

Intranasal Challenge of Cynomolgus Monkeys with *Ascaris*, Ragweed, Bradykinin, and Histamine

Ernest P. Tanner, M.S., David P. Skoner, M.D., William J. Doyle, Ph.D., and Philip Fireman, M.D.

ABSTRACT

Seven feral monkeys with *Ascaris* hypersensitivity confirmed by intradermal skin test were challenged with single doses of *Ascaris* extract (10^5 PNU/nostril), ragweed extract (10^3 PNU/nostril) and diluent; with increasing doses of bradykinin (0.1, 1, and 5 mg/nostril) and histamine (0.1, 1, and 5 mg/nostril), and with repeated doses of PBS diluent. Total mean nasal resistance (TMNR) was measured by rhinomanometry and eustachian tube function by the forced-response test. For single dose challenges, testing was conducted before and 5, 15, 40, and 50 minutes after the challenge and for multiple dose challenges before, 5 and 15 minutes after each dose, and 40 and 50 minutes after the final dose. Mean TMNR (cm H₂O/L/sec) was increased from 28.9 ± 9 at baseline to peak at 124 ± 46 after *Ascaris* challenge. In contrast, TMNR was unaffected in six of seven monkeys challenged with diluent and in all monkeys challenged with ragweed. Progressive TMNR increases from baseline (25 ± 8) were observed for bradykinin (peak = 64 ± 46) and histamine (peak 108.4 ± 59) challenges. Eustachian tube function data showed no significant changes after intranasal provocation with either allergens or mediators. Provocative intranasal allergen challenge in actively sensitized monkeys is an effective method for evaluating the pathophysiology of allergic rhinitis and the effect of inflammatory mediators on nasal resistance.

Intranasal challenge with an appropriate antigen results in increased nasal resistance, i.e., decreased

nasal patency.¹ Many substances, such as histamine, prostaglandins, and bradykinin have been implicated as mediators of the inflammatory process.² Both normal subjects and patients with allergic rhinitis have been challenged with these mediators and exhibited increases in nasal resistance similar to those experienced after antigen provocation.³ In addition to the changes in nasal resistance, many patients also experienced impaired eustachian (ET) function, resulting in an inability to open the ET as measured by sonotubometry⁴ or tympanometry.⁵

Experiments conducted in passively sensitized monkeys showed similar increases in nasal and ET obstruction after intranasal provocation with appropriate antigens⁶ and inflammatory mediators.⁷ The purpose of this study was to develop an actively sensitized monkey model for evaluating the changes in nasal patency and ET function after intranasal challenge with allergens and inflammatory mediators.

MATERIALS AND METHODS

Seven feral, juvenile *Ascaris suum*-sensitive cynomolgus monkeys (Worldwide Primates, Inc.) were sedated with ketamine (0.7 ml) and Nembutal (15 mg/kg) before challenge. Challenges consisted of the administration of 0.1 ml of the appropriate substance [*A. suum* extract: 190,000 protein nitrogen units (PNU), Greer Labs.; ragweed extract: 4000 PNU, Hollister-Stier; skin test diluent, Hollister-Stier; histamine diphosphate: 0.1, 1, and 5 mg, Sigma; bradykinin: 0.1, 1, and 5 mg, Boehringer-Mannheim; or Dulbecco's phosphate-buffered saline (PBS), repeated] per nostril. Nasal resistance was measured by active anterior rhinomanometry using a computer-assisted system previously

Department of Pediatrics, Department of Otolaryngology, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA15260-0001

described, and ET function was measured using the forced response test and instruments developed in our laboratory.^{4,8}

For challenges with *Ascaris*, ragweed and diluent (single dose challenges) rhinomanometric measurements were made before challenge (3 times) and then 5, 15, 40, and 50 minutes postchallenge. In the analysis presented below, measurements at 5 and 15 minutes postchallenge were defined as early responses, while those at 40 and 50 minutes postchallenge were defined as late responses. Assessments of the passive parameters of ET function (opening and closing pressures) were made at baseline (2 times) and then at 10, 20, 30, and 55 minutes. The challenge sessions were separated by at least 2 weeks.

For dose-response challenges with mediators, rhinomanometric measurements were made at 5 and 15 minutes and ET function measurements at 10 minutes after each dosing, and then as described above after administration of the highest dose.

RESULTS

Significantly increased average total mean nasal resistance (TMNR) was evident when *Ascaris*-hypersensitive cynomolgus monkeys were challenged intranasally with a single dose of *Ascaris*, but not with ragweed (irrelevant allergen) or diluent. Both early ($p < 0.01$) and late ($p < 0.001$) measurements were greater than those at baseline, the TMNR increasing from 28.1 ± 8.7 to 57.1 ± 45.1 and 124.4 ± 46.4 cm H₂O/L/sec, respectively. Neither ragweed nor diluent caused significant changes from baseline through late measurements. When compared to ragweed and diluent, *Ascaris* early average TMNRs were significantly higher than the corresponding ragweed TMNR (27.2 ± 10.9 ; $p <$

0.05), but not the corresponding diluent TMNR (37.4 ± 7.1). However, the *Ascaris* late average was significantly higher than both the ragweed and diluent (29.2 ± 15.6 and 34.7 ± 5.6 ; $p < 0.001$ for both) TMNRs (Fig. 1).

After PBS challenge, average TMNR measurements were 29.7 ± 11.9 , 37.9 ± 22.3 , 43.4 ± 41.7 , and 30.9 ± 21.3 , which were not significantly different from baseline. Intranasal challenge with histamine significantly increased average TMNR at all concentrations relative to baseline measurements. Average TMNR increased from 27.6 ± 6.0 to 61.6 ± 38.7 (0.1 mg, $p < 0.02$), 85.1 ± 56.8 (1 mg, $p < 0.01$), 108.4 ± 59.0 (5 mg early, $p < 0.001$), and 99.5 ± 60.1 (5 mg late, $p < 0.01$), respectively. From a baseline of 25.5 ± 7.9 rhinomanometric measurements after bradykinin challenge increased to 28.2 ± 11.8 (0.1 mg), 33.1 ± 14.2 (1 mg), 63.8 ± 46.1 (5 mg early, $p < 0.001$) and 36.4 ± 21.8 (5 mg late, $p < 0.05$). Only the early and late challenge doses were significantly higher than baseline measurements, but not significantly different from the appropriate PBS measurements. Compared to bradykinin average TMNRs, histamine evoked significantly higher average TMNRs, except for the 5 mg early measurement ($p < 0.01$) (Fig. 2).

The passive parameters of ET function were not affected by intranasal provocation with either allergens (Fig. 3) or mediators (Fig. 4). While the measurements after histamine challenge were elevated relative to baseline, the differences were not statistically significant.

DISCUSSION

These studies indicate that provocative intranasal allergen challenge in hypersensitive monkeys, coupled with computer-assisted rhinomanometry is a pow-

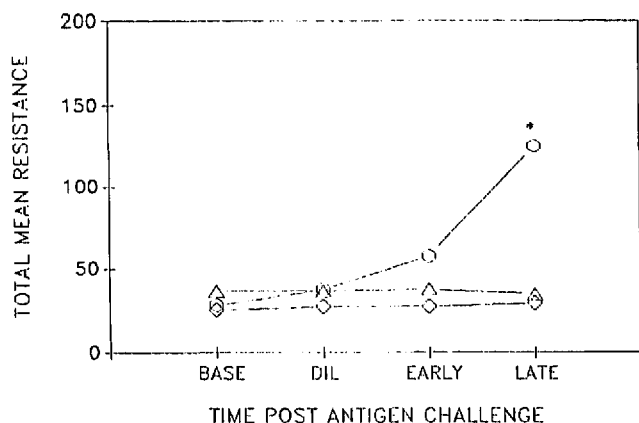


Figure 1. Average total mean nasal resistance after intranasal *Ascaris* (○—○), ragweed (◇—◇), or diluent (△—△) challenge. $p < 0.001$ relative to both ragweed and diluent.

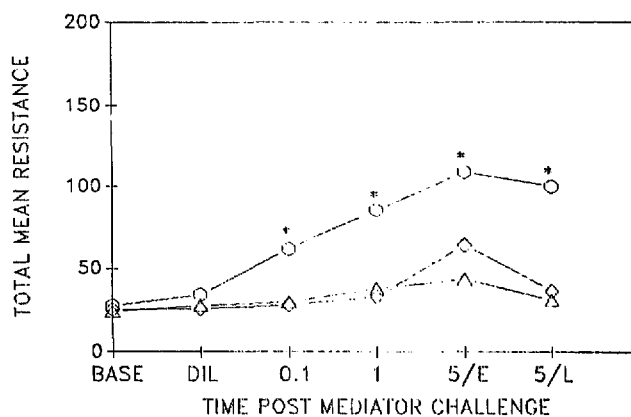


Figure 2. Average total mean nasal resistance after intranasal bradykinin (◇—◇), histamine (○—○), or PBS (△—△) challenge. $p < 0.02$ relative to PBS.

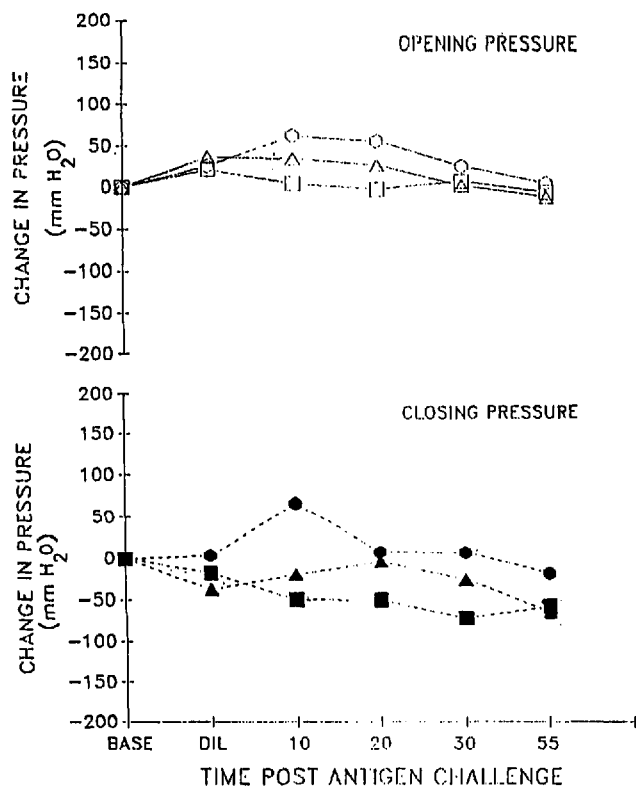


Figure 3. Changes in opening and closing pressures from baseline after intranasal *Ascaris* (○ ●), ragweed (△ ▲), or diluent (□, ■) challenge.

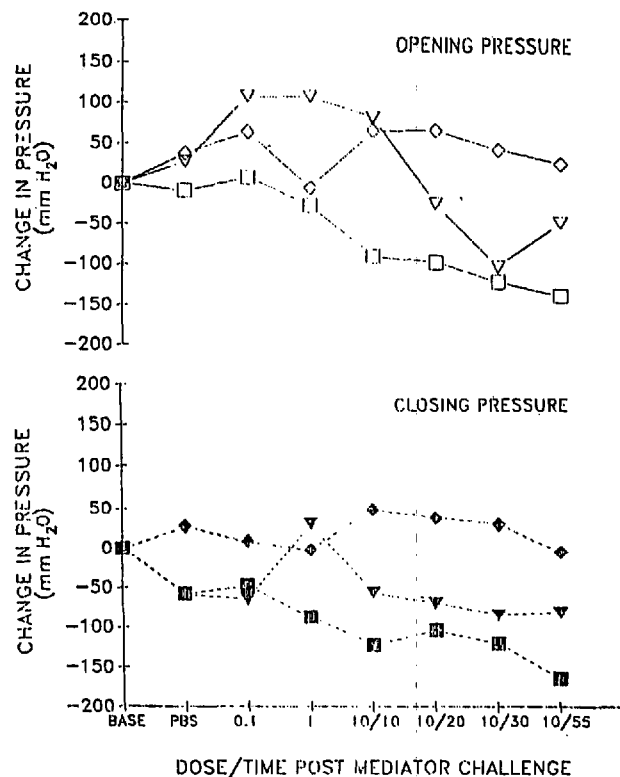


Figure 4. Changes in opening and closing pressures from baseline after intranasal bradykinin (▼, ◇), histamine (◇, ◆), and PBS (□, ■) challenge.

erful tool for evaluating the pathophysiology of allergic rhinitis and the effects of inflammatory mediators on nasal resistance. *Ascaris* challenge in sensitized monkeys produced significantly more nasal congestion than challenges with either diluent or ragweed (irrelevant allergen). Histamine challenge provoked significantly greater increases in nasal resistance than either bradykinin or PBS. Similar results have been previously reported for patients with allergic rhinitis after intranasal allergen challenge.⁴ Nasal conductance approached 0 with increasing doses of ragweed. Monkeys passively sensitized with serum from ragweed or timothy grass allergic individuals also displayed increases in rhinorrhea and nasal obstruction after challenge with the appropriate allergen.⁶ Earlier experiments by Kelly *et al.* using rhesus monkeys naturally sensitized to *Ascaris* and Patterson *et al.* using experimentally induced active *Ascaris* sensitization produced increased pulmonary resistance after bronchial *Ascaris* challenge^{9,10} or histamine.⁹ Similarly, increased nasal airway resistance followed histamine challenge in humans¹¹ and rhesus monkeys.⁸

There were no statistically significant changes in opening or closing ET pressures; however, complete nasal obstruction in many of the animals precluded

challenge with higher doses of mediators to better differentiate changes in ET function. Both open and closing pressures increased after *Ascaris* challenge. Previous results obtained in humans after intranasal provocative challenge tests with ragweed or timothy grass pollen showed that 96% of the ears studied experienced ET obstruction, manifested by an inability to equilibrate positive and negative pressures applied to the external auditory canal.¹² Slight increases in the passive parameters of ET function and significant impairment of active ET function were observed after antigen challenge in passively sensitized monkeys.⁶ Histamine challenge of rhesus monkeys produced changes similar to those found after antigen challenge.⁷ Although the present study shows a trend toward similar passive parameter dysfunction, there, again, the changes in ET function were not statistically significant ($p < 0.05$).

Evaluation of pharmacological agents is readily accomplished with a naturally sensitized animal model. Experiments conducted in our laboratory showed that rhesus monkeys orally pretreated with either cetirizine or chlorpheniramine effectively inhibited histamine-induced increases in nasal airway resistance.⁸ A natural extension of these findings would be to evaluate the effectiveness of various antihistamines in inhibiting

Ascaris-induced nasal obstruction in sensitized monkeys.

Further study utilizing this model may elucidate any physiologic variants inherent in cynomolgus monkeys that would account for any differences between them and rhesus monkeys or humans. On the other hand, additional study measurement using a larger cohort of subjects may enhance the observed changes in ET function to a point where these changes become significant. Also, the role of allergic mechanisms in the pathogenesis of otitis media can be assessed through long-term exposure of the animals to allergen and prospective examination of both passive and active ET function and middle ear physiology. These advantages are not currently available using passively sensitized models.

REFERENCES

1. Skoner DP, Doyle WJ, Boehm S, Fireman P. Response of the nose and eustachian tube to allergen challenge. In: Lim DJ, Bluestone CD, Klein JO, Nelsen JD eds. 4th International Proceedings: Recent Advances in Otitis Media. Philadelphia, Brian C. Decker, Inc., 1988, p. 96.
2. Mathews KP. Mediators of anaphylaxis, anaphylactoid reactions, and rhinitis. *Am J Rhinol* 1:17, 1987.
3. Solomon WR, McLean JA. In: Spector S, ed. Nasal Provocative Testing in Provocative Challenge Procedures: Bronchial, Oral, Nasal, and Exercise. Boca Raton, FL: CRC Press, 1983, vol. II, p. 133.
4. Stillwagon PK, Doyle WJ, Fireman P. Effect of an antihistamine/decongestant on nasal and eustachian tube function following intranasal pollen challenge. *Ann Allergy* 58:442, 1987.
5. Friedman R, Doyle WJ, Casselbrant ML, Bluestone CD, Fireman P. Immunologic mediated eustachian tube obstruction: A double-blind cross-over study. *J Allergy Clin Immunol* 71:442, 1983.
6. Doyle WJ, Friedman R, Fireman P, Bluestone C. Eustachian tube obstruction after provocative nasal antigen challenge. *Arch Otolaryngol* 110:508, 1984.
7. Doyle WJ, Ingraham AS, Fireman P. The effects of intranasal histamine challenge on eustachian tube function. *J Allergy Clin Immunol* 76:551, 1985.
8. Fireman P, Skoner D, Tanner E, Doyle W. A primate model for the evaluation of antihistamines. *Ann Allergy* 59:9, 1987.
9. Kelly JF, Cugell DW, Patterson R, Harris KE. Acute airway obstruction in rhesus monkeys induced by pharmacologic and immunologic stimuli. *J Lab Clin Med* 83:738, 1974.
10. Patterson R, Harris KE, Pruzansky JJ. Induction of IgE-mediated cutaneous, cellular, and airway reactivity in rhesus monkeys by *Acaris suum* infection. *J Lab Clin Med* 101:864, 1983.
11. Britton MG, Empey DW, John GC, McDonnell KA, Hughes DTD. Histamine challenge and anterior nasal rhinometry: Their use in the assessment of pseudoephedrine and triprolidine as nasal decongestants in subjects with hayfever. *Br J Clin Pharmacol* 6:51, 1978.
12. Ackerman MN, Friedman RA, Doyle WJ, Bluestone CD, Fireman P. Antigen-induced eustachian tube obstruction: An intranasal provocation challenge test. *J Allergy Clin Immunol* 73:604, 1984. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.