

# Induction of nasal late-phase reactions by insufflation of ragweed-pollen extract

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*We studied changes in NAC in 17 ragweed-sensitive individuals after intranasal ragweed-challenge testing. All patients experienced immediate symptoms of sneezing, rhinorrhea, and nasal congestion that were associated with marked decreases in NAC (mean = 68%). In 10 trials patients also experienced late (>0 hr) symptoms of nasal congestion with or without rhinorrhea; the mean late NAC decrease in this group was 42%. In contrast, no late symptoms were noted in nine trials, and the mean NAC decreased 5% in this group ( $p < 0.003$ ). Attempts to passively transfer immediate or late nasal sensitivity to one individual by spraying the nasal cavity with IgE antibody-containing serum, by packing the nose with cotton pledgets soaked in serum, by injecting serum directly into the inferior turbinate, and by transfusion with IgE-containing serum were not successful. We conclude that symptomatic late-phase reactions occur in the nose after intranasal challenge in about 50% of patients and that these symptomatic reactions can be confirmed objectively by rhinomanometry. (J ALLERGY CLIN IMMUNOL 73:363-368, 1984.)*

Although IgE antibodies can mediate a prolonged inflammatory skin reaction after intradermal antigen injection in most sensitive patients,<sup>1-4</sup> such LPR have not been studied extensively in the nose. Taylor and Shivalkar<sup>5</sup> reported that 37% of 54 patients experienced a recurrence of nasal symptoms that commenced 2 to 3 hr after intranasal antigen challenge with symptoms persisting for 12 to 14 hr. Nasal obstruction with increased NAR was most prominent with only slight rhinorrhea and little or no sneezing or nasal irritation. However, the magnitude of the increase in NAR was not reported, and no data were provided regarding NAR in individuals who did not experience late reactions. Schumacher and Pain<sup>6</sup> reported four patients (out of 24 studied or 16%) who experienced late reactions involving nasal obstruction,

headaches, and rhinorrhea after nasal-challenge tests with rye-grass pollen, whereas Richerson et al.<sup>7</sup> found that only seven of 21 patients (33%) who were undergoing nasal challenge complained of nasal stuffiness. Pelikan<sup>8</sup> studied 600 patients by nasal-challenge testing, and 497 patients experienced an immediate reaction. Of this group, 17 (3.7%) complained of recurrent nasal symptoms 4 to 48 hr later. These 17 patients were rechallenged. Fifteen patients developed an "immediate" response within 2 hr, eight patients developed a "late" response within 12 to 14 hr, and 10 patients developed a "delayed" response 26 to 54 hr later. Thus earlier studies have demonstrated a variable frequency of nasal LPR ranging from 3% to 37%.

We sought to confirm by rhinomanometry the occurrence of a nasal LPR that corresponded to the late phase of the immediate wheal-and-flare skin reaction. We developed a computerized rhinomanometric system and subsequently used this system to measure NAC sequentially in ragweed-sensitive patients after nasal-challenge tests with large quantities of ragweed-pollen extract. In the majority of challenges, a symptomatic LPR occurred that was associated with a significant decrease in NAC as compared to NAC measurement in patients who did not experience nasal LPR. Our attempts to transfer passively either an immediate or LPR in the nose were not successful.

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*Abbreviations used*

|      |                          |
|------|--------------------------|
| NAC: | Nasal airway conductance |
| LPR: | Late phase reaction(s)   |
| NAR: | Nasal airway resistance  |
| NAF: | Nasal air flow           |
| TNP: | Transnasal pressure      |

**METHODS**

**Patients.** We enrolled seventeen adult, nonsmoking, ragweed-sensitive volunteers with typical histories of seasonal rhinitis. None had received prior ragweed immunotherapy, and all had positive immediate wheal-and-flare skin tests to ragweed-pollen extract. Measurements of IgE-antibodies to ragweed pollen by the radioallergosorbent test were performed in 12 patients, and IgE antibodies were elevated in all. The nasal cavity was free of septal deviations, nasal spurs, and nasal polyps. The challenge tests were performed in midwinter when ragweed pollen was absent from the air; all patients remained indoors for the first 12 hr of the study.

**Ragweed extract.** A lyophilized powder made from an extract of short ragweed pollen (Lot 18-52-72, Greer Laboratories, Lenoir, N. C.) was dissolved in Dulbecco's phosphate-buffered saline at concentrations of 10, 1, and 0.1 mg/ml

**Nasal challenge tests.** A baseline NAC measurement was obtained after insufflation of 0.1 ml of phosphate-buffered saline into each nostril. The short ragweed extract was aerosolized (Model 15, DeVilbiss Co., Somerset, Pa.) by use of compressed air at 12 psi through a dosimeter (Model 2A, Rosenthal-French). A setting of 0.3 sec provided 0.1 ml solution per activation of the dosimeter. The maximum tolerated immediate reaction was induced in all patients by administering one to five sprays of increasing pollen-extract concentrations. The amount of antigen that was administered initially was more variable until we gained experience in determining how much antigen could be rapidly and safely administered. NAC was measured 10 min after insufflation of buffer or ragweed, and a semiquantitative (0 to 3) symptom score that corresponded to number of symptoms that were present (nasal congestion, sneezing, and rhinorrhea) was recorded. Measurements of NAC were made every 2 to 4 hr for 12 hr as permitted by the subjects' schedules. In no case did more than 4 hr pass between measurements.

**Symptomatic LPR.** Our criteria for a symptomatic LPR were the presence of nasal congestion alone or nasal congestion with sneezing and/or rhinorrhea. We report the number of these symptoms that each subject experienced in Table I.

**Measurement of NAC.** Two apparatuses were used in these experiments. The first apparatus was similar to the face mask described by Solomon et al.<sup>9</sup> An Acme full vision no. 8 firefighter's mask was modified to include a fine mesh pneumotachograph and connections to two Validyne MP-45 differential transducers (Validyne Engineering Corp.,

Northridge, Calif.), two Hewlett-Packard 8805A transducer carrier preamplifier demodulators (Hewlett-Packard, Waltham, Mass.), and a Tektronix 603A storage monitor oscilloscope (Tektronics, Inc., Beaverton, Ore.). The second apparatus (Fig. 1) was an adaption of the first and was designed to allow interfacing to a desktop computer (Hewlett-Packard Model 9845B), taking advantage of the data storage and processing capabilities of the computer. Each of the voltages that was originally transmitted only to the storage oscilloscope was also received by a digital conversion and interface device designed by the Section of Engineering at Mayo Clinic. After digitization, the signals were transmitted to the computer. The sample rate was controlled by the operator; we used a rate of 30 samples/sec for each variable.

**Calculations.** NAC was calculated as the quotient of NAF divided by TNP at an arbitrarily selected reference inspiratory nasal pressure of 0.4 cm of water. The value for a single breath was used with the initial apparatus, which allowed the recording of only one breath at a time. With the computerized technique, data from several consecutive breaths were averaged. NAC results were expressed as a percentage change from baseline NAC after insufflation of buffer:

$$\% \Delta \text{NAC}_{\text{obs}} = \frac{\text{NAC}_{\text{obs}} - \text{NAC}_{\text{baseline}}}{\text{NAC}_{\text{baseline}}} \times 100$$

Changes in NAC and differences in ragweed dose were analyzed statistically by use of the Mann-Whitney rank sum test (one-tailed).<sup>10</sup>

**RESULTS**

After the TNP-NAF relationship was obtained and recorded, a mathematical index was derived from that relationship that served as a measure of nasal airway patency. Under the conditions of our experiments, patients were often sufficiently obstructed (sometimes totally so) as to be unable to achieve a reference flow rate of 0.5 L/sec at which the NAR (or NAC) could be calculated as suggested by Solomon et al.<sup>9</sup> Therefore we elected to use a fixed reference pressure rather than a fixed reference flow rate. Such a pressure could be generated irrespective of nasal airway caliber in that even a patient with total obstruction could create a pressure differential across the nose in the absence of flow. We used a figure of 0.4 cm of water during the inspiratory phase as our reference, and this value was achieved easily by all subjects. Inspiration was chosen because inspection of the curves revealed less variation from breath to breath during inspiration than during expiration. We calculated NAC rather than NAR because in totally obstructed patients, the conductance was zero, whereas the resistance was infinite.

The baseline NAC in 21 subjects that were studied (17 reported in this paper and four observed for other

TABLE I. Maximum late reduction in NAC

| Patient             | Ragweed dose ( $\mu$ g) | % $\Delta$ NAC   |                  | % $\Delta$ NAC |          |
|---------------------|-------------------------|------------------|------------------|----------------|----------|
|                     |                         | Immediate        | Late             | Time (hr)      | Symptoms |
| Group I LPR present |                         |                  |                  |                |          |
| 1                   | 100                     | -60              | -73              | 6              | 1        |
| 2                   | 600                     | -99              | -61              | 4              | 1        |
| 3                   | 400                     | -99              | -60              | 12             | 2        |
| 4                   | 600                     | -20              | -52              | 4              | 3        |
| 5                   | 400                     | -100             | -37              | 4              | 3        |
| 5                   | 600                     | -61              | -31              | 8              | 3        |
| 6                   | 600                     | -74              | -32              | 6              | 1        |
| 7                   | 400                     | -73              | -27              | 8              | 3        |
| 8                   | 400                     | -33              | -27              | 12             | 1        |
| 9                   | 600                     | -26              | -23              | 4              | 2        |
| Group mean          | 470 <sup>*a</sup>       | -65 <sup>b</sup> | -42 <sup>c</sup> | 6.8            |          |
| Group II LPR absent |                         |                  |                  |                |          |
| 10                  | 20                      | -40              | +12              | 8              | 0        |
| 10                  | 800                     | -74              | -45              | 8              | 0        |
| 11                  | 500                     | -99              | -37              | 6              | 0        |
| 12                  | 20                      | -47              | -20              | 4              | 0        |
| 13                  | 400                     | -77              | -20              | 4              | 0        |
| 14                  | 300                     | -75              | -5               | 4              | 0        |
| 15                  | 100                     | -76              | -1               | 4              | 0        |
| 16                  | 1200                    | -79              | +25              | 8              | 0†       |
| 17                  | 1000                    | -75              | +45              | 4              | 0        |
| Group mean          | 480 <sup>d</sup>        | -71 <sup>e</sup> | -5 <sup>f</sup>  | 5.6            |          |

a vs. d, NS; b vs. e, NS; c vs. f;  $p = 0.003$ .

\*Statistical comparisons by Mann-Whitney rank sum test (one-tailed).

†Symptoms of cough and tightness in chest; immediate improvement with isoproterenol nebulization.

reasons) was  $0.69 \pm 0.24$  (mean  $\pm$  SD) liters per second per centimeter of water. The corresponding average nasal resistance was  $1.46 \pm 0.54$  cm of water per liter per second. These results are comparable to those of McLean et al.<sup>11</sup> who recognized that a different reference point on a nonlinear curve will produce somewhat different values.

The experimental data are shown in Table I; patients 5 and 10 were tested on two different occasions. Patients were separated into two groups dependent on whether they did (group I) or did not (group II) experience a symptomatic LPR. For those studies during which LPR were observed, the change in NAC at the time of the last-phase symptoms is recorded. If symptoms were present during more than one measurement, the maximum change in NAC that occurred when symptoms were present is reported. For those studies during which no late symptoms were reported, the maximum decrease in NAC during the period of observation is reported.

In all patients severe immediate reactions occurred that were characterized by sneezing, rhinorrhea, and nasal congestion. The magnitude of the reduction of NAC immediately after challenge was nearly identical

in both groups. In all group I patients the predominate symptom was nasal congestion; sneezing and rhinorrhea were less prominent. Although there was a greater variation in the quantity of ragweed that was administered to group II patients, the average quantity of ragweed that was administered did not differ significantly between the groups.

In group I patients the change in mean late NAC was significantly decreased as compared to the mean late NAC for group II. In group II the last NAC occasionally (3 of 10 studies) improved to values greater than the baseline that was obtained after insufflation of buffer (e.g., patients 10 and 16 who demonstrated 12% and 25% improvement). However, insufflation of the buffer alone caused a variable immediate change in NAC with a mean decrease of 1% and a standard deviation of 15%. Thus the increases in NAC in patients 10 and 16 are comparable to the change that occurs after instillation of a mist. In patient 17, however, there was a 45% improvement in late NAC over the baseline buffer value that suggested the patient probably initially had been studied when some congestion was present. Even when patient 17 was eliminated from the analysis, there still remained

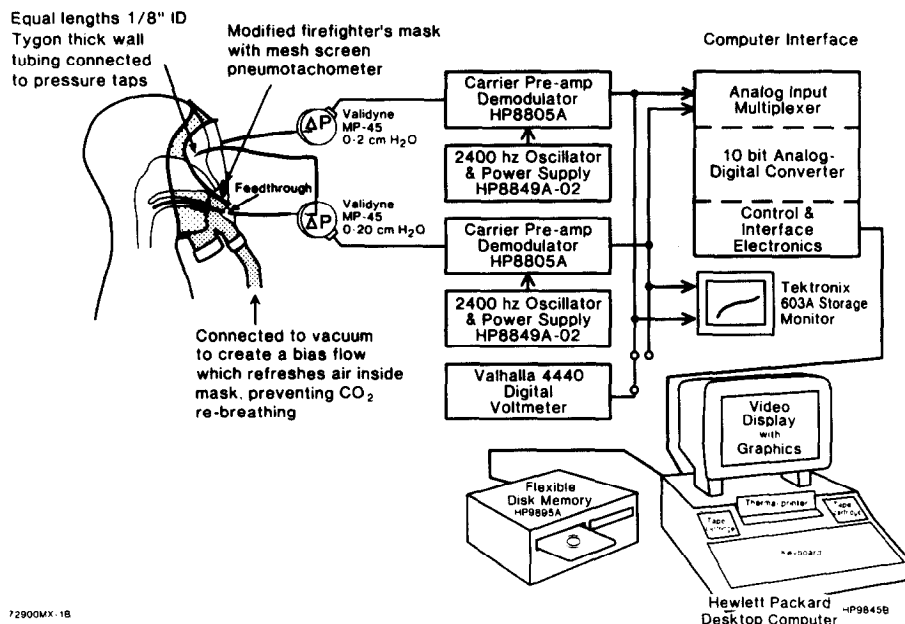


FIG. 1. Diagram of computerized posterior rhinomanometry apparatus.

a statistically significant difference ( $p = 0.006$ ) in the last NAC change in group I patients as compared to group II patients.

IgE antibodies to ragweed pollen were measured in five patients in group I and seven in group II, and they were elevated in all patients. There was no difference between the groups in the level of IgE antibody.

We made several attempts in a single individual (G. J. G.) to transfer passively nasal sensitivity to allergen. In the first series of experiments, one nostril was sprayed with serum from a ragweed-sensitive patient; this serum had previously been demonstrated to transfer passively immediate and late skin test reactivity to a nonallergic individual.<sup>4</sup> The other nostril was sprayed with diluent as a control. The next day both nostrils were challenged with aerosolized ragweed-pollen extract, but neither symptoms nor a decrease in NAC was observed. In an attempt to intensify exposure of nasal tissue to IgE antibody, cotton pledgets were soaked in the same serum and applied to one nostril. The pledgets were left in place for 4 hr. However, this maneuver also did not passively sensitize the nasal cavity, as nasal challenge tests the next day were again negative. In a third series of experiments, one inferior nasal turbinate was injected with the same serum, after which ragweed extract was aerosolized into the nasal cavity. Again neither immediate nor the nasal LPR was provoked. Injection of 1.0 ml of this serum into the nasal turbinate followed by direct injection of 0.1 ml ragweed-pollen extract into the same site 18 hr later also failed to elicit either symptoms or signs of inflammation. In

a final attempt to transfer passively nasal sensitivity, fresh plasma (300 ml) from an individual with grass-pollen sensitivity was transfused. Forty-eight hr later the recipient was reactive to grass-pollen extract by skin test, but neither nasal nor bronchial reactions were provoked after challenges with grass-pollen extract.

## DISCUSSION

The classic type I immunologic reaction is the wheal-and-flare response elicited in allergy skin-sensitization testing. This reaction is maximal at 15 to 20 min and then subsides in an hour or two. If sufficient antigen is injected to produce a wheal greater than 8 to 10 mm in diameter, there is often a subsequent response termed the late allergic reaction or LPR.<sup>1-4</sup> Beginning at 2 hr, there is gradually enlarging erythematous swelling at the reaction site that is maximal at about 6 to 12 hours and gradually subsides over a day or two. This LPR is dependent upon IgE antibody. In some allergic asthmatic individuals, inhalation of antigen leads to a late asthmatic response that follows the early response and becomes maximal over 6 to 12 hr and may last for 2 to 3 days. Although the mechanisms that underly the late asthmatic responses are as yet unknown, they may be comparable to those in the cutaneous LPR that have similar timing. Indeed the observations of Shampain et al.<sup>12</sup> that rabbits producing only IgE antibodies to *Alternaria* show a pulmonary LPR are in keeping with the hypothesis that IgE-mediated events may trigger a LPR in the respiratory tract.

We confirm earlier reports that a LPR often occurs in the nose after an immediate allergic reaction,<sup>5-8</sup> and we detected this response that occurs anywhere from 4 to 12 hr later with an average about 6 hr. In contrast to our results, Richerson et al.<sup>7</sup> did not find a correlation between late responses in the nose and in the skin. At least two variables must be considered in explaining these differences in findings. First, Richerson et al.<sup>7</sup> used sufficient ragweed pollen to produce an "acute reaction," whereas we used the maximum tolerated amount of pollen extract to produce a profound acute reaction. Second, Richerson et al. used a blockage index previously described by Taylor et al.<sup>13</sup> based on the peak expiratory flow rate through the nose. Data of Taylor et al.<sup>13</sup> demonstrate a correlation coefficient of only 0.52 that compares the blockage index (which was the objective index of nasal patency of Richerson et al.) to NAR as measured by Solomon et al.<sup>9</sup> at a reference point of 0.4 L/sec. Therefore we feel that the tremendous variation in nasal patency as measured by these two methods may also contribute to the inability to objectively demonstrate a nasal late phase response according to the method of Richerson et al.<sup>7</sup> Our patients were studied about every 4 hr for the first 12 hr, and during that time, they were indoors and were not exposed to any environmental changes that would be expected to produce both the symptoms that they described and the degree of nasal obstruction that was recorded. The frequency of observations, nevertheless, is probably insufficient for us to estimate the duration of the reaction. In two of the patients who reported late phase symptoms, studies were repeated at 24 hr. Congestion was reported, and there were changes in the NAC. However, these individuals also had been exposed to winter air in Minnesota, which could have accounted for the variations noted.

All 10 patients who experienced nasal LPR complained of congestion. Two patients complained of congestion and sneezing, and four complained of congestion, sneezing, and rhinorrhea. The immediate reaction in all these patients was accompanied by profound symptoms of congestion, sneezing, and rhinorrhea. Thus the characteristics of the LPR may differ from those of the immediate reaction in that nasal congestion is more prominent. The nasal LPR occurred in nine of the 17 patients tested or 53%. As noted above, others<sup>5-8</sup> have reported frequencies from 3% to 37%. Overall, it is surprising that the nasal LPR is not observed in a higher proportion of patients. In studies of the cutaneous LPR, we observed that almost all patients demonstrated the LPR, providing sufficient allergen was injected and providing the patient had not received immunotherapy.<sup>4</sup>

The sensitivity of the patient, as judged by the level of IgE antibody to ragweed, did not appear to determine whether or not a patient experienced a nasal LPR, and it is clear that certain patients with quite high levels of IgE antibody did not demonstrate a LPR. Overall, there was no relationship between the development of a nasal LPR and the level of IgE antibody.

One must conclude that differences exist between the capacity of the nasal cavity and the skin to express the LPR. The nature of such differences remains obscure. Clearly, failure to administer sufficient allergen could be a cause of a low LPR frequency. However, as shown in Table I, the immediate reactions were marked in our patients, and patient 16 had a mild attack of asthma after nasal provocation. Qualitatively, both patients who experienced and patients who did not experience the LPR had similar immediate reactions. Quantitatively, the immediate decrease in NAC was comparable in both groups. Thus we do not believe that administration of insufficient allergen is the reason for our inability to detect a nasal LPR in patients 10 to 17. Alternatively, the nasal LPR may frequently be mild enough to be asymptomatic and, therefore, not detectable by the methods that we used.

In 1919 Ramirez<sup>14</sup> reported a patient who experienced asthma after transfusion with blood of an asthmatic individual who was allergic to horses. In 1937 Sherman et al.<sup>15</sup> demonstrated that the nasal mucous membranes of the inferior turbinate and septum can be passively sensitized by injection of reagin-bearing serum. Reactions were elicited at these sites by parenteral or mucosal allergen injections or by topical application of dry pollen. The reactions consisted of swelling of the turbinate and usually were not associated with subjective symptoms; in only four of 25 positive reactions did the patients sneeze or experience any itching of the nose. We were not able to transfer passively nasal sensitivity to ragweed, as judged by symptoms or rhinomanometry, even though immediate and late cutaneous reactions were consistently transferred by the same serum and evoked by the allergen preparations that were used in these studies of the nose. Neither were we able to transfer nasal or bronchial sensitivity by transfusion of plasma that contained IgE antibodies to grass pollen. The reasons for our inability to transfer sensitivity are not clear; the quantity of IgE antibody could be responsible. The recipient (G. J. G.) in these experiments was consistently sensitized for the cutaneous LPR, indicating that the capacity to express the LPR was present. We suspect that passive sensitization of the respiratory tract infrequently produces symptoms in the transfused persons because blood from persons with

elevated IgE antibodies to pollen allergens is probably transfused quite commonly. Clearly, further studies of the transferability of nasal and bronchial LPR are warranted.

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