

Granulomatous Encephalitis, Intracranial Arteritis, and Mycotic Aneurysm Due to a Free-living Ameba

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Summary. Primary amebic meningoencephalitis and granulomatous amebic encephalitis are well recognized clinicopathological entities caused by free-living amebas. Associated arteritis and "mycotic aneurysms" with infiltration of intracranial arteries by lymphocytes, amebic trophozoites and cysts have not been previously reported.

A 26-month-old girl had a 3-week history of encephalitis, characterized, initially, by vomiting and low-grade fever. Subsequently, she developed ataxia, generalized weakness, lethargy, and esotropia. The first CSF showed 490 RBC/ μ l, 705 WBC/ μ l with 90% mononuclears. Her pupils reacted briskly to light. Moderate nuchal rigidity, nystagmus, fixed downward gaze, anisocoria, bilateral 6th nerve palsy, left arm monoparesis and left Babinski were present. CAT scan revealed slight symmetrical dilatation of anterior horns of lateral ventricles and an area of abnormal enhancement above the 3rd ventricle. She died 14 days after admission, 5 weeks after onset of symptoms.

The brain showed focal necrotizing encephalopathy, involving thalami, cerebellum, brain stem, and cervical and upper thoracic spinal cord. Numerous free-living amebic trophozoites and cysts were present within a chronic granulomatous encephalitis. There were thrombosis of basilar, posterior cerebral, and vertebral arteries with profuse chronic panarteritis, fibrinoid necrosis, and mycotic aneurysms.

Key words: Free-living amebas — Encephalitis — Granuloma — *Acanthamoeba* — *Naegleria* — "Mycotic aneurysm"

Increasing interest in the pathogenicity and the biological behavior of free-living amebas belonging to the genera *Naegleria* and *Acanthamoeba*, have been noted recently in the medical literature. However, the disease may be very old and unrecognized cases may have occurred [8].

The most frequent etiologic agents responsible for primary amebic meningoencephalitis (PAM) and granulomatous amebic encephalitis (GAE) are free-living amebas of the genera *Naegleria* [4, 5, 9, 20] and *Acanthamoeba* [2, 10, 11, 13, 18, 27, 28] or closely related genus [7]. To date, more than 100 cases of PAM and nearly 20 cases of GAE have been reported. Some "extracerebral" infections have also been found due to *Acanthamoeba* sp. [3, 12, 16, 25].

PAM usually occurs in healthy, young individuals with a recent history of swimming in man-made lakes, heated swimming pools, and warm springs. Apparently the infecting cysts or trophozoites might be transported by the air [15]. Free-living amebas have been isolated from the air, [14] from drinking water [22] from the nasal passages and throats of clinically normal individuals [30, 33], or as contaminant in bacterial [29] and tissue cultures [24]. *Naegleria fowleri* produces an acute, fulminant, necrotizing hemorrhagic meningoencephalitis affecting the olfactory bulbs, base of the frontal and temporal lobes, and cerebellum. The portal of entry is through the olfactory neuroepithelium by direct penetration of sustentacular cells and invasion of the amyelinic submucosal nervous plexuses by the trophozoites. No cysts are found in the lesions.

GAE due to *Acanthamoeba* sp. is probably an opportunistic infection [21] of the CNS which has occurred in chronically ill and immunosuppressed patients without a history of recent swimming. The portal of entry into the CNS may be from the lower

respiratory tract or from a skin lesion, reaching the CNS through the hematogenous route. The predominant host reaction is a subacute or chronic granulomatous encephalitis composed of lymphocytes, plasma cells, monocytes, and multinucleated giant cells, hence the name granulomatous amebic encephalitis. Cysts, as well as trophozoites, may be found within the CNS lesions. The most affected areas are the midbrain, basal ganglia, and posterior fossa structures.

Because pathogenic free-living amebas are widespread in nature, and man is frequently exposed to them, public health implications are obvious.

We report a case of granulomatous amebic encephalomyelitis in a 26-month-old girl, which was not caused by *Naegleria* and probably not by *Acanthamoeba*.

Report of a Case

A 26-month-old girl was admitted to the hospital with abrupt vomiting and low-grade fever.

Two days before her illness began, she waded in Natchez river, Texas. Three weeks before admission, her coordination was impaired and she became less active. Eight days before admission, she was admitted to a hospital for a day because of vomiting, lethargy, weakness, and esotropia of the left eye. She was given Donnatal and discharged. Four days later she was readmitted with persistent signs and symptoms, and generalized, red skin welts. Two days after admission the skin lesions disappeared.

The cerebrospinal fluid (CSF) examination showed 490 RBC/ μ l and 705 WBC/ μ l with 90% mononuclears. On the second hospital day the CSF revealed 97 RBC/ μ l, 122 WBC/ μ l with 99% mononuclears; protein, 322 mg/dl; and glucose, 20 mg/dl. She failed to improve after administration of Doxycycline and was transferred to another hospital.

Examination at the children's hospital revealed a temperature of 38.4°C, pulse 116/min, respirations, 36/min, and blood pressure, 90 mm/Hg systolic. Moderate nuchal rigidity was noted. The left pupil was 1 mm and the right was 2 mm; they reacted briskly to light. There was downward nystagmus, downward gaze deviation, bilateral 6th nerve palsy and a Babinski sign on the left. She was obtunded. The hematocrit was 33%; WBC, 8,200/ μ l with 61% segmented neutrophils; electrolytes, glucose, and BUN were normal. The CSF had an opening pressure of 225 mm/H₂O; 62 RBC/ μ l, 280 WBC/ μ l with 94% mononuclears; protein 350 mg/dl and glucose, 24 mg/dl. Gram stain and india ink preparation were negative. Amebas were searched for, but not identified in a wet preparation of CSF. Counter immunoelectrophoresis on serum and CSF was negative for *Haemophilus influenzae* b; *Neisseria meningitidis* A, C, and D; and *Pneumococcus* Omni A through F. Fluorescent staining of smears of sediment of urine, CSF and gastric aspirate were negative for acid fast bacilli. Complement fixation titers for cytomegalovirus, herpes, and mycoplasma were 1:32, 1:8, and 1:8, respectively. The spot screen and presumptive tests for infectious mononucleosis were negative. Blood and CSF cultures were subsequently negative. Chloromycetin, 100 mg/kg in four daily doses, Isoniazid, 20 mg/kg/day in two daily doses, and Rifampin, 20 mg/kg/day in two daily doses were administered.

An EEG 1 day after admission showed diffuse slow wave activity without focality. Computerized axial tomography (CAT) showed asymmetrical ventricular dilatation and, with the addition of contrast material, a midline area of enhancement was present above the third ventricle consistent with an inflammatory or vascular lesion.

She continued to deteriorate, responded only to deep painful stimuli and required assisted ventilation on the third day of hospitalization. Decadron, 3 mg, four times a day, was administered without relief. Chloramphenicol was discontinued. The following day, the serum electrolytes were as follows: sodium, 125 mEq/l; potassium, 3.8 mEq/l; chloride, 88 mEq/l, and CO₂, 24 mEq/l. Serum osmolality was 262 mOsm/l; urine electrolytes and osmolality were compatible with SIADH, which persisted for 5 days. The CSF, 6 days after admission revealed an opening and closing pressure of 100, 72 mm H₂O, respectively; WBC, 110 with 100% mononuclears, red cells, 1; glucose, 17 mg% (simultaneous blood glucose 91); and protein, too high to read. Indirect immunofluorescent antibodies for *Herpes simplex* were negative.

Chloramphenicol was reinstated on the 7th hospital day. A second CAT scan showed an area of low density which did not enhance in the temporoparietal area. The right lateral ventricle was effaced. The midline abnormal enhancement above the third ventricle remained unchanged.

On the 10th hospital day, she remained unresponsive without brain stem reflexes. Rifampin was discontinued, and Streptomycin, 20 mg/kg/day was administered.

On the 13th hospital day, no evidence of cerebral electrical activity was detected on the EEG.

She died on the 14th hospital day, 5 weeks after the onset of fever and vomiting.

Autopsy Findings

The major pathological findings were limited to the central nervous system (CNS) and lungs.

The brain weighed 1,310 g. The external appearance of the cerebral hemispheres show large areas of encephalomalacia. The leptomeninges were transparent on the convexities and thick and opaque at the base. Both olfactory nerves were necrotic at their tips.

The diencephalon and walls of the third ventricle were necrotic. The thalamus showed two abscesses, the largest measured 1.5 cm in diameter. Horizontal sections of the brain stem reveals encephalomalacia of the superior and inferior colliculi. The cerebral aqueduct contained necrotic debris. The lower portion of the brain stem exhibited hemorrhagic necrosis of the entire floor of the fourth ventricle, the latter being occupied by necrotic tissue from the cerebellar vermis. The cerebellum shows diffuse encephalomalacia, more prominent in the anterior portion with protrusion into the fourth ventricle. Horizontal sections of the spinal cord showed some foci of myelomalacia at the cervical and upper thoracic levels.

Histopathological Findings

There was a necrotizing "granulomatous" encephalitis involving the tegmentum, associated with perivascular cuffing by lymphocytes and occasional plasma cell. There was minimal chronic leptomenigitis.

The basilar, posterior cerebral, and portions of the vertebral arteries revealed "mycotic aneurysms" with panarteritis (Fig. 1). In addition, there were segmental

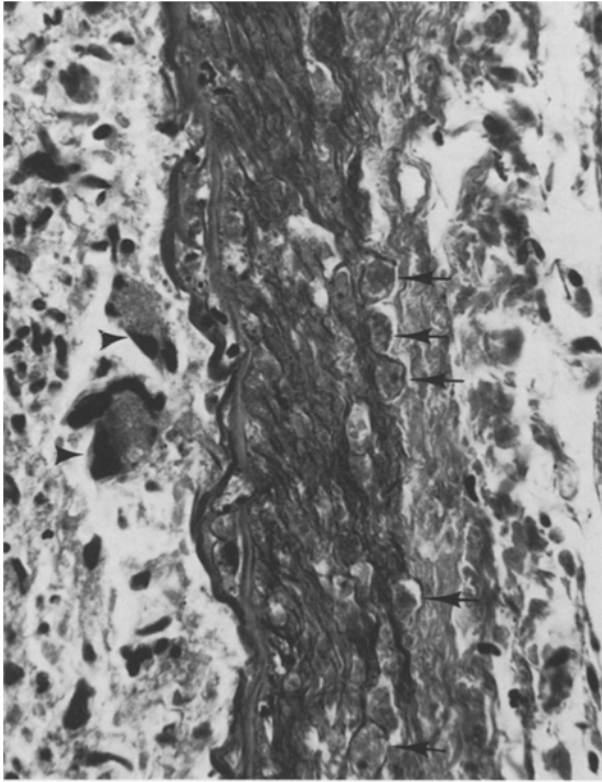


Fig. 1. Portion of the wall of the basilar artery. Two multi-nucleated giant cells are seen in the lumen (*arrow heads*). Amebic trophozoites are seen (*arrows*) between spaces of the media. The interna elastica lamina is intact in this segment. H.-E. $\times 490$

organizing thrombosis with multinucleated giant cells and numerous amebic trophozoites within the arterial wall, mainly in the adventitia and muscularis. There were numerous multinucleated giant cells in the thrombus and in the adventitia (Fig. 1).

Foci of necrotizing granulomatous encephalitis, on the posterior and lateral portions of the medulla oblongata and in one inferior olivary nucleus were identified. There was “fibrinoid” necrosis of blood vessels. Clusters of trophozoites around blood vessels and within neuropil with virtual absence of inflammatory reaction was also a striking feature. Well preserved amebic trophozoites were seen measuring 15–20 μm in diameter. Occasional spherical amebic cysts were seen with slight wrinkled walls, measuring 10 μm in average diameter. The roots of the 12th cranial nerve were surrounded by lymphocytes and few plasma cells.

The cerebellar peduncles and the central white matter of the cerebellum, revealed a necrotizing granulomatous encephalitis with multinucleated giant cells, occasional amebic trophozoites, and a few amebic cysts. Fibrinoid necrosis of medium-sized arteries was also seen.



Fig. 2. Ultrastructural features of an amebic trophozoite. $\times 5,400$. *Inset:* Amebic trophozoites and modest lymphocytic infiltration in thalamic lesion. H.-E. $\times 360$

The thalamus showed a large area of necrotizing “chronic” granulomatous encephalitis with clusters of amebic trophozoites (Fig. 2, *Inset*). The choroid plexus was undergoing necrosis.

Amebic trophozoites were seen in the deeper portions of the Virchow-Robin spaces.

The cervical and upper thoracic spinal cord disclosed focal necrotizing granulomatous myelitis with mild leptomeningitis. Trophozoites were seen in these regions. Perivascular cuffing by lymphocytes was prominent. The roots of the cauda equina showed mild chronic inflammation.

General Histopathological Findings

The lungs showed foci of atelectasis and occasional foci of chronic pneumonitis, one of them containing an amebic trophozoite. The rest of the organs showed no significant pathology.

Special Pathology Studies

Despite postmortem autolysis, trophozoites and cysts were adequately preserved, permitting ultrastructural

evaluation. The technique used was described elsewhere [7].

Ultrastructurally, the trophozoites possessed the characteristic dense nucleolus surrounded by granular chromatin and abundant cytoplasm containing short cytoplasmic processes bound by a thin, well-defined membrane (Fig. 2). The cytoplasm contained round mitochondria, free ribosomes, and different-sized vesicles and vacuoles. Some trophozoites revealed abundant, parallel stalks of endoplasmic reticulum, lysosomes, and glycogen-like granules. Golgi apparatus was not detected. Their nuclei were centrally or eccentrically located, and contained a dense nucleolus. "Vacuoles" and "dense" bodies were seen within the nuclear chromatin. The nuclear membrane was double, but was absent in the dividing trophozoites. In some instances, the nucleus contained a dividing nucleolus without nuclear membrane and at other times, two or three well defined nucleoli were seen. The nuclear divisions appear to be mesomitotic or at the end of the telophase. Some trophozoites which may be in a pre-encystment stage disclosed a condensation of cytoplasm at the periphery and the presence of numerous, small electron-dense vacuoles, perhaps representing fragments of cytoplasmic membranes. No interzonal bodies or nuclear caps were detected.

Cysts were rounded or slightly ovoid, with a prominent, thick, slightly wrinkled poreless wall often composed of partially "split" concentric layers of electron dense material. In between the splitting there was finely granular material. Smooth and rough endoplasmic reticula were barely visible. Abundant electron-dense lysosomal-like bodies were seen in the cysts.

Microbiologic Studies

The sediment of the CSF collected on the patient's sixth hospital day was placed into bacterized agar plates at 37° C and 25° C; no growth was obtained after 14 days. Fragments of brain taken at autopsy were placed in Fletcher's medium and then into bacterized agar plates at 37° C and 25° C. No growth was obtained in either case.

Indirect Immunofluorescent Antibody Analyses (IFA)

The histologic sections of the patient's brain were deparaffinized before application of the diluted antisera. The following antisera, which were produced in rabbits, were used in this case: (1) Whole cell antisera [35]; anti-*N. fowleri* (ITMAP 359), anti-*A. polyphaga* (P 23), anti-*A. astronyxis* (Ray), anti-*A. palestinensis* (Reich), anti-*A. royreba* (Oak Ridge), anti-*A. rhyodes* (CCAP 1534), anti-*A. griffini* (CCAP 1501/4), anti-

Table 1. Results of indirect immunofluorescent antibody test on cerebral tissue

Antisera	Dilution	Result ^a	Homologous end-point titer
1. Whole cell antisera:			
anti- <i>N. fowleri</i>	1/32	0	1/512
anti- <i>A. polyphaga</i>	1/16	0	1/128
anti- <i>A. astronyxis</i>	1/8	0	1/64
anti- <i>A. palestinensis</i>	1/16	0	1/128
anti- <i>A. royreba</i>	1/16	0	1/128
anti- <i>A. rhyodes</i>	1/16	0	1/128
anti- <i>A. griffini</i>	1/16	0	1/64
anti- <i>H. vermiformis</i>	1/32	0	1/128
anti- <i>V. avara</i>	1/32	0	1/128
anti- <i>E. histolytica</i>	1/64	0	1/512
2. Plasma membrane antisera:			
anti- <i>A. culbertsoni</i>	1/32	0	1/512
anti- <i>A. castellanii</i>	1/64	0	1/512
anti- <i>A. rhyodes</i>	1/64	0	1/1024

^a 0 = no fluorescence

H. vermiformis (CCAP 1534/7), anti-*V. avara* (CCAP 1588/A), and anti-*E. histolytica* (HK 9); (2) Plasma membrane antisera [31]; anti-*A. culbertsoni* (A-1), anti-*A. castellanii* (Neff), and anti-*A. rhyodes* (CCAP 1534/3).

The histologic sections were incubated and the immune complexes were visualized after incubation of the sections with fluorescein-conjugated rabbit anti-immunoglobulin (1:50) (Institute Pasteur, France). The sections were read with a Leitz Ortholux microscope equipped for fluorescence with a ploemopak 2 and a super-pressure mercury lamp HBO 6, 100 W. The filter system for FITC combined with two interference filters KP, 490, K 510, and K 445 were used.

The results of the indirect immunofluorescence analyses are given in the Table 1. None of the antisera used stained the ameba in the brain sections.

Discussion

The clinical picture and the histopathological reaction in CNS resembled those of *Acanthamoeba*, i.e., chronic granulomatous inflammation with multinucleated giant cells, lymphocytes, and plasma cells. Such pathologic findings have not been described in proven cases of *Naegleria*. Human and animal experiments are consistent with these findings [6, 17, 19]. Also, the presence of cysts has not yet been described in PAM due to *Naegleria*; however, the absence of such may be related to the lack of an identified chronic stage of this disease.

A very important feature of this case is the arterial involvement. In some places "organizing" thrombi

were seen with a moderately severe panarteritis associated with lymphocytic infiltration and the presence of foreign body giant cells. There were at least two places (basilar artery and posterior cerebral artery) in which "mycotic" aneurysms were present. Molinari [23] stated that infection of the arterial wall in cases of "mycotic" aneurysms is mediated through stasis and sepsis in the vasavasorum. In addition, the inflammation appears to proceed from the adventitial surface toward the media. Bell and Butler [1] believe that embolic material, usually from the heart, occludes the vasavasorum allowing bacterial growth within the wall of the artery. The inflammatory response weakens the vessel wall resulting in aneurysmal dilatation and possible rupture. Some intracranial "mycotic" aneurysms, usually of extravascular origin, may become thrombosed and may decrease in size or spontaneously disappear [32]. It appears that free-living amebas, in particular *Acanthamoeba* sp., have a predilection for attacking vascular walls, thus leading to necrotizing arteritis with thrombosis and aneurysm. The nuclear division of the amebas in this case appeared to be mesomitotic; that is, the nuclear membrane disappears throughout mitosis and polar caps are absent. Mesomitosis division is consistent with *Acanthamoeba* or *Hartmannella*. The absence of interzonal bodies during mitosis serves to exclude the *Naegleria*, as well as *Vahlkampfia* as the causative organisms. The presence of the cyst in the patient's CNS also points against *Naegleria* since, characteristically, this ameba does not form cysts in tissues. Furthermore, the cysts present in the patient's CNS had wrinkled, concentrically laminated walls without identifiable pores. *Naegleria* cysts are smooth and contain pores in their double wall. However, such cysts do not completely exclude *Naegleria*, as Page [26] has pointed out that the morphology of cysts of *Naegleria* can vary considerably, from smooth to rough to angular and may even be wrinkled. The large dark karyosome excludes, with the exception of *Iodamoeba*, members of the family *Entamoebidae*. If the organism identified belonged to the *Entamoebidae* family, ultrastructural evidence of peripherally located chromatin within the nuclei, as well as an absence of mitochondria, should have been noted. Such was not the case [26]. The genus *Iodamoeba* cannot be completely excluded, but the cysts were not typical of that organism.

The results of the immunological studies gave further evidence that the unknown pathogenic ameba was neither *Naegleria* nor *Acanthamoeba*. Other genera belonging to the family of Vahlkampfiidae, such as *tetramitus* and *paratetramitus*, might be considered also, as they have the same mitotic pattern. Organisms in the tissues of the patient reported herein did not fluoresce when exposed to anti-*N. fowleri* fluorescent

tagged antisera. Tissues containing *N. fowleri* obtained from autopsy specimens of culturally proven *Naegleria* PAM cases stained well with anti-*N. fowleri* fluorescent antisera. One still cannot exclude the possibility that some alteration of antigens occurred, possibly related to fixation of tissues, deparaffinization, or time from death until autopsy. To date, all isolates of *Naegleria* obtained from cases of PAM throughout the world have shown remarkable antigenic homology. Since cross reactivity between non-pathogenic *N. gruberi* and pathogenic *N. fowleri* is incomplete, it is certainly possible, at least on an immunologic basis, that the organism still could be a species of *Naegleria* other than *N. fowleri*, either *N. gruberi* or an as yet undescribed species [34, 36].

Thus, the organism responsible for this patient's death was atypical of both *Naegleria* and *Acanthamoeba*, but was a free-living ameba belonging to an unidentified genus. It may be another species of *Vahlkampfia* (other than *V. avara*) as suggested by the most reliable characteristics available for study, viz., the frequent mitosis and division patterns.

Although the responsible pathogen was not precisely identified in this instance, this case is important because it illustrates that serious diseases may be produced in humans (and probably in many other animals) by free-living ameba other than *Naegleria*, *Hartmannella*, *Acanthamoeba*, and *Vahlkampfia*. It emphasizes that the variety of free-living ameba capable of producing disease is as yet undefined, and that it may be considerable [5, 6]. These organisms are numerous, varied and ubiquitous in nature [15, 22]; they are distributed world-wide; they multiply rapidly and appear in a variety of forms; and some species either prefer or readily invade mammalian tissues. Thus, the public health implications are obvious, and the scope of this problem needs to be defined.

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