

Allergic rhinitis augments the response to a bacterial sinus infection in mice: A review of an animal model

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ABSTRACT

Background: Sinusitis is a poorly understood disease. Despite the significant morbidity and the enormous cost of treating sinusitis, little progress has been made at improving our understanding of its pathophysiology. One reason for restricted progress in understanding the disease is the lack of a satisfactory animal model that mimics sinusitis in man.

Objective: We review data establishing the development of sinusitis in mice after instillation of *Streptococcus pneumoniae*, the most common pathogen responsible for acute sinusitis in man. We also review data showing that allergic inflammation in mice worsens a subsequent bacterial sinusitis. We use this data to hypothesize how allergic inflammation worsens a bacterial sinus infection.

Methods: Different strains of mice were made allergic and/or infected.

Results: We show our ability to generate an allergic reaction in the nose after sensitization to ovalbumin. Our data further show that an ongoing allergic nasal reaction worsens acute sinusitis.

Conclusion: A mouse model has been created for a study of the interaction of allergic rhinitis and acute bacterial sinusitis.

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Sinusitis defies a straightforward definition. At its most basic level, sinusitis is defined as an inflammation of the lining membrane of any of the paranasal sinuses. In human subjects, this definition has limited intellectual, pathological, and clinical significance. As a clinical entity, sinusitis has manifestations varying from an incidental CT scan finding^{1,2} to an unremitting illness associated with a genetic abnormality in the epithelium of patients with cystic fibrosis.³

Clinicians and patients frequently diagnose sinusitis. More than 31 million Americans describe chronic sinusitis as a health problem, making it the most frequently reported chronic disease in the United States.⁴ It affects all age groups, including 17% of people >65 years of age.⁵ In 1991, 11.6 million office visits were made for sinusitis.⁶ The number of restricted activity days per year increased from 50 million to 73 million between 1988 and 1992.⁷ The number of antibiotic prescriptions increased from 5.8 million in 1985 to 13 million in 1992.⁸ ~200,000 sinus operations were performed in 1994.⁶ In 1996, \$5.8 billion was spent on the care of patients with sinusitis.⁹ Thus, each year, billions of dollars are spent on direct medical costs for the treatment of this enigmatic illness.

Despite the high prevalence of sinusitis and the fact that billions of dollars are spent on treatment, there have been essentially no definitive studies of the etiology, pathogenesis, and therapy of this condition. The major limitation lies in the types of studies that can be done in human subjects. To date, the best information has been obtained from punctures of maxillary sinuses during acute episodes of sinusitis. These

studies have shown that *Streptococcus pneumoniae* is the most common causative organism.¹⁰ Treatment of acute sinusitis with antibiotics is superior to treatment with placebo in that antibiotics lead to a faster resolution of the illness. It is also important to note that placebo treatment still leads to resolution of symptoms in 60% of patients.¹¹ This observation points to the limited morbidity associated with the typical case of acute sinusitis.

The best-studied animal model of sinusitis is the rabbit.^{12–15} The large size of the animal permits easy surgical manipulation. However, the induction of acute sinusitis in this model requires complete mechanical obstruction of the ostia. The inability to manipulate the rabbit genetically and the lack of availability of reagents and antibodies for the study of inflammation in greater detail in this species prompted us to develop another animal model. We chose the mouse because this animal offers a greater potential to study inflammation. To date, we have focused on modeling acute sinusitis, an illness of <2 weeks duration. We chose to begin by inducing acute sinusitis with *S. pneumoniae*.

The pathogenesis of *S. pneumoniae* infections in the sinuses of mice has not been investigated, and the pathogenesis of infection in other sites has not been established completely. Nasal inoculation of animals can lead to meningitis, otitis, pneumonia, rapidly fatal disseminated disease, or to an asymptomatic carrier state.¹⁶ A number of virulence factors can affect the response to infection, including the strain of *S. pneumoniae*.^{17,18} Because sinusitis in man often is self-limited and rarely leads to complications, we chose a strain of *S. pneumoniae* that leads to inflammation and infection, rather than death, to establish our model.

In addition to the systemic immune system, the mucosal immune system undoubtedly plays a role in the response to *S. pneumoniae* inoculation.¹⁹ The innate immune system is the result of a steady-state resistance to infection provided by physical barriers and the activation of multiple cellular and soluble factors that lead to the release of acute phase reactants, which promote phagocytosis and stimulate further cytokine

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and chemokine production targeted at fighting infection. The mucosal immune system also includes such elements as B cells that produce IgA, lamina propria T cells specialized in regulating the local mucosal immune system, intraepithelial lymphocytes that contain $\gamma\delta$ -T-cell receptors, M cells that allow the passage of antigen directly to follicles, dendritic cells that contribute to antigen presentation, and a unique pattern of cellular trafficking. In human subjects, these factors have not been suggested to be major contributors in the reaction to sinusitis, except for contact areas interfering locally with mucociliary transport—the target of surgical intervention.^{20,21}

A number of factors in human subjects are believed to be associated with an increased incidence of sinusitis, although none have been proved. In any large group of patients with sinusitis, allergic inflammation appears to be one factor that plays an important role in both acute and chronic sinusitis.²²

Although there are no controlled evaluations comparing the prevalence of chronic sinusitis in allergic and nonallergic populations, there is a strong clinical impression suggesting an association. van Dishoeck believed that allergy was an underlying factor in 40–67% of patients with chronic sinusitis.²³ Benninger, in studying outpatients in an otolaryngology clinic, found that 54% of those with chronic sinusitis also had allergic rhinitis.²⁴ Grove and Farrior found a 50% incidence of positive skin tests in patients undergoing sinus surgery, whereas Friedman reported an incidence of atopy in 94% of patients undergoing sphenoidectomies.^{25,26} Savolainen found the incidence of allergy to be 25% in a group of 224 patients with acute maxillary sinusitis, which was significantly greater than a 16% incidence in a control group.²⁷ Complementing the foregoing data that allergic rhinitis occurs frequently in patients with chronic sinusitis are data showing a high prevalence of sinus disease in patients with allergic rhinitis. Abnormal sinus radiographs occur in >50% of adults and children with perennial allergic rhinitis.^{28,29} In contrast, Enberg reported a low incidence of sinus abnormalities in patients with perennial nonallergic rhinitis.³⁰ MRI scans showed increased evidence of sinus mucosal abnormalities during major pollinating seasons.³¹ In human subjects, acute sinus infections often occur during allergen exposure and are a common reason for the failure of allergy treatment.³² How allergic rhinitis affects the course of sinusitis is not clear. The purpose of this study is to present a detailed review of a mouse model of sinusitis. We will review how the model was established and how it was used to show that ongoing allergic inflammation worsens the response to an acute bacterial sinus infection with *S. pneumoniae*.

MATERIALS AND METHODS

Mice

Six- to 8-week-old pathogen-free control and knockout (KO) mice were purchased from the Jackson Laboratory in Bar Harbor, ME. They were maintained in the biohazard suite of the Carlson Animal Facility in microisolator-top cages. The Animal Care Committee of The University of Chicago approved the studies.

Induction of Acute Sinusitis

Mice (18–22 g) were anesthetized with 50 mg/kg of ketamine and 5 mg/kg of xylazine. Because mice are obligate nose breathers, we placed small droplets of solution onto the external nares and allowed the fluid to be drawn into the nasal passages during inhalation. The inhaled solution was soy broth for control animals or soy broth containing *S. pneumoniae* for experimental animals. Approximately 20 μ L of solution were administered. In prior experiments, 20 μ L of Evans blue dye was observed to be widely distributed throughout the nasal passages. Careful monitoring of breathing rate and skin color during fluid inhalation prevented respiratory failure. The animals recovered within 10–20 minutes of final droplet administration.

Nasal Culture

Animals were killed with 120 mg/kg of Nembutal (Ovation Pharmaceuticals Inc., Deerfield, IL). As respiratory depression occurred, the animal's head, external nose, and oropharynx were disinfected with a swab of 70% isopropyl alcohol. Nasal lavage with 100 μ L of sterile saline was performed. Then, 10 μ L was inoculated onto blood agar plates. The incubated cultures were used for assessment of the bacteria growing in nasal passages and sinuses at the time of death. In some experiments, we used a standard technique of estimating growth, by cross-streaking plates and grading growth as few, moderate, and many. In other experiments, we quantified the bacteria obtained by nasal lavage by using serial dilutions on blood agar plates.

Sinus Culture

After the mice were anesthetized before killing, the skin was removed from the skull. A surgical scalpel was used for severing the skull in the midline and posterior to the orbit. The tissue was then weighed and homogenized. Serial dilutions were made on blood agar plates for quantifying the number of bacteria per milligram of sinus tissue.

Harvesting of Sinus Mucosa

After lavage, the animal was first perfused transcardially with lactated Ringer's and then with a solution of 4% formaldehyde and 0.5% glutaraldehyde in 0.1 mmol/L of phosphate buffer. When the experiments demanded it, the lungs were harvested also. The animal was decapitated, and the head was placed in fresh fixative overnight. The following day, the external soft tissue was removed, and then the skull was ready for histological processing.

Histological Processing

The fixed skulls were decalcified in Surgipath Decalcifier II (Surgipath Medical Industries, Richmond, IL) for 48 hours and then washed in PBS three times. Tissue was rapidly dehydrated at 4°C through a series of ethanol and xylene and then infiltrated with low-temperature paraffin under vacuum. Sections of the nasal passages were cut at 8 μ m on a microtome and then mounted on glass slides. At this point, sections were stained with either hematoxylin and eosin (H&E) or Luna (Surgipath Medical Industries).

Cytological Analysis

Individual H&E-stained sections were analyzed by use of a computer-aided microscope in conjunction with a reconstruction and image analysis system (Neurologic; Microbrightfield, Williston, VT). At 400 $\times$ magnification, this system allowed us to map precisely and to calculate the area of both the paranasal sinuses and the neutrophil clusters. An investigator blind to the experimental conditions interpreted all sections.

Ovalbumin Sensitization

Mice were sensitized by i.p. injections of 20 μ g of ovalbumin (OVA) together with an alum (aluminum hydroxide) adjuvant. The alum adjuvant induced a nonspecific T-cell response. Eleven days later, the mice received a second injection. Three days later, the animals were exposed to OVA (6% solution in 0.1 M of PBS) by intranasal inoculation daily for 5 days. At initiation of the experiment and at the time of death, serum was collected by ocular puncture for measurement of total IgE.

Total IgE Assay

The levels of total IgE antibodies were detected by ELISA. On day 1, 100 μ L/well of 4 μ g/mL of rat anti-mouse IgE in 0.1 NaHCO₃ buffer (pH 8.2) was used for coating the plate and was left to incubate overnight at 4°C. On day 2, the wells were aspirated and washed with PBS-Tris buffer (pH 7.4; wash buffer) four times. All wells were blocked with 200 μ L of 1% bovine serum albumin (BSA) in PBS for 3 hours at 25°C. The wells were aspirated and washed three times. Purified mouse IgE (range of curve, 100–2000 ng/mL) was used for the standard curve. Standard samples (100 μ L of mouse IgE) or 100 μ L of the experimental samples were added to the wells and incubated overnight at 4°C. On day 3, the wells were aspirated and washed five times. One hundred microliters of biotinylated anti-mouse IgE (4 μ g/mL) in assay buffer was added to wells containing standards. The wells were aspirated and washed six times, and 100 μ L of streptavidin-horseradish peroxidase (1:900 in blocking buffer) was added to each well and incubated for 1 hour at room temperature. Just before the end of incubation, a 10-mg tablet of O-phenyldiamine (OPD) was added to 25 mL of citrate phosphate buffer. The wells were aspirated and washed six times. Ten microliters of 30% H₂O₂ was added to the OPD mixture and vortexed, and then 100 μ L of the mixture was added to each well. The color was allowed to develop for 10 minutes before the reaction was stopped with 50 μ L of 2 N sulfuric acid added to each well. The plates were read at 490 nm. Experimental sample values were extrapolated off the standard curve, and values below 93 ng/mL were assigned a value of 50 ng/mL.

Statistical Analysis

The experiments had a similar design, and the primary analysis consisted of two-sample *t*-tests of the group differences at 5 days. Parametric statistics were used, and the results were expressed as mean $\pm$ SEM.

RESULTS

Because the details of mouse sinuses had not been well described, we began by systematically studying the anatomy

of their sinuses. We chose C57Bl/6 mice that were 5–8 weeks old because they would have had minimal exposure to environmental stimuli and limited immunologic memory, and they can be obtained pathogen free. This strain also has been used for numerous immunologic studies involving mice.

The sinuses of the mice show similarities to sinuses of human subjects.³³ The ethmoid and maxillary sinuses are air spaces with connections to the nasal cavity. As in human subjects, 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 similar lining as in human subjects, containing submucous glands. Air passes from the nasal cavity into the nasopharynx, which contains lymphoid tissue.

We began our studies with a simple, direct approach. The mice (C57Bl/6) were anesthetized, and then the inoculum of *S. pneumoniae* (type 59) or broth (the control for the inoculation procedure) was introduced into the nose, and the mice were allowed to recover. We chose the nasal route because it is believed to lead to nasopharyngeal colonization and subsequent spread to the sinuses, mimicking the course of illness in human subjects.¹⁶ The type 59 strain chosen for study is antigenically similar to type 19, the serotype most frequently recovered during episodes of community-acquired maxillary sinusitis and acute otitis media.¹⁶ This strain is much less frequently recovered during sepsis and is considered less virulent. It was our goal to mimic community-acquired sinusitis, which is mostly a self-limited infection with few systemic signs.

We observed the mice for clinical signs of illness. The mice did not appear clinically ill in the days after the inoculation. They ate and drank well. Their weight changed little, and those surviving the longest gained the most weight. There was no difference in weights before inoculation or at the time of death, between mice inoculated with broth or with *S. pneumoniae*. Sneezing, nasal discharge, cough, or nose rubbing did not appear different between the infected and control mice.

After some preliminary experiments, we chose to kill the mice on day 5 to examine their sinuses. The histological appearance in response to *S. pneumoniae* differed markedly from that of mice inoculated with broth. The obvious differences were the appearance of neutrophil clusters within the ethmoid sinuses and an increase in the number of neutrophils within the mucosa. Gram stains of the clusters showed the *S. pneumoniae*. The presence of a positive Gram stain implies at least 10⁵ organisms; in human subjects, the recovery of 10⁴ organisms/mL is considered evidence of a bacterial infection.³⁴

We then evaluated mice on days 2, 5, and 14 after inoculation, and we quantitated the inflammatory response within the sinuses by the percent of the surface area of the sinuses occupied by neutrophil clusters and by the number of neutrophils per square millimeter of sinus mucosa in areas of the sinuses occupied by clusters.³⁵ The results for neutrophil clusters (Fig. 1) and neutrophils per millimeter squared of mucosa are shown (Fig. 2). Although the percent area with neutrophil clusters may seem small, it is similar to the sinus area involved by mucosal thickening in patients with symptomatic episodes of acute sinusitis.

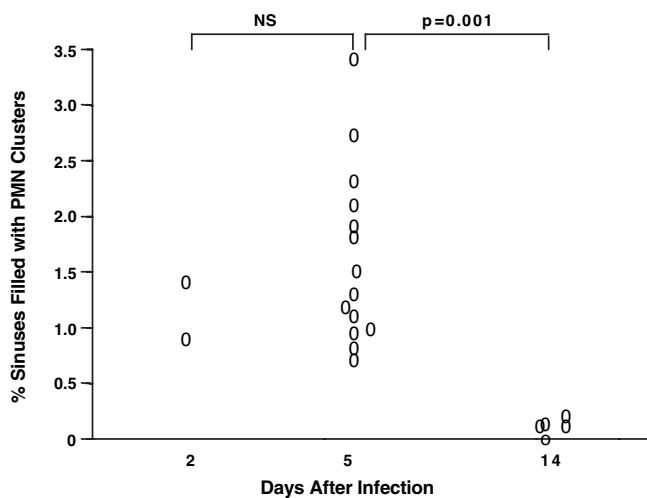


Figure 1. Percentage of sinuses filled with neutrophil clusters in mice infected with *S. pneumoniae*. The percentage of the sinuses occupied by clusters appears to peak on day 5 and to decrease by 2 weeks. Mice challenged with broth only, used to deliver the *S. pneumoniae*, and examined on days 5 ($n = 6$) and 14 ($n = 2$), did not show any neutrophil clusters (data not graphed). The difference between controls and infected mice on day 5 is significant at $p = 0.001$. NS = not significant. (Reproduced with permission from Ref. 35.)

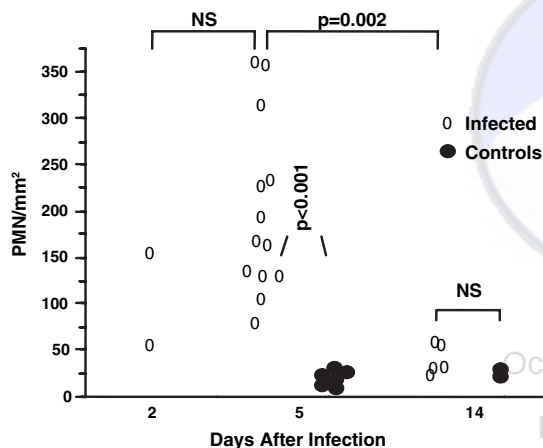


Figure 2. Neutrophils per millimeter squared of sinus mucosa in mice exposed to *S. pneumoniae* or broth (controls). The number of neutrophils appears to peak on day 5 and to decrease by 2 weeks. The difference between controls and infected mice on day 5 is significant. No day 2 controls have been evaluated to date. NS = not significant. (Reproduced with permission from Ref. 35.)

We explored the kinetics of the response to infection. The inoculum was grown to 10^9 organisms/mL. This implies that 10^7 were delivered to the mouse, because $10 \mu\text{L}$ of the inoculum was placed in the nasal cavity. After 1 hour, on average 6×10^5 organisms were recovered by lavage, which underestimates the number of organisms because of dilution.³⁶ This recovery is remarkable, considering that mucociliary clearance would be expected to reduce nonbinding, nonproliferating organisms by 90% in 1 hour. Over the next several days,

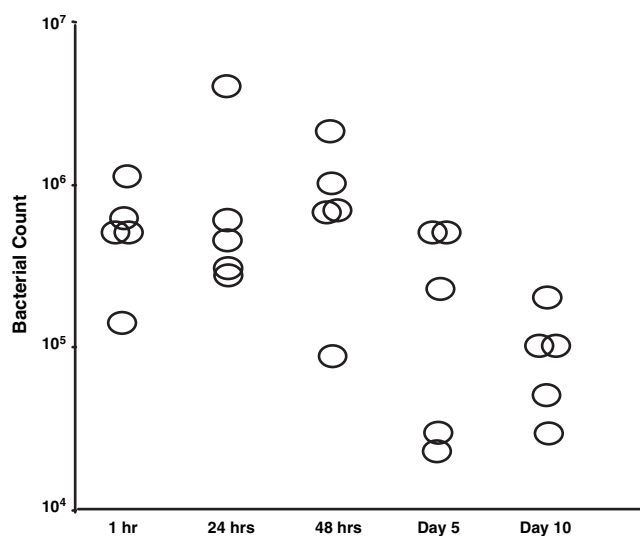


Figure 3. Kinetics of recovery of organisms from the murine sinuses. All mice were inoculated with *S. pneumoniae* and their noses lavaged at various time points after inoculation, and the lavage was examined for bacterial counts. Individual data for each animal are graphed. (Reproduced with permission from Ref. 36.)

the recovery of organisms remained fairly constant (Fig. 3), further supporting proliferation of the organism. In 17 mice, we cultured the nose by lavage after inoculation with *S. pneumoniae* and then cultured the sinuses after death by removing sinus mucosa and bone as described previously. The correlation between the number of bacteria obtained by lavage and from tissue cultures was robust and highly significant ($r = 0.848$; $p < 10^{-6}$).³⁶ Because of the strong correlation, we feel comfortable using the lavage technique to quantify the infection and keeping the sinuses intact for histological evaluation of the degree of infection. The number of bacteria per gram of tissue ranged between 10^5 and 10^6 organisms, levels compatible with those of an infection in human subjects and with the findings on Gram stain of the clusters (*i.e.*, the presence of organisms on Gram stain implies at least 10^5 were present). The results also are consistent with animal models of *S. pneumoniae*-induced acute otitis media, where bacterial counts plateau around 10^4 and persist between 14 and 21 days before being cleared.³⁷ Like sinusitis, acute otitis media rarely leads to mastoiditis or systemic disease.^{38,39}

Because of concerns about the high initial inoculum, we performed studies dosing with *S. pneumoniae* over 4 log concentrations beginning with 10^9 and going to 10^6 . The mice were cultured on day 5.³⁶ Despite a decrease in the initial inoculum, the number of organisms recovered on day 5 was similar (Fig. 4). When the lowest dose was used, the organisms had to proliferate to reach the same level as with the higher inoculum. The number of neutrophils per millimeter squared also is shown in Fig. 4 and shows large numbers of neutrophils in the sinus mucosa comparable with those seen in the previous infection experiments with the higher dose of the inoculum in Fig. 2.

As another control to show the presence of an infection, we studied the response to an antibiotic. We hypothesized that treatment with an appropriate antibiotic would reduce the

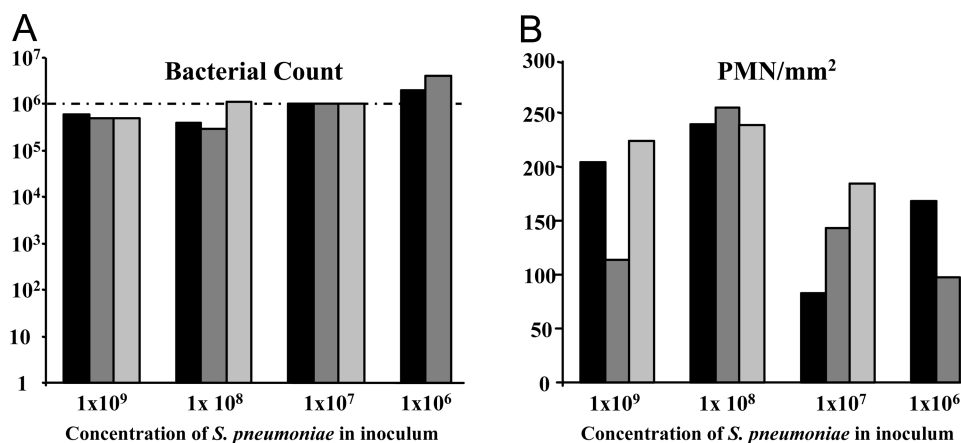


Figure 4. Bacterial recovery and sinus inflammatory response with decreasing doses of *S. pneumoniae* inoculum. Animals were inoculated with decreasing doses of *S. pneumoniae* (seen on the x-axis), and the noses were lavaged and bacterial recovery was assessed. (A) The bacterial count is graphed in and (B) the number of neutrophils per millimeter squared of sinus mucosa is shown. Each column represents data from a single animal at the specified initial inoculum dose. Recovery of organisms and the degree of inflammation within the sinuses was similar irrespective of the concentration of the initial *S. pneumoniae* inoculums.

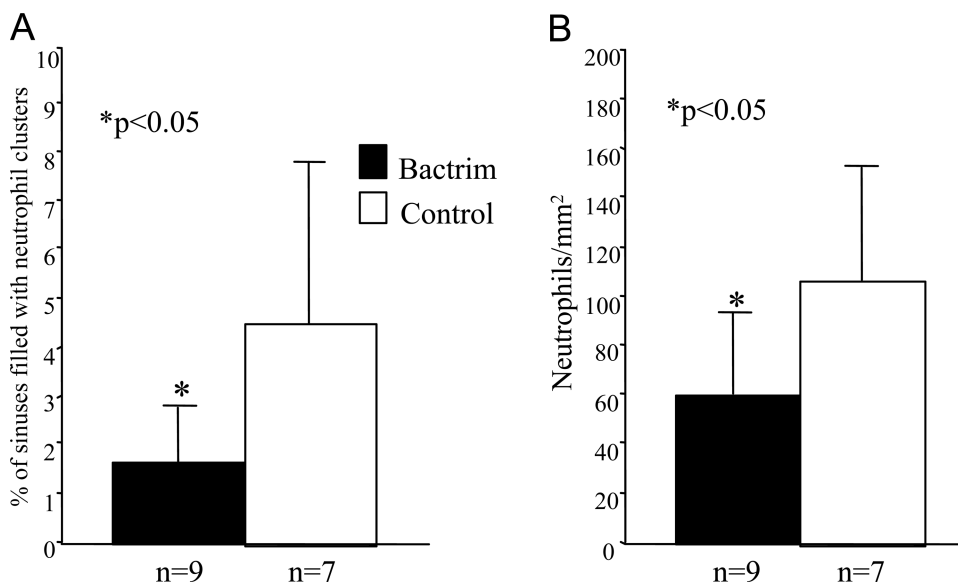


Figure 5. Treatment with Bactrim significantly reduced the percent of sinuses occupied by neutrophil clusters and the number of neutrophils per millimeter squared. (Reproduced with permission from Ref. 40.)

infection and the secondary inflammatory response. We randomized 16 mice to treatment with Bactrim i.p. (30 mg/kg q.d.) or to no treatment.⁴⁰ All mice began treatment 2 days before inoculation with *S. pneumoniae*. Treatment was continued daily for 5 days, and then the mice were killed. Bactrim, an antibiotic to which *S. pneumoniae* is sensitive, significantly reduced the objective measures of infection: neutrophil clusters, neutrophils in the mucosa, and culture density (Fig. 5). From these results, it was apparent that we could induce an infection in mice with the most common organism that causes acute sinusitis in human subjects, and we could inhibit the infection and secondary inflammation by pretreatment with an appropriate antimicrobial agent.

Previous investigations have shown that mice with different Th lymphocyte backgrounds respond differently to

leishmaniasis (a parasitic disease), with BALB/c mice showing severe systemic infection when compared with C57Bl/6 mice that have only a localized reaction.⁴¹ We next studied the impact of infection in BALB/c mice, which often are used in allergic studies because of their Th2 background, and compared the response to that of C57Bl/6 mice, which primarily favor a Th1-mediated lymphocyte response. Both groups were inoculated with *S. pneumoniae* and then killed on days 2, 5, 10, and 14. The results for the neutrophil infiltrates and the percent of sinuses occupied by neutrophil clusters were similar between the groups but much greater than in control (noninfected) mice (Fig. 6). There were no clusters in the control mice. In subsequent experiments, we found the mice differed in their ability to resolve the infection at later time points; i.e., C57Bl/6 mice

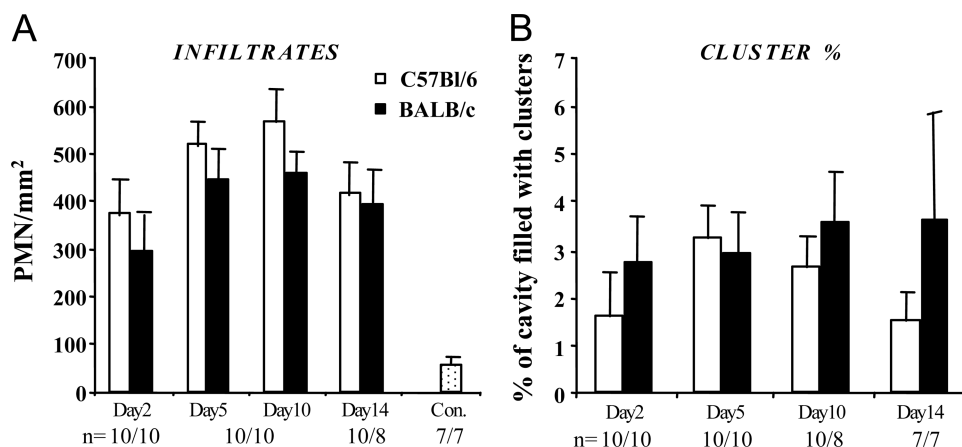


Figure 6. Time course of the response to infection in C57Bl/6 and BALB/c mice. No significant differences between the two strains of mice were noted. No clusters were identified in control mice (data not plotted). Responses in both strains are significantly greater than in controls. (Reproduced with permission from Ref. 49.)

resolve the infection completely by day 28, whereas BALB/c did not.

To begin to understand the role of the innate and acquired immune system in response to infection, we inoculated Rag 1 KO mice (mice devoid of T cells) with *S. pneumoniae* and analyzed the mice at days 2, 5, and 10.³⁶ There were two mice at each time point. One C57Bl/6 mouse was inoculated and 1 Rag 1 KO was not inoculated, and both were harvested at the same time points and served as positive and negative controls, respectively. None of the control Rag 1 KO mice grew bacteria or developed a neutrophil influx. The C57Bl/6 mice responded as before. The infected Rag 1 KO mice grew bacteria on all days at $\sim 10^5$, and the number of bacteria in the C57Bl/6 mice was similar through the first two time points but decreased >10 -fold on day 10 (Fig. 7). The neutrophil clusters in the sinuses are graphed also in Fig. 7 and show a larger response on days 2 and 5 in the RAG 1 KO mice compared with the C57 mice. The number of neutrophils per millimeter squared mirrored the cluster data. These exciting

preliminary results are consistent with containment of the infection by the innate response and then resolution of the infection and inflammation at later time points only in the animals with an intact adaptive immune system.

We then made BALB/c mice allergic to OVA.⁴² We chose mice 6–8 weeks old and injected them i.p. with OVA in alum suspension twice at a 2-week interval. To show a specific IgE response, we performed skin testing of sensitized and non-sensitized mice. We tested compound 48/80 (a mast cell degranulating agent), OVA (the sensitizing antigen), and BSA (an irrelevant antigen). The results show reactivity to compound 48/80 in both groups, no reactivity to BSA in both groups, and reactivity to OVA in the sensitized group only (Fig. 8). To document an allergic response further, we developed an assay for total IgE and showed that allergic sensitization led to a dramatic increase in total IgE (Fig. 9).

We then performed a series of experiments whereby we made mice allergic and exposed them to OVA aerosols or intranasal inoculation with OVA for 6 consecutive days.⁴² The

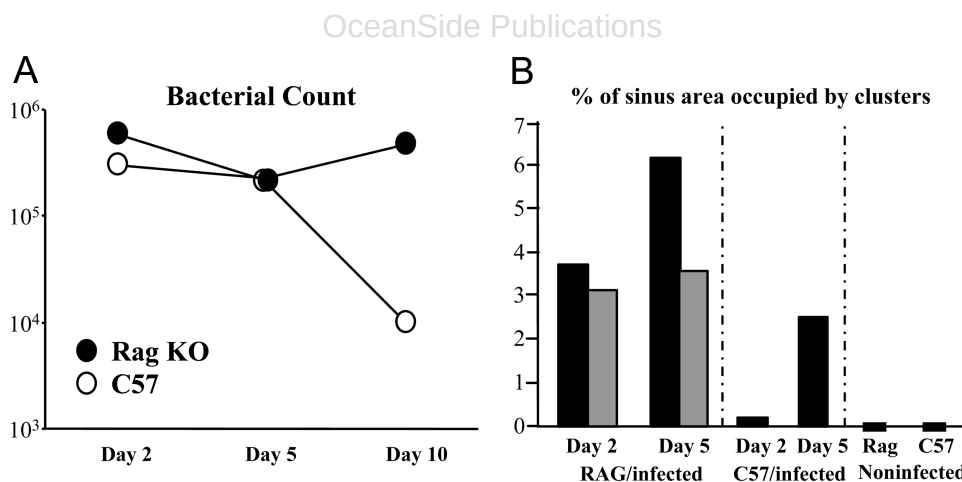


Figure 7. Response of Rag 1 KO mice to inoculation of the nasal cavity with *S. pneumoniae*. (A) Recovery of organisms from nasal lavage 2, 5, and 10 days after inoculation. Mean values are depicted. (B) The percentage of the sinus area occupied by neutrophil clusters is shown; each column represents individual animal data. The panel shows the response in RAG mice 2 and 5 days after inoculation, the response in control C57 mice 2 and 5 days after inoculation, and the response in 2 control mice: RAG KO and C57, which were both noninfected. (Reproduced with permission from Ref. 42.)

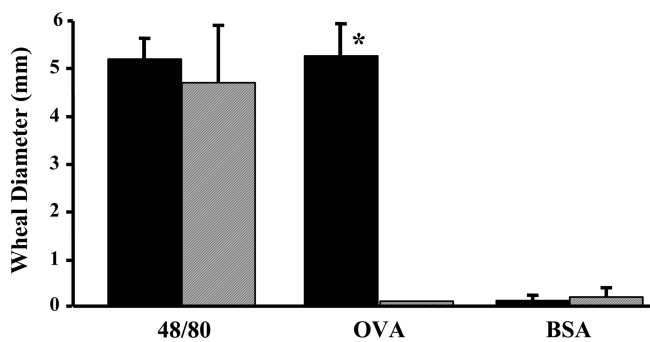


Figure 8. Skin testing of mice made allergic to OVA shows increased reactivity to the relevant antigen compared with nonallergic control mice. The difference between the groups to OVA is significant, $n = 8$, $*p < 0.01$. The 48/80 is a nonspecific mast cell degranulating agent. BSA is bovine serum albumin, an irrelevant antigen. Black bars = allergic mice; gray bars = nonallergic mice. (Reproduced with permission from Ref. 42.)

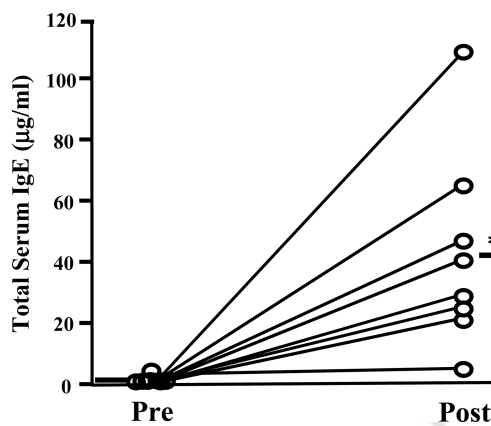


Figure 9. Serum drawn before (pre) i.p. injection of OVA in alum and after (post) i.p. and intranasal inoculation of OVA shows a significant increase in IgE ($*p < 0.01$). The lines represent individual mice. The solid bars represent means. (Reproduced with permission from Ref. 42.)

two different methods of mucosal exposure were chosen because they result in a deposit of OVA on different mucosal surfaces. Aerosolization creates particles of $<1 \mu\text{m}$ that are deposited primarily in the lungs, whereas intranasal drops are primarily deposited in the nasal cavity.⁴³ We monitored our ability to induce an allergic reaction in either the lung or the nose/sinus cavity by quantifying the number of eosinophils accumulating in these sites after allergen challenge (Table 1). The results showed our ability to induce allergic inflammation in either the nose or the lungs, depending on the method of antigen delivery, and only a slight neutrophil response during the allergic reaction.

Next, we asked whether an allergic reaction or sensitization would augment the inflammatory response to the inoculation of *S. pneumoniae*.⁴² We challenged three groups of BALB/c mice. The first group consisted of mice inoculated with *S. pneumoniae* that were not made allergic. The second and third groups were made allergic, with one being challenged with antigen intranasally and the other by aerosolization. One day

after the first antigen exposure, *S. pneumoniae* was inoculated intranasally. Daily antigen exposures to either the nose or the lungs followed until the mice were killed on day 5. The BALB/c-infected mice showed evidence of infection (Fig. 10). The allergic mice exposed to aerosolized antigen showed evidence of infection, similar to the nonallergic mice. In contrast, the allergic mice with an ongoing allergic reaction in their nose showed an approximately threefold increase in the percentage of sinuses with neutrophil clusters and a twofold increase in the number of infiltrating neutrophils. The mice with an intranasal allergic reaction also had more colonies of *S. pneumoniae* recovered at death.

To control for the possibility that the OVA by itself was augmenting the infection, we placed OVA in the nose of nonsensitized mice daily for 5 days.⁴⁴ The mice were inoculated with *S. pneumoniae* on day 1. The recovery of organisms ($6 \pm 5.5 \times 10^5$ versus 1×10^5 ; OVA plus infection versus infection alone) and the degree of inflammation in the sinuses (similar to that of mice infected without OVA exposure) suggest that it is the allergic reaction to OVA that is important and not just exposure of the nasal/sinus cavities to OVA.⁴⁴

DISCUSSION

Our results show that nasal instillation of *S. pneumoniae* induces sinusitis in mice and that ongoing allergic inflammation at the site augments the infection and the inflammatory response.

The exact pathogenesis of *S. pneumoniae* pneumonia is not completely known.⁴⁵⁻⁴⁷ The effect of this organism in the lungs has been investigated in mice and might provide insight into the pathophysiology of infection in the sinuses. Initially, there is alveolar edema combined with bacterial proliferation. This phase is followed by red hepatization, in which an influx of neutrophils is combined with a blood-tinged fluid. Gray hepatization follows, with continued influx of neutrophils and increased fibrin deposition. In most studies, the serotype of the *S. pneumoniae* chosen for study causes death within 48 hours; thus, little information exists on the local inflammatory response beyond 48 hours.

A number of virulence factors contribute to the course of the infection, such as the capsule, the cell wall, pneumolysin, pneumococcal surface protein A, complement, autolysin, neuraminidase, peptide permeases, hydrogen peroxide, and IgA1 protease.⁴⁷ These factors also affect the type of infection.

The infection that we have induced in the sinuses causes minimal symptomatology and a localized inflammatory response. This pattern is consistent with the typical episode of acute sinusitis in human subjects caused by less virulent serotypes of *S. pneumoniae*.⁴⁸

Using our model, we will investigate the mechanism by which allergic rhinitis worsens acute sinusitis. We hypothesize that the innate immune system contains the infection and then the acquired immune system eliminates the infection and the inflammatory response to the infection. The local allergic response in the nose affects both the innate immune response and the acquired immune response essential to clear the infection and the inflammatory response to that infection. Furthermore, we hypothesize that IL-4 released by Th2 cells recruited during the allergic reaction interferes with the recruitment, development, and activation of Th1 cells that con-

Table 1 Eosinophil responses of BALB/c mice to different antigen exposures

Sensitization	Inoculum	Sinus Eos (mm ² ± SEM)	Lung Eos (mm ² ± SEM)
Saline	Saline	12.4 ± 2.8	5.0 ± 1.4
OVA/alum	Saline	12.0 ± 1.2	25.0 ± 6.5
Alum	Saline	5.9 ± 0.6	9.7 ± 0.5
Saline	Nasal OVA	14.5 ± 2.3	13.4 ± 1.6
OVA/alum	Nasal OVA	380.3 ± 25*	15.6 ± 3.3
Alum	Nasal OVA	16.3 ± 1.4	24.4 ± 7.3
Saline	Aerosol OVA	17.4 ± 1.5	45.5 ± 7.7
OVA/alum	Aerosol OVA	20.0 ± 7.6	234.3 ± 70*
Alum	Aerosol OVA	18.7 ± 6.2	41.1 ± 3.6

Eos = eosinophils.

**p* < 0.01 compared with controls.

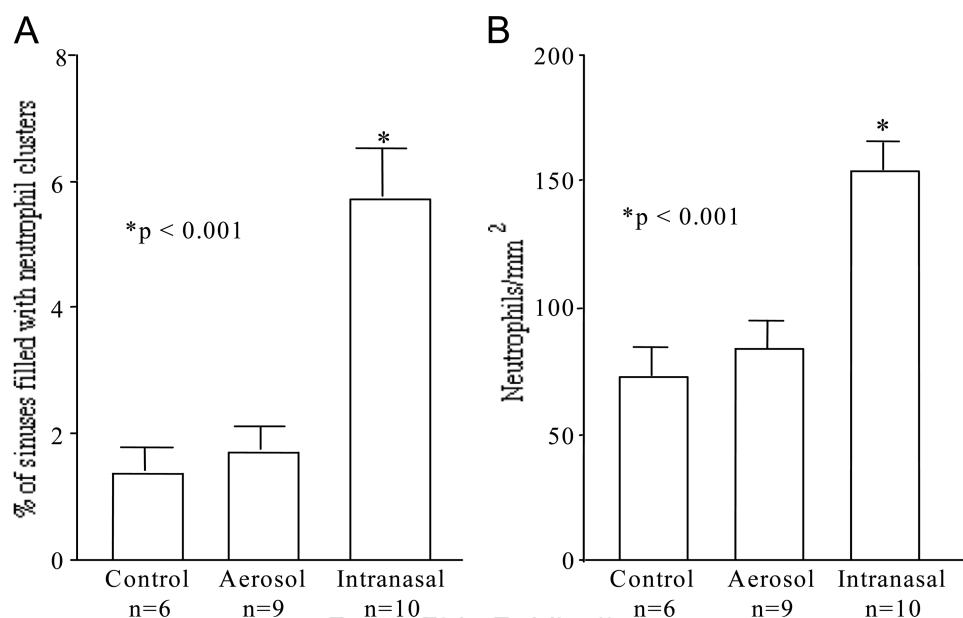


Figure 10. (A) Percent of sinuses filled with neutrophil clusters and (B) the number of neutrophils per millimeter squared in sinus mucosa. Control represents infected, nonallergic mice, aerosol, infected allergic mice with an ongoing allergic reaction in the lung, and intranasal, infected allergic mice with an ongoing allergic reaction in the nasal cavity. An ongoing nasal allergic response increases the inflammatory response to infection. **p* < 0.001 versus control and aerosol. (Reproduced with permission from Ref. 42.)

tribute to the suppression of infection and inflammation caused by bacterial infection.

Many other potential mechanisms for the worsening of sinusitis in allergic rhinitis patients exist. It is conceivable, particularly in subjects with underlying anatomic defects, that allergen-induced nasal congestion could obstruct sinus drainage and enhance a subsequent bacterial infection, although in our model obstruction of sinus ostia is not seen. Systemic differences between allergic and nonallergic individuals might influence their response to infection; however, to date, our mice have not shown such a difference. Allergic inflammation, which increases the number of mucosal mast cells, combined with C3a and C5a generated secondary to complement activation in response to *S. pneumoniae* infection, could lead to increased mast cell mediator

release, causing greater transudation of fluid and increased proliferation of bacteria in the sinus cavities. Effects of inflammatory mediators released by eosinophils during an allergic reaction could alter the epithelium so that *S. pneumoniae* binds better, leading to an augmented response. The ciliary movement from the sinuses could be altered by allergic inflammation, reducing the clearance of bacteria and hence leading to an amplified infection. Allergic inflammation could “prime” circulating leukocytes such that, when there is recruitment of such primed cells to the sinus mucosa, an enhanced response to bacterial infection occurs. Alternatively, allergic inflammation in the nasal cavity could induce neurogenic inflammation within the sinuses *via* central, as well as axonal, reflexes. These are only some of the multiple potential mechanisms by which

allergy could worsen the inflammatory response to *S. pneumoniae*.

The potential to test and develop successively more sophisticated models of other aspects of sinusitis in mice is, in our view, the most exciting future prospect of this model. Transgenic and KO mice provide opportunities to focus on the defects that prolong and exacerbate acute infections, potentially leading to chronic sinusitis. Thus, our model has the potential to address the pathophysiology of sinusitis, a common, but underexplored disease.

REFERENCES

- Calhoun KH, Waggenpack GA, Simpson CB, et al. CT evaluation of the paranasal sinuses in symptomatic and asymptomatic populations. *Otolaryngol Head Neck Surg* 104:480–483, 1991.
- Iwabuchi Y, Hanamura Y, Ueno K, et al. Clinical significance of asymptomatic sinus abnormalities on magnetic resonance imaging. *Arch Otolaryngol Head Neck Surg* 123:602–604, 1997.
- Kingdom TT, Lee KC, FitzSimmons SC, and Cropp GJ. Clinical characteristics and genotype analysis of patients with cystic fibrosis and nasal polyposis requiring surgery. *Arch Otolaryngol Head Neck Surg* 122:1209–1213, 1996.
- Collins JG. Prevalence of selected chronic conditions: United States, 1983–1986. National Center for Health Statistics. *Vital Health Stat* 10:1–87, 1993.
- Hahn B, and Lefkowitz D. Annual expenses and sources of payment for health services. National Expenditure Survey Research Findings 14, Agency for Health Care Policy and Research, Publication No. 93–0007, Public Health Service, Rockville, MD, 1994.
- Kaliner MA, Osguthorpe JD, Fireman P, et al. Sinusitis: Bench to bedside. *Otolaryngol Head Neck Surg* 116:S1–S19, 1997.
- Benson V, and Marano MA. Current estimates from the 1993 National Health Interview Survey. National Center for Health Statistics. *Vital Health Stat* 10:190, 1994.
- McCaig LF, and Hughes JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. *JAMA* 273:214–219, 1995.
- Hamilos D. Chronic sinusitis. *J Allergy Clin Immunol* 106:213–227, 2000.
- Wald ER. Microbiology of acute and chronic sinusitis in children and adults. *Am J Med Sci* 316:13–20, 1998.
- Lindbaek M, Hjortahl P, and Johnsen U. Randomized, double blind, placebo controlled trial of penicillin V and amoxycillin in treatment of acute sinus infections in adults. *BMJ* 313:325–329, 1996.
- Fukami M, Norlander T, Stierna P, et al. Mucosal pathology of the nose and sinuses: A study in experimental maxillary sinusitis in rabbits induced by *Streptococcus pneumoniae*, *Bacteriodes fragilis*, and *Staphylococcus aureus*. *Am J Rhinol* 7:125–132, 1993.
- Scharf KE, Lawson W, Shapiro JM, and Gannon PJ. Pressure measurements in the normal and occluded rabbit maxillary sinus. *Laryngoscope* 105:570–574, 1995.
- Stierna P, and Carlsoo B. Histopathological observations in chronic maxillary sinusitis. *Acta Otolaryngol (Stockh)* 110:450–458, 1990.
- Bolger WE, Leonard M, Dick EJ Jr, and Stierna P. Gram negative sinusitis: A bacteriologic and histologic study in rabbits. *Am J Rhinol* 11:15–25, 1997.
- Moxon ER. Experimental infections of animals on the study of *Streptococcus pneumoniae*. *Rev Infect Dis* 3:354–357, 1981.
- Alonsodevelasco E, Verheul AFM, Verhoef J, and Snippe H. *Streptococcus pneumoniae*: Virulence factors, pathogenesis, and vaccines. *Microbiol Rev* 59:591–603, 1995.
- Boulnois GJ. Pneumococcal proteins and the pathogenesis of disease caused by *Streptococcus pneumoniae*. *J Gen Microbiol* 138:249–259, 1992.
- Kelsall B, and Strobbler W. Host defenses at mucosal surfaces. In *Clinical Immunology*. Rich R (Ed). St. Louis, MO: Mosby, 299–332, 1996.
- Medzhitov R, and Janeway C. Innate immunity. *N Engl J Med* 343:338–343, 2000.
- Kennedy DW, Zinreich SJ, Rosenbaum AE, and Johns ME. Functional endoscopic sinus surgery: Theory and diagnostic evaluation. *Arch Otolaryngol Head Neck Surg* 111:576–582, 1985.
- Spector SL. The role of allergy in sinusitis in adults. *J Allergy Clin Immunol* 90:518–520, 1992.
- van Dishoeck H, and Franssen M. The incidence and correlation of allergy and chronic maxillary sinusitis. *Pract Otolaryngol* 19:502–506, 1957.
- Benninger M. Rhinitis, sinusitis and their relationships to allergies. *Am J Rhinol* 6:37–43, 1992.
- Grove R, and Farrior J. Chronic hyperplastic sinusitis in allergic patients. A bacteriologic study of two hundred operative cases. *J Allergy* 11:271–276, 1990.
- Friedman W. Surgery for chronic hyperplastic rhinosinusitis. *Laryngoscope* 85:199–211, 1975.
- Savolainen S. Allergy in patients with acute maxillary sinusitis. *Allergy* 44:116–122, 1989.
- Rachelefsky GS, Goldberg M, Katz RM, et al. Sinus disease in children with respiratory allergy. *J Allergy Clin Immunol* 61:31–314, 1978.
- Binder E, Holopainen E, Malmberg H, and Salo OP. Clinical findings in patients with allergic rhinitis. *Rhinology* 22:255–260, 1984.
- Enberg R. Perennial nonallergic rhinitis: A retrospective review. *Ann Allergy* 513–516, 1989.
- Conner BL, Roach ES, Laster WS, and Georgitis JW. Magnetic resonance imaging of the paranasal sinuses: Frequency and type of abnormalities. *Ann Allergy* 62:457–460, 1989.
- Karlsson G, and Holmberg K. Does allergic rhinitis predispose to sinusitis? *Acta Otolaryngol (Stockh) Suppl* 515:26–29, 1994.
- Wagenmann M, and Naclerio RM. Anatomical and physiological considerations in sinusitis. *J Allergy Clin Immunol* 90:419–423, 1992.
- Evans FO Jr, Sydnor JB, Moore WEC, et al. Sinusitis of the maxillary antrum. *N Engl J Med* 293:735–739, 1975.
- Bomer K, Brichta A, Baroody F, et al. A mouse model of acute bacterial sinusitis. *Arch Otolaryngol Head Neck Surg* 124:1227–1232, 1998.
- Blair C, Naclerio RM, Yu X, et al. Role of TH₁ cells in the resolution of acute streptococcus pneumoniae sinusitis: A mouse model. *J Infect Dis* 192(7):1237–1244, 2005.
- Tong HH, Blue LE, James MA, and DeMaria TF. Evaluation of the virulence of a *Streptococcus pneumoniae* neuraminidase-deficient mutant in nasopharyngeal colonization and development of otitis media in the chinchilla model. *Infect Immun* 68:921–924, 2000.
- Kaplan SL, Mason EO Jr, Wald ER, et al. Pneumococcal mastoiditis in children. *Pediatrics* 106:695–699, 2000.
- Hausdorff WP, Bryant J, Paradiso PR, and Siber GR. Which pneumococcal serogroups cause the most invasive disease: Implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 30:100–121, 2000.

40. Won Y-S, Brichta A, Baroody F, et al. Bactrim reduces inflammation response in a murine model of acute sinusitis. Rhinology 38:68–71, 2000.
41. Reiner SL, and Locksley RM. The regulation of immunity to *Leishmania major*. Ann Rev Immunol 13:151–177, 1995.
42. Blair C, Nelson M, Thompson K, et al. Allergic inflammation enhances bacterial sinusitis in mice. J Allergy Clin Immunol 108:424–429, 2001.
43. Andersen I, and Molhave L. The ambient air. In The Nose. Proctor D, and Anderson I (Eds). New York: Elsevier Biomedical Press, 306–336, 1974.
44. Yu X, Sperling A, Blair C, et al. Antigen stimulation of Th2 cells augments acute bacterial sinusitis in mice. J Allergy Clin Immunol 114:328–334, 2004.
45. Winkelstein JA. Complement and the host's defense against the pneumococcus. CRC Crit Rev Microbiol 11:187–205, 1984.
46. Kadioglu A, Gingles NA, Grattan K, et al. Host cellular immune response to pneumococcal lung infection in mice. Infect Immun 68:492–501, 2000.
47. Rubins JB, and Janoff EN. Pneumolysin: A multifunctional pneumococcal virulence factor. J Lab Clin Med 131:21–27, 1998.
48. Musher DA. *Streptococcus pneumoniae*. In Principles and Practice of Infectious Diseases. Mandell GL, Bennett JE, and Dolin R (Eds). Philadelphia: Churchill Livingstone, 2128–2147, 2000.
49. Gabr U, Won Y-S, Boonlayangoor S, et al. C57B1/6 and BALB/c mice have similar neutrophil response to acute *Streptococcus pneumoniae* sinus infections. Arch Otolaryngol Head Neck Surg 127:985–990, 2001. □

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