by anti-IgE antibodies. For this purpose, we determined IgE levels of serum M.D. using three different antibody-based immunoassays. In the first assay, IgE was determined with the commercial Phadebas IgE PRIST. Secondly, IgE was measured in a dot immunobinding assay, where the murine monoclonal anti-human-IgE antibody Le27 was bound to the solid phase as capture antibody. Bound IgE antibodies were subsequently revealed with labelled BSW17 monoclonal anti-IgE antibody. In the third assay BSW 17 antibodies were bound to the solid phase and labelled Le27 antibody was used for detection. Table 1 shows that for all three antibody-based assays, very different IgE values (corrected for dilution) were obtained depending on the serum dilution at which IgE determination was carried out. Maximal IgE levels recovered were as high as 4900 U/ml. Additionally, the IgE determination also differed between the assays, suggesting that anti-IgE antibodies indeed interfered with the determination of IgE.

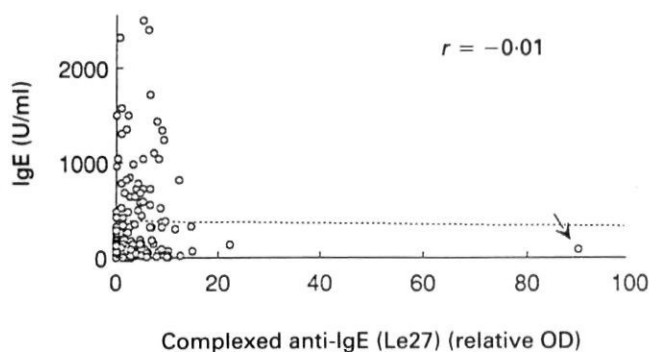


Fig. 3. Correlation between total serum IgE and IgE-anti-IgE complexes ($n = 141$). IgE-anti-IgE complexes were measured as described in the legend to Fig. 1 using the monoclonal anti-IgE antibody Le27 on the solid phase. IgE levels were determined with the commercial Phadebas IgE PRIST (Pharmacia). Data from subject M.D. are indicated ($\uparrow$). Each point represents the mean of triplicate measurements.

Purification of anti-IgE antibodies from serum MD

Anti-IgE antibodies were purified from serum M.D. by three procedures. In order to isolate 'free' anti-IgE antibodies, serum was loaded on an IgE affinity column. Eluates containing anti-IgE antibodies were combined in pool I. Subsequently, IgE-anti-IgE complexes present in the effluent of the IgE column were purified by anti-IgE affinity chromatography. A first anti-IgE affinity column was prepared by coupling the monoclonal anti-IgE antibody BSW17 to a matrix. Eluted immune complexes were collected in pool II. The effluent of this column was loaded on another anti-IgE affinity column with the monoclonal anti-IgE antibody Le27 coupled to the column matrix. Eluted IgE-Anti-IgE complexes were combined in pool III.

Figure 4 shows that all three column eluates analysed by SDS-PAGE contained both IgG and IgE immunoglobulin heavy chains. This result shows that anti-IgE antibodies present in serum M.D. seemed to be intact immunoglobulins. Based on the staining intensity of the protein bands, it can also be assumed that isolated anti-IgE antibodies were in excess of IgE. Furthermore, it also seemed that IgE levels were high, assuming that the modified IgE determination gave truly high IgE levels.

In order to purify the isolated anti-IgE antibodies further protein A chromatography was performed. Purified IgG anti-IgE antibodies were concentrated and subsequently analysed on a SDS-gel under non-reducing conditions. Figure 5 shows that IgG anti-IgE antibodies were successfully separated from contaminating IgE.

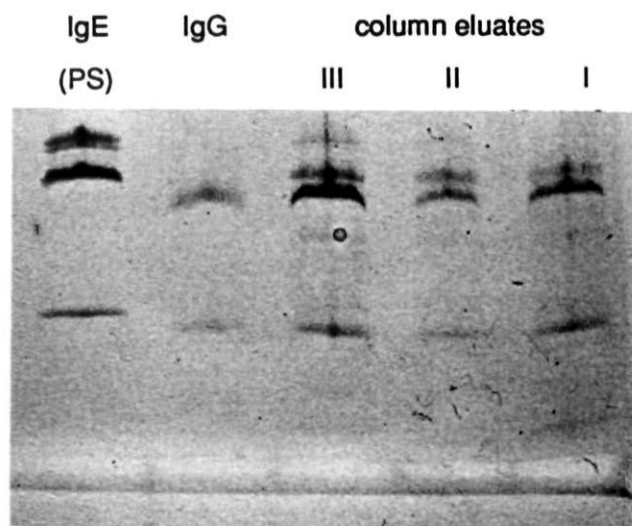


Fig. 4. SDS-PAGE of isolated IgE and anti-IgE antibodies. 'Free' anti-IgE antibodies and IgE-anti-IgE immune complexes were isolated from serum M.D. by affinity chromatography on an IgE column and two different anti-IgE columns. Eluted material was applied onto a SDS-polyacrylamide gel containing a continuous 10–15% gradient. The gel was run under reducing conditions and stained with Coomassie brilliant blue. Eluate I was derived from the IgE affinity column, whereas eluates II and III belonged to the BSW17 and Le27 anti-IgE affinity columns respectively.

IgE and anti-IgE serum levels

As shown in Table 1, IgE levels of serum M.D. determined by routine diagnostic procedures yielded 93 U/ml. However, about 4900 U/ml were determined in the same serum using the same assay or monoclonal anti-IgE antibodies, if the serum was diluted only 1/2 for IgE determination (Table 1).

Anti-IgE antibodies purified from this serum were recovered in a concentration of 56.9 $\mu\text{g/ml}$ serum (Table 2). This would mean a 6.2-fold molar excess of anti-IgE antibodies to IgE, if IgE levels were assumed to be 4857 U/ml (Table 2). Otherwise, if the 'true' IgE concentration would only be 93 U/ml serum, as determined in more dilute serum by standard procedures (Table 1), then anti-IgE antibodies would be in 325-fold excess. However, the 6.2-fold excess seemed more likely, as a similar ratio was estimated based on the IgE and IgG protein bands of the SDS-PAGE (Fig. 4).

The yield of 'free' anti-IgE antibodies obtained from IgE affinity chromatography was 4.1 $\mu\text{g/ml}$ serum (Table 2), suggesting that 7.2% of IgG anti-IgE antibodies were recovered in the form of 'free' anti-IgE, whereas 92.8% were isolated in the form of IgE-IgG immune complexes. These data confirm our results shown in Fig. 1, also

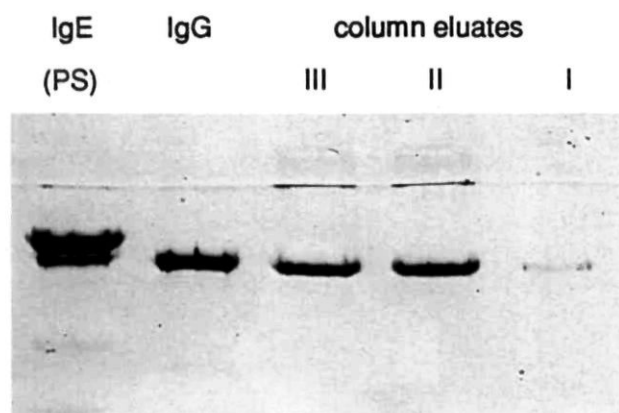


Fig. 5. SDS-PAGE of purified anti-IgE antibodies of IgG isotype. The anti-IgE containing samples as analysed in Fig. 3, were purified further by protein A chromatography and visualized on a SDS-polyacrylamide gel, which contained a continuous 10–15% gradient. The gel was run under non-reducing conditions and silver stained. Eluate I was purified by IgE and protein A affinity chromatography, while eluates II and III were purified by BSW17 and Le27 anti-IgE affinity chromatography, followed by protein A chromatography.

Table 2. Yield and ratio of IgG anti-IgE antibodies

Sample*	Total anti-IgE† (μg)	Anti-IgE (μg/ml)	Anti-IgE-IgE (molar ratio)
'Free' anti-IgE	77	4.1	0.45
Anti-IgE complexes (BSW17)	203	10.7	1.17
Anti-IgE complexes (Le27)	801	42.1	4.61
Total	1081	56.9	6.23

* Anti-IgE antibodies, isolated by IgE and protein A affinity chromatography, termed 'free' anti-IgE antibodies. Anti-IgE antibodies, isolated by anti-IgE affinity chromatography using the monoclonal anti-IgE antibodies BSW17 or Le27 respectively, and further purified by protein A chromatography, termed anti-IgE complexes.

† Total volume of serum, used for the isolation of anti-IgE antibodies was 19 ml. Serum contained about 4857 U/ml IgE (see Table 1).

reporting that in serum M.D. more complexed anti-IgE antibodies were measured than 'free' anti-IgE antibodies. Furthermore, these results suggested that anti-IgE antibodies bind to IgE in large complexes (5/1) and only few, eventually low affinity antibodies remain free.

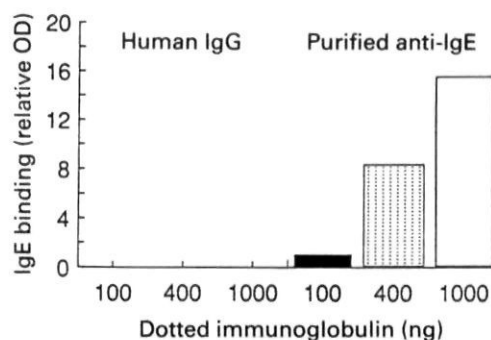


Fig. 6. IgE binding to purified anti-IgE antibodies. Purified IgG anti-IgE antibodies were dotted at maximal available concentration onto nitrocellulose and incubated with human myeloma IgE, labelled with horseradish peroxidase. Anti-IgE antibodies isolated as 'free' anti-IgE antibodies (100 μg/ml) are shown with the black bar. Anti-IgE antibodies isolated from IgE-anti-IgE complexes by affinity chromatography on a BSW17 or Le27 anti-IgE affinity column are shown with a dotted (400 μg/ml) and open (1000 μg/ml) bar respectively. Each bar represents the mean of triplicate measurements.

In order to study whether the purified IgG anti-IgE antibodies were still capable of binding IgE even after purification and dissociation from IgE, we dotted the maximally available concentrations of purified anti-IgE antibodies onto nitrocellulose. Subsequently, IgE binding capacity was revealed with human myeloma IgE (PS), labelled with peroxidase. Figure 6 shows that anti-IgE antibodies purified from both 'free' anti-IgE and from IgE-anti-IgE complexes were capable of binding IgE. Thus, intact anti-IgE antibodies were isolated by affinity chromatography, which preserved their ability to bind IgE.

In order to characterize the anti-IgE antibodies of serum M.D. in respect of their isotypes, isolated anti-IgE antibodies were bound to solid phase. Subsequently, isotypes were determined by adding labelled isotype-specific antibodies. Table 3 shows the existence of anti-IgE antibodies of IgG₁, IgG₂, IgG₄ and IgM isotypes after the first affinity chromatography step. No anti-IgE antibodies of IgG₃ and IgA isotype were observed. Furthermore, our results show that 17.2% of the isolated anti-IgE antibodies were of IgG₄ isotype, whereas 29.9% were of IgM isotype (Table 3). These results show that IgG₄ and IgM isotypes were over-represented among anti-IgE antibodies, as normal amounts of IgG₄ and IgM antibodies correspond to 2.7% and 8.1%, respectively [26]. Furthermore, Table 3 shows an enrichment of IgG₂ isotype after the second step of anti-IgE purification. This enrichment of IgG₂ relative to the other immunoglobulin isotypes is typical for the affinity of protein A.

Table 3. Isotypes of natural anti-IgE antibodies

	$\mu\text{g/ml}$ serum (%)*					
	IgG ₁	IgG ₂	IgG ₃	IgG ₄	IgM	IgA
Before protein A chromatography†						
'Free' anti-IgE	2.5 (30.5)	1.3 (16.3)	<0.1 (<1)	1.7 (20.8)	2.6 (32.4)	0 (0)
Anti-IgE from complexes (BSW17)	6.5 (33.3)	4.0 (20.2)	0.2 (1)	2.2 (11.1)	6.9 (35.4)	0 (0)
Anti-IgE from complexes (Le27)	24.1 (33.4)	18.0 (25.1)	<0.5 (<1)	14.1 (19.6)	15.8 (21.9)	0 (0)
After protein A chromatography						
Anti-IgE from complexes (BSW17)	3.8 (39.9)	4.1 (43.2)	0.0 (0)	1.6 (16.9)	0 (0)	0 (0)
Anti-IgE from complexes (Le27)	12.2 (31.3)	18.6 (47.8)	<0.5 (<2)	8.1 (20.9)	0 (0)	0 (0)

* Percentage distribution of immunoglobulin isotypes in three isolated anti-IgE antibody pools.

† 'Free' anti-IgE antibodies isolated by IgE affinity chromatography and IgE-anti-IgE complexes isolated by anti-IgE affinity chromatography using the monoclonal anti-IgE antibodies BSW17 or Le27.

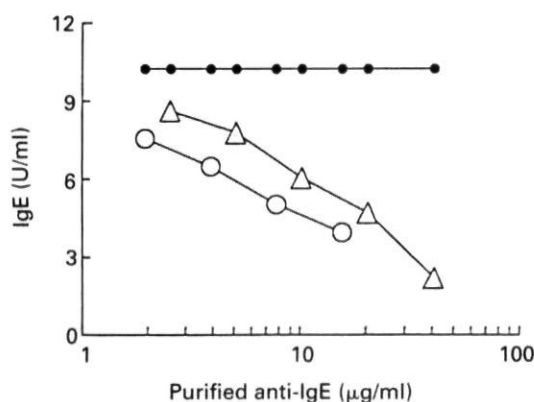


Fig. 7. Purified anti-IgE antibodies interfere with IgE measurement. Ten U/ml of IgE were measured in the presence of various concentrations of purified anti-IgE. (●) Control, where IgE was measured without addition of anti-IgE; (○, △) IgE measurement in the presence of anti-IgE, purified by BSW17 or Le27 anti-IgE affinity chromatography, followed by purification on protein A.

In order to test whether the purified anti-IgE antibodies were capable of inhibiting IgE measurement, 10 U IgE/ml were assayed in the presence of various concentrations of purified anti-IgE antibodies. IgE was determined in a radioimmunoassay, where the monoclonal anti-IgE antibody Le27 was bound to the solid phase and the ^{125}I -labelled monoclonal anti-IgE antibody BSW17 used for IgE detection. Figure 7 shows that purified anti-IgE antibodies inhibited IgE detection in a dose-dependent way. A 40-fold excess of purified anti-IgE antibodies to IgE inhibited IgE determination by 37% using anti-IgE purified by BSW17 affinity chromatography. Furthermore, we also investigated whether inhibition of an assay system

using a polyclonal antiserum, for example, the commercial Phadebas IgE PRIST, would occur. We obtained a similar inhibition of IgE determination to that shown in Fig. 7 (data not shown). Thus, purified human auto-antibodies actually interfere with diagnostic IgE determinations, questioning the use of immunological methods for precise IgE measurement.

Discussion

The existence of anti-IgE auto-antibodies in human serum has been reported previously [5–18]. In the present study, anti-IgE antibodies were isolated by their capacity to bind human IgE or in case of immune complexes, isolation was performed via murine monoclonal anti-human IgE antibodies. In a selected serum from an allergic subject with very high anti-IgE serum levels, over 90% of the purified anti-IgE was derived from immune complexes, demonstrating that a great proportion of anti-IgE was binding to IgE. This observation is in accordance with the literature, where much higher amounts of IgE-anti-IgE immune complexes than free anti-IgE antibodies have been described [8,14]. Furthermore, anti-IgE antibodies were reported to correlate with IgE serum levels [5,6,8,12,14]. In the present study by measuring IgE levels as well as anti-IgE or their immune complexes no correlation was observed. We therefore investigated whether the missing correlation might be due to an artefact of the IgE determination assay, whereby IgE in immune complexes might not be accessible for the xenogenic anti-IgE revealing antibodies. Here we showed that IgE determination could be inhibited by the purified anti-IgE auto-antibodies. At present we have to speculate why higher IgE levels were detected in relatively undiluted

serum. It is possible that IgE might still be recognized in large immune complexes, while a subsequent dilution of serum may affect the molar composition of the immune complexes leading to smaller IgE-anti-IgE complexes with a lower accessibility for xenogenic anti-IgE antibodies. Nevertheless, these results clearly show that natural anti-IgE antibodies do interfere with diagnostic IgE determinations. Based on these results, we will investigate the observation that some patients with allergic asthma or rhinitis, for example, do not show elevated IgE levels [27]. Furthermore, incidences where patients undergo an anaphylactic shock without showing positive skin tests, elevated total IgE might eventually be explained by the existence of masking but not blocking anti-IgE antibodies. In this context it might also be of interest that subject M.D., whose serum was used to isolate anti-IgE antibodies, underwent two anaphylactic shocks to prophylphenazone and aspirin within the 2 years preceding blood donation.

The technique developed here for determination of anti-IgE antibody is similar to any other immunodot assay [28] with the same reproducibility (data not shown). Our definition of 'free' anti-IgE antibody only implies that these antibodies have a free antigen binding site. As shown by the purification procedure (Fig. 4), immune complexes are present in this fraction, too. We therefore decided not to standardize such 'free' anti-IgE antibodies in terms of μg protein. Furthermore, we also did not standardize IgE-anti-IgE complexes measured in human serum as the composition of such complexes might vary widely vary between different blood donors. Indeed our data show that in some subjects more 'complexes' were detected by the Le27 and in a few subjects more by BSW17. Thus, because our detection system is always itself a complex we probably miss those complexes mainly composed of antibodies similar to the capture antibody. Interestingly, several features of rheumatoid factor parallel our findings with anti-IgE antibodies. For example, in most sera with high rheumatoid factor activity in immune complexes, only low or undetectable levels of free rheumatoid factor were described [29,30]. Furthermore, rheumatoid factors have been demonstrated to be composed of all immunoglobulin isotypes [31]. For IgG isotype rheumatoid factor it has been shown that IgG₁ and IgG₄ were the most abundant subclasses [32,33], similar to those demonstrated for anti-IgE antibodies in this manuscript. The question of whether anti-IgE antibodies are as strongly subclass-restricted as rheumatoid factor is under investigation.

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References

- 1 Fundenberg HH, Kunkel HG. Specificity of the reaction between rheumatoid factors and gamma globulin. *J Exp Med* 1961; 114:257-67.
- 2 Kunkel HG, Müller-Eberhard HJ, Fudenberg H, Tomasi TB. Gamma-globulin complexes in rheumatoid arthritis and certain other conditions. *J Clin Invest* 1961; 40:117-26.
- 3 Schrohenloher RE, Kunkel HG, Tomasi TB. Activity of dissociated and re-associated 19S anti-gamma-globulins. *J Exp Med* 1964; 120:1215-22.
- 4 Schur PH, Kunkel HG. The reactivity of 19S anti-gamma-globulins with native 7S gamma-globulins. *Arth Rheum* 1965; 8:468-72.
- 5 Williams RC, Griffiths RW, Emmons JD, Field RC. Naturally occurring human antiglobulins with specificity for gamma-E. *J Clin Invest* 1972; 51:955-1003.
- 6 Inganäs M, Johansson SGO, Bennich H. Anti-IgE antibodies in human serum: occurrence and specificity. *Int Arch Allergy Appl Immunol* 1981; 65:51-8.
- 7 Carini C, Brostoff J. An antiglobulin: IgG anti-IgE. Occurrence and specificity. *Ann Allergy* 1983; 51:251-3.
- 8 Stevens WJ, Bridts CH. IgG-containing and IgE-containing circulating immune complexes in patients with asthma and rhinitis. *J Allergy Clin Immunol* 1984; 73:276-84.
- 9 Nawata Y, Koike T, Yanagisawa T, *et al.* Anti-IgE autoantibody in patients with bronchial asthma. *Clin Exp Immunol* 1984; 58:348-56.
- 10 Nawata Y, Koike T, Hosokawa H, Tomioka H, Yoshida S. Anti-IgE autoantibody in patients with atopic dermatitis. *J Immunol* 1985; 135:478-83.
- 11 Quinti I, Brozek C, Wood N, Geha RS, Leung DYM. Circulating IgG antibodies to IgE in atopic syndromes. *J Allergy Clin Immunol* 1986; 77:586-94.
- 12 Koike T, Nawata Y, Tsutsumi A, Tmioka H. Anti-IgE autoantibody in allergic disorders. *Allergy Today* 1987; 2:4-6.
- 13 Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 1988; 90:213-17.
- 14 Carini C, Fratrazzi C, Barbato M. IgG autoantibody to IgE in atopic patients. *Ann Allergy* 1988; 60:48-53.
- 15 Paganelli R, Quinti I, Carbonari M, *et al.* IgG anti-IgE in circulating immune complexes in the hyper-IgE syndrome. *Clin Allergy* 1986; 16:513-21.
- 16 Magnusson CGM, Vaerman JP. Autoantibodies of the IgM class against a human myeloma protein IgE (DES). I. Occurrence. *Int Arch Allergy Appl Immunol* 1986; 79:149-56.
- 17 Marone G, Casolaro V, Paganelli R, Quinti I. IgG anti-IgE from atopic dermatitis induces mediator release from basophils and mast cells. *J Invest Dermatol* 1989; 93:246-52.
- 18 Nakajima K, Yang X-D, Vassella CC, *et al.* Effect of naturally occurring anti-IgE antibodies on cytophilic IgE. 1990; submitted.
- 19 Johansson SGO, Magnusson CGM, Larsson PH. Occurrence, specificity and putative roles of anti-IgE antibodies in human serum. In: Reed CE, Bellanti J Davies RJ, Fried-

- laender S, Oehling A, Slavin RG, eds. Proceedings of the XII International Congress of Allergology and Clinical Immunology. Washington DC: CV Mosby, 1986:193-7.
- 20 Johansson SGO. Anti-IgE antibodies in human serum. *J Allergy Clin Immunol* 1986; 77:555-7.
- 21 Stadler BM, Gauchat D, Vassella C. Anti-isotype regulation: cytokines and anti-IgE autoantibodies. In: Sorg C, ed. Cytokines regulating the allergic response. Basel: Karger, 1989; 2:37-50.
- 22 Knutti-Müller J, Stadler BM, Magnusson CM, de Weck AL. Human IgE synthesis in vitro: detection with monoclonal antibodies. *Allergy* 1986; 41:457-67.
- 23 Affi-gel protein A MAPS II kit instruction manual. Bio-Rad, Richmond, U.S.A.
- 24 Qiu G, Fang M, Zou M, Wu A. A highly sensitive and specific assay for human IgE: microtitre solid-phase radioimmunoassay using monoclonal antibodies. *Chin Med J (Engl)* 1989; in press.
- 25 Bio-Rad protein assay instruction manual. Richmond: Bio-Rad, 1979:1-17.
- 26 Roitt I, Brostoff J, Male D. Immunology. London: Gower Medical Publishing Ltd, 1985; Ch.5:2.
- 27 Stevens WJ, Vermeire PA. Serum IgE determination in patients suffering from asthma, rhinitis, cough and various combinations of these disorders. *Prog Respir Res* 1980; 14:51-7.
- 28 Hong CHS, Stadler BM, Wälti M, de Weck AL. Dot-immunobinding assay with monoclonal anti-IgE antibodies for the detection and quantitation of human IgE. *J Immunol Meth* 1986; 95:195-202.
- 29 Asbakk K, Iversen OJ. Rheumatoid factors in psoriatic scale, serum and circulating immune complexes detected by an isotype-specific ELISA. *Acta Pathol Microbiol Immunol Scand (Sect C)* 1987; 95:161-6.
- 30 Rodahl E, Asbakk K, Iversen OJ. Participation of antigens related to the psoriasis associated antigen, pso p27, in immune complex formation in patients with ankylosing spondylitis. *Ann Rheum Dis* 1988; 47:628-33.
- 31 Egeland T, Munthe E. Rheumatoid factors. *Clin Rheum Dis* 1983; 9:135-60.
- 32 Shakib F, Stanworth DR. Antigammaglobulin (rheumatoid factor) activity of human IgG subclasses. *Ann Rheum Dis* 1987; 37:12-17.
- 33 Yount WJ, Cohen P, Eisenberg RA. Distribution of IgG subclasses among human autoantibodies to Sm, RNP, dsDNA, SS-B and IgG rheumatoid factor. *Monogr Allergy* 1988; 23:41-56.

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