

The reagin-allergen reaction in the skin without concomitant wheal and flare

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The serum of the clinically allergic individual has been shown to contain at least three types of antibody-like constituents on the basis of its ability to give a wheal and flare reaction upon challenge with the allergen, its capacity to "block" the reaction between reagin and the allergen by passive transfer, and its ability to agglutinate allergen-sensitized red blood cells.¹⁻³ These are referred to as reagin, blocking antibody, and hemagglutinating antibody, respectively. Furthermore, it has recently been demonstrated in this laboratory that sera obtained from allergic individuals (referred to henceforth as reaginic serum or allergic serum) also possess eosinophilotactic activity^{4, 5} as demonstrated by the skin window technique of Re buck and Crowley.⁶

Through the years, it has become tacitly accepted that the reagin-allergen reaction and the pharmacologically active products released thereby, which are implicated in the induction of the wheal and flare, are responsible for the totality of symptoms seen in clinical hay fever and asthma. Fundamental to this inference is the assumption that a similar reaction occurs on the surface of mucous membranes. Further evidence in support of this concept is the fact that almost all clinically allergic individuals are capable of giving a wheal and flare reaction when challenged intradermally with the allergen and that their sera can passively transfer this whealing capacity as demonstrated by the Prausnitz-Küstner or P-K reaction.

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This investigation was supported by grants from the Department of National Health and Welfare, Ottawa, Canada, and the National Institutes of Health, Institute of Allergy and Infectious Diseases, Bethesda, Md. (Grant No. E-1322).

Received for publication Feb. 12, 1964.

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During the past few years, experiments have been carried out in this laboratory which demonstrate that reagin may react with the allergen in the skin without being accompanied by the wheal and flare reaction. These experiments are described below.

MATERIALS AND METHODS

The reaginic sera used were obtained from well-documented cases of individuals who had hay fever or asthma and were clinically sensitive to ragweed pollen. The sera were kept at -10° C. until used.

The allergen preparation used was a solution of the water-soluble extract of defatted ragweed pollen (WSR) containing from 100 to 2,000 units per cubic centimeter (1 unit = 10^{-5} mg. N).⁷

Only normal nonallergic, healthy volunteers having no history of previous liver disorders and exhibiting no immediate reaction to the intradermal injection of homologous serum were used as recipients for the passive transfer tests.

In one series of experiments a number of sites were prepared in the skin of normal volunteers by the intracutaneous injection of 0.05 ml. of a solution of reaginic serum of constant concentration. The sites were challenged by the intradermal injection of the allergen solution at varying intervals of time thereafter—5 minutes, 20 minutes, 1 hour, 2 hours, 4 hours, and 24 hours. The immediate reactions observed were graded 1+, 2+, 3+, 4+, depending on the size of the wheal, the extent of the erythema, and the presence of pseudopodia (Table I).

In a second series of experiments, different sites were sensitized with 0.05 ml. of reaginic serum of varying dilutions, and these sites were challenged at intervals of time thereafter (Table II).

In a third series of experiments, the passive transfer test was performed in reversed fashion. Constant amounts of allergen were injected into a number of sites which were later challenged with reaginic sera of varying concentrations of reagin so that some sites were in allergen excess and others in reagin excess. The sites were subsequently reinjected with the allergen, and the reactions were recorded (Table III). The concentration of allergen and reagin required in this experiment had to be determined in independent experiments. The quantity of a solution of reaginic serum capable of neutralizing one unit of WSR by the passive transfer test was considered to possess one neutralizing unit of reagin.

All experiments were performed in duplicate and repeated with several different allergic sera in different volunteers.

RESULTS

As can be seen from Table I, no reaction could be elicited upon injection of the allergen into a site passively sensitized with reaginic serum one hour or less prior to challenge. Challenge of sites from one to four hours after sensitization resulted in reactions of increasing intensity. To ascertain whether or not the reagin in the initial sites was actually neutralized by the allergen although no wheal and flare reaction was observed, the sites were rechallenged

Table I. *Challenge of passive transfer sites with allergen at varying intervals after sensitization*

	Sites*						
	1	2	3	4	5	6	7
Time of first challenge with allergen after sensitization† (0.05 ml. per injection)	5 minutes	10 minutes	20 minutes	1 hour	2 hours	4 hours	24 hours
Size of reaction	0	0	0	1+	3+	4+	4+
Time of second challenge with allergen (hr.)‡ after sensitization (0.05 ml. per injection)	24	24	24	24	24	24	24
Size of reaction	0	0	0	0	0	0	0

*All sites were prepared by the intracutaneous injection of 0.05 ml. of the reaginic serum of a constant concentration (serum with a P-K titer of 1,000 was diluted 50 fold).

†The allergen solution was used in neutralizing concentration so as to provide conditions of allergen excess in the sensitized site. The concentration usually used was 1,000 units per milliliter.

‡The concentration of allergen used was 1,000 units per milliliter.

Table II. *Shorter latent period after sensitization with reaginic serum of increasing concentration*

	Site								
	1	2	3	4	5	6	7	8	9
Dilution* of reaginic serum used for sensitization (0.05 ml. per injection)	1:2	1:2	1:2	1:10	1:10	1:10	1:100	1:100	1:100
Time of challenge with the allergen† after sensitization (0.05 ml. per injection)	10 minutes	1 hour	4 hours	10 minutes	1 hour	4 hours	10 minutes	1 hour	4 hours
Size of reaction	1+	4+	4+	0	1+	4+	0	0	1+

*Titer of reaginic serum by passive transfer is 1,000.

†Concentration of allergen used was 1,000 units per milliliter.

about 24 hours later. No reactions were observed in any of the sites, thus demonstrating that the original injection of the allergen into the sites sensitized 5, 10, or 20 minutes *did* result in neutralization of the reagin in these sites, although no wheal and flare reactions were observed at that time.

As can be seen from Table II, the "latent" period, or period of time during

which the sensitized site is refractory in its capacity to respond with a wheal and flare reaction upon challenge with the allergen, is dependent on the relative concentration of reagin used for sensitization. Sites sensitized with the particular reaginic serum diluted only twofold gave a reaction even when challenged only 10 minutes after sensitization, whereas sites sensitized with the reaginic serum diluted 100 fold gave only a slight reaction when challenged 4 hours later.

Using the reversed passive transfer technique, challenge of the allergen-sensitized site with the reaginic serum diluted only twofold did not give a wheal and flare reaction (Table III). In fact, none of the sites developed wheal and flare reactions regardless of the absolute concentration of reagin or the relative concentration of reagin to that of the allergen in the site. However, that reagin-allergen reaction had occurred in all the sites is evident by the reactions observed upon subsequent reinjection of the allergen. Sites 1 through 4 (Table III), which had been initially injected with subneutralizing quantities of reagin, did not give subsequent reactions upon reinjection with the allergen; however, sites 6 through 9, which had originally been injected with an excess of reagin, gave wheal and flare reactions upon reinjection with the allergen.

Similar results were obtained with several other allergic sera used in this study.

DISCUSSION

Since 1921, when Prausnitz and Küstner² demonstrated that the sera of allergic individuals contain a factor capable of passively transferring the wheal and flare reactivity to normal skin (P-K reaction), it has been tacitly accepted

Table III. *Reverse passive transfer test. Absence of the wheal and flare reaction*

	<i>Sites "sensitized" with allergen (100 units/ml.) (0.05 ml. per injection)</i>								
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>
Time of injection of reaginic serum (hr.)	4	4	4	4	4	4	4	4	4
Concentration of reagin* injected (units/ml.) (0.05 ml. per injection)	10	20	40	80	100	200	400	800	1600
Reaction observed	0	0	0	0	0	0	0	0	0
Time of reinjection of allergen (1,000 units/ml.) after injection of reagin (hr.) (0.05 ml. per injection)	20	20	20	20	20	20	20	20	20
Reaction observed	0	0	0	0	0	1+	3+	4+	4+

*One unit of reagin is that quantity capable of neutralizing 1 unit of the allergen preparation (see text).

that reagin is the factor in the allergic individual which confers on him the clinical hypersensitive state as well. This supposition, however, has never been proved. Although it has been demonstrated that reagin can bind easily to skin,² its capacity to react with the allergen need not be contingent upon its fixation to skin sites. In support of this latter view, it has recently been demonstrated that reagin could indeed react with the allergen in vitro, as it could be removed from solution after incubation with red blood cells or polystyrene coated with the allergen.^{3, 8, 9} Furthermore, the experiments reported upon here imply that reagin can also react with the allergen in the skin without a resultant wheal and flare reaction, provided the challenge is made within a short interval of time subsequent to sensitization. It would thus appear that reagin must undergo some form of transformation or coupling to a skin site before its reaction with the allergen is capable of resulting in the release of pharmacologically active constituents capable of eliciting the wheal and flare reaction. This fixation of reagin to the skin factor appears to occur rather slowly, but it is dependent upon the concentration of reagin in the site—the greater the concentration of reagin, the more rapid the fixation of at least a portion of the reagin in the site. That fixation to skin does occur is suggested also by the recent work of Ishizaka and his associates,¹⁰ who demonstrated that the wheal and flare reaction could be inhibited by injecting the site with normal β_2A -globulin prior to sensitization with reaginic serum.

Work is in progress to find reagents which may inhibit or compete with reagin in its fixation to skin or mucous membrane and thus be capable of preventing the clinical condition of hay fever or asthma.

SUMMARY

It has been demonstrated that reagin may react with the allergen in the skin without evoking the wheal and flare reaction. The significance of this result and its relation to clinical allergy are discussed.

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