

Characterization of the Mouse and Rat IgE Antibody Responses to Timothy Pollen by Means of Crossed Radioimmuno-electrophoresis

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The IgE antibody responses to timothy pollen allergens in one rat strain and five mouse strains were investigated by means of crossed radioimmuno-electrophoresis. The rodent strains were selected for their capacity to respond with IgE antibody production. When sensitized parenterally with the antigen mixed in alum, the animals produced IgE antibodies to essentially the same antigens in timothy pollen as timothy sensitive patients have been shown to do. Thus, the allergenicity of the complex mixture of antigens in a timothy extract is similarly expressed in animals immunized parenterally with alum as in patients sensitized by inhalation.

Key words: allergen; crossed radioimmuno-electrophoresis; IgE; mouse.

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CLINICAL ASPECTS

Atopic humans are thought to be sensitized to airborne allergens via the mucous membranes. It has been suggested that the IgE antibody response to the various components in a complex antigen mixture, e.g. a pollen, is a consequence of varying antigen penetration through the mucous membranes. However, employing crossed radioimmuno-electrophoresis (CIE), we demonstrated that the specificity of the IgE antibody responses to timothy pollen in parenterally sensitized rodents was similar to that in atopic humans. This similarity, obtained in spite of different routes of antigen presentation, suggests that the capability to induce IgE antibody response is more a consequence of molecular structure per se than of antigen penetrability through mucous membranes and of mode of antigen presentation. Further, the data support the relevance of animal models in allergy research.

Most allergenic materials, e.g. pollens, animal danders and moulds, are complex mixtures containing a variety of antigens. Of these antigens, only a limited number are allergens as defined by their capacity to stimulate the production of specific IgE antibodies in humans. It is not known why some antigens act as allergens in humans, while other antigens apparently do not.

By means of crossed immuno-electrophoresis (CIE), the various antigens in an allergen extract can be separated and identified. Antigens

separated in the CIE-plate can be characterized with regard to their capacity to fix specific IgE, employing crossed radioimmuno-electrophoresis (CIE). Using this technique, individual allergens have been identified in extracts of e.g. timothy and birch pollen (5, 15, 17) and *Cladosporium herbarum* (12). The allergens can further be classified as "major", "intermediate" or "minor", depending on the frequency and magnitude of the specific IgE antibody responses in a relevant patient group (3).

Although animal models are extensively used

in experimental allergy research, little is known about the specificity of IgE antibody responses in animals immunized with compounds containing allergens, i.e. whether the same antigens are allergenic in different species. To study this, mice and rats of different strains were immunized with a crude timothy pollen extract. Employing the CRIE-technique, we characterized the specificities of the IgE antibody responses in five mouse strains and one rat strain. The results were compared to those described in timothy sensitive allergic humans (5).

MATERIAL AND METHODS

Allergen extract

Pollen from timothy (*Phleum pratense*) (Allergon, Helsingborg, Sweden, batch No. 171135) was extracted in water at +4°C. The timothy pollen preparation (Pharmacia, Uppsala, Sweden, batch No. CD9847) was freeze-dried and stored in vials containing 0.76 mg protein nitrogen and used for all immunizations and immune chemistry work.

Animals

Ten to 15 individuals from each of five different mouse strains (CBA, C 57/Bl, C × BG, Balb/C and AKR) and one rat strain (DA) were obtained from Anticimex AB, Stockholm and the Biomedical Center, Uppsala, Sweden. Immunizations were performed by subcutaneous (s.c.) injections of the allergen extract diluted in a depot diluent consisting of 0.13% Al(OH)₃ (Pharmacia AB). In the primary immunization, 10 µg protein of the allergen extract was given. Four weeks later, the animals received 1 µg protein, which was then repeated weekly once or twice. The animals were bled retroorbitally 1 week after each booster dose and mouse sera from four to five animals of the same strain were pooled. The rats were bled individually. Sera were frozen (−20°C) for subsequent antibody analyses.

Anti-mouse IgE antibodies

Antibodies with mouse IgE specificity were produced in rabbits by the use of partly purified mouse hybridoma IgE (IgE-14-205) as the immunogen (6). The antiserum was absorbed on foetal calf serum and normal mouse serum Sepharose columns and a rat myeloma IgE (IR2) column. Antibodies against structures homologous for rat and mouse IgE (e.g. skin fixing – receptor binding site) were bound to the IR2 column and after washing desorbed at pH 3.0 (0.1 M glycine-HCl) (8). The eluted antibodies were dialysed, concentrated and then ¹²⁵I-labelled by the chloramine T procedure (7).

Anti-rat IgE antibodies

Antibodies with rat Fc-IgE specificity were isolated from rabbit antiserum against rat IgE myeloma IR162 (4). Sepharose coupled rat IgG, F(ab')₂ fragments from rat IgE myeloma IR2 and intact IgE myeloma IR2 were used for the isolation procedure (9). The isolated antibodies were ¹²⁵I-labelled as stated above.

Radioallergosorbent test (RAST)

Mouse-RAST was performed in order to screen the sera for allergen-specific IgE antibodies before the CRIE was performed. Commercially available Phadebas RAST® timothy discs were used. In the first incubation, the discs were incubated with test serum diluted 1/10 in buffer solution, and as controls, serum from non-immunized CBA-mice and rats respectively. Of the analysed serum pools, those two to three from each strain with the highest level of allergen-specific IgE antibodies were pooled for a subsequent RAST assay and CRIE analysis. Correspondingly, sera from the three individual rats with the highest anti-timothy IgE antibody levels were pooled. The amount of timothy-specific IgE antibodies expressed as the ratio between the uptake of radioactivity of the test serum and of the control serum was for the serum pools: CBA: 4.5, C 57 bl: 3.8, C × BG: 3.9, Balb C: 4.7, AKR: 3.7 and DA rat: 5.3.

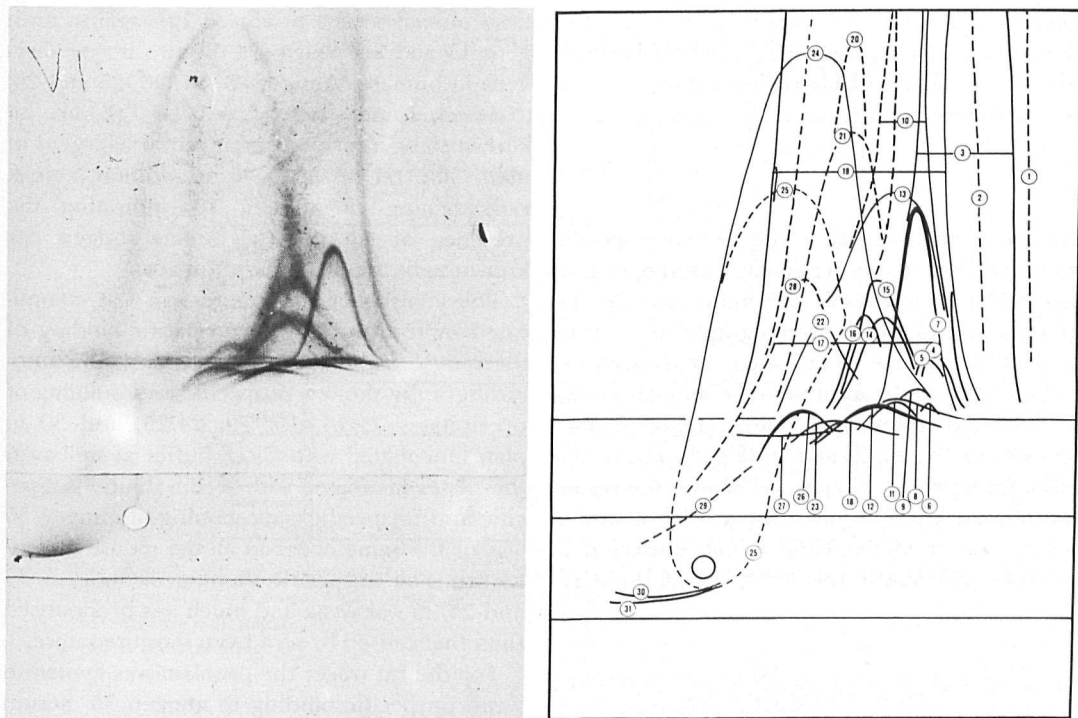


Fig. 1. Photograph and schematic drawing of the precipitate pattern with numbers of the precipitates.

Crossed immunoelectrophoresis (CIE)

This was performed as described in detail elsewhere (6, 9) using Lithex Agarose, HSB cast on a glass plate and in 1% trisbarbital buffer solution. The contents of one vial of the allergen extract were dissolved in 1.0 ml of 0.9% NaCl, and 5 μ l of the solution was applied in 2.5 mm holes for the first dimension electrophoresis. The second dimension electrophoresis was performed with 250 μ l of a rabbit-anti-timothy antibody added to 1.75 ml gel in the anodic field and 25 μ l added to 0.975 ml gel in the cathodic field. The first dimension electrophoresis was run for 25 min at 10 V/cm and the second dimension electrophoresis over night at 2 V/cm. The rabbit anti-timothy serum (Nyegaard & Co. A/S, Oslo, ref. 519/153) caused well-characterized precipitates and has been used in CRIE assays of sera from timothy allergic humans (5). A photograph and a schematic drawing of the precipitate pattern

including their arbitrary numbering are shown in Fig. 1.

Crossed radioimmuno-electrophoresis (CRIE)

Mouse and rat CRIE were performed essentially as described for the human system (16). Briefly, the CIE-plates were incubated over night with 800 μ l of serum from these pools, diluted in 6.2 ml CRIE-buffer solution (100 ml 0.5 M phosphate buffer pH 7.4, 9 g NaCl, 3 g dextrane 10, 0.2 g merthiolate and 2 ml Tween 20 per 1,000 ml). As controls other CIE-plates were simultaneously incubated with 800 μ l serum from a pool of non-immunized CBA-mice or DA-rats in CRIE buffer and in 7 ml of the CRIE-buffer solution without serum. After washing, the plates were reincubated over night with 0.5×10^6 cpm ^{125} I-anti-mouse and anti-rat IgE respectively in 7 ml of buffer solution. After washes, the plates were laid on X-ray films, which were exposed for 3, 6 and 12 days before

development. The radiostaining was graded semiquantitatively; + = barely visible radiostaining; ++ = clearly visible radiostaining; +++ = strong radiostaining.

RESULTS

All the animal sera gave rise to heavy specific radiostaining of the precipitates of antigens 19 and 25. A typical result is demonstrated by the photograph of the autoradiography pattern of one of the mouse strains after 12 days of exposure (Fig. 2). In addition some sera gave weak specific radiostaining of one or more of antigens 3, 10, 21, 26, 28 and 29 (Fig. 3). In Fig. 3 is also, for a proper comparison, shown the results of human CRIE studies using CIE plates in which was used the same rabbit antiserum to timothy pollen as in this study (5). All the sensi-

tized animal strains produced IgE against antigens 19 and 25, which are the two major allergens in humans. Antigens 3, 21, 26, 28 and 29, to which some strains produced IgE, are all known to be intermediate or minor allergens in man. The rat serum gave in addition a weak radiostaining of antigen 10, indicating the presence of specific IgE to an antigen not known to be an allergen in humans.

The analysis of the mouse sera was complicated by the presence of unspecific binding of the mouse tracer to the plates. Thus the autoradiography showed unspecific radiostaining of precipitates 7, 14, 15, 19, 24/25, and 30 in plates incubated with CRIE buffer as well as in the plates incubated with serum dilutions. Specific and unspecific radiostaining of antigen 30 was of the same order in all the mouse experiments. The unspecific staining of antigens 19 and 24/25 was weak and much less pronounced than that caused by sera from sensitized mice.

For the rat tracer the problem was limited to weak unspecific binding to antigen 30. Serum from the sensitized rat caused a stronger radiostaining of this antigen than did the buffer or control serum, demonstrating the presence of IgE antibodies specific to antigen 30 in the sensitized rats.

DISCUSSION

By the term "allergen" is usually meant a complex substance, e.g. a pollen, which may induce specific IgE antibodies in humans. It may also be used more restrictively, denoting the particular antigen in the mixture to which IgE synthesis is directed. In either sense of the word, allergens may be defined by their ability to induce IgE antibody synthesis in man. Only little is known about the molecular characteristics of an allergen, but most of the studied allergens are of protein nature (1).

Specific IgE antibody serum concentrations to whole substance allergens are easily assayed, e.g. by RAST. By the use of CRIE, it is possible to characterize in the individual patient, the IgE antibody response to the different components of an allergen extract, i.e. to identify the antigens to which the IgE in individual patient

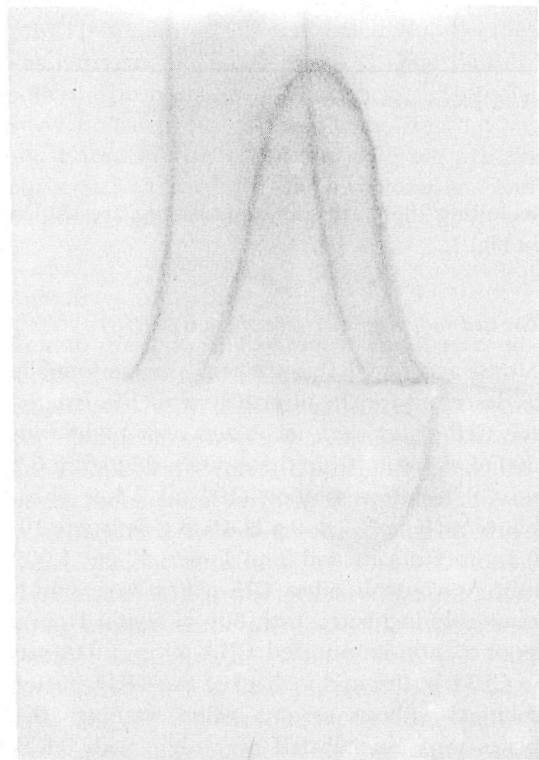


Fig. 2. Photograph of the autoradiography pattern in crossed radioimmuno-electrophoresis of one of the mouse strain serum pools (C × BG) after 12 days of exposure. The picture has been slightly retouched to improve clarity when printed.

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Fig. 3. Radiostaining to timothy pollen antigen precipitates after 3, 6 and 12 days of exposure in crossed radioimmuno-electrophoresis of four mouse strain serum pools and one rat strain serum pool.

+ = barely visible radiostaining.

++ = clearly visible radiostaining.

+++ = strong radiostaining.

sera bind (15, 16). Studies using this method have revealed large individual variations in human IgE antibody responses to allergen extracts (2, 5). These studies also showed that only a limited number of the antigens in a complex crude allergen extract acted as allergens. Studies of timothy pollen extracts, using the same rabbit anti-timothy pollen antiserum as we used in this study, have demonstrated that among approximately 30 CIE precipitates, only 10, antigens 3, 12, 16, 19, 21, 24, 25, 26, 28, 29 and 30, are potential binders of human IgE (5). Of these antigens, two, antigen 19 and 25, fulfill the criteria of major allergens (5), i.e. a majority of the patient sera cause some specific radiostaining to the antigen and at least 25% of the sera give a radiostaining of CRIE-class D or stronger (3).

The results of this study demonstrate that the mouse and rat IgE antibody responses to timothy pollen antigens are similar to that in humans. Only one of the animal serum pools investigated contained specific IgE antibodies to an antigen not known to be allergenic in man. The only

two timothy antigens fulfilling the criteria of major allergens in human CRIE (7) were strongly positive in the mouse and rat CRIE. Serum pools of certain mouse and rat strains contained IgE antibodies to antigens 3, 21, 26, 28, 29 and 30, all known to be minor or intermediate allergens in man, i.e. appear only in a minority of the patients.

In addition to the clearly visible specific radiostaining, unspecific radiostaining was observed in the plates incubated with a control serum dilution and a buffer solution. This was particularly obvious in the mouse CRIE system, in which background radiostaining to antigen 30, which gave the heaviest precipitate in the CIE plates, appeared already after 3 days of exposure. After 6 and 12 days, there was also unspecific radiostaining of other precipitates. The specific radiostaining could easily be distinguished from unspecific, since it appeared earlier. The appearance of unspecific radiostaining, however, resulted in reduced sensitivity of the assays. Therefore, weak specific radiostaining to these antigens cannot be totally

excluded. This was also the technical reason for the choice of mouse strains all known to be high IgE responders.

Great individual differences exist in the tendency to form IgE antibodies in man as well as in animals. Previous studies in mice have demonstrated that the degree of IgE antibody response depends on genetic factors, mode of antigen presentation, the antigen dose, dose intervals and type of adjuvant used (11, 13, 14). Whether such factors influence the specificity of the IgE antibody response is not known.

In man, sensitization is thought to occur via the mucuous membranes. Varying penetration of different antigens has been offered as one explanation as to why only some of the antigens in e.g. a pollen are allergenic (10). However, this study demonstrates a close similarity of the IgE antibody response to an allergen extract in mice, rats and atopic humans. Thus subcutaneously sensitized rodents respond with IgE synthesis to the same antigens in timothy pollen as do atopic humans sensitized via the mucous membranes. This suggests that allergenicity of a certain protein is mainly a consequence of molecular structure, and less a consequence of how the antigen is presented.

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