

Delayed response to antigen challenge in induced delayed reactivity

A clinical and cytologic study in man

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It has been previously shown that a high percentage of nonatopic subjects who receive an injection of pollen antigen in emulsion develop delayed skin reactivity to that antigen.¹⁻³ It is uncertain, however, whether this reactivity has any clinical significance. The possibility that symptoms of rhinitis might result from delayed hypersensitivity is suggested on the following grounds. The occurrence at times of delayed skin reactions to inhalant antigens in the absence of immediate reactions in cases of seasonal rhinitis was claimed by Vaughan.⁴ Another suggestion is made in the report by Simon and Rackemann⁵ of rhinorrhea and nasal blocking occurring several hours after a nasal pack saturated with diluted guinea pig serum, as the first sign of development of hypersensitivity.

The objectives of the present investigation were to study more fully the delayed response to pollen antigens, to repeat the experiment of Simon and Rackemann, and to extend it with cytologic studies. Before studying these delayed reactions, it was planned to observe and to correlate symptoms, physical

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This investigation was supported by Research Grant AI-04234 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service.

Presented in part at the Twentieth Annual Meeting of the American Academy of Allergy, San Francisco, Calif., Feb. 10 to 12, 1964.

Received for publication April 20, 1964.

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examination, nasal smears, and nasal biopsies after nasal application of aqueous ragweed in individuals with spontaneously acquired ragweed allergy. Thus, we could compare the temporal and cytologic responses of immediate and delayed reactions.

PROCEDURES AND RESULTS

Nasal insufflation of ragweed by naturally ragweed-sensitive subjects

A nasal spray of 1:100 aqueous ragweed pollen extract was administered to ten subjects naturally sensitive to ragweed. The subjects showed only slight variation in their responses, as indicated in Table I. While all other observations were made repeatedly on the same subjects, nasal biopsies were performed only once on each subject. Thus the temporal changes described in nasal biopsies are from *different* subjects. Within one minute after exposure, rhinorrhea, sneezing, ocular discharge, and itching began. At this point examination of the nose was normal. Only polymorphonuclear neutrophils were seen on nasal smears, and nasal biopsy of specimens appeared normal. In 5 minutes some edema of the nasal turbinates was evident and by 10 minutes this was maximal. Symptoms were still marked at this time. The nasal smear still showed only polymorphonuclear neutrophils, but eosinophils were seen in and around blood vessels in the nasal mucosa on biopsy. In 15 minutes the turbinates were less swollen and by 20 minutes they appeared normal; at the same time symptoms were lessening and by 1 hour were gone. At 1 hour eosinophils began to accumulate in the submucosa and to infiltrate the epithelium. Eosinophils were numerous at 6 hours and 24 hours in the nasal smear, and by 48 hours they were gone.

Table I. *Nasal findings following insufflation of ragweed pollen in naturally sensitive subjects*

	<i>Symptoms</i>	<i>Appearance of turbinates</i>	<i>Smear</i>	<i>Biopsy</i>
Immediate	Rhinorrhea + sneezing	Normal	Polymorphonuclear neutrophils	Negative
5 minutes	Prominent	Mildly swollen		
10 minutes	Prominent	Markedly swollen	Polymorphonuclear neutrophils	Eosinophils in and around blood vessels
15 minutes	Prominent	Less markedly swollen		
20 minutes	Less	Normal		
1 hour	Normal		Polymorphonuclear neutrophils	More eosinophils infil- trating epithelium
6 hours			Polymorphonuclear neutrophils + eosinophils	Eosinophils in submucosa
24 hours			More eosinophils	Eosinophils
48 hours			Normal	

Sensitization of atopic and nonatopic subjects with guinea pig serum by intradermal and nasal routes

Atopic and nonatopic subjects were each divided into two groups, one receiving weekly nasal packs of 1:10 guinea pig serum for 10 minutes, and the other weekly intradermal injections of 0.05 ml. of 1:10 guinea pig serum. When sensitivity was induced it usually occurred in 2 to 4 weeks. The results are summarized in Table II.

Table II. *Delayed skin or nasal sensitivity induced by repeated nasal applications or intradermal injections of guinea pig serum (1:10)*

Route of sensitization	Challenge positive*	
	Intradermal	Nasal
<i>Nonatopic</i>		
8 Intradermal	5	2
7 Nasal	0	1 (?)
<i>Atopic</i>		
12 Intradermal	7	4
14 Nasal	4	8

*Subjects negative on skin test after intradermal sensitization were not challenged nasally, nor were negative nasal reactors after nasal sensitization challenged intradermally.

Of eight nonatopic individuals who received weekly intradermal injections of guinea pig serum, five developed delayed skin reactions.* At that time these five subjects were challenged via the nasal route with a pack of 1:10 guinea pig serum and two showed delayed symptoms, that is, a correlation between delayed skin reactivity and delayed symptoms. Of seven nonatopic persons sensitized by the nasal route, one developed questionable delayed symptoms and had a negative skin test.

Twelve atopic subjects received guinea pig serum intradermally weekly and eight developed delayed skin reactions. Of these, four had marked delayed symptoms after intranasal challenge.

Of fourteen atopic persons who received weekly nasal packs of guinea pig serum, eight developed delayed symptoms and, of these eight, four had delayed skin reactions after intradermal challenge. Nasal smears of the subjects with delayed symptoms at 24 hours showed a preponderance of mononuclear cells.

Nasal packing with ragweed extract in subjects with induced delayed skin reactions to ragweed

Ten nonatopic individuals who had developed delayed skin reactions to ragweed and two who had developed delayed skin reactions to timothy following injection of these antigens in emulsion were selected for intranasal challenge.

*"Positive delayed skin reactions" throughout this paper refers to erythema and induration of at least 10 mm. in diameter appearing 6 to 12 hours after challenge and reaching a maximum at 24 to 48 hours.

The method of emulsion administration has been previously described.² All twelve subjects manifested delayed skin reactions to a 1:1,000 aqueous concentration of the respective antigens and the intensity of the reactions varied from 20 to 30 mm. in diameter. A 1:100 aqueous concentration of the respective antigen was applied by nasal pack for 10 minutes in one side of the nose, while a pack of diluting fluid or another pollen antigen to which the subject was not sensitive was placed on the other side. Eight of the ten ragweed reactors and

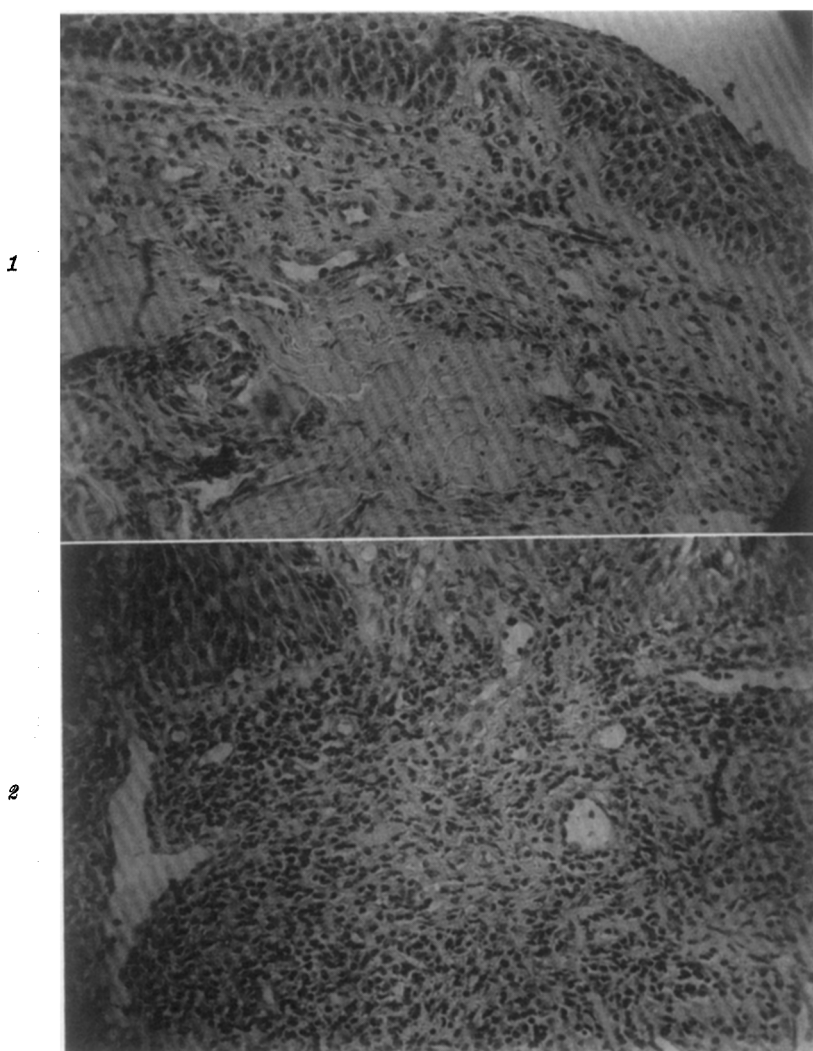


Fig. 1

Nasal biopsy taken 6 hours after ragweed pack in subject with induced delayed reaction to ragweed. The mucosa appears normal.

Fig. 2

Nasal biopsy taken 24 hours after ragweed pack in subject with induced delayed reaction to ragweed. Marked infiltration of mononuclear cells is seen.

one of the two timothy reactors responded with delayed symptoms confined to the side of the nose where the antigen to which the subject was sensitive was placed.

Table III shows the temporal sequence of the response. No changes at all were seen until 6 hours after the removal of the pack, when the subjects began to note unilateral rhinorrhea and congestion. At this time, there was definite swelling of the turbinate on that side. Nasal smears showed many polymorphonuclear neutrophils. Nasal biopsy at 6 hours was normal (Fig. 1). Symptoms continued throughout the night and were still markedly present the next morning. The turbinates remained edematous and a nasal smear at 24 hours showed neutrophils with increasing numbers of mononuclear cells. A nasal biopsy at this time revealed extreme cellularity with many mononuclear cells (Fig. 2). At 48 hours, symptoms, physical examination, nasal smear, and nasal biopsy were negative.

Table III. *Nasal findings following packs of ragweed pollen in subjects with delayed skin reactivity to ragweed*

	<i>Symptoms</i>	<i>Appearance of turbinates</i>	<i>Smears</i>	<i>Nasal biopsy</i>
10 minutes	None	Normal	Normal	Normal
1 hour	None	Normal	Normal	Normal
6 hours	Rhinorrhea + congestion, mild	Mildly swollen	Polymorphonuclear neutrophils	Normal
24 hours	Rhinorrhea + congestion, marked	Markedly swollen	Polymorphonuclear neutrophils + mononuclear cells	Extra cellularity Many mononuclear cells
48 hours	None	Normal	Normal	Less

DISCUSSION

In patients who present typical atopic symptoms but who do not manifest a positive immediate skin test, the possibility of delayed hypersensitivity playing a causative role was suggested many years ago.⁴ While delayed skin reactivity to inhalant antigens such as mold, house dust, and insects is not uncommon, its possible clinical significance has been virtually ignored in recent years.

The ease and reproducibility of inducing delayed skin reactivity to pollen antigens in nonatopic subjects by injection of pollen in emulsion has been previously reported.^{2, 3} Such a group of subjects provides an opportunity to study the possible clinical significance of delayed skin reactivity under appropriate antigenic challenge. To compare the temporal and cytologic responses of immediate and delayed reactors, it was necessary to challenge naturally atopic subjects with the appropriate antigen. As can be seen from Table I, symptoms were produced several minutes before any change in the appearance of the nasal mucosa. In addition, symptoms persisted long after the mucosa had returned to

normal. Eosinophils did appear but were quite delayed, occurring in the nasal secretions from 6 to 24 hours after challenge.

Induction of delayed hypersensitivity by means of intradermal administration of guinea pig serum was about the same in both atopic and nonatopic subjects. This was similar to the findings of Simon and Rackemann.⁶ Induction of delayed hypersensitivity by means of nasal applications of guinea pig serum was considerably different in the two groups, the atopic group having a much greater number of reactors. A similar finding with regard to induction of immediate reactivity to ribonuclease was reported by Salvaggio and colleagues.⁷ They suggest a greater mucosal permeability in the atopic individual, thus permitting better contact of inhaled antigens with immunologically competent cells.

The final step was the demonstration that, under appropriate conditions, clinical symptoms were produced in subjects with delayed skin reactions to pollen antigens. Since these reactions were all induced, it is uncertain that this occurs in nature. Experiments on subjects with natural delayed sensitivity to other antigens are being conducted at this time.

Some differences have been noted in the response of the immediate and the delayed reactors. The symptoms in the delayed reactors were predominantly rhinorrhea and nasal congestion. Notable was the absence of sneezing, itching, and ocular discharge. The absence of eosinophils and presence of mononuclear cells in the nasal smears and nasal biopsies of the delayed reactors also afforded a clear difference from the immediate reactors. A further interesting finding in the delayed reactors was the disappearance of all nasal changes by 48 hours after removal of the pack, at a time when the delayed skin reaction is still in evidence.

As yet there is no evidence of clinical difficulties during the corresponding pollen season in nonatopic persons who develop delayed skin reactions after receiving antigen in emulsion. However, it is shown here that under appropriate experimental conditions delayed symptoms can be produced in these subjects.

We have previously demonstrated that some naturally sensitive ragweed subjects who receive ragweed in emulsion possess the delayed hypersensitivity transfer factor in their peripheral leukocytes.⁸ It is possible that such a delayed reactivity might augment the inflammatory reaction in the mucosa from the immediate response and thus actually increase clinical symptoms. On the other hand, this possibility appears to be contradicted by the fact that such individuals do not show a delayed inflammatory reaction when the pollen is injected intradermally.

SUMMARY

The delayed sensitivity mechanism was investigated as a possible factor in the production of rhinitis. The influence of the nasal route of sensitization on the intradermal reaction and the influence of the intradermal sensitization on the nasal reaction to guinea pig serum were observed in atopic and nonatopic subjects. In natural immediate reactors and those with induced delayed reactions to pollen antigens, clinical and cytologic comparisons were made. The results indicate that nasal blocking and rhinorrhea, associated with cytologic changes

in the mucosa compatible with a delayed hypersensitivity state, can be produced in the presence of delayed, and in the absence of immediate, skin reactivity. The clinical significance of these findings is not yet certain.

The authors acknowledge the assistance of Dr. Jack D. Kerth, Department of Otolaryngology, Northwestern University Medical School, who performed the nasal biopsies. We also wish to thank Warden Frank J. Pate, the Administrative Staff, and inmates of the Illinois State Penitentiary, Stateville, Illinois, for their cooperation in this study.

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