

Cellular histamine release, specific and total serum IgE levels in hay fever patients and controls

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Summary

The amount of histamine released from blood leucocytes by allergen, the amount of allergen required to release 50% histamine and the total IgE and IgE specific for *Dactylis glomerata* are compared in nine hay fever patients and five control individuals.

The variation with time of total IgE, of specific IgE and of the percentage of histamine released at a fixed allergen concentration, corresponding to the cellular sensitivity, is shown for one control individual with positive *in vitro* and skin tests, but without clinical symptoms. For four patients with a low total IgE level the evolution of these parameters during a 1 year treatment period is presented.

Introduction

During the last ten years, many authors have published results on the development of clinical signs during pollen desensitization and the variation of cellular and serological parameters during this treatment. Double-blind trials strongly support the efficiency of hyposensitization, especially in ragweed pollinosis (Lowell & Franklin 1965; Norman, Winkenwerder & Lichtenstein, 1968); thus these patients are suitable for further *in vitro* studies. Recently, results in the changes in total IgE, specific IgE, histamine release, blocking antibodies and antigen-binding capacity were reported (Levy *et al.*, 1971; May & Aduna, 1971; Nelson *et al.*, 1976; Anfosso-Capra, Autran & Charpin, 1975; Stahl Skov, Norn & Weeke, 1977; May *et al.*, 1970), but the correlations between the clinical symptoms and these *in vitro* findings remained unclear. The heterogeneity of the conditions of treatment, the method of selecting patients, the diversity of allergen preparations used and the different techniques of *in vitro* measurements make the comparison of those results very difficult.

A very interesting approach is the study of the changes in time of serum ragweed-binding activity, reaginic activity and cellular sensitivity during the treatment (Evans *et al.*, 1976; Melam *et al.*, 1975; Irons, Pruzansky & Patterson, 1975).

In this paper, we have determined the conditions for similar studies. The *in vitro* diagnostic features (specific IgE, total IgE, histamine release) in five controls and in nine grass pollen-sensitive patients are reported. The variation of these parameters

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during a 1 year period is shown for one special case, a control, presenting positive *in vitro* tests, but without any clinical symptoms, and for four of the pollen-sensitive patients, treated with whole grass extract.

Materials and Methods

Patients

Adult and untreated patients were selected on a typical case report of hay fever and on positive cutaneous tests with grass pollen extract. Control individuals were volunteers of the Allergen Department who presented no clinical symptoms in the pollination period.

The patients answered an identical questionnaire after two consecutive pollination seasons. For each patient, the sum of the positive answers in this questionnaire is referred to as the symptom score. The results of the cellular histamine level variation during time and treatment were reported earlier (David & Weyer, 1976).

Allergens

The grass pollen extract used for skin testing and histamine release experiments is an aqueous mixture of extracts of four grasses: *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense* and *Secale cereale*, in which *D. glomerata* is the predominant component (90%). To prepare the stock allergen the pollen was extracted during 72 hr in Coca's solution (1 g pollen per 5 ml extracting liquid). A 1:10,000 dilution of this extract was used for immunotherapy.

Treatment of patients was made with the mixed grass pollen extract adsorbed on $\text{Al}(\text{OH})_3$ (Alipagène Pollen; Pasteur Production, France).

For histamine release experiments, this extract was stored in 50% glycerol, and for skin tests in 5% glycerol and 0.5% phenol.

Treatment

The treatment of patients was followed over a 1 year period from March 1975 until April 1976, with an interruption during the pollination season. Weekly injections were given at increasing doses. The starting dose was based on individual sensitivity according to case history and skin test results. Cumulative doses are indicated in the Results section.

Histamine release

The histamine release experiments were performed according to the technique of May et al. (1970). All the incubations were made at leucocyte concentrations ranging between 2×10^6 and 4×10^6 per ml. The dilutions of the stock allergen were made at the time of the experiment and each incubation was made in triplicate. Histamine release results are expressed as a percentage of the total available histamine.

Total IgE

Two methods have been used to determine the total IgE level in the serum. The immuno-enzymatic method was currently used for IgE levels ranging between 100 and 600 u/ml (Guesdon, Thierry & Avrameas, 1976). All the results were confirmed by the radioimmunological method (PRIST, Phadebas). This latter method makes it

possible to measure IgE levels lower than 100 u/ml. The concentrations are expressed in u/ml of serum, according to a WHO standard serum.

IgE specific for D. glomerata

The level of specific IgE was measured by the RAST method on undiluted sera (Johansson, Bennich & Berg, 1971). For the time course or the treatment evolution studies, the sera collected at various times in one individual were all titrated in a single experiment. Results are expressed as a percentage of the total input radioactivity.

Results

Specific IgE, total IgE and histamine release in controls and in patients before immunotherapy

In preliminary experiments, we examined the total IgE, the specific IgE and histamine release in the controls presenting no clinical symptoms (Table 1a).

Table 1b shows the similar characteristics in nine patients with hay fever and presenting positive skin test reactions. Samples of blood were examined in the pre-pollen period, before treatment started, or in the first month of the hyposensitization treatment.

The amount of histamine released, and the amount of allergen required to release 50% histamine from the blood leucocytes were established for each of the patients by two independent dose-response histamine release experiments. For each experiment, five ten-fold serial dilutions of the allergen were performed. The results presented in Table 1 are obtained from the mean dose-response curves.

Development of specific IgE, total IgE and histamine release in control No. 5

The variation of the *in vitro* parameters were followed up in control No. 5, who presented no clinical symptoms and thus was not treated.

Fig. 1 shows the results from person No. 5 of five histamine release experiments. Two of the assays were made out of the pollen season and three during the pollen season. In two out of the five experiments, we confirmed the presence of an inhibition portion of the curve due to an excess of allergen; the ascending part alone being sufficient for the determination of cell sensitivity to allergen. The sensitivity varied between a 5×10^{-8} and 5×10^{-7} concentration of stock allergen.

Fig. 2 presents the cell sensitivity to allergen variation for control No. 5 and the evolution with time of specific and total IgE levels. An increase in total and specific IgE levels was observed from the end of the pollination season and during the post-pollination season. The histamine release values at a fixed allergen concentration vary between 56% and 37% during the 1 year period of observation.

Evolution during treatment of specific IgE, total IgE and histamine release in hay fever patients with a low IgE level

Fig. 3 shows the development during immunotherapy of specific and total IgE levels, and the variation of the cell sensitivity to allergen for patients Nos 6, 8, 10 and 14, presenting low total IgE levels (Table 1).

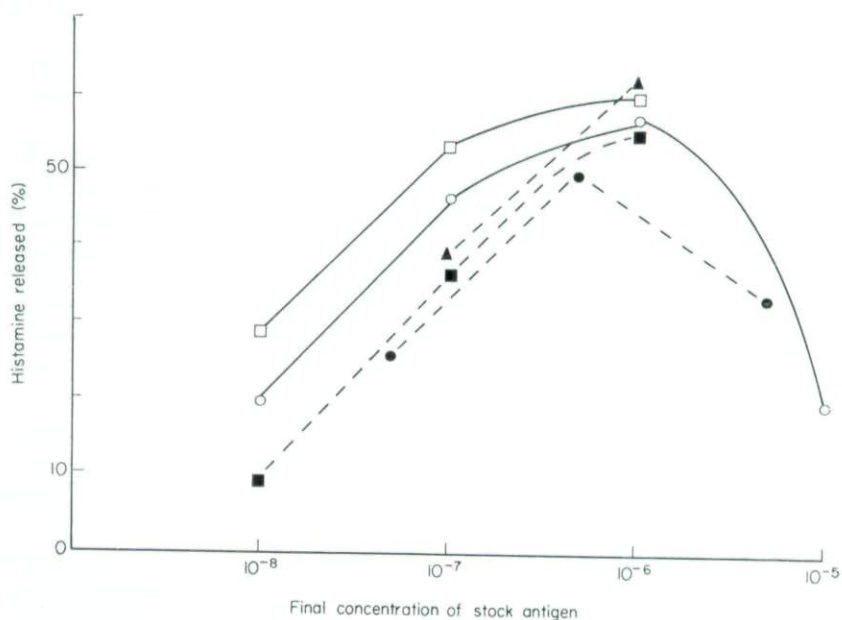


Fig. 1. Histamine release results in (●, ■, ▲) and out of (○, □) the pollen season for control patient No. 5 (see Table 1a), presenting positive *in vitro* tests but without clinical symptoms.

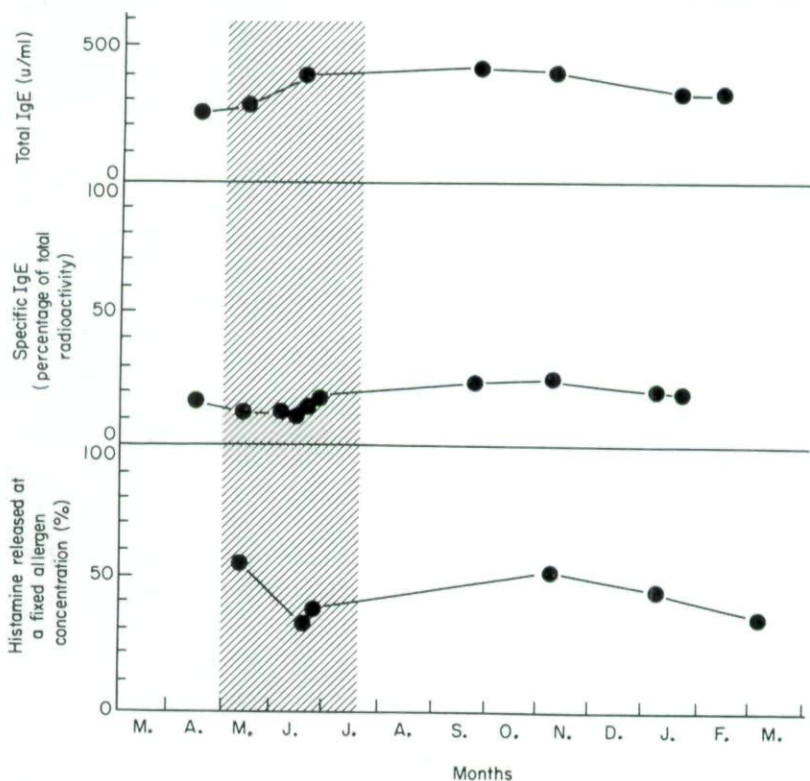


Fig. 2. Total IgE, specific IgE and cellular sensitivity in control No. 5 (see Table 1a) over a 1 year period. Hatched area shows pollen season (the allergen concentration for the histamine release was 10^{-7}).

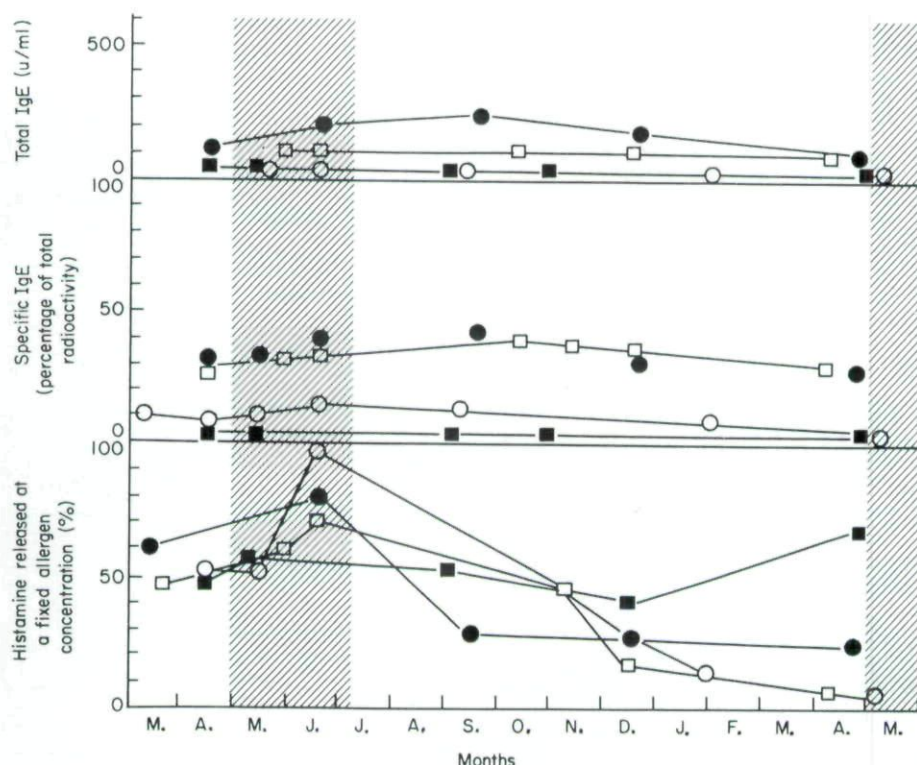


Fig. 3. Evolution of total IgE, specific IgE and cellular sensitivity in patients Nos 6, 8, 10 and 14 (see Table 1b) over a 1 year period of immunotherapy. The cumulative doses after the 1 year treatment are respectively 2.5 ml, 30 ml, 9 ml and 3.5 ml of the allergen extract (see the Materials and Methods section). Patient number (see Table 1b) and concentration of stock allergen used for histamine release experiments are respectively: (○) No. 6 at 5×10^{-8} ; (■) No. 8 at 5×10^{-7} ; (□) No. 10 at 10^{-8} ; (●) No. 14 at 5×10^{-9} . Hatched area shows pollen season.

An increase is observed for the specific IgE values in patients Nos 10 and 14 and for the total IgE values in patient No. 14.

Three of the four patients (6, 10 and 14) show an increase in cell sensitivity to allergen during the pollen season and afterwards a diminution of histamine release values, which decrease below significant 25% after a treatment of 6 months.

Symptom score

Clinical scores were compared between the first and the second pollination period. The four treated patients presented an improvement during the second pollen season.

Discussion

In the controls shown in Table 1a specific IgE was not detectable by the method used, except for person No. 5. The total IgE levels of controls Nos 1, 2, 3 and 4 are in the range of total IgE levels in a normal population (Nye *et al.*, 1975).

Similarly, the maximum percentage of histamine released (cellular reactivity) is lower than 25% (the value taken as the threshold for significant histamine release), except for person No. 5, who presented a maximum histamine release exceeding 50%.

Table 1. Specific IgE, total IgE, cellular sensitivity and reactivity in five controls and nine hay fever patients

Patients	Specific IgE (percentage of total radioactivity)	Total IgE (u/ml)	Cellular reactivity (maximum percentage of histamine released)	Cellular sensitivity (allergen dose inducing 50% histamine release)
(a) Controls				
1	1	5	7	—
2	1	25	12	—
3	1	10	0	—
4	1	15	0	—
5	19	220	55	10^{-7}
(b) Patients				
6	10	30	91	5×10^{-8}
7	16	500	83	10^{-8}
8	4	45	74	5×10^{-7}
9	36	370	55	10^{-8}
10	30	110	71	10^{-8}
11	28	310	68	10^{-8}
12	4	400	46	10^{-6}
13	3	300	35	—
14	31	100	100	5×10^{-9}

thus enabling us to determine the cell sensitivity to allergen (minimal dose of allergen provoking a 50% histamine release).

Person No. 5 had a positive skin reaction to grass pollen (prick test), so we investigated the possibility that serum factors inhibited the clinical symptoms in this person, but we did not observe any inhibition in the histamine release experiments by pre-incubating the autologous serum at a final 10% concentration with the allergen, before the histamine release experiment (May *et al.*, 1970).

There is a similarity between this case and the positive *in vitro* test of allergen-immunized non-allergic volunteers showing no clinical manifestations (March, Lichtenstein & Norman, 1972).

The determination of precise values of cell sensitivity to allergen requires three-fold serial dilutions, and thus large blood samples are necessary (Norman, Lichtenstein & Ishizaha, 1973). To save blood for serum as well as for cellular investigations, the dose of allergen inducing 50% histamine release was estimated by intrapolation in histamine release experiments with ten-fold serial dilutions of allergen. The results are shown in Table 1. The variation of the percentages of histamine released at this latter allergen concentration was followed during time or immunotherapy (Figs 2 and 3). An assay with a ten-fold more concentrated allergen was made to ensure that the corresponding percentage of histamine released was still on the ascending part of the dose-response curve.

In order to follow the variations of those biological characteristics during the treatment, we took into account the total IgE levels and the cell sensitivity to allergen of the patients: as has been reported, a high total IgE level often denotes sensitization to several antigens (Stenius, Wide & Seymour, 1972). Choosing patients Nos 6, 8, 10 and 14 (Table 1b), in whom the total IgE level was less than 250 u/ml, we believed

that there would be little interference by other sensitizing allergens besides pollen in our study of the *in vitro* characteristics during the pollen treatment. Those same patients displayed, moreover, a relatively high cellular sensitivity.

The increases observed in the specific IgE values in patient Nos 10 and 14 and in total IgE values in patient No. 14 (Fig. 3) are similar to that reported in Fig. 2 for person No. 5, and it is difficult to ascertain whether seasonal changes or treatment are responsible for these variations.

The cell sensitivity to allergen decreased significantly after treatment for 6 months in patients Nos 6, 10 and 14 (Fig. 3), as seen in other tests on patients treated with various allergens (Levy *et al.*, 1971; May & Aduna, 1971). In these same three patients we observed a parallel change in the specific IgE and cellular histamine release. For patients Nos 10 and 14, however, the decrease in the percentage of histamine released in the post-pollination season preceded the decrease in the amount of specific IgE.

Further comparative studies on the chronology of cellular and serological events occurring in selected patients are being undertaken in this laboratory.

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