

Human Serum IgE against Two Major Allergens from Cow Hair and Dander

Determination of Affinity and Quantity of Antigen-Specific IgE

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The quantity and quality of antigen-specific IgE against purified major allergens from cow hair and dander was measured by a double-antibody radioimmunoassay. In serum from patients with allergic asthma to cow the affinity constant varied from 1 to 150×10^9 l/mol and the number of antibody combining sites from 0.01 to 0.4×10^{-9} mol/l serum for each antigen.

Key words: affinity constant; cow dander, double-antibody assay.

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CLINICAL ASPECTS

Binding of antigen to specific cell bound IgE triggers a series of events which ultimately leads to the release of histamine, vasoactive amines and other substances responsible for the clinical manifestation of the allergic type I reaction. Cell bound IgE and circulating IgE interchange, and it is thus possible to characterize the antibody by analysis of peripheral blood. Antigen-specific IgE occurs in very low concentrations (10^{-9} mol/l), and until now an exact quantitation of the quality and quantity has not been performed.

The allergic type I reaction is mediated by IgE antibodies present on basophils and mast cells. Cell bound and circulating IgE antibodies interchange, and because of this, quantitation of allergen-specific IgE in human serum can be used as a diagnostic tool. Methods presently in use are semiquantitative, but the recently introduced procedure for quantitation of quantity and quality of antigen-specific IgG antibodies (blocking antibodies) has reinforced the desire for similar details for IgE (1).

This paper reports the first attempt to study the quality and quantity of antigen-specific IgE. In serum from patients with allergic asthma to cow dander the affinity constant for two purified major allergens from cow hair and dander varied from 1 to 150×10^9 l/mol and the

number of antibody combining sites from 0.01 to 0.4×10^{-9} mol/l serum for each antigen.

MATERIAL AND METHODS

The antigens

Two major allergens (Ag 1 and Ag 3) from cow hair and dander identified and purified according to Prahl et al. were used (9, 10). The activity of the antigens was ensured by RAST and prick test (10). Judged from quantitative immunoelectrophoresis and from polyacrylamide gel electrophoresis the antigens contained less than 1% of impurities. The same antigen batch was used for all experiments. The substance concentration of the antigens

used for iodination was determined by quantitation of protein content from the optical density at 215 and 225 nm (14) on a Unicam SP 1800-spectrophotometer and the relative molecular mass of the antigens (Ag 1 = 24,500, Ag 3 = 21,000) (10).

Preparation of radiolabelled antigens

Ag 1 and Ag 3 were labelled with sodium ^{125}I by the chloramine-T technique according to Hunter (6). Briefly 0.2×10^{-9} mol of purified antigen were oxidized by 10×10^{-9} mol chloramine-T in the presence of 1 mCi sodium ^{125}I in a total volume of 120 μl . After 40 s agitation 52×10^{-9} mol of sodium metabisulfite in 20 μl was added and 15 s later the reaction mixture was diluted with 110 μl 0.1 mol/l sodium phosphate buffer, pH 7.5, containing 14.5×10^{-6} mol/l human serum albumin as carrier protein and potassium iodine 0.03 mol/l. Nonprotein bound iodine was removed by gel filtration on a Sephadex G-50 column (Pharmacia, Sweden). Bed volume was 50 ml and flow rate 40 ml/h. The elution was performed with a phosphate buffer (pH 7.5) containing 14.5×10^{-6} mol/l human serum albumin and 0.1 mol/l sodium chloride.

Fractions of 600 μl were collected. Pooling of the iodinated protein was based on precipitability by a serum sample (G.E.) containing significant amounts of IgG against allergens from cow hair and dander. The pooled ^{125}I -labelled antigens were 95–100% precipitable by trichloroacetic acid 0.6 mol/l. Specific activities achieved were 2×10^{18} cpm/mol for Ag 1 and 1.5×10^{18} cpm/mol for Ag 3. The immunological activity of the tracers was the same as that of the unlabelled antigens as determined by dilution curves.

Radioimmuno precipitation technique

Anti-IgE, covalently coupled to paper discs (Phadebas IgE PRIST, Pharmacia, Uppsala, Sweden) was allowed to react with IgE in 100 μl serum samples (diluted 1 + 1 in IgE-free horse serum) for 3 h at room temperature. After seven washings with 2.5 ml 0.15 mol/l saline

insolubilized IgE was allowed to react with 50 μl purified ^{125}I labelled antigens from cow hair and dander (Ag 1 or Ag 3) in test buffer (sodium borate 55 mmol/l, sodium chloride 100 mmol/l, human serum albumin 14.5×10^{-6} mol/l, sodium azide 0.015 mol/l, pH 8.0). Approximately 10,000, 5,000, 2,500, 1,250 and 600 cpm were added to duplicate tubes and incubated overnight at room temperature. Bound and free radioactivity was separated by washing the discs seven times with 2.5 ml 0.15 mol/l saline. The bound ^{125}I -activity was measured in a gamma counter. Nonspecific binding in the assay was evaluated by replacing the patients' serum with normal human serum. The nonspecific binding was subtracted from all assay results and the net cpm used for calculations. All results were based on duplicate determinations at two different occasions.

Binding analysis

Sera were studied by analysis of their ability to bind radiolabelled antigen at concentrations ranging from 0.106 to 0.006×10^{-9} mol/l. The average equilibrium constant K_A was calculated from the plot of the Scatchard equilibrium:

$$\frac{B}{F} = K_A (Ab_t - B) \quad (12),$$

where B and F are the molar concentrations of the bound and free antigen respectively and Ab_t is the molar concentration of binding sites on the antibody molecules at the dilution of antiserum employed. A graph of B/F against B represents a straight line in which K is determined by the slope and Ab_t by the intercept on the X-axis (Fig. 1).

Patients' sera

The analyzed sera were obtained from eight patients with allergic asthma to cow judged from anamnesis, bronchial provocation test, RAST and prick test (16). Two of the donors (G.E. and A.M.F.) had been immunized with an extract from cow hair and dander for 6 and 2 years respectively.

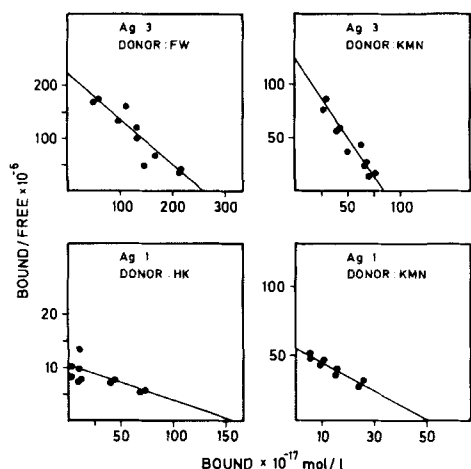


Fig. 1. Scatchard analysis of antigen binding data obtained in two antigen systems. Cow hair and dander Ag 1 and Ag 3 binding curves are shown for sera diluted 1 + 1 from donors with cow dander-induced asthma.

Total serum IgE was measured by the PRIST assay (a double antibody assay) using the Pharmacia kit (3, 7).

Specific IgE was estimated by the RAST assay with a dialysed aqueous extract from cow hair and dander (St.C-75) (10) coupled to the discs according to Ceska et al. (2). The results were expressed in sorbent units (SU) (2 SU = allergy class (a.c.) 0, 4 SU = a.c. 1, 20 SU = a.c. 2, 100 SU = a.c. 3 and 100 SU = a.c. 4) as described by Wide et al. (15).

Quantitation of antigen specific IgE

The amount of IgE bound to the PRIST discs was determined in two ways:

Diluted 1 + 1 the IgE antibody content in six of eight sera exceeded the capacity of the discs. To know the capacity of the discs, binding analyses were performed with ¹²⁵I-anti-IgE (¹²⁵I anti-human IgE- specific activity = 3.1×10^{17} cpm/mol, 87% precipitable in trichloroacetic acid 0.6. mol/l, Pharmacia, Uppsala, Sweden). The number of molecules bound was calculated from Scatchard plots. All sera were assayed undiluted and diluted 1 + 1. Identical results were obtained, thus indicating that the discs had been

saturated with IgE when the sera were diluted 1 + 1. The number of mol IgE bound on the PRIST discs was 0.180×10^{-12} mol/disc. The correction factor for antigen-specific IgE determined by Scatchard plots for the six sera able to saturate the discs was: IgE mol in 50 μ l serum / 0.180×10^{-12} mol (bound IgE mol per disc).

Total IgE of two sera (F.W. and M.P.) could be assayed directly diluted 1 + 1. The individual amount of IgE fixed to the discs was assayed in two steps. Step 1 was an ordinary PRIST assay and in step 2 the IgE antibody content of the supernatant from step 1 was assayed in a second PRIST. The amount of IgE fixed to the paper discs in PRIST₁ was calculated as = mol IgE/50 μ l PRIST₁ minus mol IgE/50 μ l PRIST₂. The correction factor for antigen-specific IgE determined by Scatchard plot was then IgE mol/50 μ l serum bound IgE mol/disc.

RESULTS

Methodological evaluation

The lack of reactivity between the anti-human IgE antibody employed and IgG was deduced from the identical results obtained with two serum samples tested directly or after removal of IgG. Both samples contained measurable amounts of cow antigen-specific IgG (9). Non-specific binding made up 0.5 to 5% of the total activity added.

The reproducibility of the assay was 20% as determined from assaying the eight sera at two different occasions, whereas the mean coefficient of variation within experiments was 7% ($n = 36$). The antigen-antibody reaction reached equilibrium after 80 min for Ag 3; some increase of the specific binding of Ag 1 was seen as the time of incubation was prolonged up to 24 h.

Measurement of antigen-specific IgE

In order to obtain data that could be utilized to calculate the affinity constant (K_A) and the serum concentration of the antigen-specific IgE, experiments were carried out over a wide range of very low antigen concentrations, i.e.

Table 1

Analysis of the affinity and quantity of serum IgE to antigens from cow hair and dander in the sera of eight patients with allergic asthma to cow dander

Donors	s-Anti Ag 1 - IgE		s-Anti Ag 3 - IgE		s-IgE $\times 10^9$ mol/l	s-Anti St.C-75 specific IgE* SU
	K_A $\times 10^9$ l/mol	Binding sites $\times 10^{-9}$ mol/l	K_A $\times 10^9$ l/mol	Binding sites $\times 10^{-9}$ mol/l		
K.M.N.	150	0.04	135	0.07	3.0	50
A.M.P.	52	0.05	84	0.15	3.6	29
F.W.	—	—	104	0.04	0.6	40
G.E.	8.4	0.45	54	0.25	2.6	47
M.P.	4.6	0.02	20	0.01	0.6	38
H.K.	5.0	0.19	—	—	2.8	30
K.L.	6.6	0.07	—	—	5.1	15
H.E.J.	1.0	0.16	—	—	2.9	37
Median	6.6	0.06	84	0.02		

* RAST. St.C-75 is an aqueous extract from cow hair and dander (10); SU, sorbent units, see text; —, non detectable.

$0.006-1 \times 10^{-9}$ mol/l. In most assays the range of bound to total antigen varied from 1 to 10%. The Scatchard plots obtained for three sera are shown in Fig. 1.

Data from additional assays are presented in Table 1. It can be seen that the K_A values for IgE anti-Ag 1 varied over a range of $1-150 \times 10^9$ l/mol and for IgE anti-Ag 3 over a range of $20-135 \times 10^9$ l/mol.

Estimates of the antibody concentration obtained from the Scatchard plots (X-intercept) are shown in Table 1. The quantity of antigen-specific IgE binding sites in undiluted serum varied from undetectable to 0.45×10^{-9} mol/l with a median of 0.06×10^{-9} mol/l for Ag 1 and from undetectable to 0.25×10^{-9} mol/l with a median of 0.02×10^{-9} mol/l for Ag 3. In most cases the total amount of antigen-specific IgE made up about 5% of total serum IgE (Table 1).

Two of the patients (G.E. and A.M.P.) had been immunized with extract from cow hair and dander for 6 and 1 year respectively. The substance concentration of antigen-specific serum IgE was rather high for G.E., whereas that of A.M.P. did not differ from those of untreated patients.

The content of antigen-specific IgE was further estimated by RAST assays performed

with whole extract from cow hair and dander coupled to the paper discs.

All sera belonged to allergy class 3 and showed little variation in sorbent units compared with the range of values found by the present methodology (Table 1).

DISCUSSION

We have used a double-antibody assay combined with binding analysis to calculate the average equilibrium constant and the number of antibody-combining sites of antigen-specific IgE. The binding analysis calculations were performed according to the theory for molecules in solution. In theory one would expect kinetics differences between solid phase molecules and molecules in solution. In practice this is not the case (5, 8). Unlike most double-antibody assays, serum IgE was bound to insolubilized anti-IgE before incubation with iodinated antigen. Through this, one of the weak points of the RAST assay has been eliminated, namely the competition between antigen-specific IgE and IgG for the binding of the antigen. Further, the assay may have the advantage that the IgE antibody is presented to the antigen in the same way as it is presented by mast cells and basophil leukocytes. The average equilibrium constant of

the IgE to purified Ag 1 from cow hair and dander ranged from 1 to 150 (median 7) $\times 10^9$ l/mol. Measurements of antigen-specific IgG in our laboratory and by others has shown an equilibrium constant of IgG antibody against several antigens of clinical interest ranging from 1 to 100 (median 5) $\times 10^9$ l/mol (1, 9). These data indicate the same range as the values observed for Ag 1-specific IgE. In contrast Ag 3-specific IgE showed a considerably higher median K_A of 84, – range 20–135 $\times 10^9$ l/mol.

The number of antibody-combining sites of antigen-specific IgE obtained from the Scatchard plots ranged from 0.01 to 0.4×10^{-9} mol/l with a median for the sum of both antigens of approx. 0.12×10^{-9} mol/l. Similar experiments for antigen-specific IgG show this class of antibody is present in a concentration around 5×10^{-9} mol/l in untreated patients (9). Cow major Ag 1 + Ag 3-specific IgE made 1.3–27% (median 6%) of total IgE. Similar experiments have been made by others. By measuring the difference of total IgE in serum before and after absorption of specific IgE with solid phase antigen Gleich & Jacob found approximately 30% of total IgE to be specific for grass and ragweed pollen antigens in patients with allergic rhinitis to these antigens before season, rising to about 40% after the season (4). These results are similar to those of Schellenberg & Atkinson (13), who employed alkaline elution of specifically bound antibody in the RAST followed by measurement of total IgE of the eluate in the serum from ragweed allergies. The amount of total IgE in the experiments mentioned above corresponds to that in our study. Compared to pollen-sensitive patients the amount of antigen-specific IgE on total IgE was low in serum from patients with allergy against cow dander. This difference might be due to the dissimilarity of the methods employed. Another explanation could be that allergy to cow was usually accompanied by allergy against several other atopic allergens, whereas patients often are only allergic to grass or ragweed. This explanation is supported by the fact that in serum from G.E., who is only allergic to cow dander, Ag 1 + Ag 3-specific IgE made up 26% of total serum IgE.

The present study describes an easy method for determination of quality and quantity of antigen-specific IgE. This together with similar measurements of antigen-specific IgG are likely to be important tools for studying the competition between these two antibody classes and thus for the development of more rational hyposensitization schemes. It should, however, be emphasized that a complete understanding of this competition demands further characterisation of the kinetics of the antigen binding to the two antibody classes.

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