A Mouse Model of Acute Bacterial Rhinosinusitis

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Objective: To develop a mouse model of acute bacterial rhinosinusitis.

Design: Study mice (C57BL6/J) were inoculated intranasally with Streptococcus pneumoniae, ATCC 49619 suspended in trypticase soy broth, and controls were inoculated with trypticase soy broth alone. After 2, 5, or 14 days, intranasal cultures were obtained and mice were killed. The sinuses were prepared for histological investigation.

Setting: Animal care facility at a tertiary, academic institution.

Method: The histological sections of the sinuses were examined in a blinded manner for the percentage of sinus cavity occupied by neutrophil clusters, and for the number of neutrophils per square millimeter of sinus mucosa.

Results: Infected mice killed at 5 days had significantly more sinus area occupied by neutrophil clusters, significantly more neutrophils within the mucosa, and significantly more S pneumoniae growth in the intranasal cultures compared with controls (15/15 vs 0/6; P<.01). The amount of inflammation had decreased at 2 weeks.

Conclusion: Streptococcus pneumoniae induces acute bacterial rhinosinusitis in C57BL6/J mice as measured by culture and influx of neutrophils, and can be used as a model of acute bacterial rhinosinusitis.


The most basic definition of rhinosinusitis is inflammation of the lining membrane of any of the paranasal sinuses. This definition, however, has limited pathologic and clinical significance. Rhinosinusitis has manifestations ranging from an incidental computed tomographic scan finding1,2 to an acute illness after an upper respiratory tract viral infection3 to an unremitting illness associated with a genetic abnormality in the epithelium of patients with cystic fibrosis.6

More than 31 million Americans describe chronic rhinosinusitis as a health problem, making it the most frequently reported common disease in the 1993 National Health Interview Survey.7 It affects all age groups, including 17% of individuals older than 65 years. In 1991, 11.6 million office visits were made for rhinosinusitis.8 The number of restricted-activity days per year increased from 30 million to 73 million between 1988 and 1992,7 and the number of antibiotic prescriptions for rhinosinusitis increased from 5.8 million in 1985 to 13 million in 1992.9 Approximately 200 000 sinus surgeries were performed in 1994. Thus, each year, billions of dollars are spent on direct medical costs for treatment of this enigmatic illness.

Rhinosinusitis is usually divided into acute, subacute, and chronic conditions, based on the reported duration of the illness.10 Although no known pathologic correlates are associated with this classification, it is assumed that the organisms and the underlying pathologic mechanisms differ. Acute rhinosinusitis implies an illness of less than 2 weeks’ duration, whereas the subacute condition lasts longer than 2 weeks without treatment. Chronic rhinosinusitis usually implies the presence of upper respiratory tract symptoms for more than 12 weeks, despite medical treatment, with radiographic evidence of mucosal thickening within the sinuses.

Rhinosinusitis is also a problem throughout the animal kingdom.11 The best-studied animal is the rabbit.12-15 The large size of the animal permits easy surgical manipulation. The induction of acute rhinosinusitis in this model, however, requires mechanical obstruction of the ostia. The inability to manipulate the rabbit genetically and the lack of reagents and antibodies for...
METHODS

MICE

C57BL/6J female mice aged 4 to 5 weeks and weighing 16 to 18 g were purchased pathogen free from Jackson Laboratory (Bar Harbor, Me) and kept in the Carlson Biocontainment Suite Facility at the University of Chicago in Chicago, Ill. We chose the strain and age of mice because they would have had minimal exposure to environmental stimuli, including bacterial infections and, thus, limited immunologic memory. This strain has also been used for numerous immunologic and genetic studies, thus preparing the groundwork for future studies. All protocols were approved by the Animal Safety Committee of the University of Chicago. All manipulations of the animals before killing were conducted in a class II biosafety hood following strict biosafety control measures as outlined by our university’s Animal Resource Center.

INOCULATION

Mice were weighed before inoculations and before killing. The animals were sedated with 80 mg/kg of ketamine and 8 mg/kg of xylazine by intraperitoneal injection. Inhalant anesthesia was avoided for prevention of potential irritation of the nasal mucosa. Intranasal inoculation was achieved by placement of small droplets of solution onto the external nares; these were then drawn into the nasal passages during inhalation. We carefully monitored the breathing rate and skin color during fluid inhalation to prevent respiratory failure. Mice were able to tolerate 0.02 to 0.025 mL of solution. The solution for control animals was trypticase soy broth, whereas the infected group received tryp- ticase soy broth in which a 24-hour culture of Streptococcus pneumoniae was suspended at an inoculum equivalent to a No. 3 McFarland turbidity standard, which corresponds to 1.2 × 10^8 colony-forming units per milliliter. The American Tissue Culture Collection, Rockville, Md, strain of S pneumoniae was obtained from the Clinical Microbiology Laboratories of the University of Chicago Hospital.

TERMINATION PROCEDURE

On the day chosen for killing, mice were sedated with a respiratory-failure dose of 120 mg/kg of pentobarbital sodium (Nembutal) given by intraperitoneal injection. While sedation ensued, the external nares, oral cavity, and head were disinfected with a moist alcohol swab and allowed to dry. Nasal lavage with 0.1 to 0.2 mL of sterile normal saline was performed by filling of the nasal cavities. The lavage liquid was allowed to drip out directly from the nares onto Columbia blood agar and chocolate agar plates. Culture results were graded according to standard microbiologic techniques. The nasal lavage sample was streaked, for isolation, perpendicularly 3 times across the plate. Streptococcus pneumoniae growth on the first streak represented few colonies, growth on the first and second streaks represented moderate colonies, and growth on the first, second, and third streaks represented many colonies. The growth rates were assigned values of 0 to 3, with 0 representing no growth.

The thoracic cavity was then quickly opened to provide exposure of the still-beating heart. An incision was made in the left ventricle, and a blunt butterfly needle (25 gauge) was inserted with care taken not to damage the intraventricular septum for perfusion of the fixative. An incision was then made in the right atrium, and the intravascular system was flushed of blood volume with lactated Ringer solution until the liver had blanched and blood no longer drained from the atrium. The mouse was then perfused with fixative (% paraformaldehyde and 0.5% glutaraldehyde in a 0.1-mmol/L phosphate buffer), which produces an immediate contraction of all muscles, with good perfusion.

HISTOLOGICAL PREPARATION

Decapitated heads were then soaked in fixative overnight. The heads were then stripped of eyes, skin, and muscle under low magnification, and the mandibles and tongue were discarded. The heads were then decalcified until soft (16-24 hours) in commercial decalcifying agent (Surgipath Decalifier II, Surgipath Medical Industries, Rockford, Md), which contains hydrochloric acid, EDTA, and water. The heads were trimmed with a fresh razor blade, with excision of the anterior portion of the nose and the brain, leaving a portion of the nasal sinus approximately 8 mm in length from anterior to posterior. The resulting blocks were embedded in paraffin, sectioned anterior to posterior at 5-µm thickness, and stained with hematoxylin-eosin.

Three anatomically similar sections were chosen from each mouse for analysis. The first section, the most anterior, was at the level of the maxillary sinuses; the second section, more posterior, was at the end of the maxillary sinuses and the beginning of the complex ethmoid turbinates; and the third section, most posterior, contained the brain superiorly. A computer-assisted microscope using software (Neurolucida software, Microbrightfield, Colchester, Vt) was used for analysis of the tissues. The percentage of sinus cavity area occupied by neutrophil clusters (original magnification ×10) and the density of neutrophils infiltrating the mucosa (original magnification ×400), as represented from a sampling of 4 mucosal areas, were determined for each of the 3 sections per mouse. The mucosa chosen for sampling was adjacent to neutrophil clusters, if these were present. All tissue sections were examined blindly with respect to the source of the tissue.

STATISTICS

Nonparametric statistical methods were applied to the data. Kruskal-Wallis analysis of variance was first applied to the data. If significance was obtained, the individual time points were compared by the Mann-Whitney U test. A 2-tailed P<.05 was considered significant.

We started with the induction of acute rhinosinusitis from intranasal inoculation with Streptococcus pneumoniae, the most common pathogen obtained from antral punctures of patients who have acute maxillary rhinosi-
nusitis. Although the anatomy of the mouse sinuses is not identical to that of humans, mice possess the same respiratory epithelium and their sinus cavities are composed of complex ethmoid turbinals that drain from small openings. Mice were observed for clinical signs of infection.

RESULTS

Because the details of the histological and anatomical features of mouse sinuses have not been well described, we began by systematically studying the anatomical features of the nasal cavities (Figure 1). The findings in mice show similarities to those in human sinuses. The ethmoid and maxillary sinuses are air spaces with connections to the nasal cavity. As in humans, the bone outlining the sinuses is covered with a pseudostratified columnar epithelium with some goblet cells. There are blood vessels and nerves, but there is no ongoing inflammation. The nasal mucosa has a lining similar to that in humans, containing submucous glands. The differences between the 2 species are related to structure. Mice do not have frontal and sphenoid sinuses. In addition, a septal perforation that creates a communication between the left and right nasal cavities is consistently found in mice.

After the inoculation of \( S\) pneumoniae or broth, mice were allowed to recover. Mice did not appear to be clinically ill in the days following the inoculation. They ate and drank well. There was no difference between weights before inoculation and those at the time of killing. Similarly, there was no difference in weight between mice inoculated with broth and those inoculated with \( S\) pneumoniae.

After some preliminary experiments, we chose to kill mice on day 5 to examine their sinuses. The histological appearance in response to \( S\) pneumoniae differed markedly from that of mice inoculated with control broth (Figure 2). The obvious differences were the appearance of neutrophil clusters within the ethmoid sinuses and an increase in the number of neutrophils within the mucosa.

In total, we have evaluated mice on days 2, 5, and 14 after inoculation. The results for neutrophil clusters (Figure 3) and neutrophils per square millimeter of mucosa are shown (Figure 4). There was significantly more sinus cavity occupied by neutrophil clusters in infected animals killed at 2 and 5 days than in controls and those killed at 2 weeks (Figure 3). In infected mice killed at 2 and 5 days, the mucosa had a significantly greater density of neutrophils (108.3 ± 50.3 and 185.0 ± 26 neutrophils per square millimeter, respectively; Figure 4) compared with controls and those killed at 2 weeks (Figure 4).

All mice given \( S\) pneumoniae, but none of those inoculated with broth, yielded the bacteria. Infected mice killed at 2 and 5 days had moderate colony growth, whereas those killed at 2 weeks had little colony growth. The density of colony growth in culture at the time of killing did not correlate with the number of neutrophil clusters or neutrophils per square millimeter (Figure 5).

COMMENT

Although most cases of rhinosinusitis in humans are acute and self-limited, a significant number of individuals develop chronic rhinosinusitis. In the middle of the 1980s, a renewed interest in chronic rhinosinusitis emerged in the United States because of the development of the techniques of functional endoscopic sinus surgery and coronal computed tomographic scans. These advances led to a renewed appreciation of the role of anatomical problems in precipitating sinus disease and resulted in a tremendous increase in the number of surgical interventions performed for this condition. However, it has become increasingly clear that anatomy is not the only factor involved.

It is not known why some individuals develop chronic rhinosinusitis. A small number have cystic fibrosis, in which there is a genetically determined abnormality of epithelial ion transport. Some individuals have atopic disease, and others have abnormalities of host defense, including antibody deficiencies and ciliary dysmotility.

Despite the high prevalence of chronic rhinosinusitis and the fact that billions of dollars are spent on treat-
ment, there have been essentially no definitive studies of its etiology, pathogenesis, and therapy. The major limitation lies in the types of studies that can be performed on human subjects. To date, information has been obtained from nasal secretions, nasal mucosa, nasal polyps, sinus secretions, and sinus mucosa, and from peripheral blood. Nasal secretions, however, arise primarily from the nasal mucosa and do not necessarily reflect the conditions in the sinuses. Therefore, nasal cultures in acute rhinosinusitis do not parallel the results for cultures obtained simultaneously by antral puncture. Furthermore, the nasal mucosa differs histologically from the sinus mucosa; e.g., there are no glands within the sinus mucosa. Nasal polyps, originating in the clefts of the lateral wall of the nose, occur in conjunction with rhinosinusitis. Interesting studies have been done on the components of this inflammatory tissue. However, polyps are an end-stage phenomenon and provide little information about the steps leading to this state of chronic sinus inflammation.

Sinus secretions can be obtained only by direct puncture, an invasive process that limits the frequency with which it can be used for sampling of the sinuses. Sinus mucosa removed at surgery, like nasal polyps, comes from a group of individuals with advanced stages of rhinosinusitis in whom all other therapeutic modalities have failed. Sampling of the blood of individuals with rhinosinusitis has the potential to provide repetitive measurements of the progression of disease. However, current markers lack sensitivity and specificity for the disease. Thus, with restricted access and the limited ability to sample the sinus mucosa, or to induce rhinosinusitis experimentally, human studies cannot provide information on the transformation from a healthy epithelium to

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**Figure 2.** Examples of sinus mucosa of control mice (A and B) and those inoculated 5 days previously with broth or Streptococcus pneumoniae (C and D). The infected mice show neutrophil clusters (arrowhead) within the sinus air spaces and neutrophils infiltrated within the sinus mucosa (original magnification: A and C, ×100; B and D, ×500).

**Figure 3.** Percentage of sinuses filled with neutrophil clusters in mice infected with Streptococcus pneumoniae. The number of clusters appears to peak on day 5 and to decrease by 2 weeks. Mice challenged with broth only (used to deliver the S pneumoniae) and examined on days 5 (n = 6) and 14 (n = 2) did not show any neutrophil clusters (data not plotted). The difference between controls and infected mice on day 5 was significant (P < .001). PMN indicates polymorphonuclear neutrophils.
polymorphonuclear neutrophils. A self-limited infection in mice with the most common developed a mouse model of rhinosinusitis. To overcome the limitations imposed on the study of human subjects, we developed a viable model for studying acute rhinosinusitis.

The percentage of the surface area involved may seem small, but when one considers that in acute rhinosinusitis in humans often only a part of 1 of 8 sinuses is involved, the percentage seems reasonable. The fact that the infection is small and self-limited will allow us to attempt to augment it by immunologic, genetic, or mechanical means. Furthermore, the mechanisms involved in the acute process can be investigated by immunohistochemistry.

Clinically, chronic rhinosinusitis most often develops after recurrent attacks of acute bacterial rhinosinusitis. Now, with a model of acute bacterial rhinosinusitis in the mouse, manipulations will be directed toward altering the course of the disease to induce chronic rhinosinusitis. By identifying factors that help to induce chronic rhinosinusitis, we may obtain a better understanding of its pathogenesis.

There are obvious limitations to applying this model directly to the disease in humans. Further experimentation will determine the use of this animal model of an enigmatic human disease.

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REFERENCES


