Comparative nasal absorption of allergens in atopic and nonatopic subjects


Based on sensitization following intranasal antigen administration, previous investigations have suggested greater absorption of allergens through the nasal mucous membranes of atopic than of nonatopic subjects. In this study mucosal absorption was assessed more directly by determining the capacity of allergens applied intranasally to elicit cutaneous Prausnitz-Küstner (P-K) reactions in nonatopic persons as compared with asymptomatic atopic subjects sensitive to other allergens. Two series of reaginic human serum dilutions were injected intracutaneously in recipients' backs, and 48 hours later one series was challenged intracutaneously with test antigen. After the responses had been recorded, concentrated allergenic extract was sprayed into the nose and the second series of P-K sites observed for reactivity. Sometimes these P-K sites were rechallenged intracutaneously the following day to determine passive transfer neutralization. Two allergens were studied: bovine ribonuclease (RNase) and peanut extract. Two sera containing peanut reagins and one with RNase antibodies were each used in 10 to 11 atopic and 9 to 11 nonatopic recipients. The atopic group failed to show greater or more rapid absorption of either allergen through the nose based on the highest serum dilution reacting after nasal challenge, the speed of the reaction, the ratio of the titer by nasal challenge to the intraocular titer, or passive transfer neutralization. Controls showed that the results were not influenced by systemic absorption of allergen employed for intracutaneous tests. Drinking the amount of peanut extract applied intranasally did not elicit P-K reactions.

In previous investigations it has been found that intranasal administration of dextran and bovine RNase antigens produced larger and more promptly developing wheal and erythema skin test reactivity in atopic individuals than in nonatopic subjects. More of the atopic subjects also developed nasal symptoms and circulating reaginic antibodies, demonstrable by the Prausnitz-Küstner (P-K) technique, after repeated nasal antigen administration. On the other hand, atopic and nonatopic subjects responded similarly, in respect to these parameters, when the same antigens were administered by subcutaneous injection. The route of immunization made less difference between these groups when a high molecular weight antigen, keyhole limpet hemocyanin, was employed. One hypothesis that was advanced to explain these observations was that there is greater absorption of antigens through the nasal mucous mem-
branes of atopic than of nonatopic individuals, a difference that might be of importance in the pathogenesis of atopic diseases. However, since factors other than mucosal absorption of allergens might account for these previous observations, this study was designed to assess mucosal absorption more directly. This was done by determining the capacity of allergens applied intranasally to elicit cutaneous P-K reactions and/or neutralize passive transfer sites in nonatopic persons as compared with atopic subjects sensitive to other allergens.

**MATERIALS AND METHODS**

**P-K recipients**

Paid young adult volunteers of both sexes were used after giving informed consent following explanation of possible risks of the procedure. Subjects were classified as atopic only if they gave a personal history of allergic rhinitis and exhibited several strongly positive scratch and/or intracutaneous skin test reactions to a battery of 14 to 19 allergens commonly causing symptoms in their area of residence. Nonatopic subjects had no history of allergic rhinitis, bronchial asthma, or atopic dermatitis, and they did not give any 2+ or greater skin test reactions to the battery of common allergens. In the studies with RNase antigen, subjects with a history of atopic disease in the immediate family also were excluded from the nonatopic group. All atopic subjects had been asymptomatic for at least several weeks at the time of the study, and none was taking medication for allergic disease. In the case of the tests with peanut extract, volunteers were excluded who had a history of allergic symptoms after ingesting peanut, who had positive skin test reactions, or who developed immediate rhinorrhea after its intranasal administration.

**Antigens**

Part of the studies employed crystalline bovine pancreatic RNase, which was protease- and salt-free (Mann Research Laboratories, Inc., New York, New York). A solution containing 0.1 mg of RNase per milliliter in sterile physiologic saline solution was used for intracutaneous testing. Preliminary studies with a wide range of RNase concentrations (1.0 to 50 mg. per milliliter) indicated that intranasal administration of relatively high concentrations was necessary for eliciting P-K reactions with the anti-RNase serum employed, but doses of 50 mg. per milliliter or above resulted in some nasal mucous membrane irritant effects. Consequently, the intranasal dosage employed for this study was 0.2 ml. of a 30 mg. per milliliter solution of RNase in saline solution divided between the two nostrils. It was administered by a DeVilbiss atomizer that delivered about 0.03 ml. per spray (three sprays in each nostril).

Other studies employed peanut extract freshly prepared for intracutaneous testing by our routine procedure. Preliminary studies indicated a dilution containing 1.4 µg of protein nitrogen per milliliter seemed optimal for eliciting P-K reactions without evoking positive control responses in atopic persons or causing flare-ups of distant P-K sites due to systemic absorption of excess allergen. The concentrated peanut extract for intranasal use was prepared from the same lot of peanuts according to the routine procedure for making scratch test extracts and then dialyzed over 24 hours against three changes of phosphate-buffered saline not containing phenol or glycerin. It contained 805 µg of protein nitrogen per milliliter, and preliminary studies indicated an optimal intranasal dosage to be about 0.5 ml. in each nostril.

**Sera**

The serum used in the experiments employing RNase antigen was obtained from a volunteer injected subcutaneously with alum-precipitated RNase according to the procedure previously reported. The two sera containing peanut reagins were obtained from young adult professional persons who experienced severe urticaria and respiratory symptoms from ingesting even trace amounts of peanut. Neither serum donor had a history of hepatitis or...
FIG. 1. Skin reactions 60 minutes after intracutaneous challenge with peanut extract (left side) and 30 minutes after intranasal application of concentrated peanut allergen (reflected by reactions on right side). The lateral vertical rows are serial dilutions of one serum and the medial vertical rows are serial dilutions of a second serum containing peanut reagins.

jaundice, the sera were free of hepatitis-associated antigen by radioimmunoassay, and both sera were used for P-K tests on one of the investigators months before injecting them in volunteers. The total IgE content of these sera was 156 and 192 U. per milliliter, respectively, as measured by the method of Gleich, Averbeck, and Swedlund.7

Procedure

Two series of the reaginic human serum dilutions (0.1 ml.) were injected intracutaneously in recipients' backs* and 48 hours later one series was challenged with 0.02 ml. of test allergen solution. The results were recorded at 20 minutes. Ten minutes thereafter the concentrated allergen solution was sprayed into both nostrils by means of a DeVilbiss atomizer and the second series of P-K sites was observed for reactivity over 1½ to 3 hours. To determine passive transfer neutralization by peanut allergen given intranasally, the second set of P-K sites was challenged intracutaneously on the following day with 0.02 ml. of peanut extract, and the results were read at 20 minutes. Controls included allergen injected into normal skin and buffered saline injected into P-K sites. In many instances the skin tests were read “blind” by a second observer.

RESULTS

Fig. 1 illustrates results of a typical experiment. The two vertical rows on the left side of the back had been challenged intracutaneously with peanut

*In the experiments employing RNase antigen the following reciprocal dilutions were employed: 2, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 300, 400, and 500. Partly because two sera were used simultaneously in experiments employing peanut allergen, only threefold dilutions ranging from 1:3 to 1:243 were used.
**TABLE I.** Results of experiments in 21 subjects employing two sera containing peanut reagins

<table>
<thead>
<tr>
<th>No.</th>
<th>Mean* intracutaneous titer</th>
<th>Mean nasal titer</th>
<th>Mean ratio nasal: intracutaneous titer</th>
<th>Mean nasal reaction time (min.)</th>
<th>Mean nasal P-K neutralization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic‡</td>
<td>10</td>
<td>65 ± 34</td>
<td>15 ± 5.0</td>
<td>0.23</td>
<td>10 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 ± 7.3</td>
<td>7 ± 2.2</td>
<td>0.15</td>
<td>13 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>81 ± 14</td>
<td>18 ± 4.5</td>
<td>0.22</td>
<td>9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54 ± 6.9</td>
<td>8.5 ± 2.1</td>
<td>0.16</td>
<td>16 ± 2.4</td>
</tr>
</tbody>
</table>

*All titers are geometric means.
†Geometric mean decrease in P-K titers following nasal challenge.
‡The two sets of figures refer to results with two different sera.
§Standard error.

extract about 60 minutes before this photograph was taken, the positive reactions up to 1:81 dilutions of each serum having largely faded away during this time period. On the right side of the back, however, one can clearly see wheal and erythema skin reactions occurring at dilutions up to 1:27 of each serum as a consequence of intranasal peanut extract administration approximately 30 minutes earlier.

Table I summarizes the results of 10 such experiments on atopic recipients and 11 experiments on nonatopic subjects. It may be noted that with both sera the nonatopic persons actually had slightly higher geometric mean P-K titers from intranasal peanut extract administration than the atopic group. However, the nonatopic subjects also had higher geometric mean titers by intracutaneous challenge. Correcting for this by dividing the nasal titer by the intracutaneous titer, it is seen that the mean ratio of nasal: intracutaneous titers is almost identical in the two groups with both sera under study. Likewise the time between nasal challenge and the first definite, objective sign of a skin reaction was not significantly different between the two groups. In both instances itching generally developed a few minutes before objective evidence of a reaction was apparent. Passive transfer neutralization by nasal challenge was determined by dividing the intracutaneous titer obtained at 48 hours on the left side of the back by the intracutaneous titer obtained at 72 hours on the right side. As indicated by Table I, the nonatopic group tended to show greater passive transfer neutralization by this criterion. If median values are substituted for geometric means in Table I, the ratio of nasal: intracutaneous titers and the nasal passive transfer neutralization values were identical for the atopic and nonatopic groups with both sera.

The data also were analyzed by randomly pairing atopic and nonatopic subjects with the same intracutaneous titer before nasal peanut extract challenge. When data obtained with the two sera were pooled, the atopic subject in the pair had a higher nasal titer in 4 instances, in 6 pairs the nasal titers were the same, and in 5 the atopic person had a lower nasal titer than the nonatopic.
TABLE II. Results of experiments on 20 subjects employing RNase antigen EV

<table>
<thead>
<tr>
<th>RNase</th>
<th>Mean* nasal titer</th>
<th>Mean intracutaneous titer</th>
<th>Mean ratio nasal:intracutaneous titer</th>
<th>Mean nasal reaction time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic</td>
<td>19 ± 4.8</td>
<td>173 ± 27†</td>
<td>0.11</td>
<td>23 ± 4.6</td>
</tr>
<tr>
<td>Nonatopic</td>
<td>35 ± 12</td>
<td>338 ± 43</td>
<td>0.10</td>
<td>20 ± 3.6</td>
</tr>
</tbody>
</table>

*All titers are geometric means.
†Standard error of the mean.

When phosphate-buffered saline was sprayed into the nose instead of peanut extract, none of the P-K sites developed reactions, and intracutaneous challenge of the sites on the right side of the back the following day yielded the same titers as intracutaneous challenge on the left side of the back the preceding day. These results indicate that the reactions and passive transfer neutralization on the right side of the back were not due to systemic absorption of allergen injected in the left side, as had also been shown in preliminary studies with various concentrations of peanut extract for intracutaneous injection. They also demonstrated that apparent passive transfer neutralization was not due simply to the P-K sites being less reactive one day later. In contrast, intracutaneous rechallenge of the P-K sites on the left side of the back in a few cases at 72 hours showed negative reactions except at the 1:3 serum sites, thus indicating almost complete local passive transfer neutralization by intracutaneous challenge the preceding day.

In two experiments a P-K recipient swallowed 1 ml. of the peanut extract ordinarily sprayed into the nose. No P-K reactions ensued, but the sites were shown still to be potentially reactive by responding to intracutaneous peanut extract challenge in one instance and yielding prominent wheal and erythema skin reactions in the other instance after the recipient had eaten about 150 Gm. of peanuts. General reproducibility of results was evaluated repeating the entire experiment in one recipient; the nasal titer, intracutaneous titer, and passive transfer neutralization, read “blind,” were the same with both sera as in the initial experiment. In all experiments duplicate 1:9 or 1:27 serum dilutions were planted to provide sites for intracutaneous saline controls and further information concerning the reproducibility of reactions after intranasal challenge. In only one instance out of the 42 paired observations with the two sera was one duplicate serum site considered positive and the other negative after intranasal challenge.

Table II summarizes the results of the experiments employing RNase antigen in 11 atopic and nine nonatopic recipients. Again the geometric mean nasal titers as well as the intracutaneous titers were higher in the nonatopic group, leading to a ratio of the geometric mean nasal:intracutaneous titers that was almost exactly the same for the two groups. Likewise, the mean nasal reaction times did not differ significantly between the two groups. If median values are substituted
for means, the atopic and nonatopic groups had nasal intracutaneous titer ratios of 0.11 and 0.12, respectively, and nasal reaction times of 16 and 20 minutes. In random pairing of atopic and nonatopic recipients with approximately the same intracutaneous titer before nasal RNase challenge, the atopic subject in the pair had a higher nasal titer in two instances, in two pairs the nasal titer were the same, and in two the atopic subject had a lower nasal titer than the nonatopic subject.

**DISCUSSION**

The P-K test was used to demonstrate absorption of allergens through the nasal mucous membranes in two studies reported over 40 years ago. Although Cohen and associates attempted to compare results in atopic and nonatopic recipients, the lack of quantitative control of the amounts of serum and intranasal antigen employed, the uncertain clinical status of the atopic subjects, and failure to use serum dilutions make it difficult to interpret their results. They actually reported less nasal absorption of ragweed allergen in the atopic group, but this may have been related to active allergic inflammation or inefficient mucosal contact due to rhinorrhea and excessive secretions, in that most of the individuals showing the least absorption of ragweed allergen were reported to be allergic to house dust. It would seem unlikely, however, that local inflammation per se decreases mucosal absorption of allergens in view of the findings of Schwartz, Leskowitz, and Lowell, who determined the response of individuals with allergic and vasomotor rhinitis to intranasal instillation of bovine RNase. In these studies the allergic subjects were actually more readily sensitized than the vasomotor group in spite of the fact that both groups were presumed to have an equal degree of nasal inflammation. In addition, Walker also failed to find delayed absorption of peanut extract through the nasal mucosa of the majority of atopic subjects as determined by time required to elicit P-K reactions in these individuals when compared with nonatopic subjects. This matter also was considered in the interpretation of a recent study of nasal secretory antibody titers following intranasal tetanus toxoid in atopic and nonatopic persons.

In the present study it might have been preferable to simulate natural conditions more closely by employing pollen grains rather than antigen extracts. This was not done, however, partly because of the risk of sensitizing particularly the atopic volunteers to allergens, which cannot readily be avoided. Also, preliminary observations indicated that large amounts of intranasal allergen are required in these types of experiments, thus discouraging the use of highly purified pollen allergens available in only very limited quantities. Although the peanut extract employed undoubtedly is very heterogeneous, it is reassuring that similar results were obtained with the highly purified RNase antigen.

While no evidence of increased nasal absorption of two antigens by atopic subjects was obtained in the current studies, the possibility of this occurrence under other conditions of course has not been excluded. For example, a transient difference in allergen absorption early in life might be of pathogenetic importance. Of interest in this regard is a recent prospective study suggesting that early IgA deficiency in infants born to "allergic" parents is associated with a
significantly increased incidence of subsequent "atopic" disease at a later age.\textsuperscript{13} The molecular weight of the antigen also might be relevant, and particulate antigens might be handled differently. In addition, the passive transfer technique employed in the current study is not sufficiently sensitive to discern a subtle difference in mucosal allergen absorption or its local elimination (e.g., a functional abnormality of exocrine IgA or other exocrine mucoproteins, or a subtle mucous membrane permeability defect operative only at low antigen doses). However, masking of a difference by gastrointestinal absorption of an excess volume applied in the nose appears to be excluded by the experiments in which the extract was swallowed.

Variations in the capacity of reagins to adhere to the skin of atopic and nonatopic subjects also required consideration. Presumably because of competition between the specific reagins in the sera used for the P-K tests and the recipients' IgE for receptor sites on cutaneous mast cells, it might be anticipated that the P-K titers would be lower in the atopic than in the nonatopic recipients if the latter had lower serum IgE levels. Among the subjects in the peanut study, the mean IgE was 502 U. per milliliter in the atopic and 190 U. per milliliter in the nonatopic subjects (three persons not tested). As reported previously by others,\textsuperscript{14} the atopic subjects did have lower P-K titers (see Tables I and II), but the P-K titers did not vary so widely as the IgE levels. Relating the 18 IgE levels in the atopic and nonatopic recipients with their corresponding P-K titers, the correlation coefficients with the two sera containing peanut reagins were \(-0.205\) and \(-0.364\), neither of which is statistically significant. In addition, analysis of the data by determining the ratios of nasal : intracutaneous titers and by pairing atopic and nonatopic individuals with similar intracutaneous titers should correct for potential errors caused by differences in the capacity of atopic and nonatopic skin to bind transferred reagins.

While the present study does not support increased absorption of allergens through the nasal mucosa of young adult subjects, it does not invalidate the previous reports of increased and accelerated reagin production by atopic individuals following intranasal immunization. Several alternative mechanisms could explain the reported findings. Among these are possible local abnormalities in antigen processing, defects in certain "protective" proteins in secretions that might bind or neutralize release of soluble antigens into mucosal surfaces, or functional defects in local "protective" exocrine IgA antibody production to common inhalant allergens. All of these abnormalities in local antigen elimination or binding might facilitate stimulation of IgE-forming cells that are prominent surrounding the respiratory tract.\textsuperscript{15} A genetically determined capacity to mount an IgE response to repeated low doses of specific foreign antigens, as has recently been demonstrated for certain inbred rodent strains,\textsuperscript{16, 17} might also be most readily apparent in man under conditions of weak antigen stimulation at the mucous membrane level. In this regard, experience with intranasal immunization suggests that this route is in general an inefficient one.\textsuperscript{18}

An incidental and somewhat unexpected finding arising from this study was that passive transfer neutralization proved to be a less sensitive indicator of nasal allergen absorption than elicitation of P-K reactions. This is shown by
comparing the mean nasal titers with the mean P-K neutralization results in
Table I. It suggests that nasal absorption occurred rapidly, since a bolus of anti-
gen would be required grossly to light up P-K sites, while neutralization of the
sites could occur cumulatively over a longer period of time. The relatively short
nasal reaction times are in accord with this hypothesis. These times following
peanut extract administration are in good agreement with the values previously
reported by Chait and Walzer19 and by Walzer.11

Addendum
Since submission of this paper for publication, the following abstract has been published
in which absorption of albumin 12:1 through the nasal mucosa was compared in atopic and

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