Acute Bacterial Rhinosinusitis Causes Hyperresponsiveness to Histamine Challenge in Mice

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Objectives: To develop a physiologic test of nasal responsiveness in mice and to evaluate whether mice with acute bacterial sinusitis develop nasal hyperresponsiveness.

Design: Several experimental studies will be described. The first was a titration pilot study. The second was a randomized, placebo-controlled study. The remainder were before-and-after trials.

Species: BALB/c or C57BL/6 mice.

Interventions: For these experiments, we exposed mice to histamine intranasally, then counted the number of sneezes and nose rubs as the primary outcome measure of nasal responsiveness. First, we constructed a dose-response curve. Second, we treated the mice with desloratadine, a histamine 1 receptor antagonist, prior to histamine exposure. Third, we challenged, with intranasal histamine, mice made allergic using 2 techniques. Fourth, we infected mice with Streptococcus pneumoniae to determine whether acute sinusitis causes nasal hyperresponsiveness to histamine exposure.

Results: Nasal histamine challenge led to a reproducible, dose-dependent increase in sneezing and nose rubs. The response to histamine exposure was blocked by desloratadine ($P<0.05$). Allergic mice had a significant increase in responsiveness ($P=0.05$) over baseline after exposure to antigen. Mice with acute sinusitis had a sustained increase in responsiveness, although less severe than after allergy, compared with baseline values that lasted 12 days after infection ($P<0.05$).

Conclusions: Nasal challenge with histamine is a physiologic test of nasal responsiveness. The hyperresponsiveness of allergic mice to histamine exposure parallels the response to nonspecific stimuli during the human allergic reaction. In addition, we showed that acute bacterial sinusitis causes nasal hyperresponsiveness in mice.


NASAL HYPERRESPONSIVENESS TO IRRITANTS AND TO REPEITIVE ALLERGEN EXPOSURE IS A WELL-ESTABLISHED PHENOMENON ASSOCIATED WITH ALLERGIC RHINITIS.¹ In humans, hyperresponsiveness to allergens increases after an antigen challenge (priming).² Non-specific hyperresponsiveness to irritants such as histamine, methacholine, bradykinin, and cold, dry air also increases after allergen challenge.³ These laboratory events correlate with the response of patients to cold weather and strong odors, like that of gasoline.

How allergic exposure causes nasal hyperresponsiveness is unknown.³ There are several proposed hypotheses for the underlying mechanism. Increased epithelial permeability may allow easier access of stimuli to nerve endings.⁴ An increase in sensitivity or in the number of irritant receptors, or a decrease in the number or responsiveness of sympathetic receptors, may result in increased mucosal sensitivity. In allergic patients, an increase in the number of muscarinic receptors has been described by some investigators,⁵,⁶ whereas van Megen et al⁷ described not only a decrease in the number of muscarinic receptors, but also an increase in their sensitivity. Ishibe et al⁸ described down-regulation of adrenergic receptors in allergic patients. Local changes related to an increase in the numbers of eosinophils, neutrophils, basophils, and lymphocytes and their released mediators after antigen exposure have been implicated in the development of nasal hyperresponsiveness.⁹

When histamine contacts the nasal mucosa, it causes an increase in vascular permeability, dilation of the cavernous sinusoids, and neuronal stimulation.¹⁰ Increased nasal secretion is caused primarily by nerve-mediated parasympathetic glandular secretion and an increase in vascular leakage. Nasal congestion is mediated primarily by pooling of blood in the vascular sinusoids.
itchiness and sneezing are thought to be secondary to histamine’s actions on the trigeminal nerve.\textsuperscript{9,11} Clement et al\textsuperscript{12} showed that allergic patients have increased nasal resistance after topical application of histamine, suggesting that there is pooling of blood in the cavernous sinusoids. However, nasal airway resistance is variable in humans. There is considerable overlap between healthy and allergic patients in their response to histamine exposure; thus, it is not a reliable test of hyperresponsiveness.\textsuperscript{3} Numata et al\textsuperscript{13} found that, although patients reported a lesser degree of congestive symptoms after antihistamine treatment, this did not correlate with objective rhinomanometry. Gerth van Wijk et al\textsuperscript{14} suggested that measurements of sneezing and secretions in allergic mice. Thus, we chose histamine as a stimulus.

We investigated whether acute bacterial rhinosinusitis causes nasal hyperresponsiveness to histamine exposure in mice. Research on sinusitis in humans is limited by access to the sinuses and the types of studies that ethically can be done. Therefore, we developed a mouse model of acute bacterial rhinosinusitis.

Little is known about sinusitis-induced hypersensitivity in humans. Sampaio et al,\textsuperscript{15} in an abstract, reported decreased ipsilateral nasal secretions after intranasal histamine challenge in a few patients with chronic sinusitis compared with healthy control subjects and no difference in the contralateral secretary or the sneezing response. Whether acute bacterial sinusitis causes differences in nasal mucosal hyperresponsiveness has not been studied.

To test the hypothesis that acute bacterial sinusitis causes hyperresponsiveness, we needed to develop a non-specific test of nasal hyperresponsiveness in mice. Imamura and Kambara\textsuperscript{16} used substance P and histamine applied intranasally to measure the sneeze responses in guinea pigs. Saito et al\textsuperscript{17} applied intranasal histamine to study the kinetics of hyperresponsiveness in allergic mice. Thus, we chose histamine as a stimulus.

We first established a dose-response curve to intranasal histamine exposure in healthy mice. We then demonstrated that the response to nasal histamine challenge could be blocked by treatment with a histamine 1-antihistamine, desloratadine (Schering-Plough, Kenilworth, NJ). Next, we histamine-challenged mice that had been sensitized to ovalbumin by 2 different methods to determine whether mice, like humans, became hyperresponsive after allergen stimulation. Finally, we applied the histamine challenge to our model of acute bacterial sinusitis.

**METHODS**

**MICE**

We purchased 6-week-old, pathogen-free, BALB/c or C57BL/6 mice from Jackson Laboratories (Bar Harbor, Me). They were maintained in the biohazard suite of the Carlson Animal Facility, Chicago, Ill, in cages with microisolator tops. The studies were approved by the Animal Care and Use Committee of the University of Chicago.

**NASAL CHALLENGE WITH HISTAMINE**

Because mice are obligate nose breathers, small droplets of solution were placed on the external nares of awake mice to be drawn into the nasal passages during inhalation. The nasal challenge with histamine consisted of intranasal application of 50 µL of various concentrations of histamine (Sigma-Aldrich, St Louis, Mo) applied gradually over 2 minutes. In our initial efforts, we used 5 different concentrations: a isotonic sodium chloride (vehicle for dissolving histamine); 1-, 10-, 30-, and 100mM histamine; but, because of the stress on the mice, the challenge was changed to 3 exposures of 0.3-, 3.0-, and 30mM histamine.

After each exposure, we observed the mice for 10 minutes and counted the number of sneezes and nose-rubbing episodes. Each count was performed by the same investigator (J.J.K.) who was blinded to the treatment groups. From these data, we constructed dose-response curves.

**DESLORATADINE GAVAGE**

Mice were treated with 10 mg/kg of desloratadine by gavage in a methylcellulose vehicle 4 hours prior to nasal challenge with histamine. The controls for this experiment were given an equivalent volume of methylcellulose (Dow Chemical Co, Midland, Mich), the vehicle for desloratadine, by gavage.

**ALLERGIC SENSITIZATION**

Two techniques were used. For the first technique, mice were sensitized by intraperitoneal (IP) injection of 20 µg of ovalbumin (Sigma-Aldrich) together with an aluminum hydroxide (Pierce Biotechnology, Inc, Rockford, Ill) adjuvant. Eight days after the first IP exposure, the mice received a second injection. Two days after the second injection, the animals were exposed to a 6% ovalbumin solution in 0.1M phosphate-buffered saline (PBS) (Roche Diagnostics Corporation, Indianapolis, Ind) by intranasal inoculation daily for 5 days. For the second technique, mice were injected with aluminum hydroxide and PBS, but no ovalbumin. These mice were then exposed to either 50 µL of 6% ovalbumin or an equivalent volume of PBS daily for the duration of the experiment.

**INDUCTION OF SINUSITIS**

Soy broth was used for control animals and soy broth containing Streptococcus pneumoniae for experimental animals. The American Tissue Culture Collection, Rockville, Md, strain of S pneumoniae was obtained from the Clinical Microbiology Laboratories of the University of Chicago Hospitals. Fifty microliters of 10\(^6\) colony-forming units/mL solution was administered to each mouse over 5 minutes.

**NASAL CULTURE**

Animals were killed with 120 mg/kg of phenobarbital. As respiratory depression occurred, we disinfected the animal’s head, external nose, and oropharynx with a swab of 70% isopropyl alcohol. We then lavaged the nasal and sinus cavities with 200 µL of sterile sodium chloride. We quantified the bacteria obtained by nasal lavage by using serial 10-fold dilutions and spread the lavage fluid on blood agar plates. The plates were incubated for 24 hours, and then colony-forming units of S pneumoniae were counted. In prior experiments, there was an excellent correlation between nasal lavage correlations and cultures of sinus tissues.\textsuperscript{11}
For the control group, there was a significant difference in the number of nose rubs (A) and sneezes (B) at 30mM histamine.

**STATISTICAL ANALYSIS**

All calculations were done by use of Minitab software (Sigma Breakthrough Technologies, San Marcos, Tex). We looked for differences between groups and between treatments with analysis of variance; P≤.05 was statistically significant. If statistically significant, the Tukey test was applied.

**RESULTS**

Histamine causes a dose-dependent increase in sneezes and nose rubs in nonallergic, uninfected mice. Five C57BL/6 mice were given intranasal histamine in 5 doses: 0 (isotonic sodium chloride), 1, 10, 30, and 100 mM. We found a dose-dependent increase in the response to histamine exposure. There was a significant increase in nose rubs from the control vehicle for the 3 highest concentrations of nasal histamine challenge (P≤.05), and the number of nose rubs reached a plateau at 10mM (Figure 1). There was a significant increase in sneezes at the 2 highest doses of histamine (P≤.05), which reached a plateau at 30mM (Figure 2).

We found that the mice did not tolerate more than 3 exposures. One mouse died after the fourth dose challenge, and several of them seemed dazed after the fourth challenge, with rapid respiratory rates and absence of nose rubbing and sneezes. When given a 30-minute rest period, these mice responded again with nose rubs and sneezing in the dose-dependent manner presented in Figure 1 and Figure 2. Because the mice did not tolerate more than 3 exposures, we decreased the number of nasal histamine challenges to 3, with the highest concentration set at 30mM, where the plateau of the sneezing dose-response curve began.

The response to histamine exposure is blocked by treatment with desloratadine. To demonstrate the specificity of the response, we hypothesized that the dose-response curve generated by nasal histamine challenge would be reduced by treatment with a histamine 1 receptor blocker. Five C57BL/6 mice were treated 4 hours prior to histamine challenge with desloratadine (10 mg/kg) by gavage, and 5 were treated with methylcellulose. For the control group, there was a significant difference between the lowest doses of histamine (0.3- and 3.0mM) and the highest dose of histamine (30mM) for both sneezing and nose rubbing (P≤.05). In the desloratadine treatment group, there was no difference between the lowest and the highest dose of histamine. The difference at the 30mM concentration between the desloratadine-treated group and the control group was significant for both sneezing and nose rubs (P≤.05) (Figure 3).

Allergic inflammation increases sensitivity to the nasal challenge with histamine. We hypothesized that mice made allergic to ovalbumin would show hyperresponsiveness to histamine. For this experiment, we used 5 BALB/c mice, because these mice have a greater tendency than do C57BL/6 mice to develop Th2 or allergic responses. Baseline histamine challenges were compared with challenges performed after allergic sensitization. Nasal histamine challenge was performed prior to the sensitization process and after IP sensitization, but prior to intranasal exposure to antigen. A third histamine challenge was performed 6 days after intranasal antigen exposure, and the last nasal histamine challenge was performed 1 week after the last intranasal antigen exposure.
of intranasal ovalbumin without systemic sensitization to ovalbumin developed increased ovalbumin-specific IgE and IgG after 28 days of exposure. These mice also had goblet cell hyperplasia, recruitment of eosinophils and lymphocytes, and increased interleukin 5 in bronchoalveolar lavage fluid. We hypothesized that mice exposed to intranasal ovalbumin without IP sensitization would develop nasal hyperresponsiveness. Six BALB/c mice were exposed daily to intranasal ovalbumin. The timeline of the sensitization protocol described earlier was used; however, instead of IP injection of ovalbumin and aluminum hydroxide, the mice were injected IP with PBS and aluminum hydroxide adjuvant and given intranasal ovalbumin 5 days a week for 4 weeks. A baseline histamine challenge was performed prior to intranasal ovalbumin exposure. Histamine challenges were performed 4, 12, 19, and 26 days after the first ovalbumin exposure. Both sneezing and nose rubbing end points increased significantly in mice exposed daily for 19 days to ovalbumin. Responses to histamine challenge on day 26 were significantly higher for both parameters than on day 19 (Figure 6).

Acute bacterial sinusitis increases sensitivity to nasal challenge with histamine, but to a lesser degree than allergy. We hypothesized that mice with acute bacterial sinusitis would develop hyperresponsiveness to histamine exposure. For this experiment, we infected 6 BALB/c mice with *S pneumoniae*. This group received daily intranasal PBS after an initial IP injection with PBS and aluminum hydroxide adjuvant.

Culture results at day 14 showed $9 \times 10^5$ colony-forming units of bacteria, and this decreased to $1 \times 10^5$ colony-forming units of bacteria by 28 days ($P \leq .05$) (Figure 7). These results paralleled the change in hyperresponsiveness measured by histamine challenge. By day 4, these mice had a small, but significant increase in nasal hyperresponsiveness as measured by nasal rubbing, which persisted to day 12 (Figure 8A). At day 19, the next time point, the number of recorded nose rubs was no longer signifi-

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**Figure 4.** Mean ± SEM of the sum of the nose rubs (A) and sneezes (B) for each histamine concentration. There was a significant difference between baseline values and postallergen exposure. A, For nose rubs there was a difference between baseline values and 1 week after the last allergen exposure. B, For sneezes this difference was no longer present by 1 week after the last exposure to allergen.

There were no significant differences between the 2 baseline histamine challenges for either outcome, demonstrating the reproducibility of this test of hyperresponsiveness (Figure 4). After sensitization and 6 days of intranasal allergen exposure, there was a statistically significant increase in the number of sneezes seen at each concentration of histamine ($P \leq .05$), and a significant increase in nose rubbing at 3 mM and 30 mM histamine concentrations from baseline ($P \leq .05$) (Figure 5).

In addition to a total increase in sneezes and nose rubs, the first concentration of histamine to be statistically different from baseline was lower after allergen exposure (Figure 5). Significant sneezing and nose-rubbing response was reached at 0.3 mM and 3.0 mM histamine concentrations, respectively, vs 30 mM for both end points before allergen exposure.

One week after the last allergen exposure, the area under the curve for the number of nose rubs was unchanged from that for the third histamine challenge, still exhibiting the same increase from baseline (Figure 4A). By 1 week after antigen exposure, there had been a decrease in response for the sneezing outcome compared with the third time point (Figure 5). When we analyzed each concentration of histamine separately, only the response at the 3 mM concentration was significantly different from baseline ($P \leq .05$) (Figure 5B).

The response to nasal challenge with histamine increases when BALB/c mice are exposed to daily nasal ovalbumin without IP sensitization. McCusker et al. had observed that BALB/c mice exposed to high concentrations...
significantly increased over baseline. There was no significant increase in the number of sneezes throughout the time course of the bacterial sinusitis infection (Figure 8B).

**COMMENT**

Nasal challenge with histamine is a reproducible and easy-to-use physiologic test of nasal hyperresponsiveness. It provides a useful outcome measure in murine models of inflammatory nasal disease and should allow us to determine the underlying mechanism of increases in hyperresponsiveness to different inflammatory stimuli.

The 2 end points, nose rubbing and sneezing, have different thresholds of responses. In the first experiment, the nose rubbing measures were increased significantly from baseline at the 10mM concentration of histamine, whereas the first significant increase for sneezing was at 30mM. Mice begin to rub their noses with less irritation than it takes to generate a sneeze. In IP-sensitized allergic mice, there were maximal increases in hyperresponsiveness 5 days after the first exposure to allergen, followed by a decline. However, with the nose-rub end point, we noted that nasal irritability remained maximally elevated over baseline even 1 week after the last ovalbumin exposure. The lower threshold for nasal rubbing is analogous to the human condition where symptoms of nasal itching precede sneezing.

The lower threshold of irritability for nose rubbing was also important for measuring the nasal hyperresponsiveness associated with acute sinusitis. Although nose rubbing was significantly increased from baseline, showing nasal irritability, the mucosal hyperresponsiveness associated with sinusitis did not stimulate sneezing above baseline in our model. This could also represent the effects of different inflammatory stimuli on the manifestations of hyperresponsiveness.

Nasal hyperreactivity testing in humans derives from the concept of bronchial hyperresponsiveness testing in asthma. In fact, sensitivity to methacholine is often used...
in the diagnosis of asthma. Its use in the nose as a test for distinguishing disease states is limited because of large variability in responses.\textsuperscript{15} We found little variability in our histamine test, as even small changes were statistically significant, making this an easy-to-use, sensitive model of nasal hyperreactivity in mice.

It has been reported that changes in the nasal mucosa associated with sinusitis in humans include increased basal secretion of lysozyme and lactoferrin.\textsuperscript{19} In the same study, the quantity of enzymes in the secretions was not increased with methacholine challenge, but the patients responded to histamine challenge with increased vascular permeability and glandular secretions. Studies of nasal secretions during acute infection with rhinovirus show that the initial response to infection is increased vascular permeability, followed 2 days later by increased glandular secretions.\textsuperscript{20} Increases in IgG and antibacterial enzymes in the setting of acute infection are protective against infection. In acute infections, hyperresponsiveness with a lower threshold for irritation may cause sneezing, itching, and other avoidance behaviors.

**CONCLUSIONS**

Using nasal challenge with histamine, we showed that systemic sensitization to ovalbumin followed by nasal ovalbumin exposure caused nasal hyperresponsiveness that persisted at least 1 week after the last exposure, whereas continued intranasal exposure to ovalbumin alone caused a slower increase in nasal hyperresponsiveness than that following IP sensitization to ovalbumin. Acute bacterial sinusitis caused hyperresponsiveness as early as 4 days after infection, and whereas sinusonal bacterial infection does not result in as much hyperreactivity as did allergy, this intranasal histamine challenge test was able to detect hyperresponsiveness.

Nasal challenge with histamine provides a reproducible dose-response curve in BALB/c and C57BL/6 mice. The test is simple and can demonstrate a physiologic change in the mucosa, which complements cellular measures of inflammation. For these reasons, nasal histamine challenge should help us to study hyperresponsiveness in other murine models of nasal inflammation.

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