Reevaluation of an experimental streptococcal canine brain abscess model

HENRY KURZYDLOWSKI, M.D., CYNTHIA WOLLENSCHLAGER, M.D., FRANK R. VENEZIO, M.D., MONA GHOBRIAL, M.D., MORRIS MARC SORIANO, M.D., AND O. HOWARD REICHMAN, M.D.

Sections of Neurosurgery, Infectious Diseases, and Neuropathology, Loyola University Medical Center, Maywood, and the Research Service, Hines Veterans Administration Hospital, Hines, Illinois

An experimental cerebral abscess model in which alpha-hemolytic Streptococci were inoculated into the brain parenchyma of dogs was evaluated for assessment of antimicrobial therapy. Intracerebral ring-enhancing lesions were visualized by computerized tomography, but they resolved after time without therapeutic intervention. Histopathological study demonstrated evolution of the lesions into sterile granulomas. Quantitative cultures were performed and uniformly became sterile in the early cerebritis stage, approximately 3 days after bacterial inoculation. Therefore, this brain abscess model should not be utilized for the evaluation of new antimicrobial treatment regimens. Rather, other models which document persistent viable organisms within cerebral abscesses need to be developed.

KEY WORDS • brain abscess • alpha-hemolytic Streptococci • cerebritis • computerized tomography • granuloma • dog

To study the efficacy of newer antimicrobial agents in the treatment of brain abscess, we employed a canine model in which alpha-hemolytic Streptococci were inoculated into the brain parenchyma. Although well-circumscribed ring-like lesions were visualized by computerized tomography (CT), they resolved without therapeutic intervention. This unexpected finding prompted the present reevaluation of that experimental design. Results of timed sequential CT scans and histopathological findings similar to those of previous studies are presented. Quantitative bacterial cultures, not routinely measured in prior experiments with other alpha Streptococci, were also performed.

Materials and Methods

Healthy adult mongrel dogs were used in all experiments in accordance with the National Institutes of Health “Guide for Care and Use of Laboratory Animals.” Following review and approval by the institution’s Animal Care and Use Committees, the experiments were performed at Hines Veterans Administration Hospital, which is fully accepted by the American Association for Accreditation of Laboratory Animal Care. In addition, the animal research care groups at both Hines Veterans Administration Hospital and Loyola University Medical Center conducted bimonthly ongoing reviews of animal usage. Standard orders included administration of meperidine postoperatively. The dogs were clinically evaluated daily and monitored by a physician member of the research team for the development of local wound infection, hematoma or seroma, systemic toxicity, fever, and abnormal neurological signs. In addition, 12-hour/day coverage was provided by the Hines animal research care staff. Particular attention was given to signs of circling, head tilt, seizures, and obtundation. Blood cultures were obtained in all animals with signs of systemic infection. Postoperative attention was given 7 days a week to insure optimal care to the animals, including daily dressing changes and open drainage of subcutaneous hematomas, seromas, or local infections. Parenteral clindamycin was administered to two dogs with evidence of sepsis, and the animals were withdrawn from the experimental protocol. All dogs were sacrificed by intravenous administration of a fully anesthetizing dose of pentobarbital followed by potassium chloride.

The animals were anesthetized with intravenous pentobarbital, intubated, and placed on a Harvard respirator.* Intravenous fluids were administered. The an

mals' heads were shaved and placed in a stereotaxic apparatus, and the surgical area was prepared with povidone iodine and draped in a sterile manner. A 5-cm incision was made over the right frontal area. The temporalis fascia and muscle were incised and reflected back. A Hudson brace was used to make a burr hole in the frontal bone. *Streptococcus-MG-intermedius* (SMG), the most common alpha *Streptococcus* causing human brain abscesses, was chosen as the test isolate. The SMG utilized was the standard reference strain from the Loyola University Medical Microbiology Laboratory. The SMG was grown overnight in trypticase soy broth at 30°C, and the inoculum dose was adjusted to between $10^7$ to $10^9$ colony-forming units/ml.

One cubic centimeter of solution was added to 1 cc of 0.5% molten agarose cooled to 50°C. The mixture was drawn into a No. 25 tuberculin syringe, and 0.2 cc of inoculum was stereotaxically injected 5 cm into the frontal lobe parenchyma. The needle was withdrawn, hemostasis maintained, and the wound closed in layers.

Serial CT scans with and without contrast enhancement were obtained at various time intervals. At necropsy, the dog brains were aseptically removed. The specimens were placed in 10% formalin solution for histopathological examination or were submitted in sterile containers for quantitative cultures. Following 3 weeks of fixation, the brains were cut into 1-cm thick coronal sections and processed for histological examination. Hematoxylin and eosin (H & E) staining was performed for general morphological examination and periodic acid-Schiff (PAS) staining for detection of glycogen and mucopolysaccharides. A tissue Gram stain was used to detect the presence of bacteria. Capsule formation was evaluated using Masson's trichrome stain to detect collagen formation, and the glial fibrillary acidic protein stain was employed to demonstrate reactive astrocytosis and gliosis. Specimens for culture were weighed and placed into a known volume of thioglycolate broth. The specimen was homogenized, diluted, and serially inoculated onto blood agar plates and incubated at 35°C for 24 hours, at which time bacterial colonies were identified and counted. Care was taken to be sure that no antibiotics were contained in the dog chow.

### Results

Twenty-nine dogs received inoculum in a dose ranging from $10^7$ to $10^9$ colony-forming units/ml. The dogs underwent CT scanning prior to sacrifice on postinoculation Days 3, 5, 7, 14, 21. Not all dogs underwent scanning on each day.

Two of three dogs in which scanning was performed on postinoculation Day 3 had faint ring-like lesions noted with contrast enhancement (Table 1). The third dog died before CT scanning could be performed. At necropsy, small cavitory lesions were seen in all three brains. The dog that had died prematurely also had a lesion that ruptured through the frontal cortex into the subdural space. Quantitative cultures in that dog revealed $3 	imes 10^3$ colonies SMG/gm of tissue. Cultures obtained from the other two dogs were sterile.

Another three animals underwent CT scanning and were sacrificed on postinoculation Day 5. In two dogs, scans revealed well-defined ring-enhancing lesions surrounded by edema; cerebritis, characterized by homogeneous enhancement, was seen in the third. The two cavities averaged $1.5 \times 1.5 \times 2.5$ cm in size. Histological studies showed necrotic proteinaceous debris with a few inflammatory cells in the central area mixed with foamy macrophages, fibroblasts, and foreign-body giant cells. At the cortical surface, a dense reticular network containing collagen formed a capsule. In the dog with cerebritis, histological investigation revealed infiltration of lymphocytes and other mononuclear cells into the adventitia of blood vessels, along with neovascularization of the cerebral tissue. In all cases quantitative cultures were sterile.

Three additional dogs underwent CT scanning and were sacrificed on postinoculation Day 7. All were noted to have ring-like lesions. The cavities measured $1.5 \times 1.0 \times 1.5$ cm, and the specimens were again sterile. Histology was similar to that of the previous group.

Fifteen dogs had CT scanning performed on Days 13 to 21 postinoculation. Ten dogs had ring-like lesions, three had cerebritis, and two had no abnormalities. Four of these dogs were sacrificed after 15 days and 11 were followed for approximately 3 weeks (range 18 to 23 days). Gross sections of brain tissue revealed smaller cavities measuring approximately $1.0 \times 1.0 \times 0.5$ cm. Histopathological examination was performed on four of the animals sacrificed after 3 weeks and revealed only a granulomatous reaction. The center of the granuloma was surrounded by a dense outer fibrous capsule and an inner zone of capillary proliferation and extravasation of erythrocytes. Inflammatory cells in the central area mixed with foamy macrophages, fibroblasts, and foreign-body giant cells. At the cortical surface, a dense reticular network containing collagen formed a capsule. In the dog with cerebritis, histological investigation revealed infiltration of lymphocytes and other mononuclear cells into the adventitia of blood vessels, along with neovascularization of the cerebral tissue. In all cases quantitative cultures were sterile.

### Table 1

<table>
<thead>
<tr>
<th>Inoculum Dose (cfu/ml)</th>
<th>No. of Dogs</th>
<th>Day of Sacrifice</th>
<th>CT Findings</th>
<th>Gross Lesion</th>
<th>Quantitative Culture</th>
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<tr>
<td>$1 \times 10^7$</td>
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<td>15-16</td>
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<tr>
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<td>18</td>
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<td>negative</td>
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<td>yes</td>
<td>negative</td>
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<td>3</td>
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<tr>
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<td>$1 \times 10^9$</td>
<td>3</td>
<td>5</td>
<td>2 ring lesions</td>
<td>yes</td>
</tr>
</tbody>
</table>

*Abbreviations: cfu = colony-forming units; CT = computerized tomography.† This dog died prematurely.
Streptococcal canine brain abscess

Fig. 1. Left: Histopathological section of a canine brain abscess in an animal sacrificed 3 weeks after inoculation. There is thick capsule formation surrounded by multinucleated giant cells, lymphocytes, plasma cells, and macrophages. A single multinucleated giant cell is seen outside the capsule. H & E, × 50. Right: Higher magnification showing a multinucleated giant cell with amorphous eosinophilic material within the cytoplasm. Similar material was seen in the center of the abscess. The giant cell is surrounded by reactive astrocytes and macrophages. H & E, × 125.

Uloma consisted of amorphous eosinophilic material on H & E; it also stained faintly with PAS and lacked bacteria or inflammatory cells (Fig. 1 left). This was surrounded by large numbers of multinucleated foreign-body giant cells with nuclei at the periphery of the cell, encompassed by dense collagen fibers (Fig. 1 right). The brain parenchyma exhibited an intense reactive astrocytosis. There was minimal proliferation of small blood vessels and perivascular lymphocytic cuffing of large blood vessels in the adjacent tissues. Cultures of the cavities and burr holes were uniformly sterile. The epidural and subdural spaces were also sterile in all but two specimens, from which Gram-negative rods (not further identified) were grown. These rods were presumed to be contaminants.

Another group of five animals received a larger dose of SMG inoculum (1 × 10⁹ colony-forming units/ml). The CT scans obtained on Day 14 revealed ring-enhancing lesions in all five dogs. These dogs were sacrificed on Day 15; gross sectioning revealed larger fluid-filled cavities measuring 1.5 × 2.0 × 2.0 cm, but cultures of the "abscess" material still remained sterile. Histopathology indicated granulomas similar to those described previously.

In order to evaluate the natural history of these lesions, a subgroup of three animals were given an inoculum dose of 1 × 10⁹ colony-forming units/ml and were followed for 38 days after surgery with sequential CT scans. The scans demonstrated a large ring-like lesion early in the follow-up period (Fig. 2 left), but the lesion spontaneously regressed in size on subsequent scans until it resolved to minimal diffuse uptake (Fig. 2 right).
2 center and right). At autopsy, gross inspection of the cerebral parenchyma revealed only faint hyperemia with surrounding cerebral edema.

To assess the role of SMG in this self-limited "absscess" model, a group of five animals served as controls and received equal portions of 0.5% agarose and sterile trypticase soy broth. Serial CT scans were performed postoperatively at 7- to 14-day intervals and prior to sacrifice on Day 36. The CT scans obtained 1 week after surgery were normal in two dogs, revealed a very small (0.1 x 0.2 x 0.1 cm) ring-enhancing lesion in one animal, and demonstrated mild cerebritis in the other two. At the time of sacrifice, CT studies were totally normal in four animals and disclosed minimal contrast enhancement at the injection site in the fifth. Postmortem examination of the brain showed normal tissue or only faint hyperemia and tissue edema. Histology revealed scant amorphous material lacking inflammatory cells, surrounded by foreign-body giant cells with sparse fibrocollagenous tissue.

Discussion

Experimental cerebral absscess models have previously been described in the primate, rabbit, rodent, cat, and dog. 7,8,10-13 Most investigators have inoculated organisms, grown in a nutrient medium, directly into the brain parenchyma. Britt, et al., 1,2,5,6 have published reports utilizing alpha Streptococci, Bacteroides fragilis, or a mixed anaerobic culture of Bacteroides fragilis and Staphylococcus epidermidis in an attempt to establish a canine cerebral absscess model. After inoculating a more potent alpha-hemolytic Streptococci, Britt, et al.,9 reconfirmed that Gram stains of absscess material showed organisms only through the cerebritis stages. In original experiments using alpha Streptococcus, Britt did not perform quantitative bacterial cultures (personal communication, 1985). In the present study, the size of the bacterial inoculum dose was increased without altering the pathological or microbiological outcome. Histologically, the brain lesions resembled sterile granulomas rather than absscesses, with the centers void of organisms. Instead, only an amorphous eosinophilic material was present, surrounded by foreign-body giant cells. Outside the fibrinous capsule, the surrounding cerebral parenchyma exhibited intense reactive astrocytosis and gliosis extending into the white matter.

In summary, our present study and other investigations5 have revealed that injecting microaerophilic Streptococci, such as Streptococcus-MG-intermedius, into brain parenchyma is incapable of establishing viable cerebral absscesses. The ability to establish chronic infection in normal brain tissue is probably dependent upon several factors, including using a more virulent organism, traumatizing tissue prior to inoculation, or altering host defenses. 8-19,13 Therefore, we are developing a canine cerebral absscess model that retains viable bacteria by implanting into brain parenchyma a fibrin clot infected with Bacteroides fragilis; this investigation appears promising.

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References


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Address reprint requests to: O. Howard Reichman, M.D., Loyola University Medical Center, 2160 South First Avenue, Maywood, Illinois 60153.