

A COMPARISON OF THE IMMUNOLOGIC RESPONSES OF NORMAL AND ATOPIC INDIVIDUALS TO INTRANASALLY ADMINISTERED ANTIGEN

J. E. Salvaggio, M.D., J. J. A. Cavanaugh, M.D., F. C. Lowell, M.D., and Sidney Leskowitz, Ph.D., Boston, Mass.*

IN a previous study,¹ it was shown that atopic and normal individuals responded similarly to immunization with dextran and pneumococcal polysaccharide in respect to development of precipitating antibody and skin reactivity. In addition, no differences in skin reactivity could be shown with use of the pharmacologic intermediates histamine and serotonin or the histamine releaser 48/80. From these results a working hypothesis was developed that the immunologic potential of atopic and normal individuals to parenterally injected antigens is similar and that in this case development of skin reactivity was dependent to some extent perhaps on the intensity of the stimulus, rather than on the atopic status of the patient and the possible genetic predisposition for producing skin-sensitizing antibody. It was further suggested that the essential difference between atopic and normal individuals lay in the mode in which antigen coming in contact with mucosal surfaces was handled. The present study was undertaken as a preliminary attempt to demonstrate such differences using a purified protein, bovine ribonuclease, as antigen and an intranasal route of immunization.

MATERIALS AND METHODS

Group Selection.—Three groups of 10 subjects each were used as follows:

Group I consisted of patients from the Allergy Clinic chosen on the basis of known seasonal rhinitis, clear-cut skin reactivity by scratch test to at least 3 common inhalant allergens of the Boston area, and age between 20 and 40 years. There was no evidence of atopic rhinitis or conjunctivitis in this group during the course of the study.

Group II consisted of normal "control" individuals with no past, present,

From the Department of Medicine, Massachusetts General Hospital, and the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

Presented in part at the Nineteenth Annual Meeting of the American Academy of Allergy, Montreal, Canada, March 16, 1963.

Supported by United States Public Health Service training grants 2E-22 and E-1289.

Received for publication July 19, 1963.

*Present address: Department of Medicine and Microbiology (Medical Immunology), Louisiana State University Medical School, New Orleans, La.

or family history of atopic disease and essentially no skin reactivity to the chosen inhalants.

Group III consisted of patients from the Allergy Clinic fulfilling the criteria of the first group.

Groups I and II were given an antigen weekly as described below over a prolonged period with skin testing and bleedings at regular intervals. Group III was skin tested only, to determine the effect of repeated skin testing per se on development of any subsequent immediate wheal-and-erythema reactions or skin-sensitizing antibody in atopic individuals.

Antigen.—Crystalline ribonuclease (RNase) from bovine pancreas, salt- and protease-free, was used.* It was chosen on the basis of its purity, low molecular weight, known antigenicity in animals,² and the small likelihood of previous or future contact with it by the individuals involved in the study. The antigen was dissolved in saline at a concentration of 1.0 mg. per milliliter.

The antigen was dispensed in a No. 40 DeVilbiss nebulizer, each squeeze of the bulb delivering approximately 0.04 ml. of fluid or 40 μ g of RNase. Those in groups I and II were given 3 intranasal inhalations in each nostril weekly for a 3 month period. Approximately 240 μ g of RNase was given per week, or a total dose of 3.0 mg. in 3 months. The amount of antigen actually absorbed was not determined. At 6 months, 7 patients from group I and 7 from group II were given "booster" inhalations of approximately 500 μ g RNase, followed, in 10 days by bleedings and intradermal testing.

Skin Tests.—Intradermal tests were done with approximately 0.02 ml. doses containing 0.1, 0.01, or 0.001 mg. of RNase per milliliter. The diameter of the areas of edema and erythema were recorded at 15 to 20 minutes. Saline controls were done in all cases. Delayed reactions at 24, 48, or 72 hours were traced on sheets of cellophane by the patients and the sheets returned to us for measurement.

Antibody Analysis.—Skin-sensitizing antibody was measured by the Prausnitz-Küstner method. Blood was collected in sterile tubes and allowed to clot. After standing overnight at 4° C., sera were harvested. Before use, the sera were centrifuged at 2000 r.p.m. for 1 hour. Approximately 0.05 ml. of serum diluted 1:10, 1:20, 1:40, 1:100, 1:500, 1:1000, and 1:5000 in saline was injected into the skin of a nonsensitive recipient. Twenty-four hours later, the sites were challenged with approximately 0.02 ml. of RNase solution in a concentration of 0.1 mg. per milliliter. This was the previously determined maximum dose that would not produce reactions in nonsensitized skin sites.

Reactions were read at 20 minutes, the reciprocal of the last dilution giving a definite positive reaction being recorded as the antibody titer. Saline controls were done in all cases.

Secretor Status.—Approximately 5.0 ml. of saliva was collected, frozen at -20° C., and later boiled for 10 minutes. Samples were analyzed for their capacity to inhibit agglutination in the anti-H (Ulex-lectin) and human O cell system.

*Obtained from Mann Research Laboratories, Inc., New York.

OPERATIONAL PROCEDURE

The following steps were done in the order indicated:

- A. Procedures on initial visit.
 1. Pre-exposure blood specimen drawn.
 2. "Scratch" and intradermal testing done with RNase solutions.
 3. Individuals in groups I and II given initial inhalations of RNase.
- B. Inhalations repeated weekly for 3 months in these 2 groups and any subsequent symptoms recorded.
- C. Monthly bleedings and intradermal testing with RNase done in all groups.
- D. "Booster" inhalations given to groups I and II at 6 months, with repeat bleeding and intradermal testing in 10 days.
- E. Saliva specimens collected in groups I and II for determination of secretor status.

RESULTS

Immediate Wheal-and-Erythema Reactivity.—In Table I are seen the results of skin tests at monthly intervals on all individuals in the study. Prior to immunization, 1 patient (No. 7 in group I) demonstrated skin reactivity to RNase which remained throughout the course of the experiment. It is of some interest that this patient developed severe rhinitis following the third exposure to antigen. An additional 3 patients in group III had slight reactivity consisting of about 4 to 5 mm. edema with little erythema, but all others in the study were initially nonreactive to the skin test dose used.

At 1 month immediate wheal-and-erythema reactivity was present in 6 of the 10 patients in group I. This pattern of response was maintained throughout the course of immunization, with slight over-all increases noted at the second, third, and sixth month. By the end of the third month, all but 2 of this group had developed skin sensitivity.

In contrast to group I, none of the control individuals in group II had developed skin reactivity after 2 months, and only 1 was positive after 3 months. Following the "booster" inhalations at 6 months, 2 of 8 tested in this group showed slight but definite immediate reactivity, while 4 developed typical delayed reactions.

Of the atopic individuals in group III whose only contact with the antigen was by skin tests, 3 showed initial skin reactivity, but in 2 this was not maintained through the first and second months. By the third month, the 3 initially reacting patients and 2 others showed slight but definite skin reactivity. Thus it appeared that, while skin testing itself was capable of producing immediate skin sensitivity in occasional atopic individuals, the degree of sensitivity produced was much less marked and required more time for development than that produced by aerosolized antigen combined with skin testing.

Delayed Skin Reactivity.—It was interesting that 4 of the 10 control subjects in group II (Nos. 1, 2, 3, and 8) developed dark red indurated reactions to RNase at 24 to 72 hours. These reactions appeared 6 months after the initial

exposure and were never more than 10 mm. in diameter. None demonstrated immediate skin reactivity at any time.

Skin-Sensitizing Antibody.—Only one patient (No. 7, group I) developed an appreciable titer of antibody as measured by the Prausnitz-Küstner technique. It is interesting that this patient also had marked symptoms after the inhalations and marked pre-exposure immediate skin reactivity to RNase. The antibody was demonstrable in a titer of 5000 but only in the post "booster" serum.

Two other individuals developed titers of 10 in the post "booster" serum (No. 1 in group I and No. 7 in group III). All other sera were negative.

TABLE I. IMMEDIATE SKIN REACTIONS TO RIBONUCLEASE DURING THE COURSE OF INTRANASAL IMMUNIZATION

GROUP AND PA- TIENT NO.	SKIN REACTION DURING COURSE OF STUDY				
	PRE-IMMUNI- ZATION	1 MONTH	2 MONTHS	3 MONTHS	AFTER "BOOSTER"
Group I					
<i>Patient</i>					
1	0	7ed*14er*	10ed35er	10ed35er	12ed35er
2	0	9ed14er	8ed28er	10ed35er	—†
3	0	10ed15er	9ed15er	9ed15er	—
4	0	7ed15er	15ed55er	10ed45er‡	11ed40er‡
5	0	0	0	0	0
6	0	0	0	0	—
7	6ed28er	9ed28er	10ed28er	§	10ed30er
8	0	0	0	9ed 9er	—
9	0	0	0	8ed 8er	11ed11er
10	6ed	12ed35er	14ed30er	14ed40er	16ed16er
Group II					
<i>Patient</i>					
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	5ed 7er
6	0	0	0	0	0
7	0	0	0	5ed12er	5ed25er
8	0	0	0	0	0
9	0	0	0	0	—
10	0	0	0	0	—
Group III					
<i>Patient</i>					
1	0	0	0	0	—
2	0	0	0	4ed15sl.er	—
3	0	0	0	0	—
4	0	0	0	0	—
5	5ed5er	0	0	4ed9er	—
6	4ed7er	0	0	4ed7er	—
7	0	0	0	0	—
8	0	0	0	0	—
9	0	0	0	6ed6er	—
10	5ed7er	7ed20er	7ed20er	5ed7er	—

Test dose: 0.01 to 0.02 ml. of 0.1 mg. per milliliter RNase solution.

*All reactions recorded at 15 to 20 minutes in millimeters erythema (er) and edema (ed).

†A dash indicates test was not done.

‡Patient tested with 0.01 mg. per milliliter RNase solution because of size of previous reaction.

§Patient not tested because of severe rhinitis after previous testing.

Symptoms.—Three patients, all in group I, developed nasal symptoms immediately following the seventh or subsequent exposure, as shown in Table II. The sequential relation among the degree of skin reactivity, the occurrence of symptoms, and the development of skin-sensitizing antibody is shown in Table III. In these 3 patients, skin reactivity appeared early, followed by symptoms on exposure approximately 1 month later. In 2 of the 3 patients, skin-sensitizing antibody was detectable only after 6 months.

Secretor Status.—As a preliminary test of a possible difference in the nasal mucosa between atopic and normal individuals, a study of secretor status was done. This is a genetically determined trait which controls the ability to release blood group mucopolysaccharides into exudates from many secretory organs. Of the 16 individuals tested, 5 in group I and 6 in group II were secretors. This coincides with the expected distribution, indicating no difference between normal and atopic individuals in this respect.

DISCUSSION

Previous work^{1, 3, 4} indicates that an altered reactivity to pharmacologic intermediates released by antigen antibody reactions is an unlikely cause of atopic allergy in man. Since an essential feature distinguishing atopic from normal individuals is the altered reactivity to airborne allergens, at least three hypotheses may be considered as explanations for this difference.

- I. A hereditary peculiarity exists in the immunologic apparatus, resulting in a predisposition of atopic individuals to produce skin-sensitizing antibody.
- II. Atopic individuals, perhaps because of some enzymatic defect, retain antigens for sufficiently prolonged periods to enable antibody production to ensue.
- III. Excessive mucosal permeability exists in atopic individuals, allowing more extensive and efficient contact of airborne antigens with immunologically competent cells.

To the best of our knowledge, no differences in immunologic capacity of the kind required by hypothesis I have been clearly demonstrated. Indeed, previous work from this laboratory suggests the contrary. In these studies¹ polysaccharide antigens were used, and immunization was via subcutaneous and intracutaneous routes. Under these circumstances, little difference could be shown between normal and atopic individuals in respect to development of immediate skin reactivity.¹ It is possible that polysaccharide antigens may function in a different manner than protein and thus mask differences in response of atopic and normal individuals. Similar studies with protein antigens would be desirable and are in progress. Kuhns⁵ has recently obtained strongly suggestive evidence that protein antigens behave like carbohydrate antigens in this respect. Using diphtheria and tetanus toxoid antigen, he showed that multiple subcutaneous and intracutaneous injections were very effective in inducing immediate wheal-and-erythema skin reactivity, rise in antitoxin titers, and sera giving successful passive transfer reactions. The fact that none of these individuals gave a past or family history of atopic disease indicates that normal individuals

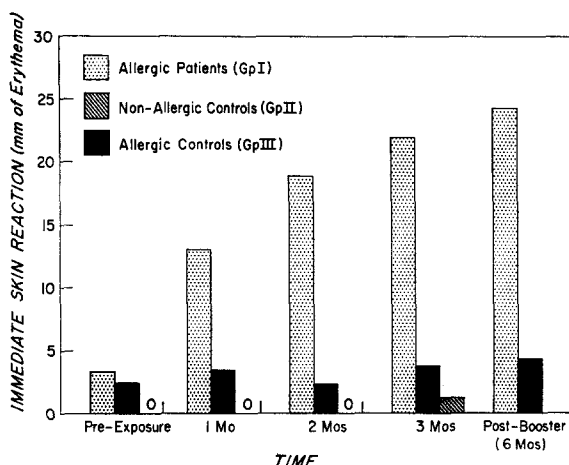


Fig. 1.—Average degree of immediate skin reactivity developing in various groups during intranasal instillation of ribonuclease.

have the ability to produce skin-sensitizing antibody to common protein as well as polysaccharide antigens, if efficient and prolonged methods of immunization are employed.

The second hypothesis (retention of antigen for prolonged periods by atopic individuals) is difficult to verify and does not appear likely, since among atopic and nonatopic subjects no difference in immune response has been demonstrable by parenteral injection of antigens.

The third hypothesis (local or general defects in mucosal tissue permeability) is the most attractive to us. Thus, while all individuals would possess the essential apparatus for production of skin-sensitizing antibody, this would depend on the usual immunologic prerequisites of sufficient antigen administered over a long enough period. As seen graphically in Fig. 1, the average immune response (as determined by skin reactivity) of group I shows a steady increase with continued mucosal exposure to antigen. That the effective antigenic stimulus was indeed administered via the intranasal route and not just as a result of the repeated skin tests is shown by the behavior of group III, in which the average response remained relatively constant and small over the course of the experiment, even in the 3 patients having some initial reactivity.

Since one difference between atopic and normal individuals is their behavior in respect to airborne antigens, it seems plausible that this route of immunization is sufficient for sensitization in the one and not in the other. Our findings with a purified protein RNase made it seem possible that no special properties need be invoked for airborne allergens in particular that do not apply to antigens in general. While our report concerns only a single protein and generalizations about other antigens may be premature, it seems likely, from the frequently observed pattern of broadening response over the years for most atopic individuals, that the process of sensitization is not selective but may encompass a host of diverse allergens, provided that they are presented in sufficient concentration for a sufficient length of time to the immunologic apparatus of the individual.

TABLE II. SYMPTOMS DEVELOPING IN ALLERGIC INDIVIDUALS FOLLOWING REPEATED INTRANASAL EXPOSURE TO RNASE

PATIENT NO.	SYMPTOMS
(Group I)	
4	Slight nasal irritation and stuffiness immediately after tenth and eleventh aerosolization.
1	a. Sneezing and "itching ears" immediately after seventh aerosolization. b. Sneezing and slight nasal discharge 10 minutes following tenth aerosolization. c. Rhinorrhea, lacrimation, and pharyngeal pruritis immediately following "booster" aerosolization.
7	a. Rhinorrhea, sneezing, nasal, and conjunctival pruritis of 2 hours' duration commencing 10 minutes after seventh aerosolization. b. Immediate sneezing paroxysm, lacrimation, and profuse nasal discharge after tenth aerosolization (1 hour duration). c. Immediate pharyngeal and conjunctival pruritis, sneezing paroxysm after lacrimation of 1 hour duration following "booster" aerosolization.

TABLE III. INITIAL APPEARANCE OF VARIOUS IMMUNOLOGIC PARAMETERS IN SYMPTOMATIC INDIVIDUALS DURING THE COURSE OF EXPOSURE TO RNASE

PATIENT NO.	TIME OF INITIAL APPEARANCE		
	IMMEDIATE SKIN REACTIVITY	SYMPTOMS FOLLOWING INHALATIONS	SKIN-SENSITIZING CAPACITY OF SERUM
(Group I)			
1	4 weeks	10 weeks	6 months
7	Pre-exposure but increased at 4 weeks	7 weeks	6 months
4	4 weeks	7 weeks	None detected

A recent study by Farr⁶ provides further support for the possibility of a general mucosal permeability defect. Using the ammonium sulfate precipitation method for measuring globulin-bound I¹³¹ bovine serum albumin, he found significant differences between groups of normal and atopic individuals in the antibody formed against orally administered antigen. Studies involving the transmucosal absorption patterns of labeled antigens would shed light on this hypothesis and are currently being planned.

The early development of immediate skin reactivity in group I in the absence of demonstrable passive transfer ability suggests that only small amounts of "fixed" antibody were present. This is in accord with the general impression that only sera from intensely sensitive individuals are capable of effecting a Prausnitz-Küstner type transfer. As shown in Table III, the appearance of symptoms after exposure seemed to occupy an intermediate position in time of appearance during the sensitization process and is in accord with the common clinical observation that skin reactivity alone is not a reliable indicator that symptoms will follow exposure. In order of increasing degree of sensitization, the sequence appears to be skin reactivity, rhinitis following mucosal contact, and skin-sensitizing capacity of serum.

Late development of small indurated delayed skin reactions in 4 nonatopic individuals was noted. None of these subjects demonstrated prior or subsequent

wheal-and-erythema reactivity. Such delayed reactions have been reported to occur prior to the development of wheal-and-erythema reactivity in persons receiving repeated injections of protein antigens.^{5, 7} However, other observers using emulsions have noted no sequential relationships between development of wheal-and-erythema reactions and delayed hypersensitivity to crude allergens.^{8, 9} One may speculate that the delayed reactions observed by us were a consequence of immunization by minute quantities of antigen permeating the normal mucosa and were comparable to delayed hypersensitivity following immunization by small amounts of antigen.¹⁰ Whether or not our normal subjects would have subsequently developed immediate skin reactivity and nasal symptoms remains a matter of conjecture.

SUMMARY

Atopic and nonatopic subjects were exposed intranasally to a protein antigen, and observations were made on the development of skin reactivity of the immediate type, the occurrence of symptoms, and the appearance of skin-sensitizing capacity in the serum. These were induced exclusively or with greater frequency in the atopic group, and this was not attributable to antigen administered in the course of skin testing.

Four among 10 nonatopic individuals developed delayed-type skin reactivity which was not observed among the atopic subjects.

The ABO blood group secretor status was observed with equal frequency in allergic and nonallergic individuals and was unrelated to appearance of skin reactivity.

It is suggested that atopic individuals differ from nonatopic in having a greater mucosal permeability to inhaled antigens.

The authors are indebted to Dr. William Stone and Miss T. Cohen of the Department of Genetics, University of Wisconsin, for their analysis of the saliva specimens for secretor status.

REFERENCES

1. Leskowitz, S., and Lowell, F. C.: A Comparison of the Immunologic and Physiologic Responses of Normal and Allergic Individuals, *J. ALLERGY* 32: 151, 1961.
2. Leskowitz, S., and Ovary, Z.: The Relation Between Molecular Weight of Antigen and Ability to Elicit Passive Cutaneous Anaphylaxis, *Immunology* 5: 1, 1962.
3. Lecompte, J.: Liberation of Endogenous Histamine in Man, *J. ALLERGY* 28: 102, 1957.
4. Farmer, L.: Evaluation of Histamine Intradermal Test as a General Indicator of Allergy, *J. ALLERGY* 16: 44, 1945.
5. Kuhns, W. J.: The Relationship of Immediate Wheal Reactions to the Repeated Administration of Diphtheria and Tetanus Toxoid, *J. Immunol.* 89: 652, 1962.
6. Farr, R. S., and Barrick, R. H.: The Increased Responsiveness of Adult Allergic Persons to an Orally Ingested Antigen. (Presented before the 19th Annual Meeting of the American Academy of Allergy, Montreal, Canada, March 12, 1963.)
7. Simon, F., and Rackemann, F.: Development of Foreign Protein Sensitization in Man Followed by Intradermal Injection of Antigen, *J. ALLERGY* 5: 439, 1934.
8. 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 the Injection of Antigen in Emulsion, *J. ALLERGY* 32: 229, 1961.
9. Feinberg, S. M., Becker, R. J., Slavin, R. G., Feinberg, A. R., and Sparks, D. B.: The Sensitizing Effects of Emulsified Pollen Antigens in Atopic Subjects Naturally Sensitive to an Unrelated Antigen, *J. ALLERGY* 33: 285, 1962.
10. Salvin, S. B.: Occurrence of Delayed Hypersensitivity During the Development of Arthus Type Hypersensitivity, *J. Exper. Med.* 507: 109, 1958.