

Studies on the Antigenic Relationship of Insect Extracts by Reagin Neutralization

Evidence for Nonspecific Inactivation of Reagin

*Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., Arthur Kaminker, M.D., and
Leo N. Meleyco, M.D., Chicago, Ill.*

CLINICAL observations have indicated that the dust of disintegrated insects might be an important cause of inhalant allergy.¹ A report has previously been made of the antigenic relationships of extracts of several insects; in that study we employed hemagglutination and gel diffusion.² In attempting to demonstrate allergenic relationships between insects by passive transfer, we made certain observations which led to a more critical study of the mechanism of desensitization of passively sensitized skin sites. As a result of these investigations, some of the limitations of this method, previously observed in this laboratory,³ were confirmed and amplified, and evidence was obtained that a specific allergen-reagin reaction in the skin inactivated unrelated reagins that were present in the same site. Nonspecific wheals caused by histamine or compound 48/80 likewise resulted in apparent destruction of reagins at the site of reaction.

Bowman and Walzer⁴ have also reported that wheals produced by immunologic or nonimmunologic means would result in hyporesponsiveness of previously sensitized skin sites. Since tissue unresponsiveness to histamine was only temporary, they concluded, apparently by the process of elimination, that the irreversible hyporesponsiveness to allergens was due to reagin destruction. In the experiments reported here, it has been established experimentally that unrelated reagins were inactivated by an allergen-reagin reaction in the skin.

METHODS

Serum containing reagins to several insects, molds, and ragweed was mixed with an equal volume of buffered diluent. Intradermal injections of 0.05 ml. of the diluted serum were made into multiple sites on the arms and backs of non-atopic persons whose skin did not react to 1:10,000 dilutions of the allergens to

From the Allergy Research Laboratory, Department of Medicine, Northwestern University Medical School.

This investigation was supported in part by Research Grant E-1226 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service, and Asthmatic Children's Aid, Chicago, Illinois.

Presented at the fifteenth annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9-11, 1959.

Received for publication Jan. 12, 1959.

be tested. Forty-eight hours later sensitized sites on the arms were titrated with 0.01 ml. of threefold dilutions of the test allergens, histamine or compound 48/80. The highest dilution producing a ++ to +++ reaction was selected for routine desensitization and subsequent testing, since this is the range of reactivity most sensitive to variations in experimental conditions. The reactions on testing were recorded as follows: +, erythema diameter less than 2 cm.; ++, a wheal of up to 3 mm. in diameter; +++, a 3 to 5 mm. wheal; +++++, a wheal of more than 5 mm.

Specific desensitization was accomplished by daily injections of 0.1 ml. of the selected dilution of allergen extract, the process being considered complete when a test dose of 0.01 ml. of the same dilution produced no reaction. This usually required two injections.* After desensitization was completed, a further 0.1 ml. of allergen was injected. Histamine and compound 48/80 were used to inactivate sites nonspecifically in the same way as described for allergen. At various times after the final inactivating injection, sites were challenged with 0.01 ml. doses of the selected dilutions of reagin or pharmacologic agent.

RESULTS

Previous work indicated that human skin might react normally again one week after depletion with compound 48/80. Several nonatopic persons were sensitized, depleted, and cross-tested eight days later. These preliminary experiments showed that all sites were hyporesponsive, regardless of whether they were tested with homologous or heterologous extracts or with histamine or compound 48/80. An extended time series was therefore planned to allow delineation of the specific and nonspecific phases of the reaction.

Three subjects were passively sensitized in eight vertical columns of eight sites each on the back and on several sites on the arms. Titration was carried out on the arm sites, and dilutions were selected as described earlier. Desensitization was then carried out as follows: rows 1 and 2, control sites, were not desensitized; rows 3, 4, and 5 were desensitized with alternaria extract; rows 6 and 7 with compound 48/80; and row 8 with histamine. Starting on the day after depletion, and at various times thereafter, sites were tested as follows: row 3, cricket extract; row 4, compound 48/80; row 5, histamine; row 6, cricket extract; row 7, compound 48/80; and row 8, cricket extract. After two or three weeks some control reactions had decreased in intensity. The next stronger solution of extract was found to produce a ++ or +++ reaction and was then used for testing.

The data obtained in one individual case are presented in Table I. The other two subjects reacted in an almost identical fashion. A definite time pattern can be traced. At first all skin reactivity was virtually abolished. In two to three weeks, sites depleted by any means became responsive to either histamine or compound 48/80, but completely normal reactivity was not usually observed for about twenty-one days. It is to be noted, in contrast, that there was a

*The sites thus desensitized were still partially reactive to histamine.

persistent impairment of response (up to seven weeks) to allergen in sites previously made unresponsive by immunologic (allergen) or nonimmunologic (compound 48/80 and histamine) means. The sites were not tested later than fifty-one days after inactivation, since by that time control sensitized sites had become much less responsive.

TABLE I. TESTING OF PASSIVELY SENSITIZED SKIN SITES AT VARIOUS TIMES AFTER INACTIVATION

TIME AFTER INACTIVATION	SITES INACTIVATED WITH:					
	ALTERNARIA	ALTERNARIA	ALTERNARIA	COMPOUND 48/80	COMPOUND 48/80	HISTAMINE
	THEN TESTED WITH:					
	CRICKET	COMPOUND 48/80	HISTAMINE	CRICKET	COMPOUND 48/80	CRICKET
One day	0	0	1+	0	0	0
One week	0	0	1+	0	0	0
Two weeks	0	1+	2+	0	1+	1+
Three weeks	0	3+	3+	0	3+	0
Four weeks	0	3+	3+	0	3+	1+
Five weeks	0	3+	2+	0	3+	1+
Six weeks	0	2+	2+	0	2+	*
Seven weeks	0	3+	3+	0	3+	0

*Technical error.

In three weeks, when tissue responsiveness had become normal, and at weekly intervals, some of the inactive sites were resensitized with the original reaginic serum and tested forty-eight hours later with allergen. Reactions of ++ to +++ were observed in each case. Similarly inactive sites injected with normal human serum did not respond to allergen. In this way it was demonstrated that reagin had been the only limiting factor in the "permanently" unresponsive sites.

DISCUSSION

The immunologic whealing response can conveniently be considered to occur in several steps: (1) allergen-reagin combination; (2) activation of mediator release mechanism; (3) release of mediator, if available; and (4) tissue response to released mediator. No skin reaction will be observed if any of these processes is inhibited. Shortly after desensitization, and for ten to twenty-one days, there was a reduced response to either histamine or compound 48/80 on any pre-treated site. It seems likely that the initial hyporesponsiveness was due to both the absence of available mediator and a refractoriness of tissue to any mediator that might have been released. After this time the sites were fully resupplied with mediator, and the responsiveness to mediator was again normal. In spite of this, all the variously depleted sites were still unresponsive to allergen. According to the above formulation, this persistent hyporesponsiveness could have been due only to either lack of allergen-reagin reaction or inhibition of the mediator release mechanism. Restoration of normal skin response to allergen after resensitizing the variously desensitized refractory sites demonstrated that the

original reagins had in some way been made unavailable for reaction with allergen. This, of course, does not point to the actual mechanism of inactivation of the reagin.

Several implications of these findings are of interest. The reliability of the passive transfer method using intradermal desensitizing injections for the study of allergenic relationship must be questioned. This criticism may not apply to the test tube "neutralization" technique. Antihistamines injected locally may help reduce wheal formation during desensitization of passively sensitized sites. The effectiveness of locally injected antihistamine on various whealing reactions has been discussed.⁵ Siegel, Bowman, and Walzer⁶ showed that antihistamines prevented the local reaction due to the sensitizing serum itself by addition to the serum of appropriate amounts of antihistamine. Our experience is that complete abolition of reactivity during desensitization would be very difficult to attain. At present there is being evolved a method for rendering sites temporarily unresponsive before sensitization and specific desensitization. Cross-testing is then delayed until normal tissue response is restored. These studies will be the subject of a future report.

The findings in the experiments reported here suggest other approaches to the study of the mechanism of the allergen-reagin reaction. The removal of reaginic activity by an extraneous allergen-reagin system or by a nonspecific agent, such as compound 48/80, must also suggest the possibility of a means of therapy. Exhausting a localized area of an undesirable symptom-producing reagin might possibly be achieved by such means, even if the allergen producing the symptoms were not known. The removal of reagins from the body as a whole, of course, is far less likely to be successful. Any of the suggested procedures would probably require continued treatment, since reagin is continuously being produced in actively sensitized persons.

SUMMARY

1. Skin sites sensitized with serum containing multiple unrelated reagins and desensitized with one allergen or treated with compound 48/80 fail to react to compound 48/80 or histamine for ten to twenty-one days.
2. Testing such sites with an unrelated allergen shows that all reaginic activity has been permanently lost at these sites.
3. That such loss is not due to tissue injury is demonstrated by the fact that the sites may be made reactive again after twenty-one days by resensitization with the original reaginic serum.
4. These findings emphasize the pitfalls inherent in some of the techniques of passive transfer in the study of allergen relationships. They also suggest certain other approaches to the study of antigen and antibody and a possible approach to therapy in some types of allergy.

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