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The Role of Bone in Chronic Rhinosinusitis

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Objectives: **To evaluate and confirm the histological inflammatory changes that occur in bone and in the overlying mucosa in experimentally induced chronic rhinosinusitis and to evaluate differences in the inflammatory patterns that may occur with different organisms.** ***Study Design:*** **Histological study of induced maxillary rhinosinusitis in 29 New Zealand White rabbits (15 with *Pseudomonas aeruginosa*, 14 with *Staphylococcus aureus*) 7 to 9 weeks after infection.** ***Methods:*** **Following maxillary sinus ostial infection, unilateral chronic bacterial rhinosinusitis was induced in 29 New Zealand White Rabbits, using *Pseudomonas aeruginosa* (n = 15) and *Staphylococcus aureus* (n = 14). The pathogenic organism was confirmed by culture, and the rabbits were sacrificed at predetermined time intervals (7, 8, and 9 wk) from the time of infection. Following harvest, en bloc sinus sections were mounted, stained, and analyzed. Specific attention was given to identifying histological changes in paranasal sinus bones on both sides.** ***Results:*** **All animals (29 of 29) demonstrated histological evidence of operative occlusion on the side of the original inoculum, and all were culture-positive for the inoculated organism at death. Histological evidence of chronic rhinosinusitis in the inoculated sinus was demonstrated in 86% of animals (25 of 29). Evidence of chronic osteomyelitis in the noninfected side was seen in 15 of 29 animals (52%) overall, or 9 of 15 animals (60%) infected with pseudomonas and 6 of 14 (43%) animals infected with staphylococcus organisms.** ***Conclusions:*** **The study provides further evidence that bacterial rhinosinusitis can involve bone at a distance from the site of primary infection, thereby suggesting that infectious agents may spread through bony structures in the pathogenesis of chronic rhinosinusitis.** ***Key Words:*** **Rhinosinusitis, pathogenesis, bacterial, bone, osteomyelitis.**

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INTRODUCTION

At an approximated societal cost of 6 billion dollars annually, chronic rhinosinusitis (CRS) is a commonly reported and often debilitating disease in the United States.[1] This disease affects approximately 30 million Americans, significantly affects quality of life, and accounts for more than 25 million physician outpatient visits annually.[2–4] The multiple etiological factors and comorbid diseases associated with CRS (asthma, allergies, rhinitis) has made understanding the pathogenesis of this disease a continuing challenge.[5] Although the relative importance of the multiple etiological factors has not been clearly delineated, a general classification of risks includes environmental, systemic, and host issues.[4] When infectious agents are identified or cultured from the sinus mucosa in chronic inflammation, whether these organisms are a primary factor in the origin of the disease or represent secondary contamination as a result of other causes of inflammation and reduced mucociliary clearance is unclear. Thus, the extent to which chronic sinusitis represents a truly infectious problem, as opposed to a nonspecific inflammatory response, remains uncertain. Recently, there has been significant publicity, some scientific study, and considerable debate about the relevance and role of fungal elements in the pathogenesis of CRS.[6–8] Although it appears that bacterial, fungal, or viral inflammation may result in an exacerbation of CRS in susceptible individuals, the extent to which any of these agents plays a significant part in the underlying disease process remains less clear.

Whether the primary underlying factors in CRS are environmental or host related, the disorder appears to become significantly exacerbated when the ostiomeatal complex (OMC) becomes obstructed and persistent inflammatory changes occur with mucociliary dysfunction and stasis of secretions, as well as the inability to clear pathogens (bacteria, fungi) from the sinuses.[9,10] This process is characterized by a prolonged inflammatory response of the nasal and sinus mucosa and the fluid within the sinuses, as well as changes in the underlying bone.[11–13] Previous investigators have primarily focused on histopathological changes of the sinus mucosa in CRS, demonstrating an influx of inflammatory lymphocytic cells, thickening of the lamina propria, polypoid changes in the mucosa, and the hallmark presence of eosinophils.[13,14]

The rabbit has been used frequently as a model of experimentally induced sinusitis because of the ease of surgical accessibility of its maxillary sinuses and the abil-

ity to occlude these sinuses and to reliably induce infections.[15,16] Histological investigations of this animal model have demonstrated epithelial desquamation, goblet cell hyperplasia, fibrosis, and polyposis as changes identified in conjunction with rhinosinusitis.[17] Inflammatory markers, mucosal metabolism, and ultrastructure and ciliary structure have all been elucidated in the rabbit model.[18–20] Furthermore, prior studies involving surgical interventions in rabbits have demonstrated that only performing a middle meatal antrostomy may be insufficient to allow recovery from maxillary sinusitis and resolve the extent of pathological disease evident in bacterially induced (*Staphylococcus aureus, Bacteroides fragilis*) sinusitis.[21–22] Such findings support the clinical observations that additional therapies are required to improve clinical outcomes in certain treatment situations.

Recent studies from our laboratory suggest that bone itself may also play a significant role in experimentally induced sinusitis.[11,15,23,24] Initial experiments involving induction of pseudomonal sinusitis infections in rabbits demonstrated bone architectural alterations, including a coordinated osteoclasis, appositional bone formation adjacent to the infected sinus followed by intramembranous bone remodeling, as rapidly as 4 days after bacterial inoculation.[15] Clinically, within the spectrum of acute to chronic sinusitis, nasal endoscopy and computed tomography (CT) studies have shown bone to undergo resorption followed by subsequent hyperostosis. In addition, in the postoperative patient we have noted that localized persistent inflammation occurs until the underlying bone is removed. Our previous work in the clinical environment has also demonstrated that these histological changes are compatible with a diagnosis of osteomyelitis.[11] Using histomorphometry and tetracycline labeling techniques, we ascertained that ethmoid bone underwent rapid remodeling in CRS that was histologically identical to the remodeling seen in osteomyelitis.[23] However, as is frequently the case in chronic osteomyelitis, we were unable to identify organisms in the underlying bone.[23]

Our most recent experiment demonstrated the ability for pseudomonal sinusitis, in the presence of surgical intervention, to involve bone both in proximity to and at a distance from the site of primary infection, without the necessity for intervening mucosal disease.[24] However, this preliminary study was performed with only one organism, and a second procedure was performed during the study that could, potentially, have affected the results. During the earlier study, some bone was harvested from the side of the infection and implanted submucosally on the noninfected side. Histologically, this implanted bone was seen to resorb without inducing adjacent inflammation. However, despite the fact that the changes within the bone could be traced in a significant number of cases directly to the experimentally infected maxillary sinus, the additional surgery also created the possibility that the inflammation seen in the bone on the noninfected side might, in some cases, result from the surgical exploration or, potentially, occur as a result of the resorbed bone. Therefore, the overall aim of the current study was to further elucidate the pathogenic role of bone in chronic rhinosinusitis.

In particular, the present study attempted to determine whether experimentally induced local sinusitis with minimal bone and soft tissue damage using either *Pseudomonas aeruginosa* or *Staphylococcus aureus* alone can stimulate inflammation at a distant site without involvement of any intervening mucosa. *S aureus* accounts for a significant percentage of aerobic pathogens in sinusitis, and *P aeruginosa* has been the most frequently cultured source of severe recalcitrant CRS infections.[9,25] In addition, *P aeruginosa*, unlike many other acute sinusitis pathogens, has demonstrated the ability to cause a long-term sinusitis in the rabbit model.

MATERIALS AND METHODS

Animal Passage

Approval for all experimental procedures was obtained from the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). Four New Zealand White rabbits were used for the animal passage portion of the study, two for each bacterial strain. Both bacterial strains, *P aeruginosa* and *S aureus*, were obtained from nasal, pharyngeal, and sinus isolates from the American Type Culture Collection (ATCC) (Manassas, VA). The animals were housed at the animal husbandry facility for at least 4 days before a surgical procedure was performed. The rabbits were anesthetized with ketamine and xylazine, and a pulse oximeter was attached to monitor oxygenation status. Vital signs were recorded by the veterinary technician, and the nasal dorsum was shaved and prepped in a sterile manner. One percent Xylocaine was injected subcutaneously along the skin on the nasal surface; then the animals were intubated and oxygen delivery was initiated and provided throughout the procedure. Inhalational anesthetic (isoflurane) was also provided throughout the procedure to keep the rabbit anesthetized. Using a No. 15 blade, a vertical incision was made approximately 0.5 cm to the right of the midline. The periosteal layer in the intended drilling region was elevated, and a small periosteal flap was excised using a scalpel. The excised periosteal tissue was placed in warm saline. With the aid of a temporal bone drill, a small opening was drilled in the maxillary bone by saucerizing a small area until a window of maxillary mucosa was visualized. This remaining mucosal layer was incised with a scalpel, and an opening approximately 5 mm wide into the maxillary sinus was exposed. Using a rongeur, a segment of bone located superolateral to the sinusotomy site was carefully removed to allow increased exposure of the maxillary ostium. To adequately occlude the semilunar maxillary sinus ostium, the mucosal surface of the ostium was abraded and a small cotton pledget was cut to size and inserted into the ostium with the aid of an alligator instrument. The periosteal flap was retrieved from the saline solution and laid over the drilling site to further ensure that complete ostium occlusion had been achieved. Valsalva maneuver was then performed by the anesthetist, and the periosteal flap was observed for any movement resulting from the inevitable air leakage around the uncuffed endotracheal tube. Any movement of the periosteum placed over the sinus opening required removal and repositioning of the cotton pledget and repetition of the flap procedure. Once the flap was immobile, the pledget was lightly moistened with cyanomethacrylate glue gel to ensure maintenance of the cotton ostial plug in the appropriate position. Next, 0.4 mL of the bacterial inoculum was carefully instilled into the maxillary sinus using a 25-gauge needle. The skin was sutured with a running 2.0 Vicryl suture to close, and the rabbits were provided with buprenorphine HCl (0.02–0.1 mg/kg intramuscularly) every 6 hours for 24 hours after surgery for analgesia.

All four animals were sacrificed 4 days after surgery with a sedating ketamine dose followed by a sodium pentobarbital solu-

tion. An aliquot of the purulent material from the right-side maxillary sinus was harvested and sent to the microbiology laboratory for bacterial culture analysis. The same day, the remainder of the collected purulent material was diluted and incubated at 37°C in an incubator on nutrient agar plates. Individual colonies were plated onto agar slant tubes overnight in the incubator at 37°C as well. After approximately 24 hours in the incubator, the surface of the agar slant tubes were rinsed 10 times, each time with a sterile pipette filled with 200 mL of a stock solution (3 mL 50% glycerol, 7 mL nutrient agar broth [Becton Dickinson]). Each individual wash was collected in a vial and stored immediately at −70°C. These vials represented the stock inoculum for inducing the infections for each of the bacterial strains. Therefore, the stock inoculum represented a human nasopharyngeal or sinusitis pathogen that had been passed through an animal sinus to become a successful animal pathogen.

Bacterial Growth

For the initial animal passage procedure, both *P aeruginosa* and *S aureus* were grown from the isolates provided by the ATCC. Following this, before each sinusotomy procedure, either pseudomonas or staphylococcus was grown from the specimens that had been harvested from the New Zealand White rabbits with infected animal passage. For these overnight growths, one aliquot of 200 mL stock inoculum was thawed and placed in a tube containing 40 mL of nutrient broth medium. This tube was placed in a rotary shaker at 37°C overnight. Approximately 16 hours after beginning the overnight incubation, 40 mL of fresh, 37°C medium in a wide-bottomed Erlenmeyer flask was placed on the shaker. Bacteria from the overnight preparation were added until the optical density (OD) was between 0.05 and 0.07. The mixture was then replaced in the incubator, and the OD for the sample was read every 20 to 45 minutes until the OD was approximately 0.3. Each time point was plotted to determine whether the bacteria had achieved logarithmic growth. The mixture was then put on ice and centrifuged and washed twice at 4000 rpm × 15 minutes at 4°C. The pellet was then resuspended in 1 mL 0.9% saline and replaced on ice to slow growth. The new mixture was added drop by drop to a cuvette holding 2 mL 0.9% saline until the OD was 0.6. The amount of 0.4 mL of this optically measured mixture was drawn into several 1-mL syringes and placed on ice until surgery.

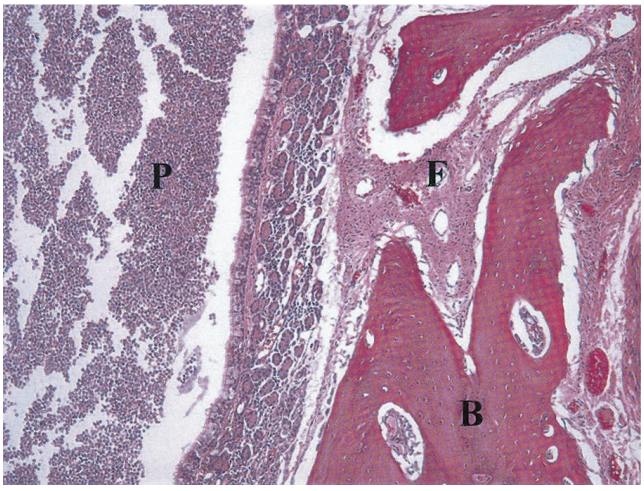


Fig. 1. Inoculated sinus cavity filled with purulent debris (P). The bone (B) immediately adjacent has prominent fibrosis (F) in the intra-trabecular spaces and in the haversian system (H&E, original magnification ×4).

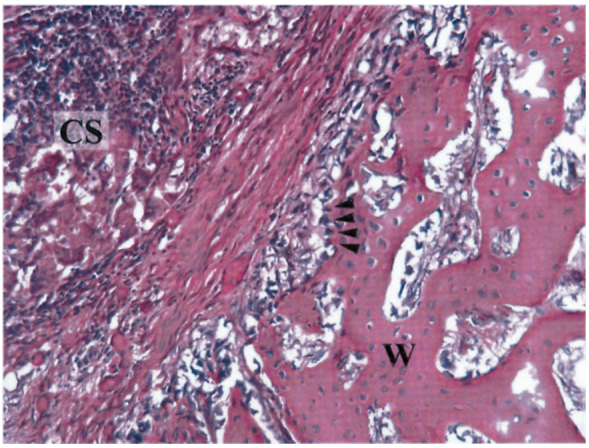


Fig. 2. Woven bone formation (W) in an area of chronic sinusitis (CS). The bone is rimmed by numerous plump osteoblasts (arrowheads) indicating high bone turnover. In the chronic sinusitis there are prominent inflammatory cells and hyperplastic mucosa (H&E, original magnification ×20).

Experimental Maxillary Sinus Inoculation

The present study used 29 Pasteurella-free New Zealand White rabbits; all rabbits were female and were weight-matched (2.5–4.0 kg). All the animals were housed at the university's animal husbandry facility for a minimum of 5 days before any surgical procedure was performed. The surgical induction of maxillary sinusitis through ostial obstruction is an identical procedure to the aforementioned animal passage procedure. Following harvest of bacteria form the animal passage, a single colony of bacteria was used to randomly inoculate the experimental animals with approximately 1.1×10^8 colony-forming units (CFU) of either *P aeruginosa* or *S aureus* (range, 1.1×10^7 to 1.16×10^9 CFU). The colony was confirmed by culture, thus ensuring con-

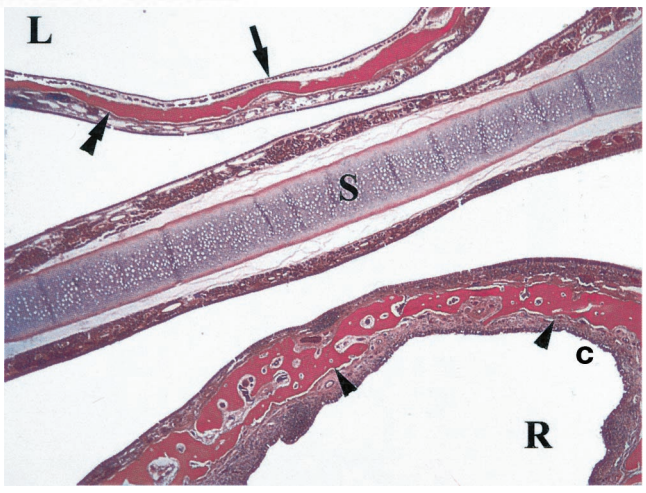
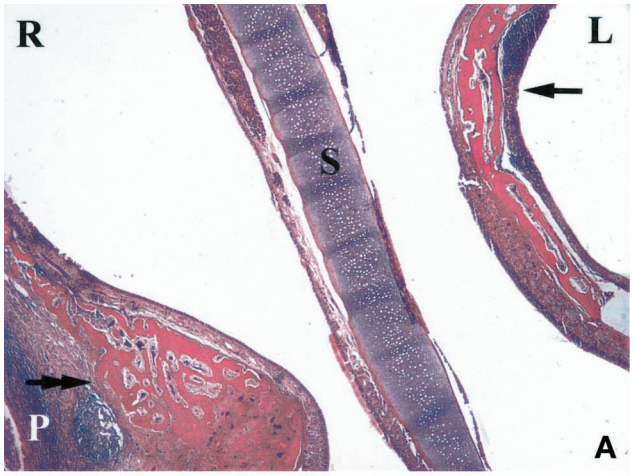
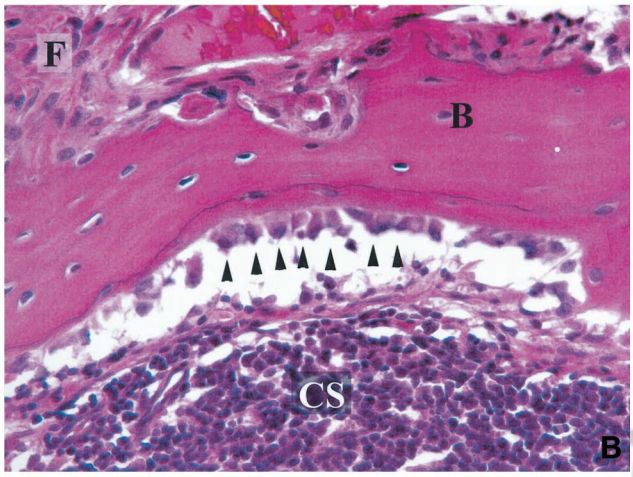


Fig. 3. The nasal septum (S) divides the full cross-section of the paranasal sinuses into right (R) and left (L) sides. In the right side (lower right, double arrow) there is chronic sinusitis, with lymphocytes and plasma cells in the mucous membranes and hyperplastic changes of the epithelium. In the left side (upper left, single arrow) the epithelium is normal. The bone in the affected side (lower right, arrowheads) shows sclerosis, disordered woven bone formation, and fibrosis of the haversian system. The bone in the unaffected side (upper left, double arrowhead) is a normal, single plate of thin lamellar bone (H&E, original magnification ×2).





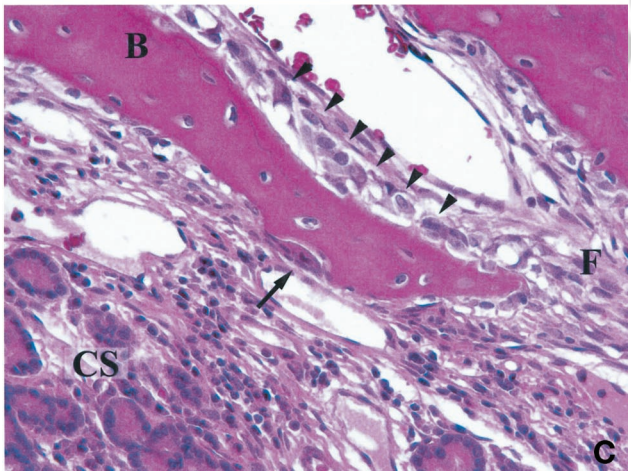
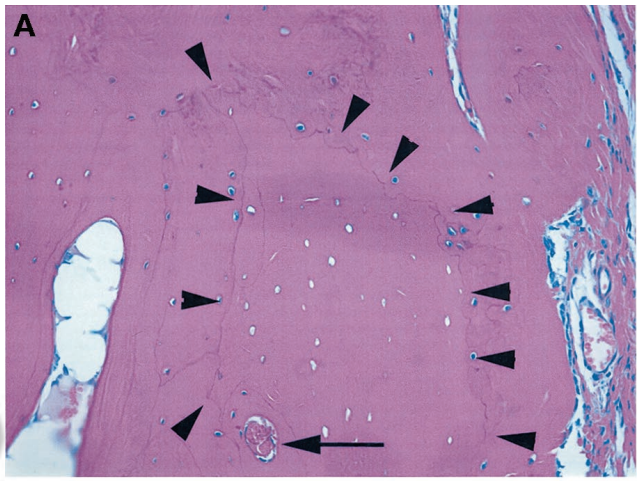


Fig. 4. (**A**) The nasal septum (S) and the bilateral medial walls of the maxillary sinus. The right (R) side is the inoculated side containing purulent material (P); the left (L) side was not inoculated. The single arrow shows mild chronic inflammation; the double arrow shows woven new bone formation (H&E, original magnification ×2). (**B**) The affected sinus (right) in one animal that demonstrates prominent chronic sinusitis (CS) and extensive bone changes (B). There is osteoblast rimming on one side of the bone trabecula (arrowheads). The marrow space has been replaced by fibrosis (F) (H&E, original magnification ×40). (**C**) The left side (not inoculated) of the animal shown in Figure 4A. Similar bone changes are evident. The osteoblastic rimming is again prominent (arrowheads), and on the other side of the trabecula, there is a multinucleated osteoclast resorbing bone in well-formed Howship's lacunae (arrow) (H&E, original magnification ×40).

sistency by requiring the bacteria to be of clonal origin. No surgical intervention or manipulation was performed on the contralateral (control) side.

Animal Killing and Specimen Harvest Procedure

All the animals were first sedated with ketamine and then sacrificed using an overdose of sodium pentobarbital intravenously at intervals between 7 and 9 weeks after initial inoculation. Five animals were killed at each of the time intervals (7, 8, and 9 wk from time of initial inoculation) for each of the bacterial strains (except that four animals were killed at the 7-wk point for the *S aureus* strain). Fifteen minutes after euthanasia, the skin over the nasal dorsum region was incised and reflected away from the bony structures. Next, the mucosa of the soft palate was separated from the hard palate by means of dissection. This maneuver allowed a bone saw to be used to cut the bone. Ana-



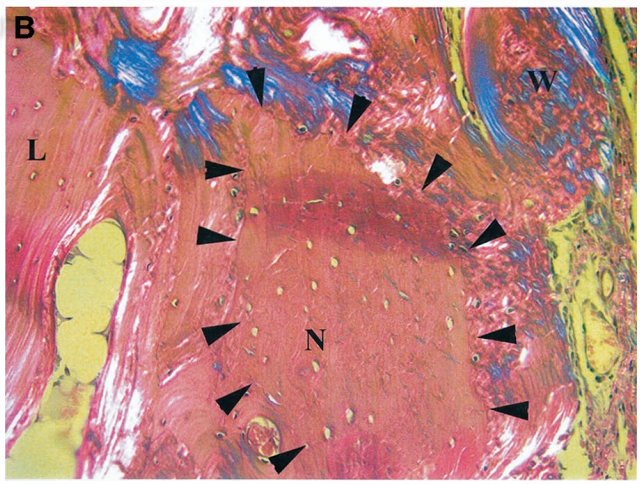


Fig. 5. (**A**) An area of central osteonecrosis (outlined in arrowheads). The osteocytes in the area do not contain nuclei. The surrounding bone is being laid down on top of the necrotic trabecula and contains viable osteocytes with nuclei. There is a dilated canal of the haversian system present, which contains fibrosis (arrow) (H&E, original magnification ×20). (**B**) The same field as shown in Figure 5A but viewed under polarized light. The central necrotic bone is again outlined in arrowheads. The collagen of the woven bone (w) has a disorderly appearance, with a lack of specific periodicity evident in the small amount of residual lamellar bone (L). The woven bone is extensive and nearly completely surrounds the osteonecrotic central core (H&E, original magnification ×20).

tomically, the sinonasal complex was separated from the rest of the animal head and then placed in 10% formalin for histological analysis.

Histological Analysis

The sinus complex specimens were fixed in formalin for more than 24 hours to ensure adequate fixation. The entire specimen was then decalcified in 10% hydrochloric acid for 8 to 12 hours. After adequate decalcification, the specimens were serially sectioned from anterior to posterior at 1- to 2-mm intervals. These tissue sections were embedded in paraffin according to standard protocols. Slides were prepared from 5-μm-thick sections of the paraffin-embedded tissue and were stained with H&E stain. The H&E sections from each animal were analyzed at low and high magnification using standard light microscopy. In each sinus complex, the site of original bacterial inoculation was identified and marked as the affected side. Histological characterization was performed to examine the sinonasal mucosa and evaluate the bony structures of the sinus complex. On average, there were five sections examined per animal, and the entire sinonasal complex was characterized histologically.

Acute sinusitis was defined as the presence of acute inflammatory infiltrate (purulent) debris located within the sinus air spaces, with tracking of the inflammatory cells into the mucosal lining (Fig. 1). Chronic sinusitis was defined as the presence of hyperplasia of the sinonasal mucosal lining associated with a dense infiltrate of chronic inflammatory cells (Fig. 2). Normal sinonasal mucosa was identified when the mucosal lining was thin, with an absence of significant chronic inflammation (Fig. 3).

The bone was examined microscopically and, in addition, with the use of polarized light. The latter was used to facilitate the identification of woven (immature) bone. Extensively remodeled bone was present when there was woven bone, increased numbers of osteoblasts (more than four per bone trabecula), and increased numbers of osteoclasts (more than one per bone trabecula) (Figs. 1–4). The bone in the unaffected and affected areas of the sinonasal complexes was examined and compared for absolute bony plate thickness, marrow space and haversian system fibrosis, and extensive remodeling. The changes were noted as significant when the bony plates were more than two times as thick as the normal-appearing sinonasal bones and had marrow fibrosis and remodeling (Fig. 4).

RESULTS

Surgeries and Bacterial Infection

Operative induction of infection of the maxillary sinus was successfully achieved in all 29 rabbits. Fifteen animals were inoculated with *P aeruginosa*, and 14 were inoculated with *S aureus* and followed in the present study.

Histological Analysis

The original inoculation site was identifiable in all animals (29 of 29). The soft tissues and skin surrounding the surgical site had moderate inflammation present, as well as focal abscess formation. However, the inflammation was contained in a localized area and did not extend beyond a 2- to 3-mm area, corresponding to the area that was directly traumatized by surgery. In most of the specimens, the cotton and glue were identified within the maxillary sinus. In some cases, cotton and glue were apparently present away from the wall of the sinus and the ostium, but this may be related to sectioning artifacts. It was not possible to confirm histologically whether the cotton was functionally occluding the maxillary sinus ostium.

Histological changes compatible with acute sinusitis induced by inoculation of the bacteria into the right-side maxillary sinus were identifiable in all of the animals (29 of 29) by the presence of an inflammatory exudate (purulent debris) within the maxillary sinus (Fig. 1). The mucosa was hyperplastic and had inflammatory cells transmigrating into the epithelium with focal ulceration of the mucosa.

Histological changes compatible with chronic sinusitis were identified in 25 of 29 animals (86%) on the side of the initial inoculum (Fig. 4A). This included both diffuse mucosal hyperplasia and thickening and chronic inflammatory cells within the epithelium and in the submucosal tissues (Fig. 4B). There was also significant edema as well as vascular congestion in the submucosal vessels. The areas of inflammation were concentrated in the more posterior sections of the maxillary sinuses, correlating to the area of experimentally induced sinusitis. Chronic sinusitis was present on the opposite side from the original inoculation in 69% and 50% of the animals, for the pseudomonas and staphylococcus organisms, respectively (Fig. 4C). The anterior sections of the sinonasal complexes in both sides did not show significant inflammation.

The bone was examined using both normal and polarized light. Bone changes on the ipsilateral side to the primary infection were found in 92% of the animals. The bone of the maxillary sinus was most significantly affected by inflammation, but other areas of bone within the region were focally involved. There was multifocal osteonecrosis, which was evident in the dropout of the osteocyte nuclei within the bone (Fig. 5A). These central, necrotic cores of bone were surrounded by new, immature bone or woven bone, which can be identified on polarized light microscopy by the disordered arrangement of the collagen fibrils (Fig. 5B). The woven bone was surrounded by hyperplastic osteoblasts and was being actively remodeled by increased numbers of osteoclasts. There was prominent marrow fibrosis as well as fibrosis within the haversian system. These findings are all indicative of chronic osteomyelitis. In some cases, cotton fibers were found to be focally embedded within a superficial segment of woven new bone, but there were no giant cells nor was there a significant foreign body reaction. The bone changes were also found on the contralateral side of the primary infection in 52% of the animals, and in the present study these animals also had contralateral mucosal inflammation. There were no apparent differences in the rates of chronic sinusitis induction or bony changes between the two different bacteria that were used.

DISCUSSION

The purpose of the present study was to further investigate the role of bone as a possible mechanism for the spread of infectious agents that cause CRS. Consequently, we investigated the presence of any histological changes within the mucosa and bone adjacent to the infection and at remote sites, including the sinuses on the opposite side

from the original inoculation. Extensive chronic sinusitis was noted on both the inoculated and the noninoculated sides of the sinonasal complex. Furthermore, there was significant evidence of chronic osteomyelitis, with multifocal osteonecrosis, prominent thickening of the bone trabeculae, increased osteoblast response, and increased osteoclastic remodeling at the site of induced infection in the animals. Interestingly, these changes not only extended to adjacent bone within the inoculated sinonasal complex, but also were observed on the contralateral side in 19 of 25 animals. Histological examination also showed increased vascularity and widened haversian canal systems lined with chronic inflammatory cells extending from the bone adjacent to the initial infection through to the opposite side.

Reactive hyperplastic changes in the mucosa of the sinuses, which is characteristic of human CRS, was evident bilaterally in 15 of 29 animals (60%). The mucosal involvement on the inoculated side is not surprising because this is a physiological response to chronic infection. On the other hand, the contralateral side, which was not exposed to any direct interventions, still demonstrated chronic inflammation of the mucous membrane, in addition to underlying bony changes. This was not seen in our previous studies and may be attributed to the use of more virulent strains of bacteria. In addition, in the present study, the animals were sacrificed at an earlier point in time following the induction of infection, which raises the possibility that the mucosal changes may resolve with time in a ventilated sinus but the bone changes may remain.

Interestingly, with the exception of the maxillary sinuses, there were no significant changes in either the bone or the mucous membrane of the other, more anterior sections of the sinonasal complexes. The fact that the inflammatory process is localized to the posterior maxillary sinus region would appear to reduce the possibility that our findings were due to incidental superinfection with Pasteurella because it is likely that this would have caused widespread mucosal disease. Thus, this adds support to the conclusion that the chronic sinusitis and chronic osteomyelitis on the contralateral side were most likely induced by the spread of pathogenic bacteria from the initial site of infection. Taken together, these findings suggest the following scenario: mucosal disease of the right-side maxillary sinus, entrance of inflammation and infectious agents into the underlying bone, subsequent activation of the remodeling process, access to the vascular network, spread through the bone to the contralateral side, and secondary mucosal inflammation on the contralateral side.

Chronic osteomyelitis in other anatomical locations has characteristic histological findings, including bone remodeling and marrow fibrosis. However, organisms rarely can be recovered from affected bone. We did not attempt to identify bacteria in the present study, but organisms were not identified in a prior human study within the bone.[23] The pathogenesis of chronic osteomyelitis remains poorly understood. The extent to which the histological changes are related to either direct pathogen effects or to cytokine effects induced by the pathogens is not clearly defined.

In prior studies, we demonstrated histological changes consistent with chronic osteomyelitis within the ethmoid bony partitions in patients with CRS.[11] In a subsequent small animal study, we found that infection of healthy sinuses caused bone remodeling and changes indicative of chronic osteomyelitis.[24] However, the surgical procedure in that study induced a greater degree of trauma than in the present study, and only one organism was studied. Based on those studies, we postulated that chronic bone involvement may be partially responsible for maintenance and spread of chronic infection after a single introduction of an infectious organism.

To evaluate the role of bone in the pathogenesis of CRS in the current study, we inoculated rabbits with two of the most common organisms implicated in the pathogenesis of human CRS. Our results demonstrate a histological congruence between *P aeruginosa* and *S aureus* in terms of their ability to produce tissue changes, thereby suggesting that there is a common pathway for the pathogenesis of CRS. In our inoculation procedure, the initial bone exposure with periosteal elevation in the presence of infectious agents did independently induce an inflammatory reaction. However, this inflammation was histologically confined to the soft tissue around the incision site. The skull bone surrounding the inoculation site did not show the changes of chronic osteomyelitis. Therefore, direct spread of infection from the surgical procedure is unlikely to be the cause of sinus disease in these animals. Also, although sampling error may account for any unobserved routes of infection, it is highly unlikely because three to five sections per animal were obtained and interpreted.

The rabbit is not the most appropriate animal model for the study of rhinosinusitis; however, it remains the most extensively studied model to date. Care must be taken in extrapolating the significance of these findings to any patient population. On the other hand, if there is in fact a potential for inflammation and pathogens to spread through the bone of patients, it could explain some of the findings seen clinically with persistent CRS. These include observations such as demineralization of the bony ethmoid septa, the potential for medial spread of ethmoid disease to the middle turbinate, persistence of localized disease until removal of underlying bone, and the recalcitrance seen in severe disease. Also, although it is tempting to extrapolate the finding of the present study to patient treatment implications, several limitations of our current study would make such actions premature. Future directions include similar studies utilizing a greater variety of infectious organisms, the use of a greater number and a diversity of animal models, cultures of the mucosal membranes to definitively rule out any superinfections, and the translation of subsequent findings to appropriate clinical trials. At the same time, it will be essential to develop treatment protocols to attempt to eradicate the introduced infection and to reverse the histological changes in both bone and mucosa.

CONCLUSION

The present study demonstrates that rabbits inoculated with bacterial organisms through a limited proce-

dure develop a chronic sinusitis and have histological evidence of chronic osteomyelitis. The mucosal and bone changes are not limited to the side of the inoculation but cross the midline and are present in the contralateral maxillary sinus. It appears that the inflammation spreads through the widened haversian canal system within the bone. The present study adds further evidence to the hypothesis that chronic infection can spread through bony structures and that this inflammation may spread through the bone to involve the opposite side. These findings may, at least in part, help to explain why conventional treatment protocols have demonstrated poor long-term results in the resolution of severe CRS.

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