C57Bl/6 and BALB/c Mice Have Similar Neutrophil Response to Acute Streptococcus pneumoniae Sinus Infections

Usama Gabr, MD; Yu-Sung Won, MD, PhD; Sue Boonlayangoor, MS; Kenneth Thompson, PhD; Fuad M. Baroody, MD; Robert M. Naclerio, MD

Background: Previous investigations have shown that mice with a tendency toward a TH1 or TH2 lymphocyte response manifest different reactions to inoculation with the parasite Leishmania major. BALB/c mice (with a tendency for a TH2 response) showed evidence of systemic infection, whereas C57Bl/6 mice (with a tendency for a TH1 response) showed only a local reaction.

Objective: To investigate whether BALB/c and C57Bl/6 mice respond differently to acute bacterial infection of the sinuses.

Methods: We inoculated the nasal cavities of C57Bl/6 and BALB/c mice with Streptococcus pneumoniae (type ATCC59), or with broth as a control. The mice were humanely killed 2, 5, 10, and 14 days after inoculation. Their heads were fixed, decalcified, and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin, and the degree of inflammation was quantified by the number of neutrophils per square millimeter of the sinus mucosa and the percentage of the sinus cavity occupied by neutrophil clusters.

Results: Both groups of mice showed evidence of inflammation that was significantly greater than controls (P = .01), with no difference between groups. There was a correlation between the number of neutrophils per square millimeter in the sinus mucosa and the percentage of neutrophil clusters (C57Bl/6 mice, r = 0.37, P < .001; BALB/c mice, r = 0.20, P < .001). In the infected mice, the number of infiltrating neutrophils was significantly greater (P < .001) in anatomically lower (dependent) areas of the sinuses compared with the upper areas.

Conclusion: Unlike leishmaniasis, acute bacterial sinusitis is not affected by the tendency of the host to favor either a TH1 or TH2 response.


A CUTE BACTERIAL infection of the sinuses is a common disorder that is often preceded by a viral upper respiratory tract infection. During acute infection, Streptococcus pneumoniae is the most common bacterium obtained from maxillary sinus punctures. Streptococcus pneumoniae has been studied extensively, and its complete genome sequence has been identified. Several virulent factors affect the host’s response to this organism, including (a) protein adhesion molecules (attach to the N-acetylgalactose complex of the respiratory mucosa), (b) IgA protease proteins (inactivate respiratory mucosal IgA), (c) antiphagocytic polysaccharide capsules (interfere with the opsonizing activity of the alternative complement pathway), (d) cell wall lipoteichoic acid (a potent proinflammatory complex capable of eliciting the production of interleukin 1 (IL-1) and tumor necrosis factor α in addition to the activation of the alternative complement pathway), and (e) pneumolysin toxin (has complement-activating and direct cytolytic activities). However, far less attention has been focused on the host’s immune response to infection.

Basic immunologic research has demonstrated functional differences between the 2 T-helper lymphocyte subsets. TH1 cells produce IL-2 and interferon γ (IFN-γ), which are important in the cell-mediated immune response directed toward the production of IgG-subclass antibodies (IgG1 and IgG2). On the other hand, TH2 cells respond by producing IL-4, IL-5, IL-10, and IL-13, which help to create a microenvironment favoring eosinophil-mediated inflammatory responses and the production of IgE and IgA antibodies. Moreover, there is reciprocal inhibition (down-regulation) between the TH1 and TH2 lymphocytes secondary to their cytokine production (ie, TH1 cytokines inhibit the development of TH2 cells, and vice...
MATERIALS AND METHODS

MICE

Black C57Bl/6 and white BALB/c mice aged 6 to 8 weeks were obtained pathogen-free from The Jackson Laboratory, Bar Harbor, Me, and housed in micro-isolation cages at the Carlson Biocontainment Facility at The University of Chicago, Chicago, Ill. All manipulations of the animals before they were humanely killed were conducted in a class II biosafety hood, following strict biosafety control measures as outlined by the university’s Animal Resource Center. The Animal Safety Committee of The University of Chicago approved the study.

DESIGN

On day 0 (the start of the experiment), the mice were given an intranasal inoculation of S pneumoniae in broth, and they were then killed on days 2, 5, 10, and 14. On each day of carnage, 1 group of C57Bl/6 mice and 1 group of BALB/c mice were included. Thirty-seven C57Bl/6 mice and 33 BALB/c mice completed the study. In addition, a control group of 4 BALB/c mice were challenged with broth only and were killed on day 5 after inoculation. The number of mice in the control group was small and consisted of only BALB/c mice because prior investigation showed no response to the broth in noninfected C57Bl/6 mice.

INOCULA

All inocula were freshly prepared in the clinical microbiology laboratory of The University of Chicago Medical Center. Streptococcus pneumoniae (type ATCC59) was suspended in trypticase soy broth at a 3.0 McFarland standard concentration equivalent to about $1.2 \times 10^{6}$ colony-forming units per milliliter. Mice in the control group were challenged with trypticase soy broth only.

METHODS

The animals were sedated with ketamine hydrochloride (80 mg/kg of body weight) and xylazine hydrochloride (8 mg/kg of body weight) by intraperitoneal injection. Their head was tilted backward, and 5 droplets of the inoculum were placed at the anterior nares of each nostril. Because mice are obligatory nasal breathers, the fluid was drawn into the nasal passages during inhalation, filling the nasal cavity and the sinus areas. To avoid choking the animal, we placed inoculum droplets very slowly, with close monitoring of the breathing rate. Animals were placed on their side until they recovered from anesthesia in about 15 to 20 minutes. They were then housed in a pathogen-free facility until they were killed.

TERMINATION PROCEDURE

On the designated day of carnage, the animals were euthanized with an intraperitoneal injection of sodium pentobarbital (120 mg/kg of body weight). While respiratory failure

RESULTS

Neither group of infected mice showed clinical symptoms of infection; however, both groups showed neutrophil infiltrates and clusters significantly greater in number than those in controls. This evidence of inflammation was significant as early as day 2 of the experiment ($P = .01$). Thereafter, the data showed an upward trend in the number of neutrophil infiltrates that reached its peak on day 10 and then declined. The inflammation was not totally resolved on day 14 (last day of the experiment). There was no statistically significant difference between groups of mice killed on the same day in either number of neutrophils or percentage of sinus cavity area occupied by clusters (Figure 4 and Figure 5).

There was a significant correlation between the number of polymorphonuclear neutrophils (PMNs) per square millimeter infiltrating the mucosa and the sinus cavity area occupied by neutrophil clusters in both strains (C57Bl/6 mice, $r = 0.37$, $P < .001$; BALB/c mice, $r = 0.20$, $P < .01$) (Figure 6).

In both strains of mice and at all time points evaluated, the number of PMNs infiltrating the mucosa showed
significantly higher numbers in the anatomically lower regions of the sinuses (dependent areas) compared with the upper areas on the same section (\(P<.001\)) (Figure 7).

HISTOLOGIC PREPARATION

Under low magnification, the skin, muscles, and eyeballs were removed from the decapitated head; the remaining tissue was immersed in the fixative solution for 24 hours. Then, the mandible, tongue, and any remaining muscles were removed. The remaining tissue was then immersed in a decalcifier solution (Surgipath II, Rockford, Ill) for 24 hours. After decalcification, the heads were soft enough to trim the anterior portion of the nose and the posterior portion of the skull with the brain. The remaining block was embedded in paraffin, and 5-µm-thick sections were cut. The sections were mounted on glass slides and stained with hematoxylin-eosin.

CYTOLOGIC ANALYSIS

Three anatomically similar sections were selected from each mouse head, at the middle portion of the sinus cavity. The sections included the posterior end of the maxillary sinuses and the ethmoid sinuses; an arbitrary transverse line at the level of the middle turbinate was used to divide the section into upper and lower portions (Figure 1). Investigators were blinded to the source groups of mice before analysis of sections. A computer-assisted microscope (×40 lens) powered with image analysis software (Neurolucida version 2.1; MicroBrightField Inc, Colchester, Vt) was used for the analysis. The 2 variables assessed were the percentage of the cross-sectional sinus cavity area occupied by neutrophil clusters (Figure 2) and the number of neutrophils per square millimeter infiltrating the sinus mucosa (Figure 3). The infiltrating neutrophils were counted in areas adjacent to the neutrophil clusters, or in 4 equivalent mucosal areas when no neutrophil clusters were present.

STATISTICAL ANALYSIS

Parametric statistical methods were used. Nonpaired \(t\) tests were applied to the data for comparison of the difference between the 2 groups of mice killed on the same day, and for comparison of differences between each group and control on day 5. We performed paired \(t\)-test analysis on the data between upper and lower areas of the mucosa to determine whether the anatomic distribution of the infiltrates varied within the same section. Numbers are expressed as mean±SEM. \(P<.05\) was considered to indicate statistical significance. The Pearson product moment correlation test was applied for evaluation of the relationship between the 2 objective variables used in the study.

In the United States, sinusitis affects about 15% of the population.\(^1\) Sinusitis diminishes patients' quality of life,
and its treatment expends a large amount of health care resources. In 1992, the estimated direct cost for the treatment of sinusitis reached $2.4 billion; by 1996, the estimate was $5.8 billion. Despite these facts, the pathophysiology of this disease is poorly understood. Consequently, we developed a murine model of acute sinusitis in an attempt to better understand the underlying mechanisms of the host involved in the pathophysiology and immunology of this inflammatory disease.

The immune system is complex and has many components that interact to eliminate foreign invaders. This defense is imposed by nonspecific mechanisms (innate immunity) and antigen-specific mechanisms (acquired immunity). Innate immunity is present at birth and consists of intact skin and mucous membrane; secretions and their components, macrophages, neutrophils, eosinophils, and natural killer cells; the lactoferrin barrier; and isoenzymes.

The response of a naive host to bacteria begins with the innate immune system, in which PMNs and macrophages are recruited. The macrophages act as antigen-presenting cells, and they process, recognize, and present the foreign antigens to other immune cells, particularly T-helper cells that continue the acute inflammatory cascade. Because PMNs play such an important role in the response to acute bacterial infection, we chose to quantify the number of PMNs infiltrating the mucosa and the percentage of the sinus cavity occupied by PMN clusters to assess the acute inflammatory response objectively. Although involved and time-consuming, this method provides reliable and reproducible data.

Figure 3. Light micrographs of the sinus mucosa. A, Sinus mucosa from a control mouse. B, Inflamed mucosa in an infected mouse, with dilated vessels (arrow), many neutrophil infiltrates, and mucosal stripping (hematoxylin-eosin, original magnification ×40).

Figure 4. Comparison of polymorphonuclear neutrophils (PMNs) per square millimeter of infected C57Bl/6 and BALB/c mice. There were significantly more PMNs per square millimeter of mucosa in the infected groups on days 2, 5, 10, and 14 compared with the control group (P<.01). There were no significant differences between the 2 strains on any day of the experiment. Data are presented as mean±SEM.

Figure 5. Comparison of polymorphonuclear neutrophil (PMN) clusters of infected C57Bl/6 and BALB/c mice. None of the mice in the control group showed clusters. The infected mice had significantly more clusters in the sinus cavities than did the control group (P<.01). There were no significant differences between the 2 mouse strains at any time point. Data are presented as mean±SEM.
Neutrophil recruitment depends on chemotactic factors related to the host and the bacteria. The results of this study suggest that the time course and the degree of inflammation were not dependent on the genetically determined differences between the 2 mouse strains. The difference between the 2 mouse strains in response to cutaneous leishmaniasis\textsuperscript{9-14} led us to investigate whether a similar difference would be seen in acute sinusitis. Our data suggest that this is not the case. Leishmania infection is an IgE-mediated phenomenon, and T\textsubscript{H}2 lymphocytes are critical to this process by virtue of the cytokines that they secrete, primarily IL-4 and IL-13, which are crucial for IgE synthesis. However, we do not know the importance of T lymphocytes and their cytokines in acute bacterial infection of the sinuses. The lack of difference in acute bacterial infection of the sinuses between the 2 mouse strains with different T\textsubscript{H} lymphocyte preponderance may suggest that T\textsubscript{H} cells are not central or critical to this process. This may be explained in part by the strong ability of \textit{S pneumoniae} to attract neutrophils independently of the host’s immune system. This ability is attributed to the T-independent antigens, which include pneumolysin, the polysaccharide capsule, and lipoteichoic acid. These antigens may act as the initiating factors for neutrophil recruitment during acute infection. Moreover, they can activate the alternative complement pathway and produce a primary IgM immune response without producing memory cells or an anamnestic secondary IgG immune response.\textsuperscript{4-7} Accordingly, these groups of antigens can directly initiate acute inflammation, independently of the host’s genetic tendency toward either a T\textsubscript{H}1 or T\textsubscript{H}2 immune profile.\textsuperscript{3}

Another possible explanation for our finding may be related to the immune mediators released locally at the infection site, which act also as chemoattractants to neutrophils; however, their release is not dependent on T-helper cells. These mediators include complement component C5a, high-molecular-weight neutrophil chemotactic factor, platelet-activating factor, IL-1 and IL-8, and leukotrienes, such as leukotriene B\textsubscript{4}.

Our data suggest that the direct interplay between the bacterial antigens and the local immune mediators may have an effect on the degree of acute bacterial inflammation of the paranasal sinuses, more than that of the genetic tendency toward either a T\textsubscript{H}1 or T\textsubscript{H}2 immune response. Furthermore, we recognize that major differences may exist between acute and chronic inflammation in regard to previous exposure to bacteria, antigen-
specific antibody production, and different cytokine profiles.\(^{17-21}\) Therefore, the host's tendency to favor a TH1 or TH2 response in chronic sinusitis may be an important determinant of the inflammatory response, but remains to be investigated.

**CONCLUSIONS**

Unlike cutaneous leishmaniasis, acute bacterial sinusitis is not affected by the genetic tendency of the mouse to favor either a TH1 or TH2 response. We speculate that the key factors affecting the course of this disease are probably dependent on the direct antigenic nature of the causative organism and its ability to stimulate the host's immune system within the local environment.

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Corresponding author: Robert M. Naclerio, MD, Section of Otolaryngology–Head and Neck Surgery, The University of Chicago, 5841 S Maryland Ave, MC 1035, Chicago, IL 60637 (e-mail: rnacleri@surgery.bsd.uchicago.edu).

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