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Passive Sensitization of Human Basophils: Evidence for Heterogeneity in the IgE Molecule¹

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Abstract. Possible functional heterogeneity of human IgE antibody was studied by passively sensitizing human basophils for antigen-induced histamine release. Six sera of known IgE anti-ragweed antigen E and total IgE content were diluted to contain 10 ng of antibody but differed with respect to the ratio of specific/total IgE. The relative ability of the sera to passively sensitize generally reflected the specific/total IgE ratio but one serum was 2.5 times more active than the remainder. This ability did not reflect the IgE antibody level nor was it due to a dialyzable or heat-stable factor. These data indicate functional IgE heterogeneity, probably due to the interaction of the Fc portion of IgE with the basophil receptor.

Heterogeneity of IgE antibody, either with respect to its ability to fix to basophil/mast cell receptors (Fc) or its ability to bind antigen (Fab), has not been described. We examined whether such heterogeneity exists by the passive sensitization of human basophils with several sera of known IgE anti-ragweed antigen E and total IgE content. The sera were diluted to contain the same concentration of antibody but differed with respect to their total IgE content. If there were no heterogeneity, histamine release would decrease as the nonspecific IgE con-

centration increased and competed for receptors with the IgE antibody. Basophil receptor differences were not a variable since a single donor was used in each experiment. Our results indicate that the ability of most sera to passively sensitize can be predicted by the ratio of specific/total IgE but that heterogeneity of the IgE molecule clearly exists.

Peripheral leukocytes from nonallergic passive recipients (with serum IgE < 10 ng/ml) were washed twice in Tris-A buffer (Tris, 25 mM; NaCl, 120 mM; KCl, 5 mM; and 0.03% human serum albumin, adjusted to pH 7.4) and resuspended in Tris-A containing 10 µg/ml heparin (Schwarz Mann, Orangeburg, N. Y.), 4 mM ethylenediaminetetraacetate (EDTA) and 1 ml of a dilution of ragweed-allergic serum in a total volume of 2.5 ml ($3-6 \times 10^7$ leukocytes/ml) [2, 3]. The

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serum dilutions were made in autologous serum so that the final serum concentration was kept constant at 16%. The passive sensitization mixtures were incubated at 37 °C for 90 min, centrifuged, washed twice with Tris-A, and resuspended in Tris-ACM (Tris-A containing 0.5 mM CaCl_2 and 1 mM MgCl_2). 0.6 ml of cells ($3-6 \times 10^6/\text{ml}$) were added to equal volumes of antigen E diluted in Tris-ACM, incubated at 37 °C for 45 min and the reaction stopped by centrifugation. The histamine released into the supernatant was measured by an automated fluorometric technique developed by *Wagman* [5, 6].

Antigen-E-specific IgE antibody was measured by the radioallergosorbent test elution technique and total IgE in the allergic sera was measured by a noncompetitive radioimmunoassay technique, both previously described in detail [1, 4]. Each IgE determination was performed in at least two assays on two dilutions of serum. The mean coefficient of variation between assays was 12%. IgG ('blocking') antibody was measured by a double antibody radioimmunoassay technique described by *Sobotka et al.* [7].

In screening sera from patients sensitive to honeybee phospholipase A or ragweed antigen E for their ability to passively sensitize basophils for histamine release, we observed several instances in which the sensitizing ability was greater than could be explained by the specific IgE antibody level or its ratio to the total IgE concentration. In

order to study this rigorously we selected six sera from ragweed-sensitive donors. The sera are characterized in table 1.

If sensitization were simply a function of specific IgE competing for basophil receptors with the total (nonspecific) IgE, then these sera, diluted to have the same final concentration of antigen-E-specific IgE antibody, should have a relative sensitizing potency which corresponds to the percentage of antigen-E-specific IgE antibody. The sera were diluted to a final concentration of 10 ng antigen-E-specific IgE and used for passive sensitization (fig. 1a). The relative potencies of sera 1, 2, 3, 4, and 6 reflected the antigen-E-specific IgE/total IgE concentrations. Serum 5, however, which contained approximately the same antigen-E-specific IgE/total IgE concentrations as serum 6 was far more efficient than serum 6 and was, in fact, more effective in passive sensitization than sera 2, 3, or 4.

The relative effectiveness of sera 5 and 6 in sensitizing the cells of this recipient was further explored in the experiment shown in figure 1b. Serum 5 was diluted to contain 10, 5, 4, 3, 2 and 1 ng antigen-E-specific IgE/ml; serum 6 contained 10 ng antigen-E-

Table 1. Analysis of sera used for passive sensitization

Serum	Antigen-E-specific IgE ng/ml	IgE total ng/ml	Antigen-E-specific IgE/ total IgE, %	Blocking antibody ¹
1	290 ± 59	1,450 ± 182	20	1/20
2	110 ± 14	1,065 ± 47	10.3	1/20
3	65 ± 12	670 ± 79	9.7	1/20
4	1,340 ± 104	18,700 ± 1,200	7.2	1/526
5	100 ± 11	1,775 ± 296	5.6	1/128
6	225 ± 14	4,260 ± 1,008	5.3	1/20

¹ Dilutions which bind 50% ^{125}I -labeled antigen E (20 ng).

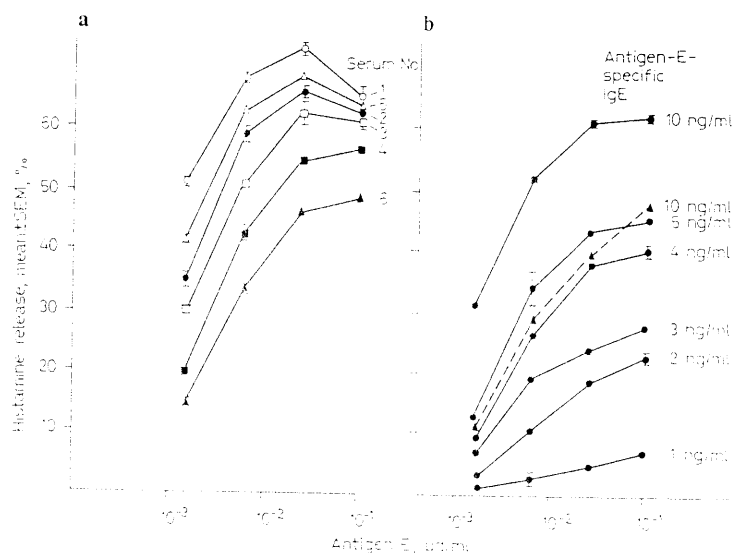


Fig. 1. a Relative effectiveness of six ragweed-allergic sera (diluted to contain 10 ng antigen-E-specific IgE/ml) to sensitize cells of a passive recipient. b Effect of further dilutions of serum 5 (solid lines) to passively sensitize the cells of a recipient for antigen E-histamine release; compare with serum 6 (broken line) at 10 ng antigen E-specific IgE/ml. Bars indicate the least accurate standard errors.

specific IgE/ml. The IgE antibody in serum 5 was almost 2.5 times more effective than that in serum 6.

In further experiments using these two sera to sensitize cells from three additional passive recipients, serum 5 was consistently 2–3 times more efficient than serum 6 (data not shown). This suggests that the heterogeneity resides within the antibody molecule itself rather than with the basophil receptor for IgE. Since serum 5 had a higher blocking antibody titer than serum 6 (1/128 compared to $< 1/20$ for serum 6), we heated serum 5 for 4 h at 56°C to destroy reagenic antibody activity. This treatment completely abolished sensitizing activity of serum 5. It should also be noted that the sensitizing activity of serum 4, which had a blocking antibody titer of 1/526, was consistent with the ratio of specific IgE/total IgE. The ability of these sera to sensitize cells did not, therefore, appear to be related to their IgG content. Extensive dialyses of serum 5 and serum 6 also did not alter their relative sensitizing abilities.

These data indicate there is heterogeneity with respect to the functional ability of IgE antibody. Whether this is due to its binding to the basophil receptor or the average affinity for antigen cannot yet be established, since techniques for establishing the average equilibrium constant (K_D) for antigen-binding sites of IgE have not been developed. We suspect, however, that the results reflect heterogeneity of the Fc portion since the IgE antibody content of the sera was defined by their ability to bind antigen and was equalized in this respect. Whatever the cause of the heterogeneity, these data indicate still another variable in our assessment of the clinical significance of serological IgE antibody measurements.

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