Role of vascular proliferation on angiographic appearance and encapsulation of experimental traumatic and metastatic brain abscesses

JAMES H. WOOD, M.D., JOHN L. DOPPMAN, M.D., WILLIAM E. LIGHTFOOTE, II, M.D., MARY GIRTON, R.T., AND AYUB K. OMMAYA, M.D.

Surgical Neurology and Experimental Therapeutics Branches, National Institute of Neurological and Communicative Disorders and Stroke, and Department of Diagnostic Radiology, National Institutes of Health, Bethesda, Maryland

Experimental traumatic abscesses were produced in rhesus monkeys by intracerebral injection of nutrient agar contaminated with *Staphylococcus epidermidis*, and metastatic abscesses were induced by intracarotid embolization of silicone cylinders contaminated with *Staphylococcus epidermidis*. All monkeys underwent preoperative and serial postoperative carotid angiography. Traumatic abscesses produced early capsular blushes and progressive anterior cerebral artery displacements. Metastatic abscesses induced transient midline shifts but no capsular stains. Postmortem studies on the monkeys showed that mean capsular thickness and segmental wall vascularity of the traumatic and metastatic abscesses were significantly different (p < 0.001), despite equal abscess ages and similar abscess volumes. In comparison to traumatic abscesses, metastatic abscesses demonstrated reduced inflammatory cell infiltration and retarded collagen formation around proliferating capsular vessels. Brain surrounding the metastatic abscesses demonstrated ischemic changes. The results suggest that 1) capsular blushes during cerebral angiography are secondary to vascular proliferation within the capsule and not to compression of surrounding brain, 2) vascular staining reflects capsular thickness, 3) capsular vascularity contributes to collagen formation, 4) encapsulation is dependent upon the integrity of surrounding brain, and 5) adjacent cerebral ischemia may impede inflammatory responses involved in capsule formation.

**KEY WORDS** • brain abscess • cerebral angiography • infection • embolic infarction • computerized tomography

In a recent review in a civilian population, approximately 70% of brain abscesses were either traumatic or metastatic in origin. The overall mortality for cerebral abscesses has not decreased, despite efforts at early detection employing cerebral angiography and computerized tomography (CT). Cerebral angiography has been reported to be 90% accurate in the localization but only 61% accurate in the diagnosis of brain abscesses. The most common angiographic finding is an avascular, rather ill-defined mass lesion with shift of midline structures. The similarity of angiographic presentation to that of intracerebral avascular tumors,
Experimental brain abscesses

hemorrhage, and edema has contributed to misdiagnosis in patients with brain abscesses. The failure of cerebral angiography to reveal multiple metastatic brain abscesses has also been reported.

Unfortunately, the angiographic “ring-sign” has been visualized in only 20% to 30% of confirmed cases. This vascular blush as described by Chou, et al., is thought to result from compression of adjacent normal brain, vascular proliferation in the capsular wall and surrounding zone of granulation tissue, or reactive hyperemia. When present, this sign has been associated with well encapsulated lesions and favors surgical resection. However, this angiographic presentation has also been associated with metastatic neoplasms undergoing central necrosis.

This study correlates the pathophysiology of capsular formation with the angiographic features of cerebral abscesses produced experimentally by direct bacterial implantation and septic embolization.

**Materials and Methods**

Twenty-one adult rhesus monkeys (Macaca mulatta), weighing 4 kg each, were sedated with 1 mg/kg phencyclidine hydrochloride (Sernylan) and anesthetized with incremental intravenous sodium thiomyylal (Surital) for each procedure. Before surgery, each animal underwent carotid angiography at 3:1 magnification by femoral catheterization using meglumine diatrizoate and sodium diatrizoate (Renografin 76). A calibration bar with 1.0-cm markings was positioned along the midtransverse plane during anteroposterior skull projections and along the mid-sagittal plane during lateral skull projections for measurement corrections.

Five animals underwent right parietal trephination and intracerebral injection of 0.05 ml of a 2% nutrient agar solution contaminated with pure cultures of Staphylococcus epidermidis (penicillin sensitive) through the intact dura mater using a No. 30 needle as described by Hassler and Forsgren. Three control animals underwent similar trephination and direct intracerebral injection of 0.05 ml of sterile 2% nutrient agar solution. After scalp closure, these intracerebrally inoculated animals received a single intramuscular dose of 600,000 units of procaine penicillin G in oil.

The technique described by Molinari and coworkers was used in the right carotid embolization of eight monkeys with 1.5 × 8-mm cylinders contaminated with pure cultures of Staphylococcus epidermidis (penicillin sensitive). Five control animals were similarly embolized with sterile silicone cylinders. These two groups of embolized monkeys received a single 600,000 unit dose of intramuscular procaine penicillin G in oil to prevent mycotic aneurysm formation and fatal subarachnoid hemorrhage.

All surviving animals that were injected with contaminated nutrient agar or embolized with septic cylinders underwent cerebral angiography at 2-week intervals until sacrifice. Those surviving monkeys that were injected with sterile agar or embolized with sterile cylinders underwent angiography as noted in Tables 1 and 2. Displacement of the anterior cerebral artery branches greater than 2.0 mm was defined as a significant shift across the midline in the anteroposterior projections.

Cerebral angiography was performed during the sixth postoperative week, then needle aspiration of the brain abscesses was attempted, and specimens, when available, were aerobically and anaerobically cultured. Thereafter, the animals were perfused with physiologic saline followed with 10% buffered formalin. After removal, the brains were stored in 10% buffered formalin for 1 week. The brains were then cut into standard 4.5 mm-thick coronal sections.

Sections were stained with hematoxylin and eosin, luxol fast blue-periodic acid-Schiff, Masson's trichrome, and van Gieson's stains. Sections from each brain slice were stained simultaneously, using uniform techniques in order to minimize errors secondary to tissue shrinkage. All stained sections were photographed at × 34, × 40, × 55, and × 80 magnification, using the same microscope. A 1.0-mm grid calibrated in 0.1-mm markings was also photographed at these same magnification settings and the negative images were used for measuring distances on the photographs of the stained sections.

Capsule thickness was determined by averaging the thickness measurements of the most superficial and deepest portions of the

---

*Microfil manufactured by Canton-Biochemical Products, P.O. Box 2017, Boulder, Colorado.*
TABLE 1

Angiographic and pathological data with respect to septic and sterile agar injection into brain*

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Cerebral Angiography</th>
<th>Aspirate Cultures</th>
<th>Abcess Volume (cu mm)</th>
<th>Capsule Thickness (mm)</th>
<th>Segmental Vascularity (vessels/mm)</th>
<th>Vascular Density (vessels/sq mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>septic agar injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X493</td>
<td>B, ND</td>
<td></td>
<td></td>
<td>817</td>
<td>1.40</td>
<td>54</td>
</tr>
<tr>
<td>X602</td>
<td>B, D</td>
<td></td>
<td></td>
<td>1376</td>
<td>1.65</td>
<td>68</td>
</tr>
<tr>
<td>X669</td>
<td>B, D</td>
<td></td>
<td></td>
<td>904</td>
<td>1.45</td>
<td>67</td>
</tr>
<tr>
<td>X670</td>
<td>B, ND</td>
<td></td>
<td></td>
<td>1047</td>
<td>1.60</td>
<td>85</td>
</tr>
<tr>
<td>X827</td>
<td>B, ND</td>
<td></td>
<td></td>
<td>660</td>
<td>1.40</td>
<td>63</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td>961 ± 121</td>
<td>1.50 ± 0.05</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>sterile agar injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A483</td>
<td>NB, ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A605</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B199</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: B = vascular blush; NB = no vascular blush; D = lateral displacement of anterior cerebral artery branches greater than 2.0 mm on anteroposterior cerebral angiograms; ND = no displacement of anterior cerebral arteries; S. = Staphylococcus; SEM = standard error of mean.

Abscess wall. Segmental vascularity was assessed by counting the number of blood vessels in a 1.0-mm section of the capsule wall as measured on the calibrated photomicrographs. Vascular density of each abscess capsule was defined as the number of vessels counted as the number of vessels counted in a 1.0-mm section of capsule wall divided by the capsule thickness and reported in vessels/sq mm.

Results

All five animals inoculated with contaminated nutrient agar developed intracerebral abscesses, and *Staphylococcus epidermidis* organisms were cultured from abscess aspirates in four of these monkeys. No brain abscesses occurred in the three animals inoculated with sterile agar and no

TABLE 2

Angiographic and pathological data with respect to septic and sterile intracarotid embolization*

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Cerebral Angiography</th>
<th>Aspirate Cultures</th>
<th>Abcess Volume (cu mm)</th>
<th>Capsule Thickness (mm)</th>
<th>Segmental Vascularity (vessels/mm)</th>
<th>Vascular Density (vessels/sq mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>septic embolization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X139</td>
<td>NB, ND</td>
<td></td>
<td></td>
<td>817</td>
<td>0.20</td>
<td>10</td>
</tr>
<tr>
<td>X381</td>
<td>NB, D</td>
<td></td>
<td></td>
<td>736</td>
<td>0.35</td>
<td>16</td>
</tr>
<tr>
<td>A320</td>
<td>NB, ND</td>
<td></td>
<td></td>
<td>697</td>
<td>0.25</td>
<td>13</td>
</tr>
<tr>
<td>A615</td>
<td>NB, D</td>
<td></td>
<td></td>
<td>1150</td>
<td>0.40</td>
<td>16</td>
</tr>
<tr>
<td>A616</td>
<td>NB, D</td>
<td></td>
<td></td>
<td>904</td>
<td>0.45</td>
<td>26</td>
</tr>
<tr>
<td>A772</td>
<td>NB, D</td>
<td></td>
<td></td>
<td>524</td>
<td>0.40</td>
<td>18</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td>805 ± 86</td>
<td>0.34 ± 0.04</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>B69</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X129</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterile embolization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A624</td>
<td>NB, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A792</td>
<td>NB, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A787</td>
<td>NB, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A316</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y209</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: B = vascular blush; NB = no vascular blush; D = lateral displacement of anterior cerebral artery branches greater than 2.0 mm on anteroposterior cerebralangiograms; ND = no displacement of anterior cerebral arteries; S. = Staphylococcus; SEM = standard error of mean.
Experimental brain abscesses

Fig. 1. Anteroposterior (left) and lateral (right) cerebral angiograms during the sixth postoperative week. Upper: Vascular stain (arrows) and displacement of anterior cerebral artery branches away from abscess produced by intracerebral injection of septic nutrient agar. Lower: Occlusion of horizontal segment of right middle cerebral artery with contaminated silicone cylinder in monkey with metastatic abscess. Note absence of vascular blush and shift of anterior cerebral arteries.

Aspirates were obtained. Among the eight animals that underwent carotid embolization with contaminated cylinders, six developed brain abscesses, and Staphylococcus epidermidis organisms were cultured from abscess aspirates from three of these animals. Abscess fluid aspirated from one septicily embolized monkey was sterile, and no aspirate was obtained from the remaining two animals before perfusion. Two monkeys died within 24 hours after septic embolization as the result of massive ischemic anterior paramedian infarctions similar to those noted by Laurent, et al.14 Among the five monkeys that underwent embolization with sterile cylinders, three surviving animals developed bland infarctions without suppuration. One monkey with massive ischemic infarction of the anterior paramedian region and another with a massive hemorrhagic infarction died 24 hours after sterile embolization. Needle aspirations in these animals were unproductive (Tables 1 and 2).

Cerebral angiography during the second postoperative week demonstrated vascular stains in all five monkeys with intracerebral injections of septic agar. Contralateral displacement of the anterior cerebral artery branches was noted in two of these animals. Cerebral angiography during the sixth postoperative week revealed mild enlargements of the capsular stains in four of these monkeys and anterior cerebral artery shifts in all five animals (Fig. 1 upper). No vascular bluses or anterior cerebral artery shifts were observed in angiograms of the three animals.
Fig. 2. Coronal brain sections through optic chiasm and basal ganglia. Left: Brain abscess produced by intracerebral instillation of contaminated nutrient agar exhibits thick capsule wall surrounding abscess and needle tract. Note massive shift of midline structures away from abscess. Right: Brain abscess induced by intracarotid embolization of septic silicone cylinder demonstrates retarded encapsulation. Note cavitation of infarcted paramedian structures surrounding abscess and absence of midline displacement.

Fig. 3. Photomicrographs of segment of abscess wall during sixth postoperative week. Luxol fast blue-PAS, × 40. Left: Prominent capsule wall induced by direct intracerebral injection of contaminated agar demonstrates central necrotic layer, thick medial band of acute inflammatory cells surrounded by layer of prolific vascularization and fibrosis, and outer layer of gliosis with prominent perivascular infiltration by inflammatory cells. Right: Meager capsule wall produced by septic embolization demonstrates few inflammatory cells in medial reactive layer and in perivascular spaces in encephalitic zone. Note ischemic changes in surrounding brain.
Experimental brain abscesses

undergoing intracerebral instillation of sterile nutrient agar.

Vascular stains were absent in cerebral angiograms obtained serially in the six animals that developed abscesses after septic embolization. Two weeks after embolization, cerebral angiography demonstrated anterior cerebral artery displacement in four of these animals; however, no shift of these midline vessels was present on later angiograms (Fig. 1 lower).

No angiographic blushes were noted in the three surviving monkeys that underwent carotid embolization with sterile silicone cylinders. Displacement of the anterior cerebral artery branches to the left was noted during angiography in each of these animals 2 weeks after embolization, however these shifts were absent on subsequent angiograms.

The mean gross estimates of abscess volumes were 961 ± 121 cu mm (SEM) in the five septically injected monkeys and 805 ± 86 cu mm in the six animals developing abscesses after septic embolization (Fig. 2). Assuming abscesses to be spherical, these mean abscess volumes were not statistically different.

Microscopic examination of stained sections of those abscesses produced by direct intracerebral injection of contaminated agar demonstrated four layers: a central necrotic core, a thick medial band of acute inflammatory cells surrounded by a layer of prolific vascularization and fibrosis, and an outer zone of gliosis with prominent perivascular infiltration by inflammatory cells (Fig. 3 left). More specifically, sections prepared with Masson’s trichrome and van Gieson’s stains revealed profound proliferation of connective tissue surrounding capsular blood vessels (Figs. 4 and 5 left). Stained sections of the abscesses produced by septic embolization also demonstrated necrotic, reactive, fibrous, and encephalitic zones; however, few inflam-

![Fig. 4. Photomicrographs of segment of abscess capsule during sixth postoperative week. van Gieson, X 40. Left: Thick capsule wall induced by intracerebral instillation of contaminated agar demonstrates extensive proliferation of connective tissue associated with capsular blood vessels. Right: Disintegra-

tion with cavitation of devitalized brain surrounding abscess capsule produced by septic embolization.](Image)

FIG. 5. Photomicrographs of segment of abscess wall during sixth postoperative week. Masson's trichrome, × 55. Left: Prominent capsule wall induced by intracerebral instillation of septic nutrient agar demonstrates profound proliferation of collagen surrounding capsular blood vessels. Right: Meager capsule wall produced by septic embolization demonstrates impedence of connective tissue formation and vascular proliferation within fibrous layer. Note condensation of neurophil surrounding abscess capsule.

Inflammatory cells were noted in the medial reactive layer and in the perivascular spaces in the encephalitic zone (Fig. 3 right). In addition, the connective tissue formation and vascular proliferation within the fibrous layer of these metastatic abscesses were diminished (Figs. 4 and 5 right) as compared with that of the traumatic abscesses (Figs. 4 and 5 left).

The mean capsular thickness of abscesses developed in animals following direct intracerebral injection of contaminated agar was 1.50 ± 0.05 mm and that of abscesses produced in the six septic embolized monkeys was 0.34 ± 0.04 mm. This difference in the mean capsular thickness for these two groups is statistically significant (p < 0.001, Student's t-test). Segmental vascularity of the capsule wall in the monkeys injected with infected agar was 67 ± 5 vessels/mm and that in the surviving animals with septic embolization was 17 ± 2 vessels/mm. This difference was also significant (p < 0.001). The mean vascular density of the capsules among the monkeys that underwent intracerebral septic agar injections was 45 ± 3 vessels/sq mm and that for the six embolized animals with abscesses was 49 ± 3 vessels/sq mm. These means of capsular vessel density were not significantly different.

Pathological examination of the four monkeys that died within 24 hours after intracarotid embolization revealed deep anterior paramedian infarctions or hemorrhage involving the pallidum, caudate nucleus, putamen, internal and external capsules, claustrum, and portions of the cortex. Massive swelling of the infarcted hemisphere with uncal herniation and secondary mid-
Experimental brain abscesses

brain lesions were also observed in these animals. The pattern of ischemic infarction occurring in the surviving animals with embolization included the right paramedian region, but spared the globus pallidus and caudate nucleus. Infarct cavitation was prominent within these devitalized areas (Fig. 4 right), but no evidence of cerebral swelling, uncal herniation, or midbrain lesions was noted even in the presence of an adjacent abscess.

Discussion

Our experimental study correlated the appearance of the "ring-sign" on cerebral angiography with pathological evidence of thick abscess encapsulation in all of our animals which had undergone intracerebral instillation of contaminated agar. In addition, all of our surviving monkeys which had been septically embolized failed to demonstrate vascular blushes during cerebral angiography and adequate capsular formation at sacrifice. The mean thickness of the wall and mean vascularity of the capsules in the former group of animals were approximately four times greater than those of the latter group; however, mean capsular vessel densities of these two groups of monkeys were similar. Thus, the appearance of this angiographic blush seems to depend upon the thickness of the capsule wall rather than upon differences in capsular vascular density. In addition, the similarity of the mean abscess volumes for these two groups of animals suggests that compression of adjacent brain does not contribute to the appearance of the vascular stain.

Experimental brain abscesses have been produced both by direct injection of bacteria into a focus of altered cerebral tissue and intracarotid injection of septic silicone emboli. Each method provides stasis of the organisms in a focus of ischemic or necrotic brain tissue. In our study, the direct injection of septic agar into the cerebrum simulated traumatic brain contamination and abscess formation. The abscesses formed by the septic intracarotid embolization technique served as a primate model for the study of metastatic brain abscesses. Although our experimental investigations demonstrated vascular blushes in only those animals injected with septic agar, clinical studies indicate that cerebral angiography cannot differentiate traumatic from metastatic brain abscesses on the basis of the presence or absence of a capsular stain.

Early experimental investigations have indicated that abscess duration and bacterial type are important variables in capsular formation. The degree of encapsulation in our monkeys which received intracarotid injections of contaminated agar was significantly greater than that in our animals with septic embolization, despite equal abscess ages in both of these experimental groups. The absence or presence of bacteria in abscess aspirate cultures did not appear to contribute to the differential encapsulation noted in our two groups of monkeys inoculated septic. These data indicate that other factors may make greater contributions to abscess capsule formation in the brain.

Agents that impede the processes of inflammation and immunity have been found to retard but not eliminate the formation of the inflammatory capsule and preclude the clearance of organisms in experimental brain abscesses. Retarded vascular proliferation, fibrosis, and inflammatory cell infiltration were noted on pathological examination of abscess walls obtained from our monkeys with septic embolization. This observation suggests the possibility that similar inflammatory or immunological deficiencies may exist in devitalized regions of brain after ischemic infarction.

The brain abscesses produced by direct bacterial injection in our animals were surrounded by thick capsules. The pathological appearance of these experimental abscesses was similar to those described in human studies insofar as the four characteristic layers were displayed: the necrotic, reactive, fibrous, and encephalitic zones. The abundant capsular collagen noted in our sections stained specifically for fibrous tissue originated from the proliferating small blood vessels in the reactive zone. Contrarily, in our septicallly embolized animals with abscesses, encapsulation and inflammatory responses appeared impeded. Local ischemia caused by the occlusion of the middle cerebral artery may not have adequately supported capillary proliferation, which in turn might have retarded fibrosis of the capsular wall. In our study, capsule wall thickness varied directly with capsular segment vascularity but
not with blood vessel density. This finding suggests the presence of a positive relationship between capsular vascularity and collagen deposition.

The proliferation of fibroblasts from the reactive vessel walls depends upon the vascularity of the surrounding brain. Gray matter is relatively more vascular than white matter. The slower progression of encapsulation in white matter is thought to account for the tendency of cerebral abscesses to extend toward the ventricles. The abscess sites in our animals were similar, thus normal variations in brain vascularity did not contribute significantly to the differences in encapsulation observed in our two experimental groups.

In accordance with human studies, the most common angiographic finding in our monkeys with brain abscess was displacement of midline structures. Among the animals with intracerebral instillation of contaminated agar, 40% had anterior cerebral artery displacement by the second postoperative week, but all had such findings at sacrifice. This displacement of midline structures appears to be the result of abscess growth because no shifts were noted in the animals with sterile agar injections.

Among the surviving monkeys with septic embolization, the anterior cerebral arterial branches were displaced by the second postoperative week in 60%. Unlike the animals with contaminated agar injection, none of the animals with septic embolization had midline shifts during the sixth postoperative week. Early shifts of midline structures were also noted to occur in those monkeys with sterile cylinder embolization, and these displacements were not present at the time of sacrifice. These data suggest that early anterior cerebral artery shifts may have been secondary to postinfarction edema. Later, cavitation of necrotic brain with resolution of edema may have spacially compensated for the expanding mass of the developing abscess.

Although this experimental study is restricted to the angiographic alterations induced by abscess capsule formation, our findings are consistent with clinical investigations by CT scanning. Since the appearance of brain abscesses seen on cerebral angiography and enhanced CT scans depends upon pathological vascular alterations, our study suggests that devitalization of brain surrounding the suppurative foci might also compromise capsular visualization during CT scanning.

References

Experimental brain abscesses


This paper was presented at the Annual Meeting of the American Association of Neurological Surgeons, Toronto, Canada, April 25–28, 1977. Address reprint requests to: James H. Wood, M.D., Division of Neurosurgery, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104.