

Determination of IgE-Containing Immune Complexes in Human Sera

Evaluation of Polyethylene Glycol Precipitation of Monomeric and Complex IgE and of the Detectability of IgE in the Complexes

R. DJURUP, E. KAPPELGAARD, P. STAHL SKOV, H. PERMIN and H. NIELSEN

Laboratories of Medical Allergology, Medical Immunology, and Immunology, Medical Dept. TA and M, Rigshospital, and Institute of Pharmacology, University of Copenhagen, Copenhagen, Denmark

We studied the polyethylene (PEG) precipitability of monomeric human IgE, and of human IgE artificially complexed with rabbit anti-human IgE. At conditions where precipitation of monomeric IgE did not occur, from 0.2 to 20% of the complexed IgE was precipitated. The PEG precipitability of the complexes was inversely related to the IgE/anti-IgE ratio used for preparation of the complexes. From 1.5 to 19.2% of the IgE in the redissolved precipitates could be detected by use of a two-site IgE immunoradiometric assay, the percentage being highest for complexes formed at equivalence. We conclude that exact quantitation of circulating IgE immune complexes (IC) probably is impossible by any PEG precipitation assay. However, the optimized assay was found to be useful for identification of IgE IC in sera with total IgE concentrations below 5,000 U/ml. IgE IC were found in 5/20 sera from patients with Felty's syndrome, in 5/39 sera from patients with extrinsic allergy and high levels of specific IgE, and in 1/17 sera from immunized wasp allergics. No IgE IC were found in 20 normal human sera.

Key words: atopic diseases; Felty's syndrome; IgE; immune complexes.

Accepted for publication 17 January 1984

CLINICAL ASPECTS

IgE-containing immune complexes may play a pathogenic role in atopic (1, 2, 3, 12), rheumatic (14, 18, 24), and infectious diseases (20). Studies of the prevalence of such complexes, and of their relation to other immunological parameters and disease activity in well-characterized groups of patients may contribute to further elucidation of their biological and pathological significance.

Studies using ultracentrifugation followed by determination of IgE in the fraction by use of a radioimmunosorbent test (RIST) have suggested that 20–100% of total IgE in sera from patients with atopic diseases (atopic eczema, allergic rhinitis, and allergic asthma) exist as complexes (2).

Low affinity Fc receptors for IgE have been found on human macrophages and monocytes (1, 12), and the number of peripheral blood monocytes with this type of Fc receptor (Fcε) is

increased in patients with severe atopic diseases (12). It has been speculated that the natural ligand for these receptors is IgE-containing immune complexes (IgE IC) (1, 12), and that circulating IgE IC may play a significant pathogenic role in atopic diseases (2, 3, 12).

As a first step in the investigation of a possible pathogenic role of IgE IC in atopic diseases, we wanted to determine the prevalence and, if possible, the amount of circulating IgE IC in various subgroups of patients with atopic dis-

eases. To evaluate whether it is possible to quantitate circulating IgE IC by a polyethylene glycol (PEG) precipitation procedure, we have studied the PEG precipitability of monomeric and artificially complexed human IgE, and the detectability of the ϵ -determinant in the artificial complexes. On the basis of these results, we have modified and optimized the PEG precipitation technique originally described by Meretey et al. (13).

The optimized PEG precipitation assay was applied on sera from normal human subjects, from patients with atopic disease, and from patients with Felty's syndrome, in whom circulating IgE IC previously have been demonstrated (13, 14, 18).

MATERIAL AND METHODS

Normal rabbit serum and antiserum

Normal rabbit serum from Gibco, Paisley, Scotland, was stored at -20°C . After thawing, sodium azide (1 g/l) was added and the serum kept at 4°C up to 2 weeks. This serum (NRS) was used as carrier serum in the PEG precipitation experiments, unless otherwise stated. Heat-inactivated normal rabbit serum (HNRS) was obtained by heating NRS at 56°C for 30 min.

Monospecific rabbit anti-human IgE (ϵ -chain specific) serum (aIgE) was from Behringwerke, Marburg, W. Germany. According to the manufacturer, the antigen binding capacity (ABC) of this antiserum was 464,748 U IgE/ml. For use in the IgE immunoradiometric assay (IgE IRMA), a crude immunoglobulin fraction (IgaIgE) of this antiserum was prepared by precipitation with ammonium sulphate (250 g/l (final volume)).

^{125}I -labelled affinity-purified rabbit anti-human IgE ($\text{D}_{\epsilon}2$ specific) was from Pharmacia, Uppsala, Sweden (Phadebas Rast Isotop[®] reagent).

Rabbit anti-human lambda light chains (a(λ)) serum with reactivity against surface as well as hidden lambda light chain determinants was

from DAKO, Copenhagen, Denmark. ABC of this serum was 0.5 mg lambda light chain protein per ml, according to the manufacturer.

*Preparation of ^{125}I -labelled monomeric, monoclonal human IgE (*IgE)*

Immunochemically pure monoclonal human IgE (IgE(λ)-JK) was isolated from a patient serum with an IgE M component by fractional ammonium sulphate precipitation, gel filtration, chromatofocusing and subtraction affinity chromatography as described elsewhere (11). The purified IgE was labelled with ^{125}I with a modification of the chloramine-T method (6). Iodinated monomeric IgE (designated *IgE) was separated from aggregates and free iodine on a 1×40 cm Sephacryl S-300 (Pharmacia) column. Elution was performed at flow rate 0.2 ml/min at room temperature and fractions of 2 ml were collected. The first two fractions of the descending part of the peak corresponding to monomeric IgE were pooled and diluted 1:5 with HNRS and stored at 4°C until use. The monomeric nature of *IgE was confirmed by gel filtration on a calibrated 2.5×98 cm S-300 column (see Results). *IgE was prepared at three different occasions, using different ratios between ^{125}I and IgE. The specific activity of *IgE was 3.5, 6.0 and 9.8 nCi/U IgE, respectively. 1 U IgE is equal to 2.4 ng IgE.

In vitro immune complex preparation

a) **IgE-aIgE IC*: To prepare immune complexes of human *IgE and rabbit anti-human IgE (*IgE-aIgE IC) at different antigen-antibody (Ag-Ab) ratios, increasing amounts of *IgE (100–3,200 U/ml) diluted in NRS were added to a constant amount of rabbit anti-human IgE, diluted in NRS. ABC of the diluted aIgE was approximately 400 U IgE/ml (final volume).

*IgE-aIgE IC for gel filtration was prepared by mixing of 2,000 U *IgE with an amount of aIgE corresponding to the ABC 2,000 U IgE. The mixture was diluted to 2.0 ml with NRS.

The mixtures were vigorously shaken, incubated at room temperature for 1 h and then

at 4°C for 24 h. Insoluble complexes were not removed.

b) IgE-aIgE IC: To prepare immune complexes of unlabelled human IgE and rabbit anti-human IgE, increasing amounts (50–3,200 U/ml (final volume)) of monoclonal human IgE(λ)-JK in the form of serum JK diluted in NRS, were added to a constant amount (ABC: 400 U IgE/ml final volume) of aIgE diluted in NRS.

Similar complexes were also formed with HNRS as diluent.

The mixtures were handled as described above.

c) IgE(λ)-a(λ) IC: Increasing amounts of purified IgE(λ)-JK (0.125 to 8.0 μ g IgE/ml, corresponding to about 0.03 to 2.0 μ g λ -protein/ml) were mixed with a constant amount (ABC: 0.25 μ g free λ chain/ml final volume) of anti-human lambda light chains serum, diluted in NRS. The mixtures were handled as described in (a).

Gel filtration

*IgE, *IgE-aIgE IC and sera were gel filtered on a calibrated 2.5 $\times$ 98 cm Sephacryl S-300 column. Calibration was performed with myoglobin (MW 16.8 kdalton), ovalbumin (MW 44 kdalton), IgG (MW 150 kdalton) and thyroglobulin (MW 670 kdalton) as markers. The correlation coefficient r for the linear regression of log MW on the elution volume, V_e , was -0.999 . Before gel filtration, sera were heated to 37°C for 45 min to dissolve any cryoglobulin. Particulate material was removed by filtration through a Millex-PF filter (Millipore, Bedford, USA) with pore size 0.8 μ m. From 0.6 to 1.5 ml filtered serum, corresponding to 4,200 to 22,950 U IgE, was applied to the column.

Elution was performed at room temperature with 0.1 M Tris-HCl, 0.2 M NaCl, 20 mg/l chlorhexidine acetate, pH 7.7. The flow rate was 0.25 ml/min and 5 ml fractions were collected after the first 180 ml had passed.

PEG precipitation

*IgE was diluted in NRS to 400 U/ml. To 50 μ l were added 2.45 ml cold (4°C) PEG (Merck, Darmstadt, W. Germany; average MW 6,000) solution in varying concentrations. PEG was dissolved in 0.1 M borate buffer, 0.02 M EDTA, 400 mg Na₃l, pH 8.4 (designated BB/EDTA). This buffer will prevent activation of the complement system (7) and solubilization of immune complexes (15), but will not hinder binding of Clq to the immune complexes (7). PEG precipitation was carried out at 0°C for 90 min. After centrifugation at $3,300 \times g$ at 4°C for 30 min, the precipitates were washed 2 times with 2.5 ml cold PEG solution of the same concentration as used for the precipitation. The precipitates were counted 1 min in a gamma-counter (counting efficiency 0.45).

The *in vitro* prepared *IgE-aIgE IC were treated similarly.

In vitro prepared IgE-aIgE and IgE(λ)-a(λ) IC and sera were diluted 1:50 with 2.45 ml cold 3.5% (w/v) PEG in BB/EDTA, precipitated and washed as described above. The precipitates were dissolved by vortex mixing with 0.1 ml 0.2 M potassium phosphate buffer, pH 7.5, for 1 h. The redissolved precipitates were further diluted with 0.2 ml 10% (v/v) NRS in 0.2 M potassium phosphate buffer, pH 7.5.

*PEG precipitation of fractions obtained by gel filtration of *IgE-aIgE IC*

1.25 ml of each fraction obtained by Sephacryl S-300 gel filtration of *IgE-aIgE IC formed in the Ag-Ab ratio 1:1 was precipitated by 1.25 ml 7% (w/v) PEG in BB/EDTA. NRS was added to 2% (v/v) final concentration. After precipitation for 90 min at 0°C, the precipitates were washed 2 times and counted for 1 min.

Quantitation of IgE in the redissolved PEG precipitates

A highly sensitive immunoradiometric assay for IgE quantitation (IgE IRMA) was developed. This assay is a modification of previously published methods (4, 24).

Maxisorb test tubes (Nunc, Roskilde, Denmark) were incubated with 0.3 ml immunoglobulin fraction of rabbit anti-human IgE (IgaIgE) (10 µg/ml 0.01 M phosphate buffer, 0.15 M, 100 mg sodium azide/l, pH 7.5 (PBS)) at room temperature overnight. Any remaining binding sites were blocked by incubation with 0.5 ml 2% (v/v) NRS in PBS at 37°C for 1 h, and the tubes were washed 6 times with PBS containing 0.1% (v/v) Tween 20 (PBS/Tw).

The IgaIgE coated tubes were incubated with 0.25 ml 10% (v/v) NRS in 0.2 M phosphate buffer, pH 7.5 (10% NRS), standard IgE solutions (0.01–1.0 U IgE/ml) obtained by dissolving of IgE standards from the PRIST kit (Pharmacia) in 10% NRS, or redissolved PEG precipitates. After 4 h incubation at 37°C, the tubes were washed 6 times with PBS Tw.

The Phadebas Rast isotop reagent was dissolved in 10 ml 10% NRS, and 0.25 ml was added to each tube. After overnight incubation, the tubes were washed 6 times with PBS Tw and counted for 2 min.

Quantitation of IgE in sera and fractions obtained by gel filtration

Total IgE in sera was determined by the Enzygnost IgE assay (Behringwerke) or by the PRIST assay. The IgE concentration in the fractions obtained by gel filtration of sera was determined by the PACIA method (9) which employs a rabbit anti-human IgE serum with D_e1 specificity.

*Assessment of binding of *IgE-αIgE IC to IgaIgE coated tubes*

Tubes coated with IgaIgE were incubated overnight at room temperature with about 10,000 cpm of each fraction obtained by gel filtration of *IgE-αIgE IC. The exact amount of radioactivity added to each tube was determined by counting during the incubation. After incubation, the tubes were washed 6 times in PBS/Tw

and counted for 1 min. The bound activity was expressed in percent of the amount added.

Patients and controls

Sera from 20 normal human subjects were kindly supplied by the hospital's Blood Bank. No history concerning the donors was obtained.

Sera from 39 patients with extrinsic allergy, defined by serum IgE RAST class 4 (scale 0–4) to at least one allergen, were obtained from patients referred to the Department of Allergy, Rigshospital.

Sera with high IgE concentrations (5,000–23,500 U/ml) were obtained from four patients with severe atopic eczema (described in (16)).

Sera from 17 wasp-allergic patients who had been in immunotherapy with wasp venom for at least 1 year were kindly supplied by Dr. H.-J. Malling, Department of Allergy, Rigshospital.

Sera from 20 patients with Felty's syndrome were examined both by the PEG precipitation technique and by an *in vitro* phagocytosis method described in (18).

Table 1
Gel filtration of sera from four patients with severe atopic eczema

Patient	Amount of IgE loaded on column (U)	Amount of IgE recovered in fraction 1–30 (U)	Amount of IgE in fraction 1–12 in U and percent of the amount recovered	pd-IgE U/ml serum
CA	22,950	23,750	735 (3.1)	0.48
CS	6,600	5,960	130 (2.2)	0.34
FS	4,200	4,750	0 (0.0)	bdl
KH	16,450	13,510	395 (2.9)	0.66

bdl: below detectable limit.

The amount of IgE in fraction 1–12 was considered to be complexed IgE (IgE IC), see Discussion.

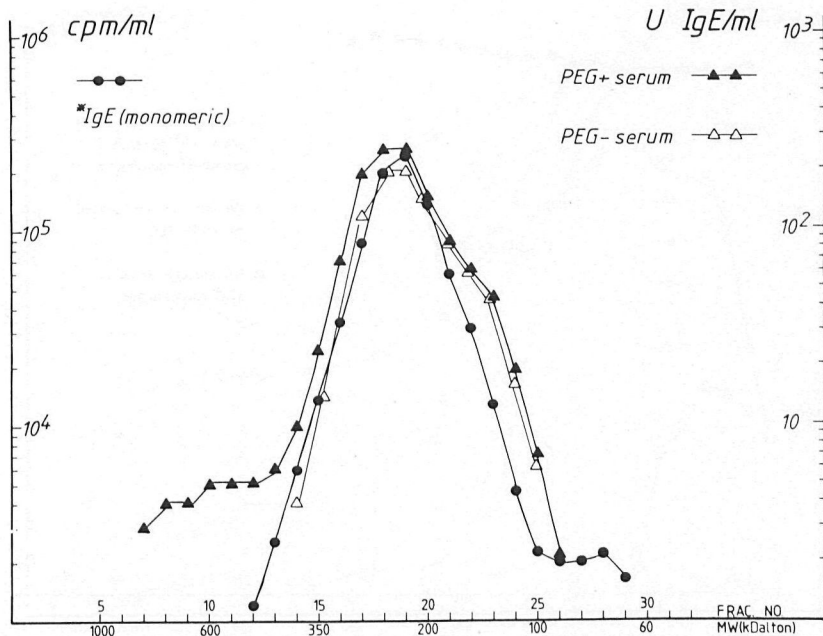


Fig. 1. Gel filtration of ^{125}I -labelled monomeric human IgE (*IgE) and of sera from two patients with severe atopic eczema. 1,400 U *IgE (specific radioactivity 3.5 nCi/U IgE), 0.84 ml serum (4,200 U IgE) without 3.5% PEG precipitable IgE (PEG-), or 0.6 ml serum (6,600 U IgE) with PEG precipitable IgE (PEG+) was gel filtered on a calibrated 2.5×98 cm Sephacryl S-300 column. The first 180 ml were discarded and 30 fractions of 5 ml collected. All fractions were examined for radioactivity or IgE content. *Abscissa*: Upper level: Fraction number. Lower level: Approximate MW in kilodalton. *Ordinate*: Left: Radioactivity (cpm/ml). Right: IgE concentration (U/ml).

Statistics

The correlation between the amount of IgE IC, expressed in pd-IgE U/ml, and total IgE in the patients' sera was tested by calculation of the Kendall rank correlation coefficient τ . The level of significance was selected as $\alpha = 1.0\%$ (two-sided).

RESULTS

Gel filtration of *IgE, *IgE-aIgE and sera

By gel filtration *IgE eluted as homogeneous, symmetrical peak with maximum in fraction 19 (Fig. 1), corresponding to MW 230 kilodalton. Less than 0.5% of the peak value (fraction 19) was found in fraction 12, and no significant

radioactivity was detected in fractions with lower number.

A serum sample from four patients with severe atopic eczema was gel filtered. From 2.2 to 3.1% of the IgE recovered was found in fraction 1–12, when sera positive in the PEG precipitation assay (see below) were gel filtered (Table 1, Fig. 1). No IgE was found in these fractions, when a serum without PEG precipitable IgE was gel filtered.

The elution profile for *IgE-aIgE IC, formed at equivalence, is shown in Fig. 2. 63% of the radioactivity recovered was found in fraction 1–12.

Binding of *IgE-aIgE IC of different size to IgA/IgE coated tubes

From 58 to 87% of the added radioactivity remained bound after 6 washes (Fig. 2). Increase in MW resulted in decrease in binding.

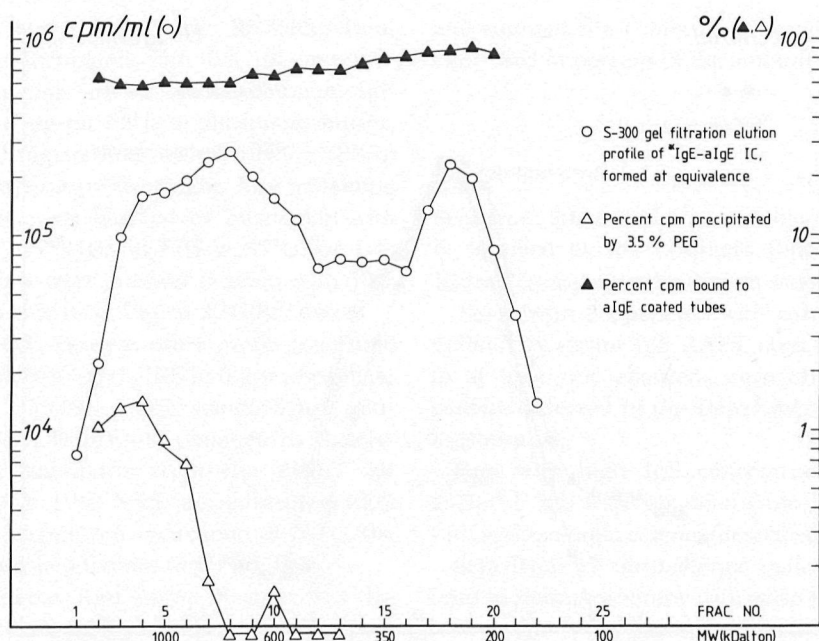


Fig. 2. Gel filtration of preformed ^{125}I -labelled immune complexes. ^{125}I -labelled IgE-aIgE IC (*IgE-aIgE IC) formed by incubation of radiolabelled human IgE and rabbit anti-human IgE at equivalence, were fractionated on a calibrated 2.5×98 cm Sephacryl S-300 column. The first 180 ml were discarded, and 22 fractions of 5 ml were collected. Fractions 2–20 were examined for 3.5% PEG precipitability (Δ) and for binding to tubes coated with rabbit anti-human IgE ($\blacktriangle$). No PEG precipitable IgE was found in fraction 14–20. *Abscissa*: Upper level: Fraction number. Lower level: Approximate MW in kilodalton. *Ordinate*: Left: Radioactivity (cpm/ml) in the fraction ($\circ$). Right: Percent cpm precipitated by 3.5% PEG (Δ) or bound to aIgE coated tubes ($\blacktriangle$).

PEG precipitation of *IgE and *IgE IC

The results obtained when *IgE and *IgE-aIgE IC were precipitated with varying concentrations (w/v) of PEG in BB/EDTA are shown in Fig. 3. It appears that insoluble complexes, i.e. complexes which are precipitable by simple centrifugation, were formed at the Ag-Ab ratio 1:4 only. For the precipitation of complexes formed at higher Ag-Ab ratios PEG was necessary.

Significant precipitation of *IgE did not occur until concentrations of 4–5% PEG were reached. We therefore selected 3.5% PEG for further studies.

The results obtained when *IgE-aIgE IC of different sizes, obtained by gel filtration, were precipitated by 3.5% PEG, are shown in Fig. 2.

Quantitation of IgE in the redissolved PEG precipitates by the IgE IRMA

The sensitivity of the IgE IRMA, defined as the IgE concentration corresponding to $\bar{x} + \text{SEM}$ of the binding obtained with buffer, was about 0.01 U IgE/ml (Fig. 4). As the dilution factor was 6, the detection limit for IgE in serum was 0.06 U/ml.

For IgE which is both *precipitable* by 3.5% PEG and *detectable* in the IgE IRMA, we introduce the term "pd-IgE" and we state the amount of IgE IC in sera as pd-IgE U/ml.

Based on the variation in background binding obtained with normal human sera, only sera containing 0.3 or more pd-IgE U/ml were considered positive.

The amount of pd-IgE found when artifici-

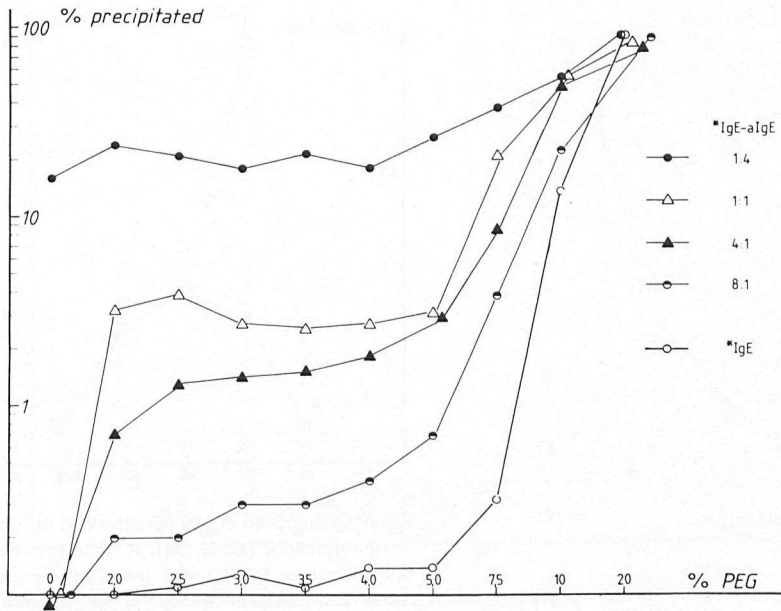


Fig. 3. PEG precipitation of ^{125}I -labelled monomeric (*IgE) and complexed (*IgE-aIgE IC) human IgE. Radiolabelled monomeric human IgE (*IgE) and *IgE-containing IC, formed by incubation of *IgE and antiIgE in the Ag-Ab ratios stated in the figure, were precipitated with varying concentrations of PEG 6,000. *Abscissa*: Concentration (w/v) of PEG 6,000. *Ordinate*: Percent of the added radioactivity recovered in the precipitates.

ally formed IgE-aIgE and IgE(λ)-a(λ) IC were precipitated with 3.5% PEG is shown in Fig. 5. Redissolving the complexes at pH 11.4 did not increase the amount of detectable IgE (results not shown). When IgE-aIgE IC were prepared with HNRS, the amount of pd-IgE in the re-dissolved precipitates was insignificant (results not shown).

The relation between Ag-Ab ratio during the formation of IgE-aIgE IC, PEG precipitability and IgE detectability is shown in Table 2.

The amount of pd-IgE found in the patients' sera is shown in Fig. 6. No IgE IC was found in the 20 normal human sera.

The five positive sera from patients with Felty's syndrome were also positive for IgE IC when examined for *in vitro* phagocytosis by normal polymorphonuclear leukocytes (18).

The amount of pd-IgE in the 80 patients' sera was not correlated to total IgE concentration ($n = 80$, $\tau = 0.1313$, $z = 1.72$, two-sided $p = 8.7\%$). Neither was the amount of pd-IgE in the

14 positive sera correlated to total IgE concentration ($n = 14$, $\tau = -0.1564$, $z = -0.779$, two-sided $p = 43.4\%$).

DISCUSSION

Nature of IgE-containing immune complexes and relevance of our model systems

Circulating IgE-containing immune complexes conceivably may be composed of a) IgE and anti-IgE antibodies, including anti-idiotypic antibodies, and b) IgE antibodies (IgE Ab), including IgE rheumatoid factor (IgE RF), and exogenous or endogenous antigens. Mixed complexes (e.g. containing specific antibodies of several immunoglobulin classes) may also occur (21), and as IgE can activate complement both by the classical and by the alternative pathway (19), complement components may also be parts of the complexes.

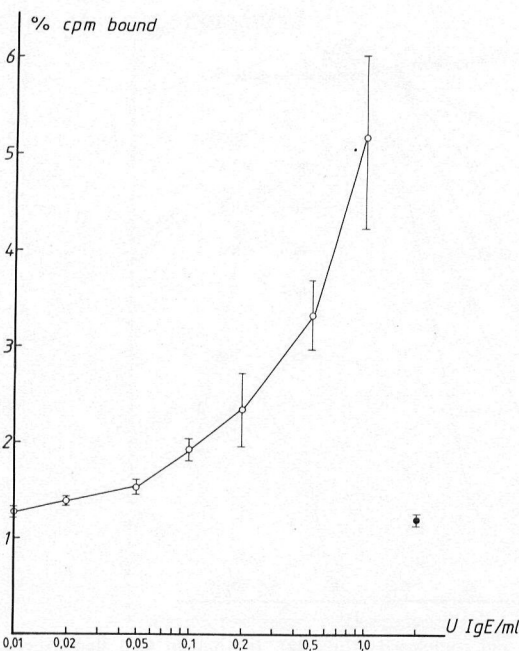


Fig. 4. Standard curve for IgE determination in the IgE immunoradiometric assay. *Abscissa*: IgE concentration (U/ml). *Ordinate*: Radioactivity (cpm) bound (○). Mean $\pm$ 2 SEM ($n = 2$). (●): background obtained with buffer.

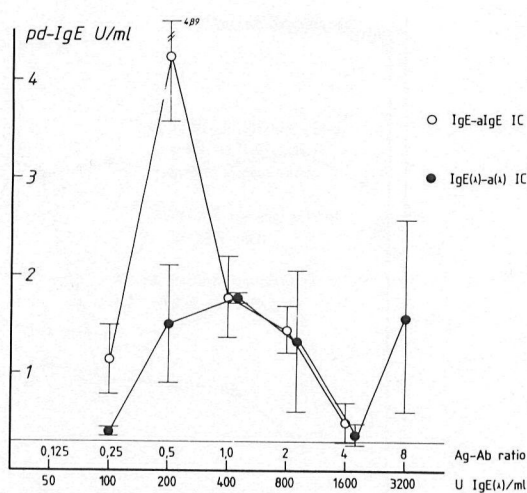


Fig. 5. Quantitation of IgE in redissolved PEG precipitates of *in vitro* prepared IgE IC. IgE IC were prepared by incubation of human IgE (λ) with rabbit anti-human IgE (○) or rabbit anti-(λ)-chain (●) in the Ag-Ab ratios stated. The mixtures were precipitated with 3.5% PEG 6,000. IgE in the redissolved precipitates was quantitated by the IgE IRMA. Values below 0.3 pd-IgE U/ml were considered insignificant and have not been plotted. *Abscissa*: Upper level: Ag-Ab ratio. Lower level: Ag (IgE) concentration (U/ml). *Ordinate*: Units PEG precipitable and detectable IgE per ml incubation mixture (mean $\pm$ 2 SEM; $n = 2$).

Table 2

Detectability of IgE in redissolved 3.5% PEG precipitates of IgE-algE IC formed at different Ag-Ab ratios

Ag-Ab ratio	Amount of IgE precipitated (U/ml)	Amount of IgE detected (U/ml)	Percent IgE detectable
1:4	21.3	1.35	6.3
1:1	10.0	1.92	19.2
4:1	24.0	0.52	2.2
8:1	10.6	0.16	1.5

Unlabelled or labelled IgE (Ag) was incubated with rabbit anti-human IgE in the Ag-Ab ratios stated. The percent of IgE precipitated by 3.5% PEG was determined in the experiments with labelled IgE (Fig. 3), and the amount of IgE precipitated calculated by multiplication with the IgE concentration (column 2). In parallel experiments with unlabelled IgE, run on a different day, the PEG precipitates were redissolved, and the amount of detectable IgE quantitated by the IgE IRMA (Fig. 5) (column 3).

Both IgM (19, 23) and IgG (8) anti-IgE auto-antibodies have been found in sera from allergic patients as well as in sera from some non-allergic persons. Inganäs et al. (8) have provided evidence that IgG anti-IgE immune complexes exist in sera from some allergic patients. Observations made by Ullmer et al. (21) indicate that complexes consisting of IgG and IgE antibodies and complementary food antigens may exist in sera from food-allergic patients immediately following relevant challenge, but complexes consisting of IgE antibodies and inhalant allergens or insect venom allergens have not been unquestionably demonstrated in human sera. We therefore considered complexes consisting of animal anti-human IgE antibody and human IgE to be a relevant model system for the IgE-containing immune complexes to be found in sera from allergic patients and used this type of com-

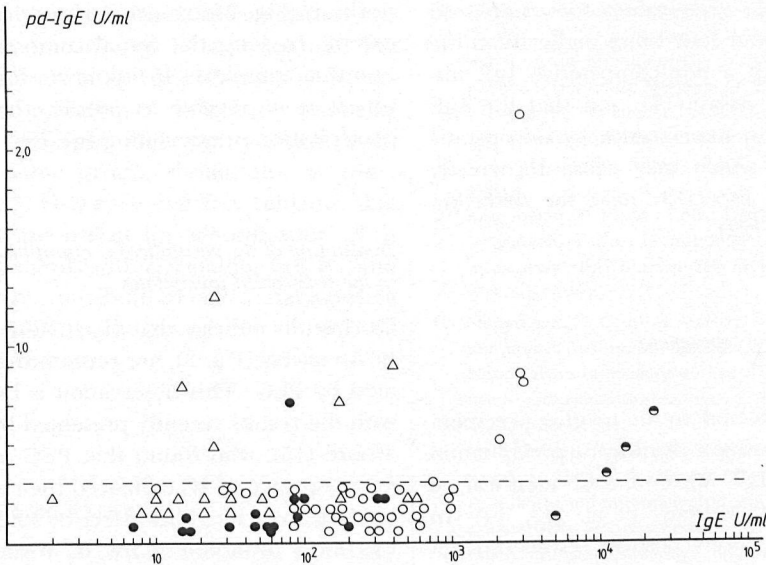


Fig. 6. Amount of IgE IC in patients' sera as function of total serum IgE. The amount of IgE IC in sera from immunized wasp venom allergic patients (●), from patients with extrinsic allergy (○), severe atopic eczema (◐), or Felty's syndrome (△) was determined by precipitation of sera with PEG followed by quantitation of IgE in the redissolved precipitates. Values below 0.3 pd-IgE U/ml (dotted line) were considered insignificant, and no attempt was done to differentiate values in this region. *Abscissa*: Total serum IgE concentration (U/ml). *Ordinate*: Amount of IgE IC (pd-IgE U/ml).

plexes for optimization of the PEG precipitation.

IgE-containing immune complexes in sera from patients with rheumatoid arthritis and Felty's syndrome may contain IgE RF (14, 24). When the PEG precipitation technique was applied to sera from these patients, we found perfect agreement with the results obtained by an *in vitro* phagocytosis technique (18). In this case therefore the PEG precipitation technique is also useful for detection of IgE-containing immune complexes in which IgE is the antibody.

Gel filtration of monomeric human IgE and IgE-containing immune complexes

Gel filtration of ^{125}I -labelled monomeric human IgE (*IgE) defined the zone broadening of the monomeric IgE peak to be expected, when the sera were gel filtered under identical conditions (Fig. 1). On the basis of this experiment, occur-

rence of more than 0.5% of the peak value of the IgE concentration in fraction 12, or in any fraction with a lower number, was considered evidence for the existence of IgE IC or IgE aggregates in the serum, and the amount of IgE recovered in fraction 1–12 was used as an estimate of the IgE IC content of the serum. According to this definition, less than 3% of serum IgE eluted as complexes when sera from four patients with severe atopic eczema were gel filtered (results partly shown in Fig. 1 and summarized in Table 1). This figure is substantially lower than that found by Brostoff et al. (2) (30–100% complexed IgE in sera from patients with atopic eczema). Further studies, using IgE IC of different types and defined composition, are needed to clarify whether this discrepancy is due to different handling of serum samples, use of different separation techniques, or use of different methods for quantitation of the complexed IgE. In this context it should be noted that use of a competitive radioimmunoassay,

such as the RIST, gives values for complexed IgE which are about four times higher than the values found with a non-competitive IgE immunoradiometric assay (17), and that the IgE RIST is subject to interference by nonspecific blocking factors which may cause incorrectly high IgE values, especially near the detection limit of the RIST (22).

PEG precipitation of monomeric and complexed IgE

The conditions found by us to give precipitation of IgE IC without significant precipitation of monomeric IgE were almost identical to those applied by Meretey et al. (13, 14). In these conditions falsely positive results did not occur when serum IgE was below 5,000 U/ml (Fig. 6). At IgE concentrations higher than 11,000 U/ml, the amount of IgE IC tended to increase linearly with total IgE concentration, indicating non-specific precipitation of monomeric IgE at extremely high IgE concentrations (Fig. 6).

However, the lack of correlation between the amount of IgE IC and total IgE in patients' sera confirms that significant precipitation of monomeric IgE does not occur at low and moderate serum IgE concentrations in our assay. In contrast, Stevens et al. (20), using serum dilution 1:4 at the PEG precipitation, found a strongly positive correlation between IgE IC and total IgE in sera from patients infected with *Schistosoma* species. No data were provided to exclude the likely possibility that the observed correlation was due to non-specific precipitation of monomeric IgE, caused by the low serum dilution.

Although 63% of the IgE incubated with aIgE at equivalence formed IC (Fig. 2), only 3% was precipitable by 3.5% PEG (Fig. 3). Apparently, only a small fraction of the IgE IC is precipitable at the assay conditions used. (Partial denaturation of IgE during labelling with ^{125}I , storage and incubation may explain why only 63% of the IgE forms complexes at equivalence). Furthermore, complexes of different sizes (Fig. 2) and different Ag-Ab com-

position (Fig. 3) are precipitated to a different extent. In sera, the actual composition of the immune complexes is unknown, for which reason it is impossible to predict the degree of precipitation of circulating IgE IC.

Evaluation of the possibility for quantitation of IgE in the redissolved precipitates

Our results indicate that big IC (Fig. 2), formed in Ab excess (Fig. 3), are preferentially precipitated by PEG. This observation is in agreement with the results recently presented by Cooper & Moore (15), who found that PEG treatment of *in vitro*-prepared IC enhanced their size and Clq binding capacity. This effect of PEG treatment was most pronounced for IC formed at equivalence and in Ag excess. It is thus very likely that the precipitated IC have a low Ag-Ab ratio, even if the IC were formed in Ag (IgE) excess. In such Ab excess IC, the accessibility of the ϵ -determinants was found to be very limited (Fig. 5), also in the IgE(λ)-a(λ) IC, in which the ϵ -determinants are not directly blocked by immunochemical interaction with an anti-IgE antibody. Thus, preferential precipitation of IC with low Ag-Ab ratio, in which the ϵ -determinants are sterically blocked, may explain the low detectability of IgE in the redissolved precipitates (Table 2).

By use of an acid pepsin digestion of the complexes (10) it would probably be possible to obtain a true quantitation of the precipitated IgE and thereby to eliminate one source of error in the quantitation of IgE IC by PEG precipitation assays.

CONCLUSION

Due to the different PEG precipitability of different types of IC it will probably not be possible to obtain an exact quantitation of the amount of IgE IC in sera by any PEG precipitation assay. This implies that although numeric expressions (percent radioactivity bound,

pd-IgE U/ml) are obtained, a correlation between the amount of IgE IC detected and the amount of circulating IgE IC cannot be assumed, and the finding of different amounts of IgE IC in sera from different patients, even within the same group, should not be over-interpreted. However, the fact remains, that such assays are useful for identification of at least some patients with circulating IgE IC, and their use may contribute to our understanding of the possible pathogenic role of IgE in various diseases.

ACKNOWLEDGEMENTS

We wish to thank Dr. Vagn Andersen and Dr. Bent Weeke for their inspiring interest in the work and for reviewing this paper. The skillful technical assistance of Mrs. Ulla Minuva is also acknowledged. This work was supported by Sygekassernes Helsefond and by the National Danish Association against Rheumatic Diseases. R. Djurup is research fellow at the University of Copenhagen.

REFERENCES

- Anderson, C. L. & Spiegelberg, H. L.: Macrophage receptors for IgE: Binding of IgE to specific IgE Fc receptors on a human macrophage cell line, U937. *J. Immunology* 126, 2470-2474, 1981.
- Brostoff, J., Johns, P. & Stanworth, D. R.: Complex IgE in atopy. *Lancet* *ii*, 741-742, 1977.
- Brostoff, J., Carini, C., Wraith, D. G. & Johns, P.: Production of IgE complexes by allergen challenge in atopic patients and the effect of sodium chromoglycate. *Lancet* *i*, 1268-1270, 1979.
- Cheung, N.-K. V., Reid, M. J., Page, A. R. & Lewiston, N. J.: A microtiter solid phase radioimmunoassay for total serum IgE. *Ann. Allergy* 46, 132-136, 1981.
- Cooper, K. M. & Moore, M.: Critical aspects of immune complex assays employing polyethylene glycol. *J. Immunol. Methods* 60, 289-303, 1983.
- Djurup, R., Søndergaard, I., Minuva, U. & Weeke, B.: A nylon ball solid-phase radioimmunoassay (NBRIA) for determination of specific antibodies in human sera. Application to measurement of IgG antibodies to pollen allergens (IgG RAST). *J. Immunol. Methods* 62, 283-296, 1983.
- Hack, C. E., Eehrenberg-Belmer, A. J. M., Hannema, A. J., Out, T. A. & Aalberse, R. C.: Polyethylene glycol enhances the binding of Clq to circulating immune complexes. *J. Immunol. Methods* 44, 211-221, 1981.
- Ingenäs, M., Johansson, S. G. O. & Bennich, H.: Anti-IgE antibodies in human serum: Occurrence and specificity. *Int. Arch. Allergy Appl. Immunol.* 65, 51-61, 1981.
- Magnusson, C. G. M., Collet-Cassart, D., Merrett, T. G. & Masson, P. L.: An automated particle-counting immunoassay (PACIA) for serum IgE. *Clin. Allergy* 11, 453-461, 1981.
- Magnusson, C. G. M. & Masson, P. L.: Particle counting immunoassay of immunoglobulin E antibodies after their elution from radioallergosorbents by pepsin: An alternative to radioallergosorbent test. *J. Allergy Clin. Immunol.* 70, 326-336, 1982.
- Malling, H.-J., Djurup, R., Magnusson, C. G. M., Søndergaard, I., Skov, P. S., Permin, H., Søbørg, M. & Weeke, B.: Clinical and immunological aspects of a case of monoclonal hyper-IgE. Purification of IgE and relation between basophil cell-bound IgE and histamine release. Manuscript in preparation.
- Melewicz, F. M., Zieger, R. S., Mellon, M. H., O'Connor, R. D. & Spiegelberg, H. L.: Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. *J. Immunology* 126, 1592-1595, 1981.
- Meretey, K., Böhm, U., Falus, A. & Bozsok, S.: Radio-immune double PEG precipitation technique for detecting complexed IgE. *J. Immunol. Methods* 26, 223-228, 1979.
- Meretey, K., Falus, A., Erhardt, C. C. & Maini, R. N.: IgE and IgE-rheumatoid factors in circulating immune complexes in rheumatoid arthritis. *Ann. Rheum. Dis.* 41, 405-408, 1982.
- Miller, G. W. & Nussenzweig, V.: A new complement function: Solubilization of antigen-antibody aggregates. *Proc. Nat. Acad. Sci. USA* 72, 418-422, 1975.
- Nielsen, H., Thomsen, B., Djurup, R., Søndergaard, I., Kappelgaard, E., Berkowicz, A., Kjersem, H., Dahlager, J.-I., Szpirt, W., Ullman, S., Valerius, N. H. & Weeke, B.: Plasma separation in patients with bronchial asthma, atopic dermatitis and hyper-IgE. *Allergy* 39, 329-338, 1984.
- Oreste, U., Calligaris, V. & Ruffilli, A.: Detection of complexed IgE in sera of patients allergic to the pollen of *Parietaria officinalis*. *Int. Arch. Allergy Appl. Immunol.* 71, 267-270, 1983.
- Permin, H., Wiik, A. & Djurup, R.: Phagocytosis of immune complexes by normal polymorphonuclear leukocytes in patients with Felty's syndrome and rheumatoid arthritis. With special reference to IgE immune complexes. *Acta Pathol. Microbiol. Immunol. Scand. Sect. C* 92, 37-42, 1984.
- Saint-Remy, J.-M. R., Magnusson, C. G. M. & Masson, P. L.: Activation of the classical pathway of human complement by a human monoclonal IgE, IgE(Des). *J. Immunology* 130, 1341-1344, 1983.

20. Stevens, W. J., Feldmeir, H., Bridts, C. H. & Dalfalla, A.: IgG and IgE circulating immune complexes, total serum IgE and parasite related IgE in patients with mono- or mixed infection with *Schistosoma mansoni* and/or *S. haematobium*. Influence of therapy. Clin. Exp. Immunol. 52, 144–152, 1985.
21. Ullmer, J., Boulton, P. R. & Brostoff, J.: Specific IgE binding to Staphylococcal Protein A: Evidence for mixed IgE complexes. XI International Congress of Allergology & Clinical Immunology, Abstract No. 464, Macmillan Press Ltd., London, 1982.
22. Underdown, B. J., Knight, A. & Papsin, F. R.: The relative paucity of IgE in human milk. J. Immunology 116, 1435–1438, 1976.
23. Williams, R. C., Jr. Griffiths, R. W., Emmons, J. D. & Field, R. C.: Naturally occurring human antiglobulins with specificity for γ E. J. Clin. Invest. 51, 955–963, 1972.
24. Zuraw, B. L., O'Hair, C. H., Vaughan, J. H., Mathison, D. A., Curd, J. C. & Katz, D.: Immunoglobulin E-rheumatoid factor in serum of patients with rheumatoid arthritis, asthma, and other diseases. J. Clin. Invest. 68, 1610–1613, 1981.

Address:

René Djurup

Laboratory of Medical Allergology

Rigshospital TA 7542

DK-2200 Copenhagen N

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.