

The accumulation of eosinophils as an allergic response to allergen applied to the denuded skin surface

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Reports that an accumulation of eosinophils occurs approximately 24 hours after the application, in pollens-sensitive patients, of pollen extract to the superficially denuded skin were confirmed. The response was seen in patients having clinical sensitivity to grass and ragweed pollen and was absent in many patients exhibiting skin reactivity only. The intensity of the eosinophil response appeared to be correlated with the degree of clinical sensitivity. The responsiveness was transferred with difficulty to normal skin by repeated injections of serum from a clinically pollen-sensitive donor. A striking fall in eosinophil responsiveness was observed among six patients receiving therapeutic injections of pollen extract. Attempts to confirm reports that the application of histamine to denuded skin sites calls forth an eosinophil response were unsuccessful.

Knowledge of the role played by the eosinophil leukocyte is meager. Eosinophils accumulate in areas containing antigen-antibody complexes^{1, 2} and it has been demonstrated that sensitized erythrocytes³ and mast cell granules⁴ may be ingested by this cell. More recently it was shown with the electron microscope⁵ that ferritin-antibody complexes are ingested by eosinophils in peritoneal exudates obtained from immunized guinea pigs. Finally, an antihistamine role has been postulated for this cell.⁶

With the use of a "skin window" technique to study the inflammatory response,⁷ it was observed that an accumulation of eosinophils and basophils followed the application of diphtheria toxoid, Rhus oleoresin⁸ and staphylococcus organisms, or an extract made therefrom⁹ to the superficially denuded skin. In the second study⁹ responses were seen more frequently in patients with furunculosis and some other skin conditions than in normal controls. Eidinger, Raff, and Rose¹⁰ used this technique to study the tissue fluid response in

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untreated atopic patients exhibiting immediate type of hypersensitivity to fish extract, grass, and ragweed pollens. They found that the cellular response of allergic individuals included abundant eosinophils whereas the control sites exhibited the normal progression of cellular response. They were also able to confer eosinophilotactic activity on the skin of nonallergic individuals by the passive transfer technique (Prausnitz-Küstner).

The study to be described confirms the occurrence of a local eosinophilotactic response to applied pollen extracts in selected subjects and indicates that the response is related to the clinical status of the subject.

MATERIALS AND METHODS

Patients were selected from the Allergy Clinic of the Massachusetts General Hospital. Group I consisted of 13 patients who entered the Allergy Clinic of the Massachusetts General Hospital in the summer of 1963. None were included who had dermatological disease, who had received injections of allergenic extracts in the past, or who were over 60 years of age. With these exceptions, the patients were unselected. All had allergic rhinitis, and five had asthma in addition. Group II consisted of 23 patients in a "double blind" assessment of injection treatment.¹¹ These patients had been followed for one or more years in the Clinic, and were known to be clinically sensitive to ragweed pollen. For 11 of these patients, treatment with ragweed pollen extract had been stopped in March, 1963, seven or more months before the studies were made. All of these had been under perennial treatment with timothy pollen and some other extracts.

Areas of the skin on the volar surface of the forearms or the lateral surface of the thighs were shaved, scrubbed with pHisoHex, rinsed with sterile saline, and allowed to dry. Sites were prepared by scraping away the superficial layers of the skin with a sterile No. 10 Bard-Parker blade until a sheen of tissue fluid was noted. Care was taken not to cause bleeding. Each site was gently wiped free of debris with sterile gauze and saline. Ragweed and timothy pollen extract 1:10 weight by volume, and saline containing 0.5 per cent phenol (the extracting and diluting solution) were applied to different sites in duplicate, symmetrically placed on opposite limbs not less than 6 cm. apart. A sterile round glass cover slip, backed by an adherent labeled piece of cardboard, was then placed on the site and bound down with adhesive tape. Upon removal 20 to 24 hours later, the cover slips were air dried, stained with Wright's stain, and mounted on microscopic slides. The cover slips were identified by number only, and all preparations were examined "blind" so as to avoid the influence of bias.

Venous blood was drawn immediately before the procedure from all patients and immediately afterward from over one-half of the patients. A total eosinophil count, total white count, and differential count were done on each sample.

The distribution of cells was usually uneven and cells were often crowded together. No satisfactory method of determining the percentage of eosinophils in the *entire slide* could be devised. We, arbitrarily, elected to scan the preparation to locate the countable areas containing the greatest relative number of eosinophils and to determine the percentage from a total of 500 cells counted.

The preparations were examined with the high dry objective. The aggregation of eosinophilic cells on the glass surface will be referred to hereafter as the eosinophil response.

Ragweed and timothy pollen extract were chosen as test antigens because the seasons in which these pollens are prevalent are sufficiently distinct to permit correlation of the response with the patient's clinical history.

RESULTS

Striking eosinophil responses were repeatedly seen in tests done in atopic patients sensitive to timothy and ragweed pollens. A study of the time course of this response confirmed reports by others⁸⁻¹⁰ that the maximum accumulation of eosinophils occurs 18 to 24 hours after application of the allergen. In general, the more concentrated the allergen applied, the more intense was the response. Very intense responses were obtained when pollen grains were applied rather than pollen extract. In these preparations, enormous numbers of ruptured eosinophils and free eosinophilic granules were seen filling the spaces between the pollen granules. The relationship between the level of sensitivity in the patient and the concentration of allergen required to elicit an eosinophil response will require study.

The clinical relevance of skin tests in Group I as related to the eosinophil response is shown in Fig. 1. The ordinate gives the eosinophil response in per cent in tests done with saline and the two extracts. Responses obtained with

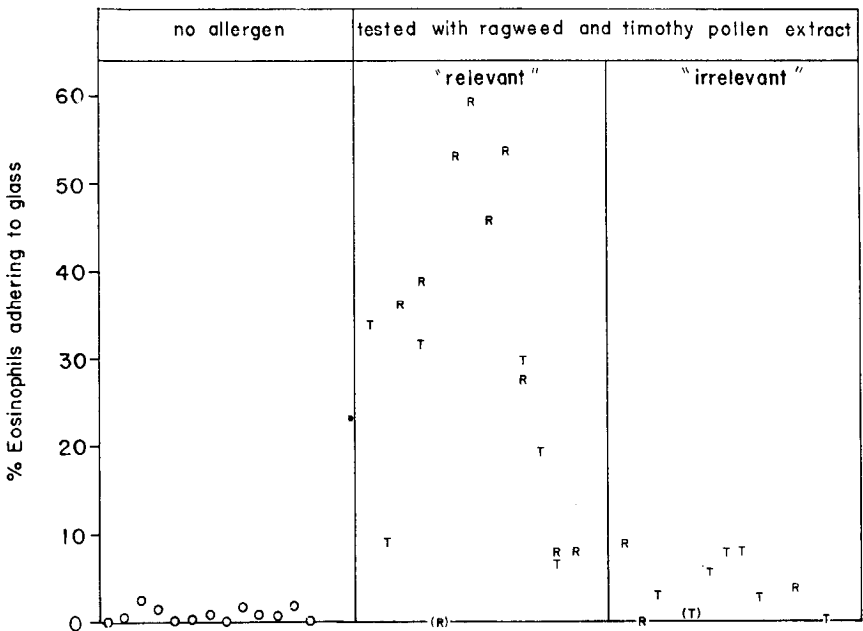


Fig. 1
Relation between the intensity of the eosinophil response and the clinical history in Group I. (See text.)

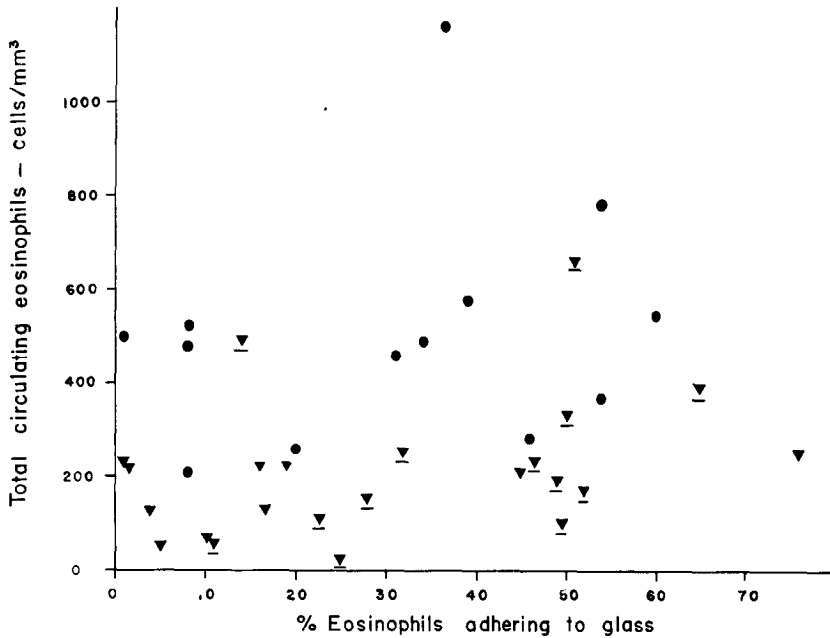


Fig. 3

Relation between the eosinophil response and the number of eosinophils in the blood. Each symbol represents a patient. Dots, Group I. Triangles, Group II. Points representing patients who were untreated (see text) are underlined.

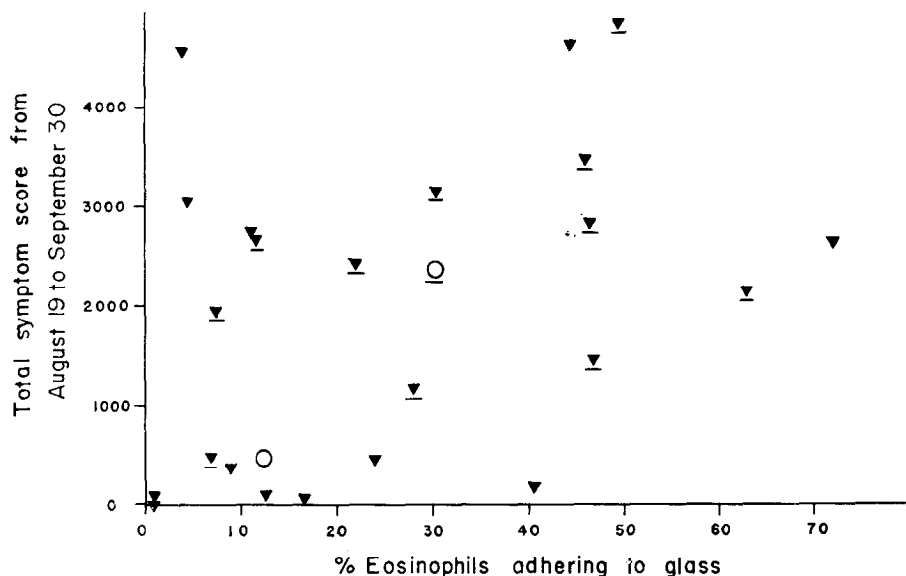
the selection of these patients was clinical sensitivity to ragweed pollen. Six were considered to be clinically sensitive to timothy pollen as well. Intense eosinophil responses were more frequent with ragweed than with timothy pollen extract as expected and, in general, the more intense responses occurred in those in whom treatment with ragweed pollen extract had been stopped.

A relation was sought between the intensity of the eosinophil responses and the level of eosinophils circulating in the peripheral blood. A total of thirty-six eosinophil counts was made in Group I and Group II immediately before the tests were applied. As is evident from what has already been presented, the eosinophil responses ranged widely (0 to 70 per cent). As shown in Fig. 3, there was little correlation between the intensity of the eosinophil response and the level of circulating eosinophils.

Among the control tests and among those done with the two pollen extracts in which the eosinophil response was less than 10 per cent there was, likewise, no relation between the eosinophil response and the level of circulating eosinophils.

Other than an elevated percentage of eosinophils in smears of the peripheral blood, the differential counts and total white counts were unremarkable.

Fig. 4 shows the total symptom score¹⁰ of patients in Group II as related to the eosinophil response to ragweed pollen extract. A relationship between the intensity of symptoms and the intensity of the eosinophil response is seen.

**Fig. 4**

Relation between intensity of symptoms during the ragweed season and the eosinophil response in Group II. Points representing patients who were untreated (see text) are underlined. The open circles represent medians for the treated and untreated (underlined) groups.

After examining all the preparations obtained in patients in Group II, but before the code was broken, an arbitrary assumption was made that patients giving eosinophil responses which exceeded 20 per cent fared relatively poorly during the ragweed season and those giving response below 20 per cent relatively well. An assessment of each patient's course was made by other physicians without knowledge as to the eosinophil response or whether the patient had or had not been treated with ragweed pollen extract and, based on the above assumptions, the manner in which the patient fared was correctly described in 21 of 23 patients.

Thus the relationship between the intensity of the eosinophil response on the one hand and the level of clinical sensitivity to a pollen on the other appears to be quite striking. It is possible, however, that rather than reflecting *clinical sensitivity*, the eosinophil response is rendered less intense by treatment of the patient with injections of allergenic extract because among these patients (Group II), those having fewest symptoms were, by and large, those who had received injections of ragweed pollen extract.

Six hitherto untreated patients were selected during the period September, 1963, to April, 1964, in order to determine the effect of weekly injections of pollen extract on the eosinophil response. Each patient gave a history of seasonal rhinitis, and exhibited intense skin reactions to ragweed pollen, timothy pollen, or both. All gave an eosinophil response exceeding 20 per cent (range: 23 to 56 per cent) to ragweed pollen and one gave such a response to timothy pollen as well. In this study, the identity of the mounted and stained preparation was not withheld in the course of estimating the response. A total of 40 tests for

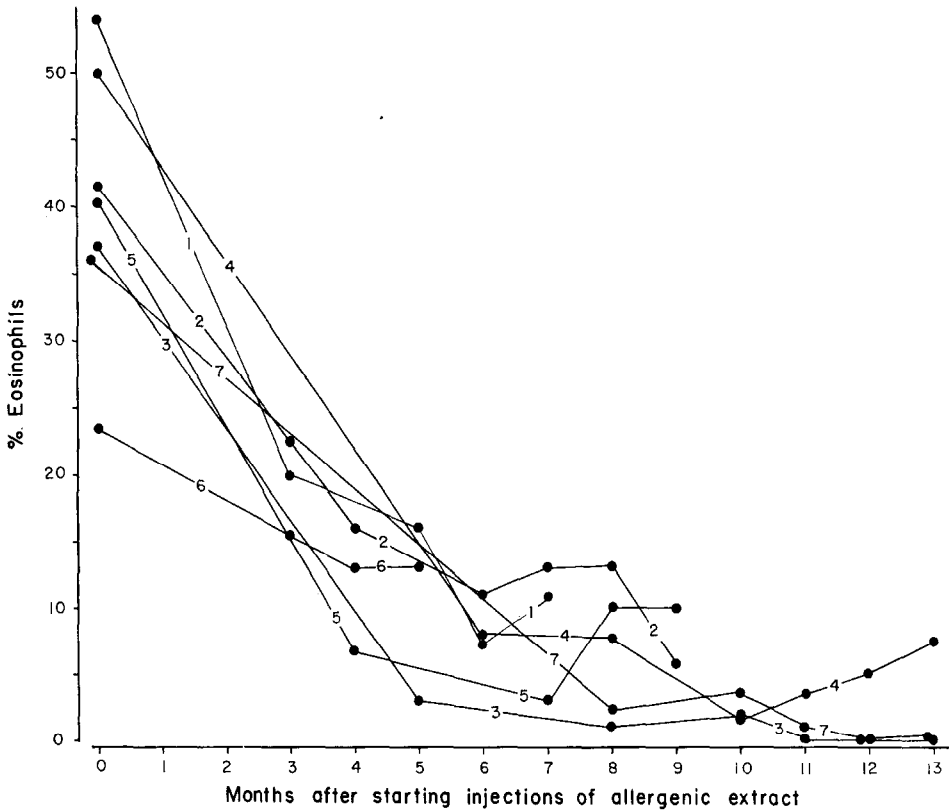


Fig. 5

Changes in the eosinophil response among six patients receiving injections of allergenic extracts. All patients received weekly injections of ragweed pollen extract. All had exhibited an initial intense eosinophil response to ragweed pollen extract. One patient had such a response to both ragweed (curve 3) and timothy (curve 7).

eosinophil responsiveness was made in succeeding months, not less than four in any one patient. As shown in Fig. 5, a progressive decline in responsiveness was observed in all instances, becoming clearly manifest within 3 to 5 months after starting the injections. At this time a cumulative dose of 0.1 c.c. or more of ragweed or timothy pollen extract 1:100 weight by volume had been given. All these patients fared well clinically. Among five patients treated for 8 months or more, the response fell to 12 per cent or less and four exhibited one or more responses of 4 per cent or less. These four patients had virtually no symptoms during the pollen season.

Recently it was reported that an eosinophil response occurs when histamine in a concentration of 100 μ g per milliliter is applied to the denuded epidermis and the cover slip is removed at 2 to 4 hours.¹² Attempts to confirm this were unsuccessful in five patients from Group I who were tested in duplicate with the above concentration of histamine. Saline controls were included as was also a test with ragweed pollen extract as a positive control. The tests with histamine were indistinguishable from the controls when removed at 4 hours, whereas

responses were obtained in each case with the pollen extract at 24 hours. Results were likewise negative when concentrations of histamine up to 10 mg. per milliliter were applied and the cover slips removed at 24 hours.

Preliminary attempts to confirm reports by others^{10, 12} that the eosinophil response could be conferred on the skin of nonatopic subjects, known to be a good recipient, with intracutaneously injected serum from sensitive (atopic) individuals were at first unsuccessful. However, a weak response was finally obtained in duplicate sites prepared with 0.2 c.c. serum from an intensely grass pollen-sensitive individual, injected three times (total 0.6 c.c. per site) at 6 to 12 hour intervals. The allergen (timothy, pollen extract 1:10 weight by volume) and cover slips were applied 24 hours after the final injection and the cover slips were removed 22 hours later. Although the cellular response (chiefly macrophages) was intense, there were only 8 per cent eosinophils. As judged by the intensity of itching and edema after the allergen was applied, a very high degree of local sensitivity had been induced by the injection of the unusually large amounts of serum. The donor of the serum consistently gave an eosinophil response of 30 per cent or higher with the same allergenic extract.

In general the cellular response following application of the appropriate allergen to the denuded skin of allergic subjects was much more intense than that in the controls without allergen or that in normal subjects with allergen. A positive response could often be predicted by mere inspection of the slide before or after staining. Since eosinophils rarely exceeded 40 per cent of the cells making up the exudate, their appearance on the slide was only one aspect of the response. Cells in some areas of most slides were neutrophils and the number of eosinophils in such areas was often small or they were absent altogether. The predominant cells in other areas, especially in those containing eosinophils, were mononuclear, contained a clear cytoplasm and a large, usually bean-shaped pale-staining nucleus, and were believed to be macrophages. Large accumulations of these cells were occasionally seen in the absence of an eosinophilic response.

In the course of assessing the eosinophil response, a large number of basophilic leukocytes and free granules were noted in some of the preparations done with pollen extracts. Especially intense accumulations of basophils were seen in two subjects recently arrived from Europe, who had suffered severe delayed local reactions to mosquito bites. They also exhibited a delayed but no immediate reaction to intracutaneous tests with mosquito extract. In one of these subjects, the application of mosquito extract elicited both an eosinophil and basophil response and, in the other, there were 29 per cent basophils and only 1.6 per cent eosinophils. In this subject, a control site to which saline was applied gave 1.2 per cent eosinophils and no basophils. Although we believe that these are basophils and not mast cells, this point remains in doubt because of the lack of cytologic detail in many of the preparations.

DISCUSSION

There appears to be a relationship between clinical sensitivity to ragweed or timothy pollen and the eosinophil response although the intensity of the

response varies widely. All patients having an eosinophil response had an intense reaction by scratch test with a 1:10 concentration (weight by volume) to the pollen extract. Patients reacting by scratch test who did not exhibit symptoms on seasonal exposure failed to give a response or responded at a low level. Although this suggests that intense skin reactivity may occur in the absence of an eosinophil response, the scratch test is not an adequate quantitative measure of skin reactivity and the relationship between this and the response will require end point titrations before a final conclusion can be drawn.

The concentration of the allergenic extracts used herein was 1:10 weight by volume. In a few experiments, more dilute extracts (1:200 and 1:2,000) gave less intense responses. On the other hand, tests done with grains of pollen thinly scattered on the denuded area of skin gave extremely intense reactions. Thus the concentration of extract used is a determining factor in obtaining the response. The relationship between the intensity of the response and the concentration of extract required to produce it will probably need careful study before the full significance of the eosinophil response can be determined.

Injections of pollen extracts, as in conventional specific treatment of pollinosis, were followed by a progressive reduction in the intensity of the eosinophil response and this reduction became manifest within a few weeks after the injections were started. Such injections give rise to both blocking¹³ and hemagglutinating antibodies,¹⁴ and since these would be expected to form antigen-antibody complexes at the test site, a recognized eosinophilotactic stimulus,^{1, 2} an enhanced rather than a diminished response, might have been expected.

Published data bearing on the capacity of skin-sensitizing antibody to confer eosinophil responsiveness on normal skin is scarce. Our own limited experience indicates that, although such transfer is possible, it is difficult to achieve, and the responses are not intense. Thus, the absence of eosinophil responsiveness in many skin-sensitive patients and the marked diminution of responsiveness observed among patients receiving therapeutic injections of allergenic extracts raises a question as to whether the presence of circulating skin-sensitizing antibody alone, accompanied by skin reactivity, is sufficient to establish eosinophil responsiveness.

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