

Thermoinactivation of human IgE: antigenic and functional modifications

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SUMMARY

The thermoinactivation kinetics of IgE were studied in experimental models revealing the antigenic properties and the basophil-sensitizing capacity of these immunoglobulins. A pool of human sera containing anti-*Dactylis glomerata* (Dg) IgE was heated from 5 min up to 4 hr at 56°. The IgE antigenicity was tested by two polyclonal ¹²⁵I-labelled anti-IgE antibodies; one anti-IgE was specific of the whole Fc ϵ region, while the other had a specificity restricted to the D ϵ 2 domain. Radioimmunoassays showed that the D ϵ 2 epitopes were more rapidly altered than the D ϵ 1 epitopes. The capacity of IgE to bind to basophil Fc ϵ receptors was assayed by passive sensitization experiments. Basophil sensitivity towards the Dg pollen extract was tested by histamine release experiments in the presence of this allergen. A progressive decrease in cell sensitivity was observed when IgE samples used for cell sensitization were heated for longer than 5 min. Thermoinactivation kinetics of IgE revealed an unexpected increase in the apparent quantity and biological activity of IgE heated for 5 min at 56°. This fact could be due to auto-anti-IgE antibodies linked to the unheated IgE and which interfere with the biological activities of IgE and their quantification.

INTRODUCTION

After the relationship between IgE and anaphylaxis was established, the raised interest in this class of immunoglobulins led to detailed studies on their structural characteristics and their biological activities. Subsequent work showed that the Fc ϵ domain is engaged in binding to the membrane Fc ϵ receptors present on mastocytes and basophils. Antigen challenge, *in vivo* or *in vitro*, may lead to the release of mediators that are partly responsible for the allergic manifestations (for review, see Metcalfe, Kaliner & Donlon, 1981).

One of the outstanding properties of IgE antibodies is their heat-lability. Heating of mouse and rat reaginic sera was reported to destroy their activity in the passive cutaneous anaphylaxis (PCA) assay (Binaghi *et al.*, 1964; Mota & Peixoto, 1966; Prouvost-Danon, Binaghi & Abadie, 1977). Nevertheless, part or all of the IgE antigenic determinants, as well as Fc-related biological activity, can be preserved after heating at 56° in particular experimental buffers (Binaghi & Demeulemester, 1983). In rat myeloma, IgE polymers were formed at 56° that could no longer bind to rat mast cells (Rousseaux-Prévost *et al.*, 1983). With human IgE, a decrease in Prausnitz-Kustner (PK) sensitizing capacity was observed after heating, due to changes in the Fc part of the molecule, whereas the Fab region was preserved (Ishizaka, Ishizaka & Menzel, 1967; Dorrington & Bennich, 1973).

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This work reports on the detailed thermoinactivation kinetics at 56° of human IgE from patients sensitive to *Dactylis glomerata* (Dg). Changes induced by this treatment on the antigenic properties of the IgE molecules were followed by radioimmunoassays and by the nitrocellulose immunoprint technique using two different polyclonal anti-IgE antibodies. The cell degranulation capacity of the serum samples heated for different times were compared in histamine release experiments after binding assays of the anaphylactic antibodies onto human basophils.

MATERIALS AND METHODS

Antigens and antibodies

The water-soluble extract of *Dactylis glomerata* (Dg) was obtained by stirring 1 g of Dg pollen suspended in 10 ml distilled water for 1 hr at room temperature. The crude extract was then centrifuged for 5 min at 9000 g. The supernatant was distributed in aliquots of 200 μ l, which were lyophilized. Before use, the aliquot was solubilized in 200 μ l distilled water and centrifuged for 5 min at 9000 g; the resulting supernatant was used for isoelectric focusing, histamine release experiments and preparation of allergen-coated paper discs. The protein concentration of this extract measured by the Lowry technique was 6 mg/ml.

Sera were obtained from patients with high anti-Dg IgE concentrations. Pools were made by mixing ten of these sera. Heat treatment was performed by placing 1-ml aliquots for

different periods in a thermostated water-bath at 56° in closed tubes.

Horse serum, anti-IgE coated discs, anti-Fcε region and anti-De2 ¹²⁵I-labelled antibodies were obtained from Pharmacia (Uppsala, Sweden). All batches were tested and gave similar results.

Total and specific IgE level determinations

Allergen (Dg)-coated paper discs and anti-IgE coated paper discs were used for specific IgE and total IgE determinations.

The coupling of the Dg extract on activated paper discs (Whatman no. 1) was based on the method described by Ceska, Eriksson & Vaga (1972).

The initial technique for IgE evaluations (Wide, Bennich & Johansson, 1967) was slightly modified: 50 μl of dilutions of patients' sera were incubated for 3 hr with the discs, followed by three washings with saline and then incubated for 16 hr in the presence of 50 μl of ¹²⁵I-labelled anti-Fcε or anti-De2 antibodies. After three more washings, the bound radioactivity was counted in a gamma counter.

For total and specific IgE determinations, serum dilutions were chosen that gave experimental values on the upper linear part of the dose-response curve obtained with the untreated serum. All assays were performed in duplicate.

Agarose isoelectric focusing (IEF) and nitrocellulose immunoprints

These experiments were performed as previously described (Peltre, Lapeyre & David, 1982), with slight modifications. The agarose IEF was performed on a plastic sheet of Gelbond 11 × 11 cm (Marine Colloids, Rockland, ME). The gel, 0.4 mm thick, consisted of 1% agarose IEF (Pharmacia), 12% sorbitol, 2% mixed ampholytes forming a pH range of 3.5–10 (LKB pH 3.5–10: 60 μl, pH 7–9: 30 μl, Pharmacia pH 4–6.5: 30 μl, pH 3–10: 60 μl, Serva pH 2–4: 15 μl, pH 4–5: 15 μl, pH 4–9: 60 μl, pH 9–11: 30 μl). Dg water-soluble extract (200 μl) was applied on the agarose gel at 2 cm from the anode, parallel to the electrodes. The IEF separation was performed at 4° at a constant power of 4 W for 90 min. A print of the agarose gel was taken on a nitrocellulose (NC) sheet (BA 85, 0.45 μm, Schleicher and Schull, Dassel, FRG) by blotting and pressing. The NC sheet was finally dried 15 min under a fan in order to bind efficiently the allergens onto the NC. The NC print was separated from the dried agarose gel and saturated in 1% bovine serum albumin (IBF, Gennevilliers, France), 0.1% Tween 20, in phosphate-buffered saline (PBS) pH 7.4 for 1 hr at room temperature. The NC print was then cut into strips; each strip was incubated overnight with 100 μl of serum pool in 900 μl PBS containing 0.1% Tween 20. The strips were washed three times for 5 min in saline, 0.1% Tween 20, and then incubated for 2 hr at room temperature with anti-Fc or anti-De2 radiolabelled antibodies (20,000 c.p.m./strip in 1 ml PBS containing 0.1% Tween 20). After washing, the NC strips were dried and exposed under close contact with a Kodak-X-Omat film and a Cronex Intensifier Screen (Dupont, Wilmington, DE) at –70°.

Cell sensitization

Leucocytes were isolated and washed as described by May *et al.* (1970). The sensitization of the cells with IgE was performed as indicated by Pruzansky *et al.* (1983) and modified by Weyer *et al.* (1986).

Histamine release assay

A volume of 0.1 ml of each dilution of Dg soluble extract was mixed with 0.9 ml cell suspension containing 1–2 × 10⁶ cells/ml. Duplicate incubations were performed with constant stirring at 37° for 40 min. The tubes were centrifuged (300 g for 8 min at 4°), except for the incubations intended for the determination of total histamine content. A volume of 0.85 ml supernatant (or cell suspension) was mixed with 0.1 ml 4 N perchloric acid and, after centrifugation, the histamine content was quantified by an automatized fluorometric method (Lebel, 1983). Percentage histamine release was calculated by the formula:

$$\frac{(e-b)}{(c-b)} \times 100$$

where *e* = mean of fluorometric readings for the experimental supernatant, *b* = mean of blanks, and *c* = mean of total histamine.

RESULTS

Total IgE assay

The human sera pools containing IgE were heated at 56° for increasing times. Then, the total IgE level was measured by radioimmunoassay. The diluted human serum pool was incu-

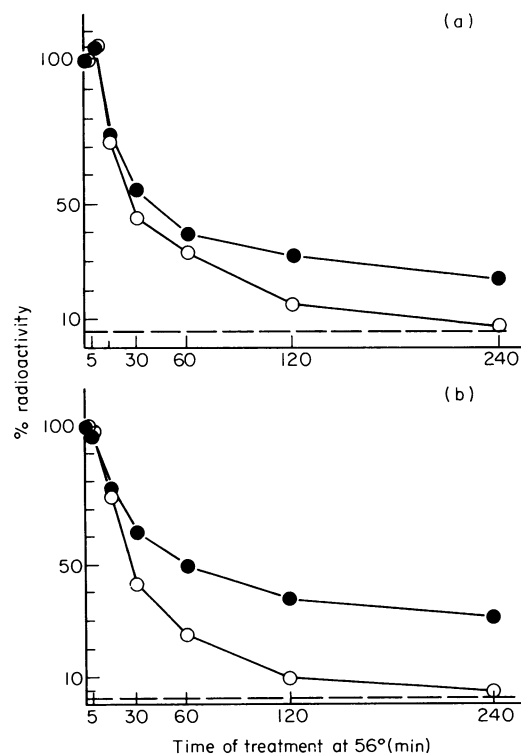


Figure 1. The anti-Dg pooled sera were heated at 56° during the indicated periods. Coated paper discs [anti-IgE in (a) or Dg soluble extract in (b)] were incubated with 50 μl of diluted pool [30-fold in (a) or four-fold in (b)] for 3 hr, washed and incubated for 16 hr with either anti-Fcε (●—●) or anti-De2 (○—○) ¹²⁵I-labelled antibodies. Results are expressed as the percentage of the c.p.m. values obtained with the unheated serum pool. Backgrounds are represented on the graphs (—).

bated with anti-IgE coated paper discs and bound IgE was revealed by anti-Fc ϵ or anti-De2 ^{125} I-labelled antibodies.

A 5% increase in detected IgE was observed after 5 min of heat treatment, as shown in Fig. 1a. This weak increase was reproducible, occurring in three independent experiments. Then a gradual decrease in detectable IgE was observed as a function of the heating time, indicating that the Fc ϵ region and the De2 domain progressively lost their antigenic binding capacity. After 4 hr of heating, 10% of the De2 epitopes and 25% of the Fc ϵ epitopes were still revealed.

Anti-*Dactylis glomerata* (Dg)-specific IgE assay

The influence of heating on De2 and Fc ϵ epitopes was studied by a radioimmunoassay in which the human serum containing anti-Dg-specific IgE was first incubated with Dg-coated paper discs. The anti-Dg-specific IgE antibodies were quantified using ^{125} I-labelled antibodies to De2 or Fc ϵ epitopes. The percentage of bound radioactivity gradually decreased as the time of heat treatment increased (Fig. 1b). In the specific IgE assay, after 4 hr of heating at 56°, 5% of De2 and 30% of Fc ϵ epitopes were still recognizable. In contrast to results obtained with total IgE assay, 5 min of heating did not provoke any increase of the bound radioactivity.

Agarose isoelectric focusing (IEF) and nitrocellulose immunoprint

The Dg constituents were separated by IEF and transferred onto nitrocellulose (NC) strips. These strips were incubated with

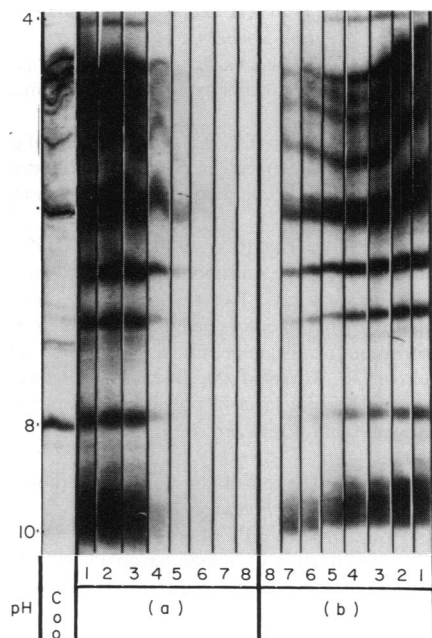


Figure 2. Immunoprints of the Dg constituents separated by IEF were performed. Strips were incubated with serum pool samples or control buffer. The numbers in front of each strip refer to the following treatments: unheated (1), heated at 56° for 5 min (2), 15 min (3), 30 min (4), 60 min (5), 120 min (6), 240 min (7), PBS (8). Autoradiography was done using either radiolabelled anti-Fc ϵ (b) or anti-De2 (a) antibodies. Control with Coomassie Blue staining is shown (Coo).

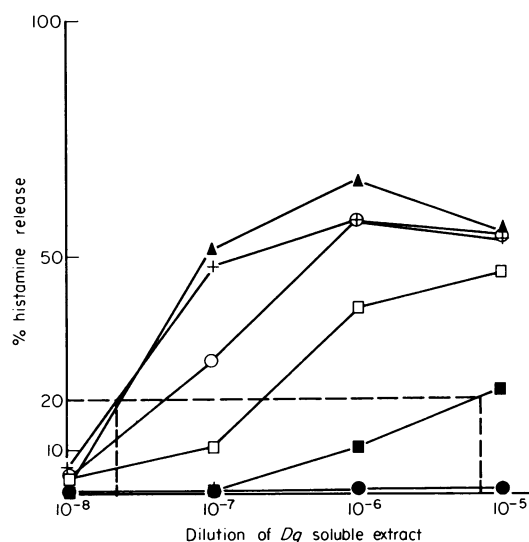


Figure 3. The leucocytes were treated and sensitized as indicated in the Materials and Methods with serum pool samples: unheated (+ — +); treated at 56° for 5 min (▲ — ▲); 15 min (○ — ○); 30 min (□ — □); 60 min (■ — ■); 120 min (● — ●). After challenge with serial dilutions of Dg soluble extract, histamine released in the supernatants was assayed by the fluorometric method.

samples of pooled sera heated for different times at 56°. The NC-bound IgE antibodies were detected by ^{125} I-labelled anti-De2 or anti-Fc ϵ antibodies and visualized by autoradiography (Fig. 2).

The positive controls with the unheated sera were identical when obtained either with anti-Fc ϵ or with anti-De2 antibodies. The intensity of all Dg bands recognized by anti-De2 antibodies was very weak after 1 hr of heating and was totally abolished after 2 hr at 56° (Fig. 2a), whereas the ones revealed by anti-Fc ϵ antibodies were still visible after 4 hr heating (Fig. 2b).

Sensitization of human basophils with unheated and heated sera containing anti-*Dactylis glomerata* (Dg)-specific IgE

The leucocytes from non-allergic donors were first treated with lactate-buffered saline, pH 3.8, in order to elute the IgE originally bound to the basophils. Then, the *in vitro* binding of IgE present in unheated or heated samples of the serum pool was performed. Figure 3 shows the results of histamine release experiments when the sensitized cells were incubated with 10-fold dilutions of the Dg soluble extract. Maximal percentage of histamine release gradually decreased with increased heating time. Cellular sensitivity, defined as the reciprocal of the allergen dilution eliciting 20% of histamine release, decreased under these conditions. The cellular sensitivity of the basophils sensitized with the 1-hr heated serum sample was 300 times lower than for the basophils coated with unheated serum. The percentage of spontaneous histamine release was 8% using unheated or heated serum samples. Basophils incubated with 2-hr heated serum did not degranulate. Histamine release did not occur in the control experiments performed with cells maintained in Tris albumin buffer alone. The 5-min heated serum led to a higher percentage of histamine release than the unheated serum. This result was obtained in three independent experiments with two different pools of sera.

DISCUSSION

In this study, changes induced in human IgE molecules by heating at 56° were first followed by the evolution of their antigenic properties. Two polyclonal anti-IgE antibodies of different specificities were used, one against the D ϵ 2 domain and the other against the whole Fc ϵ region, i.e. D ϵ 1 + D ϵ 2 domains.

Radioimmunoassay and autoradiography experiments using these two anti-IgE antibodies gave results indicating that most of the D ϵ 2 antigenic determinants were heat-labile, whereas more heat-stable epitopes were present in the D ϵ 1 domain. Indeed, in order to obtain a positive immunoprint picture, it may be assumed, first, that the paratopes of specific IgE antibodies have to recognize the Dg allergens, and second, that their epitopes in the Fc region should be detected by the ¹²⁵I-labelled anti-IgE antibodies. Ishizaka *et al.* (1967), who also performed inactivation experiments on whole serum, reported that the Fab region of anti-ragweed specific IgE conserved most of their antigen-binding activity after 4 hr of heating at 56°. We showed here (Fig. 2b) that even after 4 hr of heating, the IgE antibodies were still detected. This probably meant that both paratopes and epitopes were partly preserved. As seen in Fig. 2, the Dg bands disappeared more rapidly during the heat treatment in (a) than in (b). This confirms that epitopes in the D ϵ 2 domain are the most heat-labile epitopes among those of the Fc ϵ region.

In order to study IgE binding capacity to basophil receptors, *in vitro* sensitization of those cells with the serum samples was performed. The presence of functional membrane-bound IgE molecules was followed by histamine release experiments after incubation with *D. glomerata* soluble extract. As seen in Fig. 3, a 5-min heating period of the serum IgE induced a low but reproducible increase in basophil sensitizing capacity. Heating times between 5 min and 4 hr induced a gradual decrease in the cellular sensitivities to the allergen as stated by histamine release assay.

The increase in IgE reactivity after 5 min heat treatment of the serum was observed in histamine release experiments (Fig. 3) as well as by total IgE dosage (Fig. 1a). This fact could be ascribed to natural anti-IgE antibodies present in some human sera, as previously reported (Williams *et al.*, 1972; Inganas, Johansson & Bennich, 1981; Carini & Brostoff, 1983; Nawata *et al.*, 1984). These anti-IgE could be bound to very heat-labile antigenic determinants of the D ϵ 2 domain, so a short heating time could allow the dissociation of the IgE-anti-IgE complexes. This dissociation could reveal more epitopes on the IgE, leading to an apparent increase in IgE quantities (radioimmunoassays) and activities (degranulation assay). These results agree with previous reports showing that auto-anti-IgE were directed against the D ϵ 2 antigenic region and were dissociated from IgE after heating (Inganas *et al.*, 1981; Carini & Brostoff, 1983).

The decrease in cell sensitivity when 15 min–4 hr heated serum samples were used in the histamine release assay (Fig. 3) can be explained by less effective IgE binding to the basophil Fc ϵ receptors and/or by the inactivation of a degranulation effector site, as previously described by Stanworth (1984).

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