Pneumococcal Meningitis Threshold Model: A Potential Tool to Assess Infectious Risk of New or Existing Inner Ear Surgical Interventions

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Hypothesis: A minimal threshold of *Streptococcus pneumoniae* is required to induce meningitis in healthy animals for intraperitoneal (hematogenous), middle ear, and inner ear inoculations, and this threshold may be altered via recent inner ear surgery.

Background: There has been an increase in the number of reported cases of cochlear implant-related pneumococcal meningitis since 2002. The pathogenesis of pneumococcal meningitis is complex and not completely understood. The bacteria can reach the central nervous system (CNS) from the upper respiratory tract mucosa via either hematogenous route or via the inner ear. The establishment of a threshold model for all potential routes of infection to the CNS in animals without cochlear implantation is an important first step to help us understand the pathogenesis of the disease in animals with cochlear implantation.

Methods: Fifty-four otologically normal adult Hooded Wistar rats (27 receiving cochleostomy and 27 controls) were inoculated with different amounts of bacterial counts via three different routes (intraperitoneal, middle ear, and inner ear). Rats were monitored during 5 days for signs of meningitis. Blood, cerebrospinal fluid, and middle ear swabs were taken for bacterial culture, and brains and cochleae were examined for signs of infection.

Results: The threshold of bacterial counts required to induce meningitis is lowest in rats receiving direct inner ear inoculation compared with both intraperitoneal and middle ear inoculation. There is no change in threshold between the group of rats with cochleostomy and the control (Fisher's exact test, \( p < 0.05 \)).

Conclusion: A minimal threshold of bacteria is required to induce meningitis in healthy animals and is different for three different routes of infection (intraperitoneal, middle ear, and inner ear). Cochleostomy performed 4 weeks before the inoculation did not reduce the threshold of bacteria required for meningitis in all three infectious routes. This threshold model will also serve as a valuable tool, assisting clinicians to quantitatively analyze if the presence of a cochlear implant or other CNS prostheses alter the risk of meningitis. Key Words: Pneumococcal meningitis—Routes of infection—*Streptococcus pneumoniae*—Threshold model.

Meningitis was considered to be a rare complication postcochlear implantation. However, since 2002, an increase in the number of cases of cochlear implant-related meningitis has been reported to the U.S. Food and Drug Administration (1). The most common organism identi-
Pathogenesis of pneumococcal meningitis is very complex and is not completely understood (5). An individual’s susceptibility to pneumococcal meningitis is analyzed via the interaction between the host’s immune response and the virulence factors of S. pneumoniae. It is well documented that adults with chronic illness or conditions, individuals with a history of congenital CSF fistula or head trauma with basilar skull fracture with or without CSF leak, and adults older than 65 years old and children younger than 2 years old who are otherwise healthy are associated with increased frequency and/or severity of serious pneumococcal infections (6–12). However, an immunocompetent individual with no pre-existing risk factors can acquire pneumococcal meningitis, and this may be because of increased virulence of the bacteria in a particular host. Although the polysaccharide capsule of S. pneumoniae can spread to the meninges directly or via the bloodstream, the key virulence factors of the bacteria (13), other components, including the enzymes (e.g., hyaluronidase, pneumolysin, neuraminidases) and cellular wall components (e.g., PspA, PspC, PsA), have been implicated as important virulence factors for the pathogenesis of pneumococcal disease (14–22).

A review of the literature for patients who acquired pneumococcal meningitis and have not received a cochlear implant suggests that S. pneumoniae can spread to the meninges either directly from the middle ear or from the hematogenous seeding of bacteria. The tympanogenic or otogenic spread of infection can be further subclassified into either a direct invasion of the meninges by the bacteria (via direct communication between the middle ear/ mastoid cavity and the central nervous system [CNS]) as a result of congenital temporal bone malformation, trauma or neoplasm, or chronic infection of temporal bone) or indirect invasion via the inner ear (23,24). When meningitis occurs in the presence of OAM, there is usually insufficient evidence to suggest a direct spread of S. pneumoniae from the middle ear to the inner ear then to the meninges as the only means of infection. The role of the hematogenous spread of the bacteria from the middle ear to the meninges must also be considered.

Previous work investigating Haemophilus influenzae meningitis in rats demonstrates that a minimal threshold of bacteria is required to induce meningitis via intranasal inoculation of bacteria (25,26). The development of meningitis as a result of H. influenzae infection is analyzed via the intensity of bacteremia, which correlates with the concentration of the intranasal inoculum (26). The threshold for CNS infection for different routes of infection has not been studied in S. pneumoniae. This concept may be important in the development of pneumococcal meningitis in humans. We propose that the number of bacteria exposed to a human subject is one of the important factors for determining whether a person acquires pneumococcal meningitis. A minimum or threshold level of the bacteria is required to induce meningitis in a healthy individual. This threshold may be reduced in patients with existing risk factors for acquiring pneumococcal meningitis. We have established an animal model to examine whether a threshold relationship exists between the rate of pneumococcal CNS infection and the concentration of the infective inoculum.

Because there are different routes by which S. pneumoniae reach the meninges, our previous work showed that animals with cochlear implantation acquired pneumococcal meningitis after three separate routes of infection (27). The present study builds on this previous work by examining the threshold of bacteria required to induce meningitis for each route of infection in healthy nonimplanted animals. The understanding of these relationships is an important first step to analyze whether cochlear implantation increases the risk of pneumococcal meningitis. The threshold model will help us analyze whether cochlear implantation or other surgical interventions reduce the threshold for all possible routes of CNS infection and may provide insight into the pathogenesis of pneumococcal meningitis in patients with other CNS prostheses.

MATERIALS AND METHODS

Source of the Animals

All the experimental animals were bred and housed in the animal house within the Department of Otolaryngology, University of Melbourne. All procedures and animal handling were conducted in accordance with guidelines set by the Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital and The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes from the National Health and Medical Research Council (2004).

In total, 54 otologically normal adult Hooded Wistar rats (10 to 16 wk old) weighing between 100 and 400 g were used in the study. One cohort of 27 rats received a cochleostomy to the left ear 4 weeks before inoculation with bacteria. A second control cohort of 27 rats had no surgical procedures performed before inoculation. The cochleostomy was selected as the surgical intervention because the breach of the bony and mucosa barrier between the middle and inner ear during cochlear implantation has been considered a potential risk factor for meningitis post cochlear implantation (28).

Eighteen rats (nine operated and nine nonoperated control animals) were randomly allocated to each of the three different routes of bacterial inoculation (middle ear, inner ear, and intraperitoneal; Table 1). Within each infection route group, six rats (three nonoperated and three operated) were allocated.

<table>
<thead>
<tr>
<th>TABLE 1. Summary of meningitis threshold study</th>
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<tbody>
<tr>
<td>Routes of inoculation</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Intraperitoneal</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Inner ear</td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Middle ear</td>
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</table>

CFU, colony-forming unit.

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to one of three concentrations of the bacteria used for each route (Table 1).

**Cochleostomy Surgery**

Twenty-seven adult rats underwent a cochleostomy of the left inner ear. Under anesthesia (see below), a postauricular skin incision was made, and subcutaneous tissues and muscles of the head and neck were dissected and retracted to expose the auditory bulla. Care was taken not to damage the facial nerve, which lies anterosuperior to the bulla. A 2.0-mm cutting burr was used to open the bulla and expose the round window membrane. The bulla cavity was inspected for any abnormality of the middle ear mucosa; only animals with a macroscopically normal mucosa were used in this study. Both the stapedial artery and the round window niche were identified. The stapedial artery, which is located just below the round window niche, was cauterized with a Zencor MFI bipolar coagulator (Zencor, Melbourne, Victoria, Australia), and a cochleostomy was performed. The scala tympani was entered by using a straight Kirschner wire (diameter, 0.8 mm) (KAOR5171; Kai-sers, Perth, Western Australia), and temporalis fascia was used to seal the cochleostomy site. The animal had two doses of 10 mg/kg, s.c., prophylactic enrofloxacin antibiotics (Baytril 50; Bayer Australia, Ltd., Sydney, New South Wales, Australia) for analgesia immediately after surgery. They were assessed continuously for signs of postoperative pain and discomfort, and buprenorphine was given on an as needed basis during recovery from the surgery for fluid replacement.

**Surgical Anesthesia**

Rats were anesthetized with an intraperitoneal injection of a mixture of 8 mg/kg xylazine (Ilium Xylazil-20; Troy Laboratories Pty., Ltd., Sydney, New South Wales, Australia) and 75 mg/kg ketamine hydrochloride (ketamine; Parnell Laboratories, Sydney, New South Wales, Australia). A local anesthetic agent (0.1 mL of lignocaine hydrochloride with 0.0182 mg/mL of adrenaline tartrate, Troy Laboratories) was injected subcutaneously around the surgical incision. The animals were then placed on a heated pad maintained at 37°C throughout the surgery. The animals were given 0.03 to 0.05 mg/kg, s.c., buprenorphine (Temgesic; Reckitt Benckiser, Sydney, New South Wales, Australia) for analgesia immediately after surgery. They were assessed continuously for signs of postoperative pain and discomfort, and buprenorphine was given on an 8 to 12 hourly basis if there were signs of postoperative pain or discomfort. The animals were given 10 mL/kg, s.c., of isotonic sodium chloride solution during recovery from the surgery for fluid replacement.

**Inoculation of S. Pneumoniae**

The preparation of inoculum has been described in detail in the previous study (27). In brief, S. pneumoniae 447A, which carries Type 2 capsular antigen and was isolated from the CSF of a child with meningitis, was selected for the study (27). The bacteria were stored at -70°C in aliquots of nutrient broth containing 30% (vol/vol) glycerol. The inoculum for each experiment was prepared from bacteria grown on horse blood agar at 37°C overnight in air containing 5% carbon dioxide. The isolated colonies from these plates were thoroughly emulsified in prewarmed phosphate-buffered saline until they matched a turbidity standard equivalent (McFarland Equivalence Turbidity Standards) of the desired concentrations. Three graded concentrations of inoculum for each of the infectious routes were chosen (Table 1). The amounts of bacteria for each route of inoculation were derived from the maximum infective dose of our previous established rat meningitis model and titrated down at a decrement of 2 log scale (28).

Retrospective viable counts of the inoculum were performed by making serial 10-fold dilutions in phosphate-buffered saline using the method of Miles and Misra (29) for quantifying bacteria just before inoculation. 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 was calculated (colony-forming unit [CFU]/mL). Retrospective viable counts of the inoculum confirmed that each rat consistently received the desired amount of bacteria as indicated in Table 1.

**Methods of Bacterial Inoculation**

Bacteremia as a result of intraperitoneal inoculation was introduced to study hemogenous spread of infection without the possible confounding effect of direct invasion of the meninges from middle ear infection. Direct inoculation of the bacteria into the inner ear was introduced to study the direct route of infection from the middle ear to the meninges via the inner ear without the bacteremia of the middle ear infection. The attack rate of meningitis from middle ear inoculation is compared with the inner ear and hemogenous route of infection.

**Intraperitoneal Inoculation (Hemogenous Spread of Infection to the Meninges)**

Eighteen rats were anesthetized as described above, and three groups of six rats (three operated and three nonoperated controls)

### TABLE 2. Summary of the results

<table>
<thead>
<tr>
<th>Routes of inoculation</th>
<th>Amount of S. pneumoniae (CFU)</th>
<th>No. rats exhibiting meningitis after 5 days</th>
<th>Ratio of meningitis rats with cochleostomy versus control</th>
<th>Time from inoculation to meningitis (h)</th>
<th>No. rats with positive blood culture $\frac{\text{CFU}}{\text{mL}}$ ($S. pneumoniae$)</th>
<th>No. rats with positive CSF culture $\frac{\text{CFU}}{\text{mL}}$ ($S. pneumoniae$)</th>
<th>No. rats with positive left middle ear culture $\frac{\text{CFU}}{\text{mL}}$ ($S. pneumoniae$)</th>
<th>No. rats with positive histologic evidence of meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrapерitoneal</td>
<td>$4 \times 10^6$</td>
<td>6</td>
<td>3:3</td>
<td>14-15</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>$4 \times 10^5$</td>
<td>3</td>
<td>2:1</td>
<td>28-30</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>$4 \times 10^4$</td>
<td>2</td>
<td>0:2</td>
<td>51-82</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^5$</td>
<td>5</td>
<td>3:3</td>
<td>22-26</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^4$</td>
<td>2</td>
<td>2:3</td>
<td>50-54</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^3$</td>
<td>1</td>
<td>0:1</td>
<td>72</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle ear</td>
<td>$3 \times 10^6$</td>
<td>4</td>
<td>1:3</td>
<td>72-120</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>$3 \times 10^5$</td>
<td>2</td>
<td>1:1</td>
<td>96</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$3 \times 10^4$</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

*No rats from both cohort of control and cochleostomy exhibited meningitis.

CFU, colony-forming unit; CSF, cerebrospinal fluid.

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received three different concentrations of the bacteria in 1 mL inoculum via direct injection into the intraperitoneal cavity using a sterile 20-gauge needle and a 1-mL syringe (Table 1).

**Middle Ear Inoculation**
Under general anesthesia, the left bullae of 18 rats were surgically exposed for direct inoculation of three different concentrations of the bacteria in a 10-μL inoculum (Table 1). To retain the microorganisms in the bulla, the cavity was first filled with Gelfoam (Pharmacia & Upjohn, Kalamazoo, MI, U.S.A.). After the inoculation of the bacteria, the opening of the bulla was covered with temporalis fascia, and the wound was sutured in two layers.

**Inner Ear Inoculation**
Under general anesthesia, the left bulla was surgically exposed, and a cochleostomy to access the scala tympani was performed with a straight Kirschner wire. Two microliters of perilymph was removed, and 1 μL of bacterial inoculum was inoculated into the scala tympani for 1 minute using an infusion catheter, 5-μL microsyringe (ILS, Stützerbach, Germany), and a microsyringe pump controller (World Precision Instruments, Inc., Sarasota, FL, U.S.A.; Table 1). The cochleostomy was then covered with temporalis fascia. The opening of the bulla was covered with temporalis fascia, and the wounds were sutured in two layers.

**Postinfection Monitoring**
After the inoculation, each animal was examined twice daily at a minimum for clinical signs of meningitis during 5 days. The clinical assessment was recorded in a 12-point scored monitoring sheet as described previously (28).

**Microbiologic Specimen Collection and Tissue Preparation**
Once they developed early signs of meningitis, isoflurane was used to deeply anesthetize rats to allow collection of CSF, middle ear fluid, and blood for microscopy and culture (described in

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**FIG. 1.** Lower-power H&E photomicrographs illustrating the left (A) cochlea with previous cochleostomy and the right (B) cochleae of a rat 28 hours after intraperitoneal inoculation of 4 x 10^8 CFU S. pneumoniae. This animal exhibited clinical and histologic (CNS) evidence of meningitis. However, the scalae of both cochleae were devoid of gross infection. Higher-power photomicrograph of gram's stain from the modiolus of the left cochlea (C) illustrates the presence of bacteria (arrows). Higher-power photomicrograph of gram's stain of the subarachnoid space around the brain (D) illustrates the presence of bacteria (arrows) and inflammatory cells with phagocytosed bacteria. The approximate location of the higher-power micrograph (C) is illustrated in A. Scale bar: A and B, 200 μm; C and D, 10 μm.
detail in the previous study) (28). Briefly, a 30-gauge needle with a 1-mL syringe was used to perform a cisterna puncture to aspirate CSF using sterile techniques. Approximately 10 to 50 μL of CSF was collected for bacterial culture. Biochemistry, microscopy, and cell counts could not be performed because of the small sample of CSF collected; not performing these tests did not alter the interpretation and outcome of the results (28). One milliliter of blood was aspirated via intracardiac puncture using a 23-gauge needle and sent for blood culture. While animals were still fully anesthetized, bacterial swabs were taken from the left bulla under a strict aseptic technique and were sent for bacteriologic analysis.

The animals were then given a lethal dose of 120 mg/kg of body weight, i.m., pentobarbitone sodium (Lethabar; Virbac Pty., Ltd., Sydney, New South Wales, Australia) and were transcardially perfused with 0.9% saline then 10% neutral-buffered formalin (NBF; pH 7.4) at 4°C. The brain, meninges, and the cochleae were harvested and placed in 10% NBF for further processing.

Fifty-four brains, including meninges, were harvested and stored in 10% NBF for 48 hours then embedded in paraffin. The specimens were sectioned 10-μm thick, stained with both hematoxylin-eosin (H&E) and gram’s stain and examined under light microscopy for presence of inflammation and gram-positive cocci.

Twelve pairs of randomly selected cochleae were harvested from the temporal bones and fixed in 10% NBF. They were decalcified in a solution of 10% ethylenediamine tetra-acetic acid in 0.1 mol/L phosphate buffer (pH 7.4) on an agitation platform. Excessive bone was trimmed. They were then processed and embedded in Spurr’s resin. The embedded cochleae were orientated, and two sets of twenty-one 2-μm sections were collected at 126-μm intervals throughout the cochlea. One set of 21 sections was stained with H&E and the other set with gram’s stain.

The outcome of the study was to detect the presence of meningitis. CSF, blood, and middle ear cultures were collected to detect the presence of the bacteria. The meninges were harvested.

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**FIG. 2.** Lower-power H&E photomicrographs illustrating the left (A) cochlea with previous cochleostomy and the right (B) nonoperated cochlea of a rat 72 hours after left middle ear inoculation of 3 × 10⁸ CFU *S. pneumoniae*. This animal exhibited clinical and histologic (CNS) evidence of meningitis. In this example, there is severe labyrinthitis of the left inoculated ear (A), whereas the contralateral cochlea exhibits evidence of infection predominantly localized to the scala tympani (B). Lower-power H&E photomicrograph of a horizontal section taken at the level of the round window niche of the left (C) cochlea of the same animal illustrating the inflammatory cells mass infiltrating the round window membrane (nw). Higher-power H&E micrograph of the round window niche (D) was taken from region (D) in lower-power micrograph (C). Scale bar: A–C, 200 μm; D, 10 μm.
for histologic analysis and were used to confirm the culture results. Serotypes of \textit{S. pneumoniae} isolated from the cultures were reexamined to ensure that the strain causing the disease was the same as the initial inoculum.

**Histology Analysis**

The sections of histologic specimens 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, thickening and hyperplasia of the meningeal cells, and gram-positive cocci within the subarachnoid space and brain tissue. The cochleae were examined for the presence of bacteria and inflammatory cells.

**Statistical Analysis**

The effects of cochleostomy on the threshold of infection for the three different routes of inoculation have been evaluated statistically using Fisher's exact test, which calculates the exact probability of observing a particular $2 \times 2$ table, and those in which more extreme values would be obtained. The null hypothesis is that there is no difference in the incidence of meningitis when comparing rats with a cochleostomy to that of a control group of rats for each of the three different inoculating routes. If the sum of probabilities calculated using Fisher's exact test is less than the significance level required (i.e., $p < 0.05$), the null hypothesis is rejected, and a significant difference between the groups has been demonstrated not to have occurred by chance.

**RESULTS**

In control rats with normal cochleae, the thresholds of \textit{S. pneumoniae} required to induce meningitis differed for each of the three different routes of inoculation. The following symptoms were observed when rats acquired meningitis: tiredness, lethargy, unresponsiveness to sound and light stimulations, a hunched body posture, poor grooming, weight loss, and rectal temperature rising above 38°C. When these signs developed, the histology of the brain consistently showed evidence of meningitis with the infiltration of inflammatory cells and gram-positive diplococci within the subarachnoid space. Rats that did not exhibit the above clinical signs showed no histologic evidence of meningitis.

The attack rate of meningitis was reduced, and the time required to induce the disease increased because the number of bacteria in the inoculum was lowered for all routes of infection (Table 2). The attack rates of meningitis for rats with and without cochleostomy are also shown in Table 2. A cochleostomy performed 4 weeks before inoculation did not seem to increase the attack rate of meningitis compared with the nonoperated group.

Blood culture, CSF culture, and left middle ear swab culture results are summarized in Table 2. Serotyping of the bacteria from positive blood, CSF, and middle ear fluid cultures showed the bacteria to be serotype 2 (the same strain of \textit{S. pneumoniae} contained within the inoculum).

**Histology**

In rats with clinical and histologic evidence of meningitis, gram-positive bacteria and inflammatory cells were found within both cochleae, but the histopathologic pattern of infection was dependent upon the route of inoculation (described in detail in the previous study) (28). The amount and distribution of the inflammatory cells within both cochleae were similar in rats with intraperitoneal inoculation (Fig. 1). Bacteria were found symmetrically within the internal acoustic meatus (IAM) and modiolus but were not present within scalae. However, the inflammatory changes within the cochleae were asymmetric in meningitic animals after middle
A more severe labyrinthitis was observed in the cochlea when changes were evident in the contralateral cochlea. When intraperitoneal (Fig. 2) and inner ear inoculation (Fig. 3). In these cases, a more severe labyrinthitis was observed in the cochlea ipsilateral to the inoculation, and minimal inflammatory changes were evident in the contralateral cochlea. When inflammatory cells were present in the contralateral ear, they were found more in the scala tympani than in the scala vestibuli. In rats without meningitis, the histologic appearance of the cochlea was normal for intraperitoneal (hematogenous) inoculation. Few inflammatory cells and scant serofibrinous exudate were observed in the basal turn of the cochlea in rats that did not develop meningitis after direct middle or inner ear inoculation.

Microscopic examination of the ipsilateral middle ear mucosa in the region of the round window niche revealed evidence of inflammation in rats after direct middle ear inoculation. When present, the inflammation was mild in the ipsilateral middle ear mucosa of rats after direct inner ear inoculation. The contralateral control bullae showed no evidence of middle ear inflammation for both middle and inner ear inoculations. There were no inflammatory changes within the middle ear mucosa in rats after an intraperitoneal inoculation.

### Effects of Cochleostomy on the Threshold of Infection

Statistical analysis of the results was performed using the Fisher's exact test of independence. To analyze the effect of cochleostomy on the risk of acquiring pneumococcal meningitis, a comparison of attack rate of meningitis between a group of rats receiving cochleostomy and a control group for each of the inoculating routes was performed (Table 3). No significant difference was observed at the 95% confidence level for all three different routes of inoculation ($p > 0.05$; two-tailed test).

### DISCUSSION

A quantitative threshold model of pneumococcal meningitis has been established in healthy rats with no pre-existing risk factors for meningitis. The results showed that the number of bacteria that healthy animals were exposed to is an important factor influencing the outcome of the infection. A minimal threshold of bacteria is required to achieve meningitis in healthy animals. Moreover, this threshold value varies depending on the route of inoculation. A cochleostomy did not seem to increase the attack rate of meningitis compared with the nonoperated group. However, it is important to stress that no implant has been used in this series so that it cannot be stated whether the presence of a cochlear implant electrode in the inner ear also increases the risk of meningitis, i.e., lowers the threshold.

Previous animal models have shown that meningitis rarely occurred after direct inoculation of $1 \times 10^3$ CFU of virulent pneumococci into the subarachnoid space (30). This observation suggests that a minimal number of bacteria must be present in the CSF to achieve meningitis. Our work supports and expands this finding by demonstrating that minimal numbers of bacteria are required to induce meningitis for hematogenous, inner ear-, and middle ear-based infection.

When the intraperitoneal inoculum of *S. pneumoniae* was reduced from $4 \times 10^6$ to $4 \times 10^4$ CFU, the attack rate of meningitis in rats dropped from 6 of 6 to 2 of 6. The number of rats with bacteremia was similar across the three groups of rats receiving intraperitoneal inocula (Table 2), suggesting that the intensity of the bacteremia must be reduced in rats inoculated with fewer bacteria, resulting in a reduced attack rate of meningitis. We hypothesize that a greater concentration of intraperitoneal inoculum leads to a greater magnitude of bacteremia and a greater number of the organisms reaching the subarachnoid space. Although the magnitude of bacteremia for each intraperitoneal inoculum was not measured, our data using *S. pneumoniae* are consistent with previous work with *H. influenzae*. The magnitude of the bacteremia with *H. influenzae* has been shown to be directly related to the size of the intranasal inoculum and the incidence of meningitis (25,31).

There is a sharp drop in the attack rate of meningitis when the inner ear inoculum was titrated down from $1 \times 10^6$ to $1 \times 10^3$ CFU. It is likely that an inoculum of *S. pneumoniae* below $1 \times 10^3$ CFU will not induce meningitis via the inner ear inoculation in this species. Similar results were observed in rats with middle ear inoculation because the attack rate dropped when the bacterial count was titrated from $3 \times 10^6$ to $3 \times 10^4$ CFU (Table 2).

The attack rate of meningitis increased when animals were exposed to a higher concentration of the bacteria. Moreover, the time required to develop meningitis was reduced as the infective dose of the bacteria was increased. This is clearly demonstrated by rats receiving the highest bacterial counts via intraperitoneal or the inner ear compared with rats receiving smaller quantities of the bacteria. Previous work with *H. influenzae* in rhesus monkeys with intranasal inoculation of the bacteria also demonstrated that time to onset of meningitis after inoculation is a function of the density of the bacteremia (32).

On the basis of our data, the threshold bacteria required to induce meningitis is lowest with direct inner ear inoculation and is higher in rats with middle ear and hematogenous infection (Table 2). As the inner ear has a

### TABLE 3. Comparison of attack rate of meningitis between rats receiving cochleostomy and control group for each of the inoculating routes

<table>
<thead>
<tr>
<th>Routes of inoculation</th>
<th>Attack rate of meningitis in rats</th>
<th>Cochleostomy</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal</td>
<td>(n = 9)</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Inner ear</td>
<td>(n = 9)</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Middle ear</td>
<td>(control group)</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Fisher's exact test: calculated $p$ at the 95% confidence interval; two-tailed test. *$p > 0.05$, there is no statistical difference between the two groups. Failure to reject null hypothesis.
close anatomic association with the CNS via the cochlear aqueduct and the modiolus through the canaliculi perforantes and the IAM, the threshold required to induce meningitis is expected to be lowest for this route. The route by which *S. pneumoniae* spread from the intraperitoneal cavity to CNS infection after intraperitoneal inoculation is via the blood circulation. Unlike the inner ear, the intraperitoneal cavity has no direct anatomic association with the CNS. Bacteremia was observed in 15 of the 18 rats receiving intraperitoneal inoculation. Eleven intraperitoneal-inoculated rats developed meningitis in the presence of a positive blood culture. This suggested a direct bacterial invasion of the blood–brain barrier as a consequence of bacteremia. Likewise, no rats developed meningitis in the absence of a positive blood culture. Finally, there are two possible routes for the bacteria to reach the CNS via the middle ear cavity: either from the local or systemic blood circulation or via the inner ear through the round or oval windows. It is also possible that the bacteria may enter the CNS via a combination of these described routes. Despite having two potential routes of infection, the attack rate of meningitis in rats with middle ear inoculation was similar to the group of rats with intraperitoneal inoculation. However, a longer time was required to induce meningitis after middle ear inoculation compared with the intraperitoneal inoculation of similar bacterial counts. There are two possible explanations. First, bacteremia as a result of AOM is not as intense compared with intraperitoneal inoculation. Second, the bony and soft tissue barriers between the inner and middle ear are effective in either preventing or reducing the amount of bacteria reaching the inner ear.

The histologic appearance of the cochleae in rats with meningitis was dependent upon the route of infection (28). A symmetric distribution of gram-positive bacteria and inflammatory cells within IAM and modiolus was found in both cochleae in rats with intraperitoneal inoculation. There were no bacteria or inflammatory cells observed within the scala tympani and vestibuli, and no evidence to suggest that bacteria traversed blood vessels to enter the scala media. An asymmetric distribution of the bacteria and inflammatory cells was observed in meningitic rats after both middle and inner ear inoculations. In a number of rats with middle ear infection and meningitis, bacteria were found to infiltrate the round window membrane to reach the scala tympani. This provides support for the notion that meningitis might have been caused by direct spread of infection through round window membrane into the scala tympani and then to the CNS. Although we cannot completely exclude the hematogenous spread to the meninges after middle ear infection, one would have expected a more symmetric distribution of the bacteria and inflammatory cells within their cochleae if this were the dominant route.

Any breach of the bony and mucosal barrier is considered to increase the risk of infection spreading from the middle ear to the inner ear (28), and this may reduce the threshold of bacteria required in the middle ear to induce meningitis as a result of AOM. A cochleostomy is a standard procedure before electrode insertion and presumably disrupts the labyrinthine architecture within a circumscribed region of the basal turn. Our data showed that any disruption did not affect the threshold for infection. Rats inoculated 4 weeks after cochleostomy surgery did not show a greater incidence of meningitis when compared with control rats without a cochleostomy. This can be explained by the fact that after 4 weeks, the cochleostomy had healed either via fibrosis of the fascial seal or, in some instances, a complete regeneration of the bony capsule. Any type of repair at the cochleostomy may act as a physical barrier to prevent infection from easy access to the inner ear and then to the CNS. Not surprisingly, a cochleostomy did not reduce the threshold of bacteria required for meningitis for the hematogenous (intraperitoneal) or inner ear routes of inoculation.

The cochleostomy group was performed to serve as a control for a subsequent study. One needs to ensure that if the implant reduced the threshold for meningitis, it is because of the implant per se rather than the effects of a cochleostomy. Furthermore, the surface features of cochlear implant design can provide a surface for bacterial biofilm infection (33,34). A biofilm containing *Staphylococcus aureus* has been observed in the depressions on the surface of receiver stimulator (33,34). Although a biofilm containing *S. pneumoniae* has not been described on the surface of cochlea implants to date, the role of biofilms associated with implant devices and their role in pneumococcal meningitis is an important issue that can be explored using the threshold model described above. Furthermore, in human cochlear implantation, surgery directly adjacent to the dura within the mastoid or deep to the temporal squama in providing a bed for the receiver stimulator may affect the blood–brain barrier. Any change may affect both the thresholds for direct and hematogenous routes of infection. Again, our model can be used to test this hypothesis.

The present experiment was conducted in rodents with previous normal hearing; therefore, the labyrinthine architecture and internal vascularity were presumably intact, and fully immunocompetent. The aim of this animal model is to examine the threshold for meningitis in healthy rats with normal ears. Significantly, many implant patients have preexisting risk factors for pneumococcal meningitis, including cochlear malformation and temporal bone fractures. It is therefore difficult to analyze whether the presence of the implant or the preexisting risk factors or the combination of both contributes to post implant meningitis. When examining the effect of cochlear implantation on the threshold for meningitis, one needs to ensure that there are no other confounding factors, hence the need to use an animal model with no preexisting risk factors in a controlled laboratory environment. Therefore, a model involving healthy animals with normal ears is an important prerequisite before examining whether interventions such as a cochlear implant alter the risk of infection. Previous research has shown that an animal model involving cats might be substantially more resistant to
spreading of infection in association with cochlear implantation (35). This emphasizes the importance of selecting a suitable animal species to elucidate mechanisms of meningitis in association with cochlear implantation (28). The use of a rat model to study human disease is reasonable because it has been instrumental in the study of bacterial meningitis. The rat model of meningitis has enabled investigators to identify the pathogenic and pathophysiologic mechanisms responsible for the neurologic damage observed in patients with bacterial meningitis in the absence of a cochlear implant (36). Rats have been shown to exhibit meningitis with features that closely resemble human disease (37,38). Furthermore, the rat is a suitable animal model for the study of pneumococcal meningitis in the presence of a cochlear implant due to the routes by which infection spreads from upper respiratory tract mucosa to the CNS in rats closely resemble that of a human infection (28), and the surgical approach to cochlear implantation in rats has been established (28,39), providing an excellent opportunity to study implant-related CNS infection.

Despite the obvious differences between the human and the rat’s inner ear anatomy related to the patency of the cochlear aqueduct and defense mechanism of the mucosa, the aim of the present study was to demonstrate the principle of threshold for meningitis. The threshold principle and the effects of surgical intervention and prostheses on threshold in an animal model will provide an important contribution to our understanding of human infection.

Whether a threshold level of S. pneumoniae is required to cause meningitis in human subjects after bacteremia or AOM has not been established, and if so, the threshold is likely to depend on the interaction between the host’s immunity and the virulence of the bacteria. Patients with pneumococcal bacteremia do not always develop meningitis because these bacteria are commonly grown from the blood of febrile infants and children in whom no obvious focus of infection is analyzed (40). Similarly, pneumococcal meningitis as a result of AOM is a very rare complication in patients with anatomically normal middle and inner ears, despite the fact that pneumococcal AOM is commonplace. Even without antibiotic treatment, the spontaneous resolution of AOM has been reported to be as high as 75% within 14 days of infection (41). Our result demonstrates that under experimental conditions, a threshold number of bacteria is required to cause meningitis. This finding suggests that the threshold may be one of the factors determining whether a human subject develops meningitis after pneumococcal bacteremia or AOM.

The principle of a threshold model, as described in the present study, can be used to assess whether the risk of pneumococcal meningitis is influenced by the presence of a cochlear implant or by the presence of other neurosurgical prostheses. Furthermore, the model can also be used to test whether the design of prostheses and/or modifications in surgical technique effectively reduces the risk of implant-related CNS infection.

CONCLUSION

There has been an increase in the number of reported cases of pneumococcal meningitis post-cochlear implantation. The pathogenesis of pneumococcal meningitis is complex even in the absence of cochlear implantation, and the relative importance of different infection routes remains unknown. Improved knowledge of these areas is an important first step in determining the effect of cochlear implantation on the risk of acquiring pneumococcal meningitis. A quantitative threshold model for pneumococcal meningitis has been established and demonstrates that a minimal number of bacteria in a healthy animal is required to achieve meningitis. The thresholds of bacteria required are significantly different for each route of infection. However, inner ear surgery (cochleostomy without cochlear implantation) performed 4 weeks before inoculation does not alter the threshold for infection. This model can be used to assess the risk of acquiring pneumococcal infection in animals with cochlear implantation or other neurosurgical implantable devices.

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