

Diminution of the eosinophilotactic response, a specific effect of injections of allergenic extracts

Carlton I. Halle, M.D., and Francis C. Lowell, M.D., Boston, Mass.,
with the technical assistance of Marsha Williams

The eosinophilotactic response was observed in seven untreated patients with pollinosis who appeared to be clinically sensitive to two pollens. The subjects reacted intensely by skin test to extracts of both pollens to which they also gave a marked eosinophilotactic response. Each patient received a course of injections with one of the two extracts giving the responses described and, after three to four months, the tests were repeated. A highly significant decrease in the eosinophilotactic response was observed in tests with those extracts given by injection, whereas no significant change was seen in tests done with the extracts withheld. It is concluded that the decrease observed is a specific response to injection therapy.

Studies have shown that an intense local eosinophilia can be produced when the appropriate pollen extract is applied to the abraded skin of an individual with seasonal hay fever or asthma.¹⁻³ This eosinophilotactic response appears to be better correlated with clinical sensitivity to pollen than either the scratch or the intracutaneous test,³ although this point has not been adequately studied.

Earlier uncontrolled observations³ indicated that conventional injection therapy with aqueous ragweed pollen extract in selected patients brought about a reduction in the intensity of this response. This observation seemed of sufficient interest to warrant re-examination under conditions of experimental control.

METHODS

Seven patients were selected who had seasonal asthma or rhinitis and positive scratch and intradermal skin tests to the appropriate pollen, and who further exhibited striking eosinophilotactic responses to extracts of two or more pollens.

From the Departments of Medicine, Harvard Medical School, and Massachusetts General Hospital, Allergy Unit.

Supported by United States Public Health Service Grant 5T1 AI 22-07.

Received for publication May 24, 1966.

The procedure used entailed the removal of the superficial layers of the skin to a point just short of bleeding, the application of pollen extract (1:10 weight by volume), and the placement of a cover slip on top, which was taped in place and removed 20 to 24 hours later. The adhering cells were then stained with Wright's stain and examined under the microscope. All 7 patients gave an eosinophilotactic response to ragweed pollen extract, and 5 also gave responses to timothy, and 2, to oak pollen extract. Timothy and oak pollen extracts were given in treatment, whereas ragweed pollen extract was withheld in order that this extract might serve as a control.

At the start and again at the end of the study, end-point titrations in the skin were done with serial tenfold dilutions of those extracts given and those extracts withheld in treatment. Extracts were freshly prepared at the start of the study in a concentration of 1:10 weight by volume and stored at 4° C. All dilutions were freshly prepared from these stock solutions on the day of testing.

No medication was taken on the days in which the end-point titrations or the tests for eosinophilotactic responsiveness were carried out.

The study was begun in February, 1965, and was continued in 5 of the patients until April and in 2 until May, 1965. Injections were given twice a week starting with a concentration of 1:10,000 weight by volume and progressing to a maximum single dose of 0.4 c.c. of 1:10 weight by volume when possible. The smallest cumulative dose was 1.6 c.c. of 1:100.

Eosinophilotactic responses were repeated in each subject with two pollen extracts, as were the end-point titrations at the end of the study. The cover slips were mounted on glass slides and labeled by code, and the percentage of eosinophils was determined without knowledge of their source. As in our earlier study, the area on each slide in which the eosinophilotactic response seemed most intense was selected, and the percentage of eosinophils was established by counting 500 cells.

RESULTS

In Fig. 1, the intensity of the eosinophilotactic response is indicated on the ordinate and the time elapsed is indicated on the abscissa. On the left half of the figure are given the results obtained with that pollen extract (ragweed in each instance) which was withheld in treatment. As can be seen, there was a random change in the response. Some or all of the large changes seen may have been caused by deficiencies of the technique rather than by real changes in responsiveness of the subjects. In any case, there was little change in the means or the medians between the beginning and the end of the study. On the right half of the figure are shown the results obtained with oak or timothy pollen extract given in treatment. As can be seen, each subject exhibited a decrease in the response at the end of the period of treatment. This decrease in the response was highly significant ($p = <0.01$).

End-point titrations in the skin at the end of the study showed an across-the-board reduction of about one tube, that is, a tenfold reduction in end point. There was no difference in this respect between titrations done with extracts given in treatment and those done with extracts withheld. The reduction in end

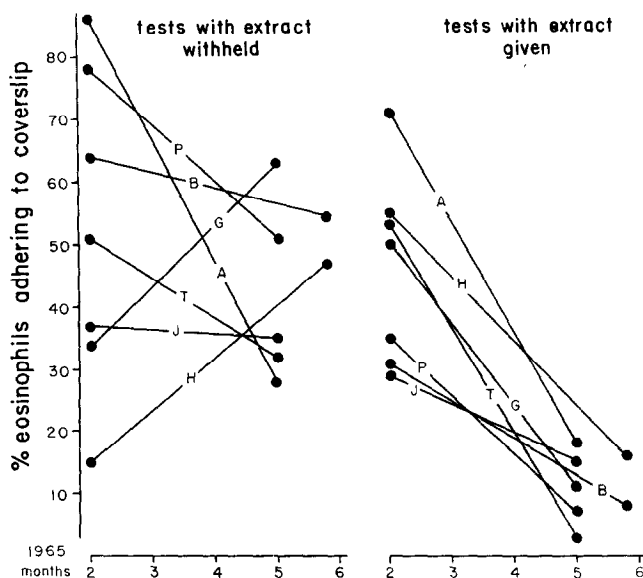


Fig. 1

Eosinophilotactic response with *extracts given* and *extracts withheld* in treatment. See text for explanation. (For method of statistical analysis, see G. Milton Smith: *A Simplified Guide to statistics for Psychology and Education*, New York, 1964, Holt, Rinehart & Winston, Inc., pp. 84-87.)

points is attributed to a falloff in activity of the extracts in the course of the 4 months that elapsed from the beginning to the end of the study.

DISCUSSION

It is unlikely that the observed difference in the eosinophilotactic response to the extracts given and extracts withheld can be explained as a peculiarity of ragweed pollen extract, which in each instance was the extract withheld, because, in the earlier uncontrolled study referred to,³ it was following treatment with injected ragweed pollen extract that the fall in response was first observed. Therefore, injections of all three pollen extracts, ragweed, oak, and timothy, can induce this fall in responsiveness which is in all probability a general phenomenon.

An attractive implication of these findings is the application of the test for eosinophilotactic responsiveness to determine the degree of clinical sensitivity, the need for injection therapy, and the effectiveness of injection therapy. We believe it is premature at this time to apply this procedure for these purposes before a more thorough study of its significance has been made. However, the possibilities seem promising. Future studies would be facilitated by a more quantitative means of eliciting the eosinophilotactic response than that used here and in earlier studies.

The striking eosinophilotactic response obtainable in atopic individuals and the decrease in this response induced by injection treatment quite naturally stimulate speculation as to what factors are involved in the eosinophilotactic response. The findings do not support some of the commonly held views; namely,

that the accumulation of eosinophils at the test site is merely a response to union of antigen and antibody, to the presence of antigen-antibody complexes,⁴ or to the presence of altered serum or tissue proteins.⁵ Although probably present at the test sites and in some degree eosinophilotactic, they do not adequately account for the observations. The reduction in the response following injections of allergenic extract demands the presence of an eosinophilotactic factor peculiar to the untreated atopic individual. Injections of extract are well known to induce production of antibodies, not only of more than one chemical type, but also with specificity directed to several different antigens in such extracts. Antigen-antibody interactions and antigen-antibody complexes would presumably be increased and skin reactivity would persist undiminished. Therefore, in the post-treatment state one might expect an enhanced rather than a decreased response. If we abide by the prevailing view regarding skin-sensitizing and blocking antibodies, then one may ascribe to skin-sensitizing antibody an especially intense eosinophilotactic capability, which is interfered with by the presence of blocking antibody induced by injections of allergenic extract.

REFERENCES

1. Eidingen, D., Raff, M., and Rose, B.: Tissue Eosinophilia in Hypersensitivity Reactions as Revealed by the Human Skin Window, *Nature* **196**: 683, 1962.
2. Eidingen, D., Wilkinson, R., and Rose, B.: A Study of Cellular Responses in Immune Reactions Utilizing the Skin Window Technique, *J. ALLERGY* **35**: 77, 1964.
3. Fowler, J. W., and Lowell, F. C.: The Accumulation of Eosinophils as an Allergic Response to Allergen Applied to the Denuded Skin Surface, *J. ALLERGY* **37**: 19, 1966.
4. Litt, M.: Studies in Experimental Eosinophilia. IV. Determinants of Eosinophil Localization, *J. ALLERGY* **33**: 532, 1962.
5. Cohen, S. G., and Sapp, T. M.: Experimental Eosinophilia. VIII. Cellular Responses to Altered Globulins Within Cutaneous Tissue, *J. ALLERGY* **36**: 415, 1965.