Pneumococcal Meningitis: Development of a New Animal Model

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Hypothesis: The rat is a suitable animal to establish a model for the study of pneumococcal meningitis postcochlear implantation. Background: There has been an increase in the number of cases of cochlear implant–related meningitis. The most common organism identified was Streptococcus pneumoniae. Whether cochlear implantation increases the risk of pneumococcal meningitis in healthy subjects without other risk factors remains to be determined. Previous animal studies do not focus on the pathogenesis and risk of pneumococcal meningitis postimplantation and are based on relatively small animal numbers, making it difficult to assess the cause-and-effect relationship. There is, therefore, a need to develop a new animal model allowing direct examination of the pathogenesis of meningitis in the presence of a cochlear implant.

Methods: Eighteen nonimplanted rats were infected with $1 \times 10^6$ and $1 \times 10^6$ colony-forming units (CFU) of a clinical isolate of S. pneumoniae via three different inoculation routes (middle ear, inner ear, and i.p.) to examine for evidence of meningitis during 24 hours. Six implanted rats were infected with the highest amount of bacteria possible for each route of inoculation ($4 \times 10^6$ CFU i.p., $3 \times 10^6$ CFU middle ear, and $1 \times 10^6$ CFU inner ear) to examine for evidence of meningitis with the presence of an implant. The histological pattern of cochlear infections for each of the three different inoculating routes were examined.

Results: Pneumococcal meningitis was evident in all 6 implanted animals for each of the three different routes of inoculation. Once in the inner ear, bacteria were found to enter the central nervous system via either the cochlear aqueduct or canaliculi perforantes of the osseous spiral lamina, reaching the perineural and perivascular space then the internal acoustic meatus. The rate, extent, and pattern of infection within the cochleae depended on the route of inoculation. Finally, there was no evidence of pneumococcal meningitis observed in 18 nonimplanted rats inoculated at a lower concentration of S. pneumoniae when observed for 24 hours postinoculation.

Conclusion: Meningitis in implanted rats after inoculation with a clinical isolate of S. pneumoniae is possible via all three potential routes of infection via the upper respiratory tract. The lack of meningitis observed in the 18 nonimplanted rats suggests that longer postinoculation monitoring periods are required to ensure whether or not meningitis will develop. Based on this work, we have developed a new animal model that will allow quantitative risk assessment of meningitis postcochlear implantation, and the assessment of the efficacy of potential interventional strategies in future studies. Key Words: Meningitis—Streptococcus pneumoniae—Cochlear implants—Animal model. Otol Neurotol 27:844–854, 2006.

Since 2002, there has been an increase in the number of reported cases of meningitis associated with cochlear implants. The most common organism identified was Streptococcus pneumoniae (1,2). The incidence of pneumococcal meningitis in cochlear implant recipients was found to be greater than that of an age-matched cohort in the general population (2). Identified risk factors included an implant with a positioner, inner ear malformations, the presence of a cerebrospinal fluid (CSF) leak after cochlear implantation, and a history of ventriculoperitoneal shunt placement. In addition, signs of otitis media have been present in implanted children with meningitis (2,3). It remains to be determined whether cochlear implantation per se increases the risk of meningitis in subjects with no existing risk factors for acquiring the disease.

The exact routes by which the bacteria reach the meninges in the presence of a cochlear implant are subject to debate (4). Because of the potential compromise of both mucosal soft tissue and bony barriers between the inner and middle ear as a result of cochlear implantation, the direct spread of infection from the middle to the inner ear and then to the central nervous system (CNS) has been proposed as a major route of infection (5). Others have postulated that pneumococcal meningitis is caused by the bacteremia that follows colonization of the upper
Therefore, it is important to develop experimental models designed to study all the potential routes of infection leading to meningitis.

This article describes the development of an animal model of pneumococcal meningitis with and without cochlear implantation. Three different inoculation routes were established to help differentiate between the potential routes of infection described above. In the presence of acute otitis media (AOM) as a result of direct middle ear inoculation, S. pneumoniae can reach the meninges via either the hematogenous route or direct inner ear invasion to the CNS. Both the hematogenous and direct routes of infection need to be studied independently to assess their relative importance in cochlear implant-related meningitis. Therefore, two additional routes of inoculation were established to study pneumococcal meningitis for both hematogenous and direct spread (via the inner ear) of the bacteria to the meninges. Hematogenous seeding of the bacteria in the CNS can be achieved by direct i.p. inoculation (7). Direct spread of bacteria to the CNS can be achieved by inoculation of the bacteria directly into the inner ear as described in this article.

A pneumococcal meningitis model examining all potential routes of infection spread from the upper respiratory tract to the meninges is the first key step to allow a quantitative investigation of the risks of CNS infection posed by cochlear implants for subsequent pneumococcal infection and to promote methods for preventing the spread of infection from the middle ear to the cochlea and CSF.

**METHODS AND MATERIALS**

A total of 24 otoscopically normal adult Wistar rats (150–300 g, 10–16 wk of age) were used in the experiment. All procedures and animal handling were approved by the Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (project No. 04-105A) and conducted in accordance with guidelines set by The Code of Practice for the Care and Use of Animals in Research in Australia from the National Health and Medical Research Council.

**Preparation of the Inoculum**

S. pneumoniae strain 447A, which carries the type 2 capsular antigen and was originally isolated from the CSF of a child with meningitis, was selected for the study based on our previous experience with it in a cat model of otitis media (8,9). The virulence of this strain was confirmed by passing it through mice, after which it was stored at -70°C in aliquots of nutrient broth containing 30% glycerol. The inoculum for each experiment was prepared from bacteria grown on horse blood agar at 37°C overnight in air containing 5% CO₂. Isolated colonies from these plates were thoroughly emulsified in sterile prewarmed phosphate-buffered saline until they matched a turbidity standard equivalent (McFarland Turbidity Standards (10) of the desired concentrations. The number of viable bacteria in the inoculum was determined by making serial 10-fold dilutions in phosphate-buffered saline for quantification just before inoculation (11). The bacteria were spotted onto horse blood agar plates (10 μL/dilution), allowed to dry, and incubated at 37°C overnight. Colonies were counted after incubation, and the actual inoculum calculated as colony-forming units per mL (CFU/mL).

The number of bacteria chosen for the present experiment (Table 1) was based on previous experience using S. pneumoniae (12–16). The maximum deliverable volumes of inoculum for the three different routes of inoculation were 1 mL for i.p., 10 μL for middle ear inoculation, and 1 μL for direct inner ear inoculation.

A cohort of implanted animals was also used to create a worst-case scenario to examine whether pneumococcal meningitis could be achieved in rats with cochlear implants. The animals were inoculated 4 weeks after cochlear implantation to allow the development of a soft tissue capsule around the electrode array. The highest numbers of bacteria for each inoculation site were used (Table 1).

**TABLE 1. Summary of the methods and results (microbiology and histology)**

<table>
<thead>
<tr>
<th>Routes of infection</th>
<th>No. of bacteria (CFU)</th>
<th>No. of rats</th>
<th>Observation period (h)</th>
<th>Blood culture</th>
<th>CSF culture</th>
<th>Middle ear swab</th>
<th>CNS histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.p. (hematogenous)</td>
<td>1 x 10⁶</td>
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<td>24</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I.p. (hematogenous)</td>
<td>1 x 10⁶</td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Middle ear</td>
<td>1 x 10⁹</td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Middle ear</td>
<td>1 x 10⁹</td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Inner ear</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Inner ear</td>
<td>1 x 10⁹</td>
<td>3</td>
<td>24</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</table>

Rats without cochlear implantation

<table>
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<th>Routes of infection</th>
<th>No. of bacteria (CFU)</th>
<th>No. of rats</th>
<th>Observation period (h)</th>
<th>Blood culture</th>
<th>CSF culture</th>
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<tr>
<td>Inner ear</td>
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<td>48</td>
<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
</tbody>
</table>

*The number of bacteria chosen for the present experiment (Table 1) was based on previous experience using S. pneumoniae (12–16). The maximum deliverable volumes of inoculum for the three different routes of inoculation were 1 mL for i.p., 10 μL for middle ear inoculation, and 1 μL for direct inner ear inoculation.

*Number of animals with positive culture for S. pneumoniae.

*Number of rats with brain histology consistent with pneumococcal meningitis, namely, the presence of inflammatory cells and gram-positive cocci within the subarachnoid space.

N/A indicates that specimens for bacterial cultures were not collected because the inoculation of 1 x 10⁶ CFU of S. pneumoniae via the inner ear was unsuccessful. The inoculum was too viscous to be delivered using the microinfusion pump system.

CFU indicates colony-forming unit; CSF, cerebrospinal fluid; CNS, central nervous system.

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ST$^\text{OM'}$;

5.0 mm

FIG. 1. (A), Dimensions of dummy scala tympani electrode array. (B), Photomicrograph of a cochlea in spur resin showing a scala tympani electrode array inserted via a cochleostomy (scale bar, 0.15 mm). ow indicates oval window with stapes footplate; P, polyimide tubing (outer diameter, 0.10 mm; length, 5.0 mm); rw, round window niche; S, silicone coating; ST, silicone tubing.

Surgical Anesthesia

Rats were anesthetized with an i.p. injection of a mixture of 8 mg/kg xylazine and 75 mg/kg ketamine. The animals were then placed on a heated pad maintained at 37°C throughout the surgery.

Cochlear Implantation

Six of 24 animals used in this study received a unilateral cochlear implant 4 weeks before inoculation with S. pneumoniae (Table 1). The dummy electrode arrays for implantation into the rat scala tympani were manufactured in-house from 5-mm lengths of 0.10-mm—outer diameter polyimide tubing. The tube was coated with a layer of medical-grade silicone to a diameter of 0.15 mm (Fig. 1). The dummy electrodes were cleaned with absolute alcohol in an ultrasonic cleaner, rinsed with MilliQ water water (three times), and bathed in MilliQ water for 10 minutes before drying, packaging, and sterilizing using $\text{H}_2\text{O}_2$ sterilization.

Using sterile technique, a postauricular skin incision was made to expose the round window membrane. The bulla cavity was inspected for any abnormality of the middle ear mucosa. The stapedial artery, which is located just below the round window niche, was cauterized with a Zencor MFI (Zencor, Melbourne, Australia) bipolar coagulator. A cochleostomy was performed just below the round window niche over the site of the cauterized stapedial artery (17). All care was taken to clear away bone dust and blood from the cochleostomy before placement of the dummy electrode array, which was inserted 2 to 3 mm into the scala tympani (Fig. 1). The cochleostomy was sealed with temporalis fascia, and the wound sutured in two layers.

After surgery, the implanted animals received two doses of prophylactic enrofloxacin 10 mg/kg subcutaneously, diluted 1:1 with saline, one dose immediately after surgery and the second dose 12 hours later. Intramuscular injection of antipamizolec hydrochloride 5 mg/mL was given to reverse the effects of anesthesia and s.c. injection of 0.03 to 0.05 mg/kg buprenorphine was given for analgesia. All animals were given 10 mL/kg of normal saline subcutaneously during recovery from the surgery for fluid replacement and assessed for signs of postoperative pain and discomfort. Additional buprenorphine was given on an 8- to 12-hourly basis as required.

Bacterial Inoculation

Intraperitoneal Inoculation

After anesthesia, 1 mL of the desired concentration of S. pneumoniae 447A was inoculated into the intraperitoneal cavity using a sterile 20-gauge needle and 1 mL syringe.

Middle Ear Inoculation

Under general anesthesia, the left bulla of each rat was exposed using sterile surgical techniques. The bulla cavity was filled with Gelfoam (Pharmacia & Upjohn, Kalamazoo Michigan, U. S. A.) into which 10 μL of the inoculum was injected. The bulla was then covered with temporalis fascia, and the wound sutured in two layers.

FIG. 2. Gram's stain illustrating the meninges and subarachnoid space of (A) a normal control rat and (B) a rat with clinical evidence of meningitis induced by i.p. inoculation. Gram-positive cocci (arrows) and inflammatory cells with phagocytosed bacteria are located within the subarachnoid space (scale bar, 10 μm). cb indicates cerebral cortex; m, meninges; sb, subarachnoid space.
**ANIMAL MODEL—IMPLANT AND MENINGITIS**

**Inner Ear Inoculation**

Under general anesthesia, the left bulla was exposed, and a cochleostomy was performed close to the round window niche using a straight Kirschner wire. Using a 5-μL microsyringe connected to a micropump, 2 μL of perilymph was removed, and 1μL of inoculum was then gradually injected into the scala tympani. The cochleostomy and the bulla were covered with temporalis fascia, and the wound sutured in two layers.

**Postinoculation Monitoring**

The postoperative management of the animals followed that described after cochlear implantation (above), except that no prophylactic enrofloxacin was given to animals after bacterial inoculation. After inoculation, each animal was examined at least twice daily for clinical signs of meningitis. The clinical assessment was recorded based on a 12-point scoring system developed in conjunction with a veterinarian. This assessment included the animal’s weight and a quantitative monitoring of the animal’s general status, as indicated by alertness (1, normal; 2, tired but still responded to light, sound, and tactile stimuli; 3, very lethargic with no movement to stimuli); grooming (1, normal; 2, abnormal); posture (1, normal; 2, hunched); movement (1, spontaneous; 2, only in the presence of light, sound, or tactile stimuli; 3, no movement with stimuli); and rectal temperature (1, normal; 2, above 37°C). Animals were killed if one of...
the following conditions was met: a score of 11 or above, a weight loss of greater than 25%, or a score of 5 to 10 with rectal temperature of greater than 41°C. Regular monitoring of the rats using this technique allowed early detection of meningitis.

**Specimen Collection**

Animals were deeply anesthetized with a mixture of isoflurane and oxygen 24 hours after inoculation (nonimplanted cohort) or once they developed clinical signs of meningitis (implanted cohort) (Table 1). A 30-gauge needle with a 1-mL syringe was used to perform a cisternal puncture to aspirate the CSF (7,15). Blood (1 mL) was aspirated from an intracardiac puncture via a 23-gauge needle and cultured using an automated blood culture system. Finally, bacterial swabs were taken from the left bulla using a strict aseptic technique.

**Tissue Preparation**

Each animal was killed with a lethal dose of pentobarbitone sodium (120 mg/kg) and transcardially perfused with normal saline and 10% neutral buffered formalin, pH 7.4 at 4°C. The brain, meninges, and the cochleae were harvested. Brain specimens, including meninges, were taken from 24 rats and were stored in 10% neutral buffered formalin for 48 hours, then embedded in paraffin. The specimens were sectioned at 10 μm, stained with hematoxylin and eosin or Gram’s stain. Cochleae of the 6 implanted rats were decalcified in a solution of 10% EDTA in 0.1 mol/L phosphate buffer (pH 7.4), and then processed and embedded in Spurr’s resin. The embedded cochleae were orientated in the midmodiolar plane, and two sets of twenty-one 2-μm sections were collected at 126-μm intervals throughout the cochlea. One set of sections were stained with hematoxylin and eosin and the other with Gram’s stain.

**Examination of the Specimens**

The main outcome measures for the study were the clinical and histological detection of meningitis. CSF, blood culture, and middle ear cultures were taken to detect the presence of bacteria. The cochleae and meninges were harvested for histological detection of meningitis. CSF, blood culture, and middle ear cultures were taken to detect meningitis. The main outcome measures for the study were the clinical and histological detection of meningitis. CSF, blood culture, and middle ear cultures were taken to detect the presence of bacteria. The cochleae and meninges were harvested for histological analysis and were used to confirm the culture results. Serotyping of *S. pneumoniae* isolated from the cultures was performed to ensure that the strain causing the pathology matched the inoculum. The presence of bacteria in the CSF was used to confirm the pathogenesis of meningitis. Because only a small volume of CSF was available from this species, contamination is a potential problem. Therefore, the histological appearance of the brain was considered the criterion standard test to detect meningitis.

Histological sections were examined under a light microscope. The brain and the meninges were examined for the presence of an inflammatory cell response within the subarachnoid space and brain tissue, for thickening and hyperplasia of the meningeal cells, and gram-positive cocci within the subarachnoid space and brain tissue. The implanted cochleae were examined for fibrous tissue response to cochlear implantation, and insertion trauma. All turns of both implanted and nonimplanted cochleae were examined for the presence of inflammatory cells and bacteria.

**RESULTS**

The eighteen nonimplanted rats receiving either 1 × 10⁹ CFU or 1 × 10⁸ CFU of *S. pneumoniae* via three different routes of infection did not develop meningitis during a 24-hour monitoring period. The inner ear inoculum of 1 × 10⁸ CFU in 1 μL was too viscous to be infused using the microsyringe pump; consequently, no infection was observed in this group of rats (Table 1).

In contrast, all 6 rats with cochlear implants acquired meningitis when given the highest doses of bacterial inocula via the three different routes of infection. Clinical evidence of meningitis developed within 12 to 48 hours after inoculation and depended on the route of inoculation. In these animals, the meninges were thickened with inflammatory cells, and gram-positive cocci were seen within the subarachnoid space (Fig. 2). The development of the infection in the CNS was successfully monitored and predicted by the use of the daily clinical monitoring sheet. The histopathological evidence of meningitis correlated closely with the clinical symptoms. Two rats exhibited cerebral edema and meningitis within 12 hours of i.p. inoculation of 4 × 10¹⁰ CFU of *S. pneumoniae*; two animals exhibited clinical and histological evidence of meningitis within 24 hours after middle ear inoculation of 3 × 10⁹ CFU of *S. pneumoniae*; and two animals exhibited clinical and

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**FIG. 4.** Photomicrograph illustrating Gram’s stain sections of an implanted rat cochlea 24 hours after middle ear inoculation with 3 × 10⁸ CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis (scale bar, 10 μm). A, Insertion trauma to the osseous spiral lamina (arrow heads) resulted in an extensive spread of bacteria (arrows) into Rosenthal’s canal. B, Higher-power micrograph of the same section showing invasion of the bacteria (arrows) into the fibrous tissue sheath surrounding the electrode array. im indicates scala tympani electrode array; rc, Rosenthal’s canal.

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histological evidence of meningitis 48 hours after direct inner ear inoculation of $1 \times 10^6$ CFU of *S. pneumoniae*.

In the implanted rats that developed meningitis as a result of bacteremia via i.p. injection, bacteria were found along the perivascular and perineural spaces within the internal acoustic meatus (IAM) and modiolus bilaterally. Bacteria were also seen in the stria vascularis on both sides of the cochleae. Although there was no evidence of inflammatory cells or bacteria in the scalae throughout the cochleae, bacteria were present in the lumen of the right stapedial artery and also found to infiltrate the thick, well-organized arterial wall (Fig. 3).

In implanted rats with meningitis after a middle ear inoculation, bacteria and inflammatory cells were found within the dense, well-formed, and matured peri-implant fibrous seal (Fig. 4). There were no bacteria or inflammatory cells in the interface between the fibrous seal and the implant. In the ipsilateral inner ear, bacteria and inflammatory cells were found within the scala tympani, scala vestibule, Rosenthal’s canal, and the canaliculi perforantes.

**FIG. 5.** Lower-power hematoxylin and eosin photomicrographs illustrating the implanted (A) and contralateral control (B) cochleae of a rat 24 hours after middle ear inoculation of $3 \times 10^8$ CFU *S. pneumoniae* ipsilateral to the implanted cochlea. This animal exhibited clinical and histological (CNS) evidence of meningitis (scale bar: A and B, 200 μm; C–F, 10 μm). In this example, there is severe labyrinthitis throughout the implanted ear (A), whereas the contralateral cochlea exhibits evidence of infection mainly localized to the scala tympani of the basal turn (B). Higher-power micrograph of Gram’s stain of the OSL (C and D) and modiolus (E) of the implanted cochlea, and the IAM (F) of the contralateral cochlea. Extensive bacterial invasion of Rosenthal’s canal (C, arrows) appears to be associated with bacteria traversing the canaliculi perforantes (D, double arrow). Bacteria were also located within the modiolus of the implanted cochlea (E), and the IAM of the contralateral cochlea (F). The approximate location of the higher-power micrographs (C–F) are illustrated in (A) and (B). bn indicates bone; sgn, spiral ganglion cells.
within the wall of osseous spiral laminae (OSL), adjacent to the scala tympani. Bacteria were located along the perineural and perivascular spaces within the OSL, modiolus, and the IAM. Extensive degeneration of spiral ganglion cells were seen within the OSL infiltrated by bacteria and inflammatory cells (Fig. 5). Few bacteria were isolated within the scala media. Serofibrinous or proteinaceous exudate was seen in some sections of the scala tympani and vestibule, whereas endolymphatic hydrops was also evident in some sections.

The contralateral cochlea of animals that received a middle ear inoculation that resulted in meningitis exhibited bacteria and inflammatory cells within the IAM, modiolus, and scala tympani. In the scala tympani, bacteria and inflammatory cells were prominent in the basal turn, very close to the undersurface of the OSL and infiltrating the contralateral round window membrane in some specimens (Fig. 6). In one animal, the cochlear aqueduct was examined in the contralateral cochlea; bacteria and inflammatory cells were found to traverse the aqueduct and within the scala tympani (Fig. 6).

In implanted rats with meningitis as a result of direct inner ear inoculation, bacteria and inflammatory cells were found to infiltrate within the fibrous tissue seal around the dummy implant. They also infiltrated the canaliculi perforantes of the OSL to enter the Rosenthal’s

![Image of histological sections showing bacterial and inflammatory infiltrates in the cochlea](image)

**FIG. 6.** A. Low-power photomicrograph illustrating a Gram's stain section from the right cochlea of an implanted rat 24 hours after inoculation with $3 \times 10^9$ CFU S. pneumoniae via the left middle ear. This animal exhibited clinical and histological (CNS) evidence of meningitis. In this example, bacteria were predominantly present in the scala tympani of the basal turn (B) (scale bar: A, 200 μm; B, 100 μm; C–F, 10 μm). Bacteria were also evident in the cochlear aqueduct, (D) modiolus, and were also observed (E) traversing the round window membrane. F. Normal control round window membrane is illustrated for comparison. ca indicates cochlear aqueduct; m, modiolus; rwm, round window membrane; st, scala tympani.
FIG. 7. Lower-power hematoxylin and eosin photomicrographs illustrating the implanted (A) and contralateral control (B) cochleae of a rat 48 hours after direct inner ear inoculation of $1 \times 10^6$ CFU of *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. Extensive labyrinthitis of the inoculated ear (A) involved all three scalae. In contrast, the contralateral cochlea (B) exhibited bacteria predominantly within the scala tympani (arrows) (scale bar, 200 μm).

canal. The distribution of the bacteria and inflammatory cells in the ipsilateral and contralateral cochlea was similar to the middle ear-inoculated cohort (Fig. 7).

Finally, the nonimplanted/nonmeningitic cohort exhibited a number of histological features that contrasted with the implanted/meningitic animals described above. First, there were no inflammatory cells or bacteria within the inner ears after i.p. inoculation. Second, there was only a mild inflammatory response in the ipsilateral cochleae after middle ear inoculation (Fig. 8). Finally, although bacteria

FIG. 8. Lower-power hematoxylin and eosin photomicrographs illustrating representative cochlear histology in unimplanted nonmeningitic animals. Left (A) and right (B) cochleae 24 hours after inoculation with $1 \times 10^6$ CFU *S. pneumoniae* to the left middle ear. Inflammatory cells were evident in the round window niche of the left cochlea resulting in a small inflammatory response within the basal turn. The remainder of the cochlea appeared normal as did the contralateral control. Left (C) and right (D) cochleae 24 hours after direct inner ear inoculation with $1 \times 10^6$ CFU *S. pneumoniae*. Bacteria and inflammatory cells were evident in the scala tympani of the basal and middle turns (arrows); however, the remaining scalae and the contralateral cochlea appeared normal (scale bar, 200 μm). Ir indicates inflammatory response; rwn, round window niche.
DISCUSSION

The present study describes a new pneumococcal meningitis model we have established in rats using a clinically relevant strain of *S. pneumoniae*. The model is unique in that meningitis is achieved via three different routes of inoculation to resemble all potential routes of pneumococcal infection from the upper respiratory tract to the CNS. The histological pattern of the spread of pneumococci from the inner ear to the CNS and then to the contralateral cochlea in the presence of a cochlear implant is delineated in this new model.

The cochlear pathophysiology of both anterograde (middle ear to inner ear) and retrograde (meninges to the inner ear) spread of infection is not well understood.

The use of the Gram's stain before (the unimplanted cohort) or after clinical evidence of meningitis (the implanted cohort) provides a means of identifying gram-positive pneumococci and to trace the possible route of spread of the bacteria.

In the two implanted rats that acquired early meningitis as a result of bacteremia after i.p. inoculation, bacteria were found to locate symmetrically in the perivascular and perineural spaces of the IAM and modiolus. No bacteria were seen within the scalae of both cochleae—probably because of the early stage of the disease. Both the cochlear aqueduct and IAM have been proposed to be the potential routes of infection from the meninges to the inner ear based on postmortem human temporal bone studies (18–20). Previous animal studies of meningitic suppressive labyrinthitis also demonstrated the cochlear aqueduct as a pathway of infection from meninges to the labyrinth (21–23). Our study also demonstrates that bacteria can infect the inner ear via perivascular and perineural spaces of the IAM to the modiolus of the cochlea.

The histology of anterograde spread of infection from the middle ear to the meninges then to the contralateral cochlea has a distinct appearance compared with cochlear infection as a result of meningitis from a hematogenous route of infection. Specimens from rats with either direct inner ear inoculation or middle ear inoculation demonstrated a more severe degree of labyrinthitis in the ipsilateral cochlea compared with the contralateral cochlea. Bacteria were found to reach the subarachnoid space from the ipsilateral cochlea by traversing the canaliculi perforantes of OSL to enter neural structures within the Rosenthal's canal, and then following the perineural and perivascular spaces of cochlear nerve within the modiolus and IAM to reach the CNS. This finding is consistent with a previous study in the chinchilla, in which the bacteria were inoculated directly into the middle ear cavity (24). However, bacteria can also reach the CNS and the contralateral cochlea via the cochlear aqueduct, as observed in the present study (Fig. 6).

A well-formed peri-implant fibrous seal was present around the electrode array and cochleostomy site 4 weeks after implantation, consistent with previous animal studies (5). The presence of bacteria infiltrating the well-formed fibrous seal to enter the scala tympani suggests infection can spread from the middle ear into the inner ear. Histological evidence from the present study also indicated that in cases of severe infection, bacteria have the ability to invade and track through the thick, organized and condensed fibromuscular wall of the stapedial artery, suggesting that the body's natural fibrous seal may not be sufficient in preventing either inner ear or CNS infection after a severe middle ear infection.

There are several reasons why we consider the rat as a suitable animal model of pneumococcal meningitis post-cochlear implantation: cochlear implantation is possible (17); the relatively low cost of the animals make it feasible to perform relatively large-scale quantitative studies; previously established pneumococcal meningitis in infant and adult rats via direct intracisternal, i.p., or nasopharyngeal inoculation (7,15); previously established pneumococcal otitis media models (25,26); and the middle ear mucosa (27,28) and function of the eustachian tube (29–31) resembles that of humans.

However, a rat model is only relevant if the animal can acquire meningitis with *S. pneumoniae*, preferably via the three possible infectious routes to resemble CNS infection in humans. An ideal strain of *S. pneumoniae* for the infection study should be able to induce disease both in humans and in rats. The route(s) by which infection spreads from upper respiratory tract mucosa (including middle ear cavity) to the CNS in rats must also closely resemble that of a human infection. These two characteristics are important to ensure that the results obtained in the rat model are also clinically applicable. However, this model is developed in rodents with normal cochlea anatomy and is therefore not suitable to evaluate the risk of meningitis that is associated with an inner ear malformation.

The selection of the correct serotype of *S. pneumoniae* was essential for the establishment of the new rat model. The virulence of several pneumococcal serotypes has been found to be dissimilar between human and some animal species. Not all clinical CSF isolates can induce meningitis in rats (23). Most studies involving the use of type 3 and 6A *S. pneumoniae* in rats have shown that animals develop acute otitis media but not meningitis from direct middle ear inoculation (32). Although Rodriguez et al (23) were able to show hematogenous pneumococcal meningitis in the infant rat after i.p. inoculation of a *S. pneumoniae* type 6 clinical isolate, bacteremia and subsequent invasion of the CNS could not be achieved in adult rats from either i.p. or intranasal inoculation (15). The present study showed that *S. pneumoniae* serotype 2 is a suitable strain because it was isolated from the CSF of a child with meningitis and caused meningitis in implanted adult rats via three different methods of inoculation. Moreover, in the present model, both the hematogenous and otologic routes can
be studied, allowing us to determine whether an intervention such as cochlear implantation influences the risk of developing meningitis via either or both routes.

The variation in the number of bacteria administered and the postinoculation monitoring period reflects the fact that the prime purpose of this work was to successfully develop an appropriate animal model of meningitis. A postinoculation monitoring period of 24 hours was initially selected for the nonimplanted rat cohort in this study because this period has been widely used in previous studies (1-4). A small number of bacteria were present in the inner ear of nonimplanted animals 24 hours after direct inner ear inoculation. Bacterial meningitis might have developed had the nonimplanted animals been monitored for longer periods, although it is also possible that the animals could eliminate the bacteria in the inner ear after extended postinoculation monitoring periods.

After failure to observe meningitis in any nonimplanted animal after 24 hours of monitoring, we extended the work to include a worst-case scenario, that is, implanted animals inoculated with the highest number of bacteria possible for each route of infection. We sought to adjust the monitoring period in the implanted animals so that they were killed at the first clinical sign of meningitis or a period of 5 days (whichever came first). As a result, clinical evidence of meningitis was evident at 12, 24, and 48 hours postinoculation, depending on the inoculation site. The meningitis model as illustrated in this study emphasized the importance of a longer duration (>24 hours) of monitoring after the inoculation. However, a prolonged period of infection should be avoided when taking the welfare of animals into consideration. We propose that all future studies using this model should extend the postinoculation monitoring period to 5 days while ensuring rigorous monitoring, so that the animal is killed at the first clinical sign of meningitis.

Many cochlear implant recipients have pre-existing risk factors (e.g., cochlear malformation, skull base fractures) for meningitis. Therefore, it is difficult to determine whether the presence of the implant in isolation or in combination with pre-existing risk factors contributes to postimplant meningitis (5). The advantage of the animal model presented here is that the effect of cochlear implant per se on the risk of meningitis can be examined in healthy animals with no pre-existing risk factors in a control laboratory environment.

There are two limitations to the present study that must be taken into consideration. First, cochlear implant electrode arrays differ from the array modeled in the present study; however, the surface of both consist mainly of medical-grade silicone. Second, it is important to point out that the present meningitis model does not provide a valid model for an immature immune system found in young children. Currently, there is no other model that does this; however, it may be possible to use the proposed model in conjunction with the use of immunosuppressant drugs to model the immature immune system.

The small numbers of laboratory animals used in most previous infection studies has made it is difficult to assess the relative efficacy of different surgical approaches in reducing the risk of cochlear infections. A quantitative study using this new rat model can overcome these limitations and show cause-and-effect relationships. The development of a quantitative model using the rat will allow us to investigate whether cochlear implantation alters the risk of acquiring pneumococcal meningitis in healthy animals via both hematogenous and otologic routes. The model will provide a means to establish whether there is an association between infection risk and surgical trauma to the modiolus and osseous spiral lamina after cochlear implantation. A demonstration that intracochlear trauma predisposes to meningitis would have implications for both the surgical technique of implantation and implant design. The effectiveness of the fibrous seal around the cochlear electrode to reduce the invasion of bacteria should also be examined further. It is clear from the present study that bacteria can track through the fibrous sheath. Modifications of the cochlear implant electrode array to prevent bacteria from tracking around the prosthesis into the inner ear and then into the CNS should also be further investigated.

CONCLUSION

An animal model in rats that allows quantitative assessment of CNS infection risk postcochlear implantation is described. Pneumococcal meningitis developed in these animals after inoculation of a clinical isolate of S. pneumoniae serotype 2 via the three most likely routes of infection: hematogenous spread, and direct spread from the middle or inner ear. This animal model will allow us to determine whether an intervention such as cochlear implantation influences the risk of developing meningitis and to develop intervention strategies to reduce the risk of infection associated with cochlear implants.

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