

STUDIES IN ABSORPTION OF RAGWEED ANTIGEN FOLLOWING THE INJECTION OF ALUM-PRECIPITATED PYRIDINE-RAGWEED COMPLEX IN MAN

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IN a previous study,¹ it was shown by means of the reverse passive transfer technique^{2, 3} that ragweed antigen is absorbed and released into the circulation following a repository injection of ragweed antigen emulsified in mineral oil.

The present study was undertaken to determine whether the absorption and release of ragweed antigen can be demonstrated following the parenteral administration in man of alum-precipitated pyridine-ragweed (APPR) complex.

MATERIALS, METHOD, AND PROCEDURE

A. *Antigen*.—The alum-precipitated pyridine-ragweed complex used in this study was kindly furnished in two concentrations, 10,000 and 50,000 PNU per milliliter, by Miss Margaret Strauss of the Allergy Laboratory, New York University, Bellevue Medical Center, who provided the following information about its preparation. Whole (non-defatted) pollen is extracted with pyridine and weak sodium hydroxide. The pyridine pollen extract is then precipitated with potassium alum solution and the supernatant portion containing excess alum, pyridine, and unconjugated and unprecipitated antigen is discarded. The precipitate is then washed four times and a nitrogen determination of the final product is made. The total nitrogen of the final product is practically identical with the phosphotungstic acid precipitable protein nitrogen since no nonprotein nitrogen is present.

B. *Serum*.—The reaginic serum used for skin testing in the reverse passive technique was obtained from a hay fever patient highly sensitive to ragweed. By the dilution method it was shown to have a reaginic titer of 1:1,536. Approximately 1/100 ml. of the undiluted serum was injected intracutaneously just prior to the depot injection of APPR and then daily thereafter for serial detection of ragweed absorption.

C. *Subjects*.—Four nonatopic subjects were carefully selected from among a large group of volunteers. Each subject gave a negative skin test with the undiluted reaginic serum. If the intensity of the reaction was found to be

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greater than a negative-plus (0) wheal with a faint circumscribed erythema (minimal reaction), the subject was not used.

D. Reverse Passive Transfer Technique.—The first 3 subjects received a deep subcutaneous injection in one arm of 20,000 PNU of APPR complex. The fourth subject received an injection of 40,000 PNU of APPR. The rate and duration of absorption of the ragweed antigen was studied by daily skin tests with the reaginic serum on the opposite arm in a definite pattern.

E. Study For Possible Sensitization.—Each subject was skin tested at onset of study and weekly thereafter with aqueous ragweed extract (0.1 mg. N per milliliter) to detect the possibility of experimentally induced sensitivity to ragweed antigen, as manifested by a delayed or immediate wheal reaction. Control tests with unrelated antigens (timothy, house dust) were also performed.

F. Direct Passive Transfer Studies.—Two additional nonatopic subjects were passively sensitized at numerous sites on the forearm with 1/20 ml. of the undiluted ragweed reaginic serum. Four days later the serum sites were tested with aqueous ragweed extract in dilutions of 0.00001, 0.0001, and 0.001 mg. N per milliliter, with APPR in isotonic saline in dilutions of 5, 50, 500, and 5,000 PNU per milliliter, and with several unrelated antigens.

G. Contralateral Passive Transfer Technique (Fernausslösung).—Three nonatopic subjects were injected intracutaneously on the forearms with 1/20 ml. of the undiluted ragweed reaginic serum. Four days later they received a deep subcutaneous injection in the opposite arm of 40,000 PNU of APPR complex. The serum site was carefully observed for the next 6 days for the presence of an antigen-antibody reaction as evidenced by erythema, wheal, or pruritus.

RESULTS

A. Technique of Reverse Passive Transfer.—Each of the 4 subjects who had received a depot injection of APPR was skin tested daily with undiluted reaginic serum for 45 days. The results were entirely negative, no reactions being obtained. After the forty-fifth day, one subject was given a subcutaneous injection of an aqueous ragweed extract (0.4 mg. N). Daily intracutaneous tests made with undiluted ragweed reaginic serum were positive for a period up to 10 days.

B. Possibility of Induced Sensitivity Following Depot APPR.—Skin tests performed with aqueous ragweed extract (0.1 mg. N per milliliter) on each of the 4 subjects employed in the above study failed to produce evidence of induced sensitivity of either delayed or immediate type. Control tests with unrelated antigens were also negative.

C. Direct Passive Transfer.—In both subjects, tests on the passively sensitized sites with aqueous ragweed extract in a concentration of 0.00001 mg. N per milliliter was positive (1+). Skin tests with ragweed extract 0.0001 and 0.001 mg. N per milliliter elicited correspondingly stronger reactions. Sites tested with timothy and house dust extracts were negative. No reactions were observed at sites tested with the various concentrations of the alum-precipitated

pyridine-ragweed complex up to that which elicited an irritative response (skin tests with 5,000 PNU per milliliter).

D. *Contralateral Passive Transfer*.—In this study, performed on 3 subjects, no evidence of an immunologic reaction could be detected.

DISCUSSION

The interpretation of the effectiveness of any form of therapy on a basis of clinical, subjective data is hazardous at best. In the evaluation of depot therapy for pollinosis, it is desirable to consider not only the effectiveness of the procedure but, also, the more basic mechanisms which are involved, including the possible sensitization potential. In a previous paper, in an attempt to clarify the controversial subject of repository therapy, the authors studied the absorption and release of antigen from a water-in-oil emulsion of ragweed pollen. They also investigated the possibility of active sensitization following a depot injection. It was shown by the reverse passive transfer technique that the ragweed antigen was released from a repository water-in-oil emulsion shortly after injection and could be detected thereafter in the circulation for a period as long as 35 days. Surprisingly enough, ragweed antigen was detected as long as 15 days following an injection of an aqueous extract.

Feinberg and co-workers⁴ have reported induction of both delayed and immediate hypersensitivity in several nonallergic individuals injected with a water-in-oil emulsion of ragweed pollen extract. Becker⁵ continued this study with similar findings. The authors¹ have also demonstrated induced hypersensitivity of both immediate and delayed type in 3 nonatopic subjects. Furthermore, in one atopic subject a minimal skin reaction to aqueous ragweed extract was intensified and subsequently was successfully transferred passively.

In the present study there was no evidence of active sensitization following depot injections of APPR. Moreover, it was not possible by various techniques employing a high titered ragweed reaginic serum (reverse, direct, and contralateral passive transfer) to demonstrate the release of ragweed antigen, as such, following a depot injection of APPR. A possible explanation for these results may be that ragweed antigen is not released in a free state but rather in a modified or conjugated form not detectable by the techniques employed in these investigations.

The authors could not confirm the findings of Fuchs and Strauss⁶ who reported the detection of ragweed antigen at specifically sensitized passive transfer sites for from 90 to 140 minutes following the injection of 5,000 PNU of APPR in a remote site. In the present study, too, it was not possible to detect ragweed antigen, even when APPR was injected directly in a site passively sensitized with a high titered ragweed reaginic serum.

In the experimental animal the following studies with alum-precipitated pyridine extracts have been reported. Kind and Roesner⁷ sensitized mice with alum-precipitated pyridine-grass complex and caused anaphylactic death following the intravenous injection of water-soluble grass extract. These authors also demonstrated significant red cell hemagglutination titers with the sensitized

serum by the bis-diazotized benzidine coupling method. Strauss⁸ sensitized rabbits with ragweed complex and the sera gave precipitin tests with aqueous ragweed extract.

Proper interpretation of the findings in human beings presented by the authors and of those in the experimental animal will await further study.

SUMMARY

1. By means of the reverse passive transfer technique it was not possible to demonstrate the absorption of free ragweed antigen from the site of injection of alum-precipitated pyridine-ragweed complex (APPR) in nonatopic subjects.

2. Direct skin tests with a strong ragweed extract in subjects injected with APPR failed to reveal induced sensitivity to ragweed antigen, as evidenced by the absence of delayed or immediate skin reactions.

3. Direct tests on specific ragweed serum-sensitized sites with APPR were negative, whereas tests with aqueous ragweed extract in comparable concentrations were strongly positive.

4. Skin sites in nonatopic subjects sensitized with a potent ragweed reagin-containing serum failed to detect free ragweed antigen following the injection with APPR.

REFERENCES

1. Grolnick, M., Pelz, H. H., and Chao, I.: Absorption Pattern of Ragweed Antigen and Active Sensitization In Man Following Repository Pollen Injection, *J. ALLERGY* **32**: 327, 1961.
2. Walzer, A., and Walzer, M.: Studies in Urticaria. I. Wheal Production Through Internal Channels, *Am. J. M. Sc.* **173**: 270, 1927.
3. Walzer, A.: A New Immunologic Wheal Produced by Reverse Technic, *Arch. Dermat. & Syph.* **38**: 1, 1938.
4. Feinberg, A., Feinberg, S., and Fisherman, E.: Repository Antigen Injections. II. *J. ALLERGY* **31**: 433, 1960.
5. Becker, R. J., Sparks, D. B., Feinberg, S. M., Patterson, R., Pruzansky, J. J., and Feinberg, A. R.: Delayed and Immediate Skin Reactivity in Man After Injection of Antigen in Emulsion, *J. ALLERGY* **32**: 229, 1961.
6. Fuchs, A. M., and Strauss, M. B.: The Clinical Evaluation and the Preparation and Standardization of Suspensions of a New Water-Insoluble Whole Ragweed Pollen Complex, *J. ALLERGY* **30**: 66, 1959.
7. Kind, L., and Roesner, L.: Toxic and Allergic Properties of Pollen Extracts in the Mouse, *J. Immunol.* **84**: 390, 1960.
8. Strauss, M.: Personal communication.