Neuropathological and computerized tomographic findings in experimental brain abscess

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The neuropathological progression of brain abscess formation was studied experimentally at sequential stages in dogs, and the findings correlated with the appearance on computerized tomographic (CT) brain scans. The evolution of brain-abscess formation was divided into four stages based on histological criteria: early cerebritis (Days 1 to 3); late cerebritis (Days 4 to 9); early capsule (Days 10 to 13); and late capsule formation (Day 14 and later). The cerebritis stage was characterized by prominent perivascular cuffing by inflammatory cells in the area adjacent to the developing necrotic center. However, the early elements of capsule formation appeared with the presence of fibroblasts by Day 5. The CT scans showed ring-shaped contrast enhancement by Day 3. Delayed scans at 30 minutes revealed diffusion of the contrast material into the developing necrotic center, forming a solid lesion. In lesions that were well encapsulated (14 days and older), five distinct histological zones were apparent: 1) a well formed necrotic center; 2) a peripheral zone of inflammatory cells, macrophages, and fibroblasts; 3) the dense collagenous capsule; 4) a layer of neovascularity associated with continuing cerebritis; and 5) reactive astrocytes, gliosis, and cerebral edema external to the capsule. The CT appearance of well encapsulated abscesses showed a typical ring-shaped contrast-enhancing lesion. On delayed scans, the “ring” did not fill in with contrast enhancement. The diameter of the ring correlated best with the presence of cerebritis (perivascular infiltrates in the adventitial sheaths of vessels surrounding the abscess). The discussion focuses on the relevance of this study to the current management of patients with brain abscess.

KEY WORDS • brain abscess • cerebritis • computerized tomography • ring-like contrast enhancement • capsule formation

Failure to identify and accurately localize brain abscesses is a major factor that contributes to the high mortality and morbidity rates associated with these lesions. The introduction of computerized tomographic (CT) brain scans has been a significant advance in localizing suspected intracranial infection. It has been assumed that the cerebritis stage of abscess formation correlates with the CT finding of a low-density lesion with little or no contrast enhancement, and the appearance of ring-shaped contrast enhancement represents an encapsulated abscess. Based on these assumptions and the accuracy of CT scanning, a new conservative approach to brain abscess management has emerged using antibiotics alone. Precise criteria as to which lesions will respond to antibiotics alone and which will require surgical aspiration or excision for cure have not been established.

We have developed an animal model for studying the CT findings associated with the evolution of brain abscesses. The histological progression of experimental brain abscess in this model is analyzed from the stage of early cerebritis through the stage of well encapsulated lesions. The dynamic aspects of contrast enhancement have been reported in a previous paper.

Materials and Methods

In 19 dogs, intracranial abscess formation was induced by intracranial injection of a bacterial strain of alpha streptococcus. For both the surgical procedures...
and the subsequent CT scanning procedures, the dogs were tranquilized using acepromazine maleate, 10 mg/15 kg injected intramuscularly, and then anesthetized with intravenous pentobarbital (60 mg/2.5 kg). The animal's head was fixed to a standard stereotaxic apparatus. The shaved scalp was prepared with a 5-minute lavage with Betadine and was aseptically draped. A scalp incision was made in the midline, and the left temporalis muscle was reflected. A single burr hole was placed using a Hudson brace with perforator apparatus. The shaved scalp was prepared with a

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The alpha streptococcus was grown in Todd-Hewitt broth in an overnight culture at a temperature of 36°C. In each experiment, the exact number of colony-forming units inoculated was determined by making serial dilutions of an aliquot of the broth culture and inoculating 0.1 cc of each 10-fold dilution on a blood agar plate.

Computerized tomographic brain scans were obtained in the coronal plane by using a custom-made Plexiglas holder and polystyrene rings to position the head. Scans were obtained both before and after injection of 66% diatrizoate meglumine and 10% diatrizoate sodium (4 ml/kg) (Renografin 76). Serial scans were made at the following time intervals: 0 to 5, 10 to 15, 20 to 25, 30 to 35, 45 to 50, and 60 to 65 minutes. Serial scans were obtained in each animal at 2- to 3-day intervals during the first 2 weeks, and then every 5 to 7 days in lesions older than 2 weeks.

Brains were removed on the day of sacrifice, and placed in 10% formalin solution for a minimum of 3 weeks. The brains were cut in 1-cm coronal sections, photographed, and representative sections were taken for processing and embedding in paraffin. Routine hematoxylin and eosin (H & E) staining was performed for general morphology. The Gram stain was used to evaluate the presence of bacteria. To evaluate the progression of encapsulation, the reticulin stain was used to evaluate reticulin or pre-collagen deposition and Masson's trichrome and hematoxylin van Giesen (HVG) stains were used to determine the presence and extent of mature collagen. Phosphotungstic acid hematoxylin (PTAH) and glial fibrillary acid protein (GFAP) stains were used to evaluate the presence of gliosis and reactive astrocytes. The gross dimensions of the lesions were compared with the size of the lesions on CT scans, assuming a 17% shrinkage artifact of the tissue due to formalin fixation. Twenty-one animals were sacrificed at the following time intervals: 1, 3, 5, 6, 7, 8, 9, 10, 14, 19, 21, 22, and 38 days.

Results

Classification of Findings

Based on the detailed histological evaluation of the material from the 19 experimental animals, four stages of brain abscess formation were defined: early cerebritis (1 to 3 days); late cerebritis (4 to 9 days); early capsule formation (10 to 13 days); and late capsule formation (14 days and later). To some extent this is an arbitrary division, since the evolution of brain abscess formation is a continuing process. However, there were salient features to be discussed that justified this division into four stages. In each of the four stages, the histological criteria for each of five zones present in all well developed abscesses were evaluated. The five zones were as follows: Zone 1, the necrotic center; Zone 2, an inflammatory infiltrate mixed with macrophages and fibroblasts surrounding the necrotic center; Zone 3, collagenous capsule formation; Zone 4, cerebritis: perivascular infiltration of inflammatory cells in the adventitial sheaths of blood vessels surrounding the lesion; neovascularity; and Zone 5, reactive astrocytes, gliosis, and cerebral edema. Table 1 summarizes the salient features of each stage described below.

Early Cerebritis (1 to 3 Days)

Twenty-four hours after inoculation with alpha streptococcus organisms, a marked inflammatory infiltrate comprised of polymorphonuclear cells, lymphocytes, and plasma cells was seen interspersed among the residual agarose (Zone 1, Fig. 1A). A Gram stain showed Gram-positive cocci scattered throughout this developing necrotic center. At this early stage, there had not been time for a significant inflammatory response to be induced in the brain peripheral to the developing necrotic center (Zone 2). However, there was early infiltration of acute inflam-
### TABLE 1

<table>
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<tr>
<th>Findings</th>
<th>Early Cerebritis (Days 1–3)</th>
<th>Late Cerebritis (Days 4–9)</th>
<th>Early Capsule Formation (Days 10–13)</th>
<th>Late Capsule Formation (Day 14 and later)</th>
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<tr>
<td><strong>Histological Features</strong></td>
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<tr>
<td>Zone 1: necrotic center</td>
<td>inflammatory cells inter-spersed among residual agarose; bacteria present on Gram stain</td>
<td>necrotic center enlarged &amp; reached its maximal size</td>
<td>necrotic center began to decrease in size</td>
<td>necrotic center continued to decrease in size with further encapsulation of abscesses</td>
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<tr>
<td>Zone 2: inflammatory border</td>
<td>acute inflammatory cells seen between the developing necrotic center &amp; brain</td>
<td>inflammatory cells mixed with macrophages &amp; fibroblasts</td>
<td>increased numbers of fibroblasts &amp; macrophages appeared during this stage</td>
<td>number of fibroblasts continued to increase in relation to inflammatory cells &amp; macrophages</td>
</tr>
<tr>
<td>Zone 3: collagen capsule</td>
<td>no evidence of reticulin formation until Day 3</td>
<td>fibroblasts appeared with rapid formation of reticulin surrounding necrotic center</td>
<td>mature collagen evolved from reticulin precursors; forming capsule was less developed on ventricular side of lesion</td>
<td>a collagen capsule was completed by end of 2nd week; during 3rd week, capsule increased in density of collagen deposition &amp; thickness</td>
</tr>
<tr>
<td>Zone 4: cerebritis &amp; neovascularity</td>
<td>perivascular infiltration of polymorphonuclear leukocytes, plasma cells, &amp; mononuclear cells (cerebritis) developed rapidly</td>
<td>cerebritis reached its maximal extent during this stage; new vessel formation rapidly increased around developing abscess</td>
<td>cerebritis was less acute; maximal degree of neovascularity developed at this stage</td>
<td>cerebritis was restricted to area immediately outside collagen capsule; perivascular infiltrate contained fewer inflammatory cells &amp; more fibroblasts; neovascularity was less marked</td>
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<td>Zone 5: reactive gliosis &amp; cerebral edema</td>
<td>marked cerebral edema surrounded lesion at this stage</td>
<td>cerebral edema continued to be prominent in surrounding white matter; reactive astrocytes began to appear late in this stage</td>
<td>cerebral edema started to regress; number of reactive astrocytes increased</td>
<td>surrounding cerebral edema regressed as capsule developed; in 3rd week, marked gliosis developed outside the capsule; large numbers of reactive astrocytes surrounded abscesses</td>
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<td><strong>Computerized Tomography</strong></td>
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<td>CT scan findings</td>
<td>partial ring contrast enhancement on Day 1 evolved to well developed ring enhancement by Day 3</td>
<td>ring enhancement with diffusion of contrast medium into the lucent center on delayed scans was salient CT finding of cerebritis stage</td>
<td>ring enhancement with less diffusion of contrast material into lucent center was seen in incompletely encapsulated abscesses</td>
<td>ring enhancement without diffusion of contrast material into lucent center was salient feature of well encapsulated abscesses</td>
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Inflammatory cells in the adventitial sheaths of vessels surrounding the inoculum (Fig. 1B). Polymorphonuclear leukocytes, small lymphocytes, and a few large mononuclear cells were present. The reticulin stain showed the splitting of the vessel walls caused by the perivascular cuffing (Zone 4, Fig. 1C). A GFAP stain showed no evidence of reactive astrocytes at this early stage (Zone 5). Meningitis was prominent at Day 1 and extended down the Virchow-Robin spaces near the site of injection (Fig. 1D).

By Day 3, there was a marked increase in the perivascular infiltration of inflammatory cells in the vessels surrounding the developing necrotic center (Fig. 1E). A few blood vessels began to show sprouting of reticulin, indicating the early presence of fibroblasts in or adjacent to the vessel walls (Fig. 1F). There was marked edema of the white matter surrounding the lesions during this phase of abscess formation.

In the early cerebritis stage, CT brain scans at Day 1 generally demonstrated low-density lesions with some degree of partial ring-like enhancement. By Day 3, there was a prominent ring enhancement, the diameter of which best correlated with the diameter of cerebritis (vessels with perivascular cuffing of inflammatory cells) present surrounding the developing necrotic center.

**Late Cerebritis (4 to 9 Days)**

The most significant histological changes began to take place on Day 4 and extended through Day 9. A
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Fig. 1. Photomicrographs showing early cerebritis (Days 1 to 3). A: On Day 1, an inflammatory infiltrate consisting of polymorphonuclear leukocytes, lymphocytes, and plasma cells was seen interspersed among the residual agarose (Zone 1). H & E, x 300. B: Early cerebritis on Day 1 consisting of a modest perivascular infiltrate as seen in this section around two vessels (Zone 4). H & E, x 300. C: On Day 1, there was no evidence of new reticulin formation in vessels adjacent to the developing necrotic center. Reticulin stain, x 300. D: There was prominent meningitis at the site of needle entry for inoculating the organisms into the brain during the early cerebritis stage. H & E, x 300. E: By Day 3, the degree of perivascular infiltrate or cerebritis markedly increased compared with Day 1 (B). H & E, x 300. F: By Day 3, there was early sprouting of reticulin, extending from the sides of a few vessels near the developing necrotic center. Reticulin stain, x 300.
Fig. 2. Photomicrographs showing late cerebritis (Days 4 to 9). A: The necrotic center had developed and consisted of inflammatory cells and debris (Zone 1). H & E, × 300. B: Along the margin of the necrotic center, a mixture of inflammatory cells, large foamy macrophages, and fibroblasts (spindle-shaped cells) was seen (Zone 2). H & E, × 300. C: New vessels formed around the necrotic center and had perivascular inflammatory infiltrates containing fibroblasts. H & E, × 300. D: In places along the periphery of the developing necrotic center, the reticulin stain demonstrated a marked proliferation of reticulin in areas comparable to photomicrographs B and C. × 300. E: Only a minimal amount of maturing collagen was associated with the neovascularity. Masson’s trichrome, × 300. F: During the cerebritis stage, a few reactive astrocytes were seen in the white matter overlying the developing abscess. GFAP, × 300.
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well formed necrotic center developed and enlarged, consisting of a mixture of tissue debris and inflammatory cells (Zone 2). The agarose was nearly depleted (Fig. 2A). At the periphery of the developing necrotic center, there was a zone of inflammatory cells, macrophages (foam cells), and scattered fibroblasts (Zone 2, Fig. 2B). The fibroblasts were characterized by oval nuclei with spindle-shaped cytoplasm. Cerebritis (Zone 4) was maximal during this time. In addition, the microscopic features of the perivascular cuffing changed with the appearance of fibroblasts in addition to inflammatory cells. The rapid increase in new vessel formation during this stage at Zones 3 to 4 is shown on H & E stain (Fig. 2C), reticulin stain (Fig. 2D), and Masson’s trichrome stain (Fig. 2E). By Day 7, there was a significant amount of reticulin bridging the neovascularity (Fig. 2D). Masson’s trichrome and HVG stains showed only modest amounts of maturing collagen in the immediate vicinity of blood vessels. There was still a considerable amount of white matter edema beyond Zone 4. Beginning on Day 7, GFAP-stained sections showed the presence of early reactive astrocytes in the area just outside the zone of cerebritis (Zone 5, Fig. 2F).

Late cerebritis was characterized on CT scan by a well formed ring 10 minutes after infusion of contrast medium (Fig. 3). The contrast medium gradually diffused into the lucent center and gave the appearance of a homogeneous lesion. This was the salient feature of cerebritis on CT in this experimental model.

Early Capsule Formation (10 to 13 Days)

At 10 to 13 days, the necrotic center (Zone 1) decreased slightly in size. There was a marked increase in the number of fibroblasts in the zone of inflammatory cells surrounding the necrotic center (Zone 2, Fig. 4A). By this time, a well developed layer of fibroblasts had been established. There was a significant difference in the amount of reticulin deposition on the cortical (Fig. 4C) and the ventricular (Fig. 4B) sides of the lesions. Mature collagen was seen bridging the spaces between vessels on Masson’s trichrome and HVG stains (Fig. 4E, Zone 3). Outside the developing capsule, there was a region of continued cerebritis and neovascularity (Fig. 4D, Zone 4). The number of reactive astrocytes in Zone 5 had increased by this time (Fig. 4F). The degree of edema in the white matter gradually began to diminish.

The CT appearance during the early stages of capsule formation was similar to that previously described for the cerebritis stage. The necrotic center seen on the CT scan was smaller, and this was verified histologically. Serial scans continued to show some diffusion of contrast material into the necrotic center.

Late Capsule Stage (14 Days and Later)

Histologically, the late capsule stage showed a continuation of the process already in progress. The necrotic center continued to get smaller in the majority of cases (Fig. 5A, Zone 1). In one lesion, however, a multiloculated abscess with daughter abscesses developed. In Zone 2, adjacent to the necrotic center, the percentage of inflammatory cells and macrophages decreased while fibroblasts increased (Fig. 5B). The capsule was well developed and was comprised of fibroblasts, reticulin, and mature collagen (Zone 3). The increased thickness of the capsule was seen on both reticulin (Fig. 5D) and Masson’s trichrome (Fig. 5E) stains. Mature collagen was present in abundance beginning in the 3rd week of abscess formation. The capsule increased in thickness due to the migration of fibroblasts migrating from new vessels adjacent to the capsule (Zone 4, Fig. 5F). Cerebritis, although present, continued to show fewer inflammatory cells (Zone 4, Fig. 5C). The number of reactive astrocytes continued to increase both in numbers and in the intensity

**Fig. 3.** Computerized tomography findings of late cerebritis. Sequential scans in a 5-day-old lesion after contrast infusion showed a gradual diffusion of the contrast medium into the lucent center forming a solid lesion at 0 to 5 minutes (left), 10 to 15 minutes (center left), 20 to 25 minutes (center right), and 45 to 50 minutes (right).
FIG. 4. Photomicrographs showing early capsule formation (Days 10 to 13). A: At the periphery of the necrotic center, the number of fibroblasts increased significantly (top right) (Zones 1 to 2). H & E, \( \times 300 \). B and C: Reticulin increased greatly, but there was an asymmetry in the majority of lesions, with more dense deposition on the cortical surface (C) compared with the ventricular surface (B) (Zone 3). Reticulin stain, \( \times 300 \). D: Cerebritis, although still present on the margin of the developing capsule, consisted of fewer inflammatory cells and more fibroblasts (Zone 4). H & E, \( \times 300 \). E: A dense deposition of mature collagen extended between new vessels which have developed (Zones 3 to 4). Masson's trichrome, \( \times 300 \). F: There was an increase in the number of reactive astrocytes in the surrounding white matter compared with the late cerebritis stage (Fig. 2F) (Zone 5). GFAP, \( \times 300 \).
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Fig. 5. Photomicrographs of late capsule formation (Day 14 and on). A: The necrotic center was filled with increasing amounts of acellular debris and was diminished in size (Zone 1). Inflammatory cells were still scattered throughout. H & E, × 300. B: At the periphery of the necrotic center, the number of fibroblasts continued to increase (top left, Zones 2 to 3). Foamy macrophages and a few inflammatory cells were present close to the necrotic center (Zone 2). H & E, × 300. C: Cerebritis was present outside the capsule associated with the neovascularity which had evolved (Zone 4). H & E, × 300. D: Reticulin increased in both density and amount in the well developing capsule. Reticulin stain, × 300. E: A thick, loosely woven collagen capsule matrix can be seen surrounding the necrotic center (Zone 3). Masson’s trichrome, × 300. F: The reticulin stain demonstrated the significant neovascularity present outside the capsule. It also demonstrated the process by which the capsule thickens: the neovascularity allowed increased numbers of fibroblasts to migrate into the area, which in turn generated reticulin and mature collagen. Reticulin stain, × 190.
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Fig. 6. Photomicrographs showing mature capsule formation. A: The degree and extent of reticulin formation continued to increase and, by the end of the 3rd week, a solid mature capsule was present surrounding a small necrotic center. Reticulin stain, x 300. B: A thick mature collagen capsule can be seen. Masson's trichrome, x 300. C: Reactive astrocytes proliferated and were most numerous in the overlying gray and subcortical white matter. Note the astrocytic foot-processes lining the blood vessel. GFAP, x 300.

The CT appearance of well encapsulated brain abscesses continued to show ring-like contrast enhancement 10 minutes after infusion. The necrotic center did not fill in by diffusion of contrast medium as seen in the lesions in the cerebritis stage on delayed scans (Fig. 7). Unfortunately, the majority of these experimental lesions had necrotic centers that were microscopic in size and therefore were below the spatial resolution of the CT scanner.

Discussion

Experimental Models of Brain Abscess

Experimental brain abscess models have been developed in the primate, dog, cat, rabbit, guinea pig and rat. The majority of authors have inoculated organisms in a suitable medium directly into the brain, but two studies utilized septic emboli to induce...
FIG. 7. Sequential computerized tomography (CT) findings from late cerebritis to a well encapsulated brain abscess. These serial CT scans were performed 10 to 15 minutes after contrast infusion. A: Day 5. The ring enhancement was thick in the late cerebritis stage and diffused into the lucent center. B: In the early capsule stage (Day 13), the ring enhancement was thinner and contrast material did not diffuse into the lucent center. C: In the late capsule stage (Day 19), the lucent center was much smaller, but it did not fill in with contrast material.

metastatic brain abscess formation. Histological evaluation of sequential stages of brain-abscess formation has been documented in relatively few of these studies.\textsuperscript{17-19,25,69,70}

In the present study, the dog was used to develop this model for two reasons: 1) the brain size was large enough to image with the resolution of current CT scanners, and 2) the response of the dog's central nervous system to infection is comparable to that of man. Although classical evolution of brain-abscess formation with encapsulation can occur in any of the animal models cited, there are species differences. In 1913, Homn\textsuperscript{e} used a mixture of streptococci, staphylococci, and Bacillus perfringens (Clostridium welchii) to obtain brain abscesses in dogs, rabbits, and guinea pigs. In the rabbit, all components of capsule formation were more poorly formed and appeared later than in the dog. It was Homn's conclusion that, in the dog, abscess formation approximated most closely the disease as it occurs in humans.

Species differences are of even more importance when it comes to manipulating an experimental model to study the effects of different treatment modalities or the influence of steroids on encapsulation.\textsuperscript{72} Long and Meacham,\textsuperscript{36} in a study using dogs, found that dexamethasone given at the time of inoculation with Staphylococcus aureus or Proteus mirabilis retarded, but did not totally inhibit, capsule formation. Dexamethasone started a few weeks after inoculation had no apparent effect on further encapsulation.\textsuperscript{36} Quar- tey, et al.,\textsuperscript{50} studied the effect of dexamethasone and antibiotics administered at the time of inoculation with Streptococcus pyogenes or Staphylococcus aureus on brain abscess formation in rabbits. In the rabbits treated with dexamethasone and antibiotics, no evidence of encapsulation was present at 10 days, and only necrotic areas could be found. The control animals had well encapsulated lesions. The difference in these two studies can be explained by immunological studies which suggest that rats, mice, and rabbits are extremely sensitive to corticosteroids with respect to lymphoid depletion, circulating antibody production, and cell-mediated immunity, whereas man, guinea pig,\textsuperscript{11} and probably dogs are more resistant to steroids.

The formation of the collagen capsule in a developing abscess is the single most important response that limits the spread of infection in the brain. The biosynthesis of collagen has largely been worked out biochemically,\textsuperscript{48,49} and is known to originate from fibroblasts derived from the connective tissue elements associated with blood vessels.\textsuperscript{10,49,50} With respect to brain abscess, two particular features of collagen formation are of interest. The first is that encapsulation is more rapid on the cortical side of the lesion compared with the ventricular side. The second is the relatively limited encapsulation seen with metastatic brain abscesses caused by septic emboli, as compared with those caused by direct extension or trauma. Both of these features of encapsulation can be explained by the fact that oxygen is required for pro-alpha chains to form the triple-helix strands of collagen.\textsuperscript{10,49} The increased vascularity of normal cortical gray matter allows for faster proliferation of new capillaries, which in turn release more fibroblasts in a relatively oxygen-rich environment compared with the more poorly perfused deep white matter. The thinner capsules of metastatic brain abscesses\textsuperscript{73,74} can also be explained by this theory. The vegetative embolus initially causes an infarct. This area of nonviable tissue is hypoxic and impedes new vessel formation and the collagen-forming process of the fibroblasts.

The glial reaction of the brain to abscess formation
has not previously been studied in detail. In this model, two stains were used to study this response: Mallory's phosphotungstic acid hematoxylin (PTAH) and the immunocytochemical localization of the glial fibrillary acidic protein (GFAP). The GFAP first described by Eng, et al., has been used extensively to identify astrocytes, both of neoplastic and non-neoplastic origins. The GFAP is found in normal fibrillary astrocytes, and questionably in normal protoplasmic astrocytes, Bergmann glial cells, reactive astrocytes, and reactive fibrillated ependymal cells. It is not found in fibroblasts, microglial cells, or macrophages. This study represents its first use to study intracranial infection and also its first use to demonstrate reactivity in dog brain. This study confirms the previously reported finding that there is not always a direct relationship between GFAP-positivity and the demonstration of glial fibers by traditional neurohistological stains (PTAH and Holzer crystal violet stains). Whereas GFAP does not stain well the intricate and closely textured network of glial cell processes and fibers of white matter, it does stain intensely the perikarya and cell processes of the reactive astrocytes in cortical gray matter. It has been hypothesized that the soluble form of GFA protein is active astrocytes in cortical gray matter. It has not previously been studied in detail. In this model, two stains were used to study this response: Mallory's phosphotungstic acid hematoxylin (PTAH) and the immunocytochemical localization of the glial fibrillary acidic protein (GFAP). The GFAP first described by Eng, et al., has been used extensively to identify astrocytes, both of neoplastic and non-neoplastic origins. The GFAP is found in normal fibrillary astrocytes, and questionably in normal protoplasmic astrocytes, Bergmann glial cells, reactive astrocytes, and reactive fibrillated ependymal cells. It is not found in fibroblasts, microglial cells, or macrophages. This study represents its first use to study intracranial infection and also its first use to demonstrate reactivity in dog brain. This study confirms the previously reported finding that there is not always a direct relationship between GFAP-positivity and the demonstration of glial fibers by traditional neurohistological stains (PTAH and Holzer crystal violet stains). Whereas GFAP does not stain well the intricate and closely textured network of glial cell processes and fibers of white matter, it does stain intensely the perikarya and cell processes of the reactive astrocytes in cortical gray matter. It has been hypothesized that the soluble form of GFA protein is a subunit or precursor of glial fibers. Indirect evidence suggests the insoluble form of GFAP is associated with glial filaments, and perhaps this explains the difference in the results obtained with the two stains. In this study, the reactive astrocytes that stained with GFAP were most marked in the cortical gray and subcortical white matter, although glial fibrils were prominently stained in some lesions at 3 weeks in areas adjacent to the capsule. In contrast, the PTAH demonstrated gliosis and cells of astrocytic origin maximally in the area immediately adjacent to the outer edge of the capsule. By Day 7, GFAP was weakly reactive in a few astrocytes, but it was not until the end of the 2nd week that significant numbers of reactive astrocytes were observed. By the end of the 3rd week, this process was even more evident using both GFAP and PTAH stains.

Clinical Correlates of this Experimental Study

Brain abscesses have been associated with a high mortality (29% to 64%) and morbidity, despite the advances of antibiotic therapy. Lack of clinical suspicion and poor localization were often cited as reasons for this high mortality. With the advent of CT brain scanning, earlier diagnosis and accurate localization of intracranial suppuration have resulted in a decreased mortality resulting from brain abscess. Management of brain abscess cases in recent years has focused on nonsurgical treatment in which antibiotics alone are used. The accuracy of CT scanning has aided in this approach, allowing the size of the lesions to be followed. Although classical "ring" enhancement has been equated with capsule formation, this study conclusively demonstrated ring enhancement during the cerebritis stage prior to any evidence of capsule formation. Ring enhancement correlated closely with the extent of cerebritis (perivascular infiltration of inflammatory cells), and is likely due to altered permeability of the normal blood-brain barrier. Rapid proliferation of new vessels associated with cerebritis was seen in this study in the area surrounding the developing abscess. Studies have shown that proliferating capillaries leak protein-containing fluid due to incomplete sealing of the endothelial lining. In a study of regeneration of brain capillaries after local freezing injury, it was demonstrated that the new vessels had endothelial cells which lacked interconnecting tight junctions.

Some preliminary clinical evidence in the literature supports the findings in this experimental study. A recent case report of fatal cerebritis in man due to Clostridium septicum showed a ring-like contrast-enhancing lesion with no evidence of capsule formation histologically. Whelan and Hila also suggested that the results of this study are applicable to man. In a series of 20 cases of brain abscess, 13 patients required surgery because of failure to improve on antibiotics alone or because of clinical deterioration. However, a firm capsule was encountered at surgery in only eight patients. In all eight cases, the symptoms had been present for longer than 2 weeks. In the five patients not having capsules at surgery, all had ring-like contrast-enhancing lesions which the authors felt represented cerebritis. Since delayed scans were not performed in these studies, it is not known whether the results would have been similar to those of our experimental model. Some of the reports purporting to have cured brain abscess with antibiotics only may have been due to the fact that the lesions were in the cerebritis stage, since the CT scans showed significant diffusion of contrast into the necrotic center.

Clinically, the cerebritis and well encapsulated stages may require different treatment. One clinical study demonstrated that patients with well encapsulated lesions, who had adequate killing levels of antibiotic in the abscess fluid, continued to deteriorate clinically and required aspiration. It was possible to grow out the offending organisms in each case. Whelan and Hila reported that despite administration of intravenous antibiotics for a minimum of 2 days, eight of 13 surgical specimens had positive cultures.
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Ideally, one would like precise criteria that separate the cerebritis stage from the encapsulated stage. Clinically, the first step is treatment with appropriate antibiotics. If a presumptive organism is not known, then one should seriously consider aspirating the lesion(s), under CT guidance if necessary, to obtain material for culture. This is particularly true in the immunocompromised host, since the offending organism may be either bacterial, fungal, or protozoan in nature. On the basis of this study, we suggest the use of delayed CT scans at least 30 minutes after contrast infusion so that the degree of contrast diffusion into the lucent center can be evaluated. If the contrast material diffuses completely into the necrotic center, then one can presume that the lesion is in the cerebritis stage and continue the antibiotics alone, providing the patient is stable neurologically. If the contrast material does not diffuse into the center, a well encapsulated lesion is probably present. The approach will then depend on the size of the abscess and the clinical condition of the patient. Abscess size may be the determining factor whether antibiotics alone will be effective or whether surgical aspiration or excision will be required. Two studies have shown that lesions greater than 4 cm in size usually require surgical decompression whereas lesions of less than 2 cm will respond to conservative therapy. Unfortunately, data are not available in these studies correlating the size of the lesion and the degree of encapsulation. It is likely that some lesions in the developing stages of encapsulation will require surgical decompression because of clinical deterioration. Future work on this experimental model will concentrate on developing additional methods for separating the cerebritis and encapsulated stages, and will hopefully lead to precise criteria for determining when surgical intervention is required or when antibiotic therapy alone will succeed in curing brain abscesses.

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