

Serum and cell bound IgE in chronic urticaria

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Summary

Basophil leucocyte-bound IgE has been investigated in patients with chronic urticaria by the method of reversed anaphylaxis. In this reaction basophil-bound IgE behaves as an antigen, the amount present being inversely proportional to the concentration of anti-IgE producing maximum histamine release. Dose-response curves in which histamine release was plotted against the concentration of anti-IgE failed to reveal any substantial difference in the optimum concentration of anti-IgE for maximum release from basophils of urticarial subjects compared with control subjects, thus suggesting there is no quantitative abnormality of basophil bound IgE in chronic urticaria. However, the magnitude of maximum histamine release by anti-IgE from basophils of urticarial subjects was reduced compared with controls and this was not related to serum IgE concentrations since the mean serum IgE concentration was slightly higher in the urticaria group. Studies of spontaneous and compound 48/80-evoked histamine release in the two groups did not reveal any differences in stability of histamine stores or in the biochemical histamine release mechanism over a wide range of concentrations of 48/80. These results raise the possibility of a qualitative abnormality of basophil bound IgE in chronic urticaria.

Introduction

Chronic urticaria is generally considered to be an allergic disease but in at least 70% of patients no cause can be identified (Green, Koelsche & Kierland, 1965; Champion *et al.*, 1969). Juhlin *et al.* (1966) found no significant abnormality of serum IgE in seventeen patients with chronic urticaria. Since IgE is specifically fixed to mast cells and blood basophils (Ishizaka & Ishizaka, 1971; Sullivan, Grimley & Metzger, 1971) it is possible that investigation of cell membrane-bound IgE will yield more information on abnormalities of IgE than determinations of serum concentrations alone. We have therefore determined basophil-bound IgE in patients with chronic urticaria by reversed

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anaphylaxis, and have compared these results with serum IgE determinations carried out in the same subjects.

Methods

Patients. Two groups of subjects were studied: patients with chronic urticaria and age- and sex-matched control subjects who were healthy volunteer members of the staff. The diagnosis of chronic urticaria was established on clinical grounds, the duration being not less than 3 months. In none of the urticarial patients was a cause identifiable either from the history or from the results of intradermal skin testing using the standard 'Bencard' range of allergens (Bencard Ltd, Brentford, Middlesex). A history suggestive of atopy resulted in exclusion from either group.

IgE. Both serum IgE and basophil leucocyte-bound IgE were studied using aliquots of single 55 ml venous blood samples. A 5 ml portion was allowed to clot and the serum separated for determination of the serum IgE concentration by the method of McLaughlan *et al.* (1971) (normal range 25–375 ng/ml).

The IgE coating of basophil leucocytes was investigated using the reversed allergic reaction (Ishizaka *et al.*, 1969). Histamine release from basophils was obtained by adding a specific goat anti-human IgE serum (Miles-Yeda Ltd). The anti-serum was raised against the Fc fragment of IgE and was monospecific on immunoelectrophoresis. Histamine release from basophil leucocytes evoked by anti-IgE serum is dependent upon the IgE fixed on the basophil cell membrane (Ishizaka *et al.*, 1969). In this reaction basophil membrane-bound IgE behaves as an antigen, the amount present being inversely proportional to the concentration of anti-IgE producing maximum histamine release (Ishizaka, Soto & Ishizaka 1973).

Preparation of basophil leucocyte suspension. Leucocyte suspensions were prepared as follows. 50 ml venous blood was mixed with 2500 units Heparin and divided into 5 equal aliquots. To each aliquot was added 3 ml of a 1% solution of methyl cellulose in 0.9% NaCl. The mixture was allowed to separate at 37°C for 20 min, the methyl cellulose phase was aspirated and centrifuged at 4°C and 1000 rev/min for 10 min. The supernatant methyl cellulose was discarded and the cell pellet washed in calcium free Tyrode solution and recentrifuged as before. After discarding the washings the leucocyte pellets were reconstituted in calcium-free Tyrode and pooled in a final volume of 10 ml. No attempt was made to increase the concentration of basophils in the leucocyte suspensions since all or almost all of both IgE and histamine is localized on or in the basophils (Ishizaka & Ishizaka 1971; Sullivan *et al.*, 1971; Graham *et al.*, 1955; Ishizaka *et al.*, 1972; Sampson & Archer, 1967).

Histamine release by anti-IgE. The leucocyte suspension was divided into 1 ml replicate samples. Anti-IgE serum dissolved in Tyrode was mixed with each replicate of the leucocyte suspension to a final concentration of 0.002–2.0 µg antibody/ml. The final reaction volume was 2 ml and the final calcium concentration was 1.8 mM. All determinations, including negative control histamine release ('spontaneous release') were carried out in duplicate. The mixture was incubated at 37°C, the reaction being terminated at 15 min by cooling. Released histamine was obtained by centrifuging each tube for 15 min at 4°C followed by decantation of the supernatant which was retained for histamine determination. The histamine in the residual cell pellet was determined by reconstituting the pellet in 4 ml Tyrode solution followed by boiling for 5 min. The histamine content of both the supernatant and the boiled residue were determined by bioassay using an atropinized guinea-pig ileum preparation and an

automatic bioassay apparatus. Results of histamine assays were expressed in terms of histamine base. Histamine releases for each replicate were calculated as a percentage of the total histamine in each tube, i.e. the sum of the histamine content of the supernatant solution after incubation and the residual histamine. In expressing the results, all anti-IgE-evoked releases were corrected by subtracting the corresponding negative control release.

Chemical histamine release. Histamine release from leucocyte suspensions evoked by the chemical histamine liberator compound 48/80 (Burroughs-Wellcome Ltd) was investigated in some experiments. The procedure adopted was as described above for release by anti-IgE except that 48/80 (final concentration 10–1000 µg/ml) was substituted for anti-IgE. Higher concentrations of 48/80 interfered with the response of the guinea-pig ileum to histamine at bioassay. Reliable histamine assays were obtained by boiling the supernatants for 3 min, which inactivated the 48/80. The histamine in these samples was unaffected by boiling for 3 min.

Basophil leucocyte counts. In some experiments basophil counts were carried out on the leucocyte suspensions by the method of Moore & James (1953) using toluidine blue for basophil staining and a Fuchs-Rosenthal haemocytometer for basophil counting.

Table 1. Leucocyte suspensions: histamine content and basophil leucocyte counts

Subject	Cell count (cells/mm ³)	Histamine (ng/ml)	Histamine (pg/basophil)
Control			
1	57.9	25.4	0.44
2	87.6	70.0	0.8
3	18.8	46.5	2.48
Urticaria			
1	17.2	30.0	1.76
2	103.3	50.8	2.03
3	165.9	10.9	0.06
4	181.5	30.5	0.16

Results

Histamine and basophil leucocyte content of leucocyte suspensions

Basophil leucocyte counts were carried out on leucocyte suspensions obtained from three control subjects and four patients with urticaria. The results are given in Table 1. The histamine content of the same suspensions was determined by boiling for 5 min followed by bioassay after cooling. From these results the mean histamine content per basophil could be calculated for each of the seven donors (Table 1). Values ranged from 0.44–2.48 pg/basophil for control donors and 0.06–2.03 pg/basophil for urticarial patients. The values for the three control donors are of the same order as those published for healthy subjects by Sampson & Archer (1967) and Ishizaka *et al.* (1972). However, basophils of two donors with urticaria (subjects 3 and 4) gave abnormally low values.

Histamine release from basophil leucocytes by anti-IgE

Leucocyte suspensions were prepared from the blood of eleven patients with chronic

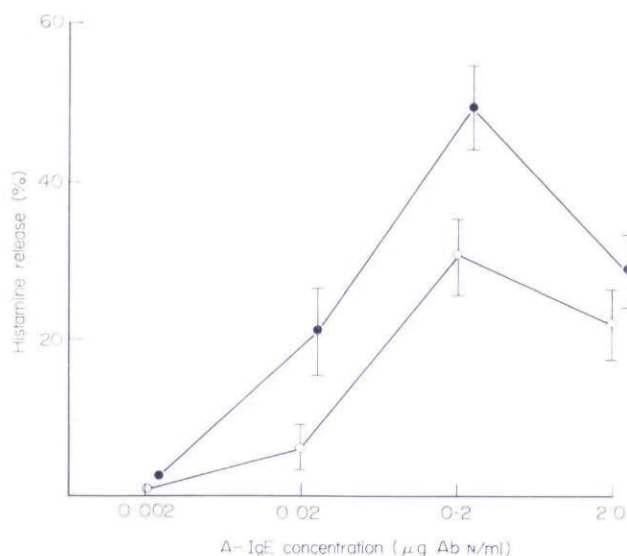


Fig. 1. Mean histamine release ($\% \pm \text{S.E.M.}$) evoked *in vitro* by 4 concentrations of anti-IgE in basophil leucocytes from eleven patients with chronic urticaria (○) and sixteen control subjects (●).

urticaria and sixteen control subjects. Dose-response curves for specific reversed allergic histamine releases by concentrations of anti-IgE ranging from 0.002–2.0 μg antibody N/ml are given in Fig. 1. Basophils from both groups of donors released little or no histamine in the presence of the lowest concentration of anti IgE (0.002 μg Ab N/ml). Release was maximum in the presence of 0.2 μg Ab N/ml, further increase in the concentration of anti-IgE resulting in inhibition. The shape of the dose response curves was similar in the two groups and the optimum concentration of anti-IgE for maximum histamine release was approximately 0.2 μg Ab N/ml in each group. However, the magnitude of histamine release from basophil leucocytes of urticaria patients evoked by 0.02 and 0.2 μg Ab N/ml anti-IgE was reduced by a mean of 70.4% and 37.8% respectively ($0.05 > P > 0.01$).

Table 2. Histamine release (%) evoked by anti-IgE

Subjects	Anti-IgE concentration (μg Ab N/ml)				Total histamine (ng/ml)	Serum IgE (ng/ml)
	0.002	0.02	0.2	2.0		
Control (n = 16)	2.4 $\pm$ 0.9	21.0 $\pm$ 2.4	49.7 $\pm$ 5.4	29.2 $\pm$ 4.5	77.0 $\pm$ 2.1	209 $\pm$ 81
Urticaria (n = 11)	0.8 $\pm$ 0.4	6.3 $\pm$ 2.9	30.9 $\pm$ 4.9	22.3 $\pm$ 4.4	63.4 $\pm$ 2.2	336 $\pm$ 70

Values for serum IgE represent mean of eight subjects (control group) and nine patients (urticaria group) $\pm$ S.E.M. Mean % negative control ('spontaneous') histamine release 2.9 $\pm$ 0.6 (control group) and 2.5 $\pm$ 0.7 (urticaria group).

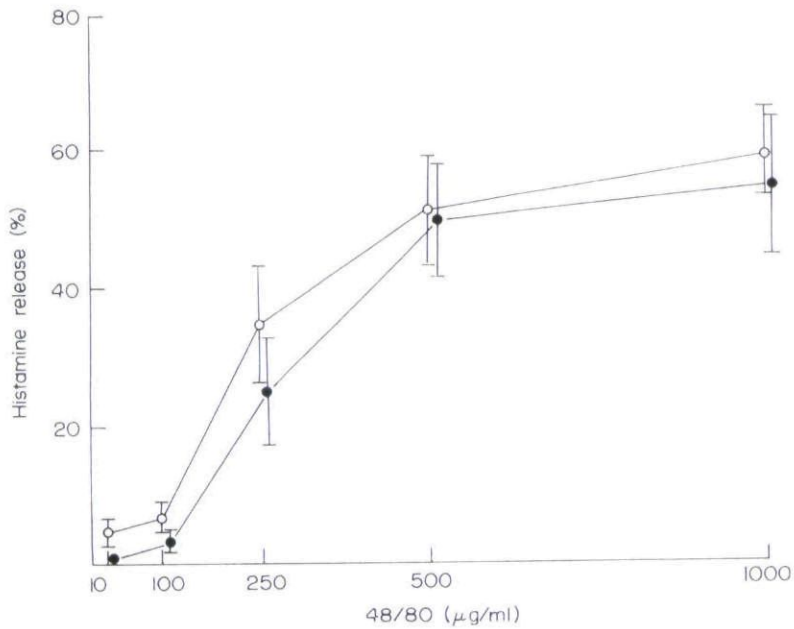


Fig. 2. Mean histamine release ($\% \pm$ S.E.M.) evoked *in vitro* by 5 concentrations of compound 48/80 from six patients with chronic urticaria (○) and five control subjects (●).

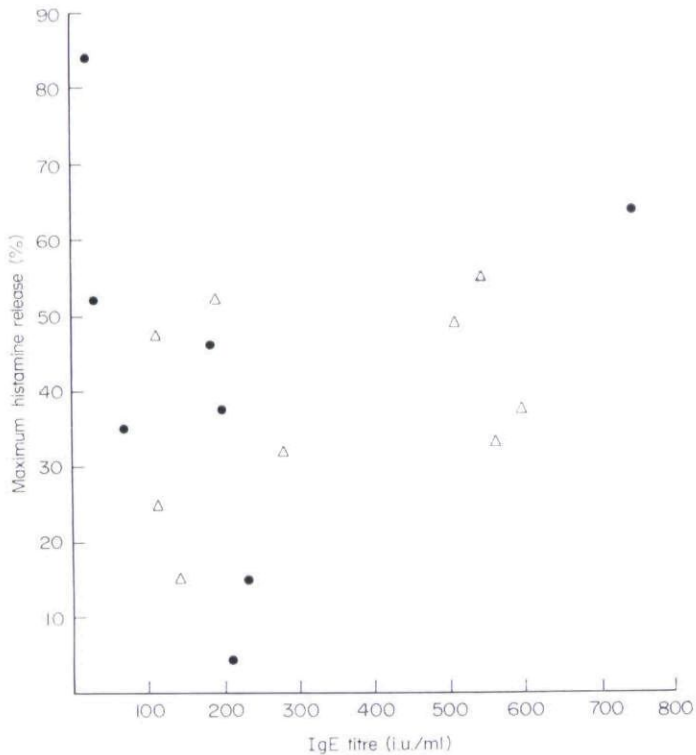


Fig. 3. Maximum histamine release evoked *in vitro* from basophil leucocytes by 4 concentrations of anti-IgE plotted against the serum concentrations of IgE in the same donors. ● = control subjects; △ = chronic urticaria patients.

Negative control (spontaneous) histamine release from basophils of both urticarial and control subjects during incubation was extremely low (less than 5% of the total histamine present in the samples) as indicated in the footnote to Table 2, which suggests that the separated basophil leucocytes had not been significantly damaged by the isolation procedure and that the histamine content of basophil leucocytes in urticarial patients is not abnormally unstable.

Histamine release from basophil leucocytes evoked by compound 48/80

The histamine-releasing effect of compound 48/80 (10–1000 µg/ml) on basophil leucocytes was studied using cells obtained from five normal donors and six patients with urticaria. The dose-response curves obtained are shown in Fig. 2. There was no significant difference between 48/80-evoked histamine release in the two groups at any of the 5 concentrations of 48/80. The shapes of the two curves were similar and the optimum concentration of 48/80 for maximum release was equal in the two groups. These results suggest that the cellular histamine release mechanism is intact in the basophils of urticaria patients.

Serum IgE

Serum IgE determinations were carried out in nine of the patients with urticaria and in eight control subjects. Although the mean serum IgE concentration for the urticaria patients was higher than the corresponding value for the controls (Table 2) this difference is not significant ($P > 0.2$) and both mean values were within the normal range. Figure 3 shows that there was no correlation between serum IgE and anti-IgE-evoked histamine release from basophil leucocytes obtained from the same sample of blood.

Discussion

Dose response curves in which histamine release was plotted against the concentration of anti-IgE failed to reveal any substantial difference in the optimum concentration of anti-IgE for maximum histamine release from basophils of patients with chronic urticaria and those of control subjects. The method of reversed anaphylaxis used in the present study is indirect and the inverse correlation between histamine release and basophil bound IgE is approximate. Thus small differences in the concentration of anti-IgE required for maximum histamine release would probably not be detected by this method (Ishizaka *et al.*, 1973).

The dose response curves show that maximum histamine release evoked by 0.02–0.2 µg Ab N/ml anti-IgE from basophils of urticarial subjects was diminished compared with corresponding values in control subjects. Although the magnitude of maximum release does not correlate with the amount of basophil bound IgE (Ishizaka *et al.*, 1973), this finding does raise the possibility of a qualitative abnormality of cell-fixed IgE which results in a functional impairment of its efficiency as an antigen in our experimental system. The reduction in release was not due to differences in basophil leucocyte counts between leucocyte suspensions obtained from patients with urticaria and control subjects or to differences in the stability of histamine stores in basophils since the shapes of the dose response curves for the two groups were identical, and there was no difference in spontaneous histamine release from suspensions during the 15 min incubation. Furthermore, the reduction was not due to a reduced sensitivity of the intracellular histamine release mechanism since dose response curves for histamine release evoked by compound 48/80 in basophils from urticarial and control subjects

were identical over a wide range of doses. The diminished sensitivity of basophil leucocytes to anti-IgE in urticaria was not accompanied by a parallel reduction in serum IgE. On the contrary, the mean serum IgE was actually higher in the urticaria group than in control subjects although we found, as did Juhlin *et al.* (1966), that serum IgE concentrations in chronic urticaria were within the normal range. A lack of correlation between serum IgE concentrations and the amount of basophil leucocyte bound IgE as determined by anti-IgE-evoked histamine release have been reported for patients with intrinsic asthma by Assem *et al.* (1971). It is of interest that both intrinsic asthma and chronic urticaria resemble allergic disorders clinically but are associated with a negative intradermal skin response and a normal serum IgE. We have not found evidence of a gross quantitative abnormality of basophil bound IgE in chronic urticaria, although studies in progress using a direct method for estimation of cell bound IgE may uncover a smaller difference. However, our results do raise the possibility of a qualitative abnormality of basophil leucocyte-bound IgE in urticaria which could be explained by an abnormal distribution of IgE on the plasma membrane of basophils or possibly by a conformational change in the IgE molecule. Whatever the explanation, our findings may have important implications in the pathogenesis of urticaria.

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