Chronic Bacterial Rhinosinusitis

Description of a Mouse Model

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Objectives: To survey normal murine sinonasal anatomy and to create a mouse model for chronic bacterial rhinosinusitis.

Design: Anatomic, histologic, and pathophysiologic study displaying normal murine sinonasal anatomy and surgically created unilateral sinonasal inflammation.

Subjects: Twenty-one 6-week-old, male C57BL/6 mice.

Interventions: Animals that underwent unilateral maxillary sinus ostial obstruction using Merocel nasal packing, animals with unilateral Bacteroides fragilis inoculation alone, and animals with both ostial obstruction and bacterial inoculation were examined at 4 weeks for histologic evidence of chronic sinonasal inflammation. Experimental interventions were compared with contralateral control sinuses within each animal and with normal and sham-operated controls.

Results: Normal mouse paranasal sinuses include maxillary sinuses, ethmoid air cells, and respiratory-type epithelium. In experimental animals, the lateral maxillary sinus wall, nasal septum, and superior turbinate of the maxillary sinus were examined histologically. Epithelial thickening and disarray, goblet cell hyperplasia, inflammatory infiltrates, and sinonasal fibrosis were present in the experimental sinuses of animals packed with Merocel alone or Merocel with bacterial inoculation. Changes seen with Merocel and bacteria were more dramatic than those with Merocel alone. Sham-operated controls and sinuses inoculated with bacteria alone did not differ significantly from the sinuses of normal animals.

Conclusion: Unilateral maxillary sinus ostial obstruction using Merocel nasal packing along with B fragilis inoculation results in a persistent, localized bacterial rhinosinusitis in mice.


Rhinosinusitis affects more than 10% of the US population and is the reason for approximately 12 million physician office visits annually. The number of antibiotics prescribed and sinus surgical procedures performed have increased significantly during the past decade.1

Sinus disease is divided clinically into acute (symptoms persisting for less than 2 weeks), subacute (symptoms persisting for more than 2 weeks but less than 3 months), and chronic (symptoms persisting for more than 3 months without return to baseline) based on the duration of disease.2 Patients with chronic sinusitis experience nasal obstruction, rhinorrhea, postnasal discharge, headache, facial pain and pressure, and a chronic cough. Obstruction to sinus drainage, immune dysregulation, and mucociliary dysfunction result in mucosal disruption, hypersecretion, and a chronic infection.3 Given the obvious clinical significance of rhinosinusitis, a systematic study of the pathogenesis and treatment of sinusitis is clearly indicated. Without a good animal model, investigation into the basic molecular mechanisms and pathophysiology of the disease is lacking. Important questions remain about the mediation of this localized inflammatory response, histologic changes, and potential therapeutic modulation.

Animal experiments in the study of sinus disease date back to the early 20th century.4-8 A variety of interventions have been attempted in dogs, cats, rabbits, and, more recently, mice. Hilding9 pioneered the development of a rabbit model for sinusitis, which subsequently became the dominant tool with which researchers studied mucosal blood flow, lactic acid accumulation, mucous substance histochemistry, cellular histomorphology, local and systemic humoral immune response, polyposis, and responses to a variety of thera-
MATERIALS AND METHODS

STUDY GROUPS

Six normal, 6-week-old, male C57BL/6 mice were used to define the relevant normal anatomy of the murine sinonasal cavity. Then, in creating the rhinosinusitis model, 15 six-week-old, male C57BL/6 mice (Jackson Laboratory, Bar Harbor, Me) were used as follows: 2 normal controls without intervention, 2 sham-operated controls, 3 animals with ostial obstruction alone using Merocel nasal packing (Xomed Surgical Products, Jacksonville, Fla), 3 animals implanted with Merocel plus 10^6 colony-forming units (CFU)/mL of Bacteroides fragilis, 3 animals implanted with Merocel plus 10^8 CFU/mL of B fragilis, and 2 animals without ostial obstruction inoculated with 10^8 CFU/mL of B fragilis. All mice were shipped directly from the Jackson Laboratory to the Animal Barrier Facility at Washington University School of Medicine in St Louis, Mo. Bacteroides fragilis suspended in isotonic sodium chloride solution at concentrations of 10^6 and 10^8 CFU/mL were obtained from the Department of Microbiology, Barnes-Jewish Hospital/ Washington University School of Medicine. Merocel nasal sponge served as the obstructive implant.

SURGICAL PROCEDURES

All procedures were performed in strict accordance with Animal Studies Committee guidelines at the Washington University School of Medicine.

The surgical procedure was done under the operating microscope using otologic microinstrumentation. After achieving a surgical plane of anesthesia (80 mg/kg of ketamine hydrochloride with 15 mg/kg of xylazine hydrochloride, intraperitoneal injection), a 5-mm midline incision was achieved, with a surgical plane of anesthesia (80 mg/kg of ketamine hydrochloride with 15 mg/kg of xylazine hydrochloride, intraperitoneal injection). A single drop of bacterial suspension from a standard pipette was sufficient to saturate the sponge during insertion. The Merocel sponge was placed in the middle third of the sinonasal cavity approximating roughly 2 to 3 mm of the total anterior-posterior length (Figure 1 and Figure 2). The sham-operated controls had their right nasal cavity opened from above in a similar manner. However, no Merocel was inserted in this group. Skin flaps were reapproximated with 6-0 polypropylene sutures, and the anesthetic reversed throughout 2 hours. Once awake and active, the animals were returned to the rodent care facility. The duration of each surgical procedure ranged from 10 to 15 minutes.

Four weeks postoperatively, a euthanasia dose (200 mg/kg of pentobarbital was administered via intraperitoneal injection. The thoracic cavity was opened, and transthoracic perfusion fixation was accomplished with 4% paraformaldehyde and 0.05% glutaraldehyde in phosphate buffer. The animal was decapitated, skin and soft tissues of the head were removed, and the mandible was excised. A coronal cut was made 1 mm posterior to the eyes using a razor blade. In this manner, the eyes and maxilla (including the sinonasal cavity) were isolated for tissue processing.

HISTOLOGIC ANALYSIS

The tissues were immersed overnight in the same fixative at 4°C. They were decalcified in buffered 0.35-mol/L tetrasodium EDTA solution at 4°C during 1 week and sinus and superior turbinelle ranges from cuboidal anteriorly to pseudostratified columnar more posteriorly. Goblet cells with characteristic morphologic features appear to be present in greater numbers posteriorly.

GOBLET CELL HYPERPLASIA AND METAPLASIA

Comparing experimental to contralateral control sinuses, the experimental sinuses of animals with both Merocel placement and either 10^6 or 10^8 CFU/mL of B fragilis inoculation demonstrated significantly increased goblet cell numbers (Figure 5). Increases were also observed in animals implanted with Merocel alone and those inoculated with bacteria alone. Within-animal comparisons showed no significant differences between experimental and control sinuses in normal or sham-operated controls.

Comparisons of goblet cell number by intervention are displayed in Figure 6. Animals having undergone Merocel placement and either 10^6 or 10^8 CFU/mL of B fragilis inoculation demonstrated significant increases in goblet cell numbers at all locations when compared with normal or sham-operated controls (Table). In addition, animals with both Merocel placement and 10^8 CFU/mL of B fragilis inoculation displayed goblet cell
decanted daily. Tissues were then rinsed in phosphate-buffered saline, dehydrated through a graded series of acetone, and embedded in a Epon-araldite mixture. In the normal animals, sequential 1.0-µm sections were taken in the coronal plane at 250-µm intervals. The animals’ snout and the anterior margin of the interocular plane defined the limits of sectioning. These were stained with toluidine blue and basic fuchsin. For the sinusitis experiment, the sinusonal cavity was divided into thirds, and 1-µm sections (at 50- to 75-µm intervals) were taken from the middle segment. This segment consistently contained the implanted Merocel, maxillary sinus, and ostium. Five representative sections within this area were chosen at random from each animal for analysis.

QUANTIFICATION

Anatomical observations were based on sequential sections through the paranasal sinuses (Figure 1). For the sinusitis experiment, the principal areas of analysis were in the middle third of the sinusonal cavity (Figure 2). The locations used for tissue morphometry include the lateral maxillary sinus wall, nasal septum, and inner margin of the superior maxillary sinus turbinelle (Figure 3). Epithelial thickness and area and goblet cell number were quantified, whereas the presence or absence of a luminal or submucosal mononuclear infiltrate, epithelial denudation or disarray, and sinusonal fibrosis was evaluated qualitatively (Figure 4). Having experimental and contralateral unoperated-on control sinuses within the same animal, within-animal and intergroup comparisons were made.

Given the presence or absence of Merocel within each section, image analysis could not be done in a blinded fashion. Quantitative histologic analysis was carried out as follows. Using SigmaScan Pro image analysis software (SPSS Science Inc, Chicago, Ill), area measurements were traced directly on the computer from digitally captured images. Once standardized to 1-mm distance along the basement membrane, these measurements correlated directly with average epithelial thickness. Goblet cell counts were then standardized to the same 1-mm distance. These were morphologically identified at ×500 magnification by their pale cytoplasm, shape, and characteristic nuclei. No special stains were used. Qualitative histologic analysis based on a plus or minus scale denoted the presence or absence of luminal or submucosal infiltrates, epithelial disarray or sloughing, and presence of either luminal or submucosal fibrosis. The experimental right-sided sinusonal cavity was compared with the contralateral control within each section. Images were evaluated at ×30, ×125, ×250, and ×500 magnifications from 5 sections chosen at random from each intervention group.

All histologic figures presented herein were digitally captured, converted to gray scale, and placed on white backgrounds in Adobe Photoshop 4.0 (Adobe Systems Inc, San Jose, Calif). Figures were subsequently organized in Corel Draw 7 (Corel, Ontario).

STATISTICAL ANALYSIS

All statistical analyses for quantitative histologic analysis were performed using SigmaStat (SPSS Science Inc) statistical software. Within-animal comparisons were evaluated with the 2-tailed t test, whereas comparisons across interventions were evaluated using a 1-way analysis of variance. Five data points from the middle third of each animal’s sinusonal cavity were used at the following locations: the lateral sinus wall, the nasal septum, and the superior turbinelle. Data were pooled within each intervention group, and means and SEs were calculated to make comparisons. For all comparisons, α = .05. Mean data, SE bars, and P values are presented in Figures 5, 6, and 7.

EPITHELIAL THICKENING

Within-animal comparisons revealed statistically significant epithelial thickening in the experimental sinuses of animals with Merocel placement and 10⁶ CFU/mL of B fragilis inoculation displayed significant increases at the lateral wall and septum. There were no differences between Merocel-only and bacteria-only animals vs normal or sham-operated controls. Counts did not differ significantly between normal controls and sham-operated controls.

INFLAMMATORY INFILTRATE, EPITHELIAL DISARRAY, AND SINONASAL FIBROSIS

Animals with Merocel placement and 10⁶ CFU/mL of B fragilis inoculation, Merocel placement with 10⁸ CFU/mL of B fragilis inoculation, and Merocel placement alone (Figure 7). No statistically significant epithelial thickening was found in intra-animal comparisons of normal controls, sham-operated controls, or animals inoculated with bacteria alone.

Compared with the experimental sinuses of normal or sham-operated controls, Merocel placement alone or Merocel placement with either 10⁶ or 10⁸ CFU/mL of B fragilis inoculation resulted in statistically significant epithelial thickening at all locations analyzed (Figure 6 and Table). Compared with Merocel alone, Merocel plus bacteria at either concentration resulted in more dramatic epithelial thickening at the lateral sinus wall and nasal septum. There were no differences between normal controls, sham-operated controls, and animals inoculated with bacteria alone.

COMMENT

Unilateral maxillary sinus ostial obstruction with Merocel nasal packing and B fragilis inoculation at either 10⁶ or 10⁸ CFU/mL resulted in a persistent, localized bacte-
Material rhinosinusitis in mice. With limited operative time and minimal morbidity and mortality, histologic changes characteristic of chronic sinonasal disease were present 4 weeks after intervention. A 4-week end point was chosen to demonstrate the chronicity of disease. A combination of quantitative and qualitative histology focused on epithelial thickening, goblet cell changes, presence of a luminal or submucosal inflammatory infiltrate, epithelial disarray, and sinonasal fibrosis in the murine sinonasal cavity. Within-animal comparisons demonstrated epithelial thickening and goblet cell hyperplasia in the experimental sinuses of animals with Merocel only and Merocel plus bacteria. Epithelial disarray, inflammatory infiltrates, and sinonasal fibrosis were present in these groups as well. Comparison across interventions qualified these findings by noting that changes seen with Merocel plus bacteria were more severe than those with Merocel alone. No such changes were noted in normal controls.
sham-operated controls, or animals with bacterial inoculation alone. This represents the first described murine model of chronic bacterial rhinosinusitis. There were no operative mortalities within the present study group, and animal caregivers did not report any evidence of postoperative suffering or behavioral dysfunction.

Merocel is used clinically in the control of epistaxis. A patient seen in the emergency department may have this material inserted into his or her nasal cavity if bleeding is diffuse and discrete sources cannot be identified and cauterized. At our institution, the Merocel is then left in place for 3 days and patients are prescribed antibiotics during that time. If the Merocel is left in longer, these patients often develop sinusitis on the obstructed side. This is only one example of clinical sinusitis arising from foreign body obstruction of the maxillary sinus ostium in humans. Another is the use of nasogastric tubes. Patients intubated with nasogastric tubes often have fluid in their maxillary sinuses. In fact, this is a frequent reason for otolaryngology consultation at our institution. Most texts define rhinosinusitis as

Figure 4. Characteristic histologic changes in chronic bacterial rhinosinusitis: luminal and submucosal inflammatory infiltrate, epithelial thickening or derangement, goblet cell hyperplasia, and sinonasal fibrosis (toluidine blue/basic fuchsin).

Figure 5. Within-animal comparisons of mean goblet cell number by intervention. P values <.05 are shown. 1 indicates left lateral wall; 2, right lateral wall; 3, left septum; 4, right septum; 5, left superior turbinate; 6, right superior turbinate; and B fragilis, Bacteroides fragilis. Error bars are SEs.
Figure 6. Mean (SE) number of goblet cells and epithelial thickness by treatment. 1 indicates normal controls; 2, sham-operated controls; 3, Merocel only; 4, Merocel plus $10^6$ colony-forming units (CFU)/mL of Bacteroides fragilis; 5, $10^8$ CFU/mL of B fragilis only; and 6, Merocel plus $10^8$ CFU/mL of B fragilis. Corresponding statistical data appear in the Table.

Figure 7. Within-animal comparisons of average epithelial thickness by intervention. Area measures correlate with average epithelial thickness. Statistically significant $P$ values are displayed. 1 indicates left lateral wall; 2, right lateral wall; 3, left septum; 4, right septum; 5, left superior turbinate; 6, right superior turbinate; B fragilis, Bacteroides fragilis; and CFU, colony-forming units. Error bars are SEs.
mucosal and/or luminal inflammation in the sinonasal cavities. “Chronic” sinusitis is further defined by duration of disease. To that extent, we believe that epithelial thickening and disarray, luminal or submucosal inflammation, increased goblet cell counts, and sinonasal fibrosis in our murine model qualify under that definition.

Both the quantitative and qualitative findings presented herein were dramatic. Therefore, quantitation required relatively few animals to discern statistically significant differences. Qualitative analysis used straightforward plus or minus designations. We believe that stratified scales (1+ to 4+) added rather than reduced subjectivity in assessment and were therefore abandoned. Although it would have been ideal to quantify the area of cellular infiltration, fibrosis, and epithelial sloughing, we believe that tissue processing (decalcification, dehydration, infiltration, and embedding) made such quantitation of luminal findings suspect. Although it seemed unlikely that all such findings would be washed away, it was certainly reasonable that some portion of it may be. The plus or minus designation, indicating the presence or absence of findings, was thought to be the most truthful representation of the data.

Although the rabbit model has dominated the scientific literature for the past 5 decades, research at the molecular and genetic levels requires a transition to murine models. Genetic knockouts and transgenic mice have revolutionized animal research. Mice are pathogen free, demonstrate minimal antigen priming, and have mini-

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### One-Way Analysis of Variance Across Interventions

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<th>Comparisons</th>
<th>Goblet Cell Number</th>
<th>Epithelial Area</th>
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<tr>
<td></td>
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<td>Right Septum</td>
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<td>Merocel + 10⁴ CFU/mL of B. fragilis vs normal control</td>
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<td>Y</td>
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<td>Merocel + 10⁴ CFU/mL of B. fragilis vs sham-operated control</td>
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<td>N</td>
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<tr>
<td>Merocel + 10⁴ CFU/mL of B. fragilis alone vs Merocel alone</td>
<td>N</td>
<td>Y</td>
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<tr>
<td>10⁴ CFU/mL of B. fragilis alone vs normal control</td>
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<td>Merocel alone vs sham-operated control</td>
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*CFU indicates colony-forming units; Y, statistically significant at P < .05; and N, not statistically significant.

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**Figure 8.** Comparison of experimental and control sinuses within the same animal. Chronic right-sided rhinosinusitis induced by foreign body ostial obstruction and Bacteroides fragilis inoculation (toluidine blue).
mal sibling genetic variability. Cost and care issues are simple. The ethics of animal experimentation oblige us to use the least sophisticated animal species possible when gathering scientifically relevant data.

To our knowledge, there is only one article in the literature on murine bacterial rhinosinusitis. Using an inhalation technique with pneumococcal suspensions, researchers successfully created an acute sinusitis in mice. An inflammatory response was noted by day 2, peaking at day 5, and subsiding by day 14 to normal levels. Although the investigators argued that recurrent acute attacks of sinusitis may progress to chronic disease, their model was unable to sustain the sinonasal inflammatory response. We have drawn on the osteomeatal hypothesis of human disease to solve this problem. In humans, obstruction of the sinonasal osteomeatal complex and infection are thought to impair sinus drainage, reduce ventilation, and generate an acute inflammatory response. With persistent obstruction to drainage, the body fails to clear the infection and chronic sinusitis develops. By providing both the infecting organism (B fragilis) and the obstruction to drainage (Mecol sponge), our localized inflammatory response persisted for at least 4 weeks. Bacteroides fragilis was used based on rabbit studies indicating that it exerted a more prolonged inflammatory response than did pneumococcus. Furthermore, anaerobes are recovered frequently in human patients with protracted sinus disease. Given that the anaerobic infection itself may contribute to the chronicity of the disease, it was a reasonable choice.

Clinical data on chronic sinusitis are abundant, but there is little information on the disease at its molecular and genetic levels. Human studies are limited by genetic and environmental variability, an inability to control the specificity of intervention, small sample sizes, limits in surgical specimens, and a lack of normal and internal controls. Murine research in this area could be the foundation for a more thorough understanding of this unique inflammatory process.

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REFERENCES