The Neuropathogenesis of *Acanthamoeba* encephalitis: Barriers to Overcome

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### Abstract

*Acanthamoeba* encephalitis exhibits broad spectrum of neuropathology depending on the localization of the lesions. Natural infection is associated with haematogenous spread leading to *Acanthamoeba* entry into the brain, most likely via the blood-brain barrier, although blood-cerebrospinal fluid barrier has also been suggested as a possible route. Experimental infection is induced by intranasal injection of parasites, followed by amoebae invasion of the central nervous system via the olfactory neuroepithelium route. The precise mechanism of entry and the factors that influence amoebae entry into the central nervous system are not fully understood. The development of strategies to combat this fatal infection requires an understanding of the interactions between *Acanthamoeba* and the central nervous system.

### Keywords:
*Acanthamoeba*; Granulomatous encephalitis; Central nervous system; Blood-brain barrier; Olfactory neuroepithelium

### Introduction

*Acanthamoeba* is an opportunistic protist pathogen that is ubiquitously distributed in the environment. It is known to produce a sight-threatening, extremely painful keratitis and granulomatous encephalitis [1-4]. *Acanthamoeba* encephalitis is a fatal brain infection with a case fatality of more than 90%, despite advances in antimicrobial chemotherapy and supportive care. This is partly due to our incomplete understanding of the pathogenesis and pathophysiology of *Acanthamoeba* infections. At present, the treatment of *Acanthamoeba* encephalitis is a hit and miss, where a mixture of drugs is administered and even then the prognosis remains extremely poor. No single drug has shown to be effective against this devastating infection indicating an urgent need for improved antimicrobial chemotherapy and/or alternative strategies for therapeutic interventions. Here, we attempt to elucidate the mechanisms of parasite entry into the central nervous system (CNS) leading to granulomatous encephalitis-associated complications.

*Acanthamoeba* granulomatous encephalitis

*Acanthamoeba* encephalitis exhibits broad spectrum of neurological signs and symptoms depending on the localization of the lesions [5-7]. The brain scans using computed tomography or magnetic resonance imaging show single or multiple space-occupying masses or ring-enhancing lesions. The clinical symptoms may include headache, fever, behavioral changes, hemiparesis, lethargy, stiff neck, agitation, aphasia, ataxia, vomiting, nausea, cranial nerve palsies, increased intracranial pressure. The later stage of the disease is irreversible and includes loss of consciousness, seizures and coma, leading to death. Post-mortem examination often shows severe oedema and hemorrhagic necrosis [5-7]. The cutaneous infections due to *Acanthamoeba* can last for months but the involvement of the CNS can result in fatal consequences within days or weeks. A granulomatous response may be absent or minimal in patients with a severely impaired immune system [5,6].

### Routes of Entry into the Central Nervous System

#### Blood-brain barrier at the choroid plexus (a barrier between the blood and the cerebrospinal fluid)

Two different structures separate the blood from the CNS:

- The blood-brain barrier and the blood-cerebrospinal fluid barrier.

The blood-cerebrospinal fluid barrier is limited to choroid plexus. The choroid plexus is highly vascularized epithelium found within each of the four cerebral ventricles and is responsible for the production of cerebrospinal fluid (Figure 1). Here, endothelial cells are fenestrated to allow penetration of the blood contents but are surrounded by epithelial cells of the choroid plexus. Like capillary endothelium, the polarized choroid plexus epithelium form tight junctions (Figure 1), however these exhibit lower electrical resistance compared with the brain capillary endothelium [8].

**Blood-brain barrier at the brain parenchyma (i.e., a barrier between the blood and the brain tissue)**

Blood-brain barrier is composed of endothelial cells, pericyte, astrocytes and neurons. Astrocytic end feet are in close association with the endothelial cells. The presence of astrocytes ensheathing the endothelium is limited to the blood-brain capillaries. Astrocytes are involved in the induction of permeability barrier properties of the blood-brain barrier. Pericytes also are closely associated with the endothelial cells and are required for capillary maturation, while basement membrane is important for blood-brain barrier differentiation. Blood-brain barrier is characterized by the presence of tight junctions. The tight junctions are primarily responsible for the barrier function and provide a high electrical resistance (∼2000Ω/cm²) making it a highly selective barrier [9,10]. The nutrients such as glucose, amino acids etc. are transported across the blood-brain barrier via transporters, while larger molecules such as insulin, leptin, iron transferrin are taken up via receptor-mediated endocytosis to support neuronal function. Junction complex in the blood-brain barrier comprise of tight junctions and...
adherens junctions (Figure 2).

**Tight junctions:** These are composed of three integral membrane proteins (i.e., claudin, occludin, junction adhesion molecule), and cytoplasmic proteins [zonula occluden-1 (ZO-1), ZO-2, ZO-3] (Figure 2). Claudins are 22 kDa phosphoproteins with four transmembrane domains that bind to claudins on the adjacent endothelial cells to form tight junctions. The carboxy terminal of claudins binds to cytoplasmic zonula proteins. Ocludin is a 65 kDa phosphoprotein with four transmembrane domains and its cytoplasmic domain is directly associated with zonula proteins. In addition, junctional adhesion molecule (JAM) and endothelial cell-selective adhesion molecule (ESAM) are localized in tight junctions of blood-brain barrier. Junctional adhesion molecules (JAM) are 40 kDa with a single transmembrane domain. The integral proteins are linked to cytoskeleton via cytoplasmic peripheral membrane proteins such as zonula-occluden-1 (ZO-1), ZO-2, and ZO-3. Zonula protein (ZO-1) is 220 kDa, ZO-2 is 160 kDa, and ZO-3 is 130 kDa provide structural support. The cytoplasmic proteins link membrane proteins to actin cytoskeleton [11-13].

**Adherens junctions:** These are composed of membrane protein, vascular endothelial-cadherins (VE-cadherin). The cytoplasmic domains of cadherins bind to catenin that are linked to actin cytoskeleton and form adhesive contacts between the cells. Adherens junctions include cadherin, actin, and vinculin (analogue of catenin). Both tight junctions and adherens junctions are present in epithelial cells of the choroid plexus. Claudins-1, -2, -11, occludin and ZO-1 are present in choroid plexus epithelial cells (blood-cerebrospinal fluid barrier), whereas claudins-1, -5, -11, occludin and ZO-1 are present in the blood-brain barrier [14] (Figure 2).

**Entry of Acanthamoeba into the Brain**

Understandings of pathways by which CNS protective barriers are breached leading to pathophysiological events are crucial for our ability to devise mechanisms for the rationale design of therapeutic interventions. The primary route of entry include lower respiratory tract leading to amoebae invasion of the intravascular space, followed by the haematogenous spread (Figure 3) [5,6]. Skin lesions may provide direct amoebae entry into the bloodstream, thus bypassing the lower respiratory tract (Figure 3). The affected tissues other than the CNS may include subcutaneous tissue, skin, liver, lungs, kidneys, adrenals, pancreas, prostate, lymph nodes, and bone marrow, which suggest haematogenous spread pre-mortem. Thus amoebae entry into the CNS most likely occurs at the sites of the blood-brain barrier [5,6]. Although it is possible that amoebae enter the brain at the sites of the blood-cerebrospinal fluid barrier, several lines of evidence suggest that *Acanthamoeba* entry into the CNS most likely occurs at the cerebral capillary endothelium (Figure 3). This is shown with the observations that lesions are most frequent in the brain parenchyma in majority of *Acanthamoeba* encephalitis-infected patients and sections of the brain tissue exhibit large numbers of amoebae in the perivascular space. In contrast, pathogens that enter via choroid plexus route will end up in the cerebrospinal fluid. Thus the pathology in such cases will first appear in the ventricles, and only at later stages with extensive inflammation it will involve the brain parenchyma, a finding that is inconsistent with the pathologic features of *Acanthamoeba* encephalitis patients. The
Figure 3: The model of Acanthamoeba granulomatous encephalitis. Amoebae enter lungs via the nasal route. Next, amoebae traverse the lungs into the bloodstream, followed by haematogenous spread. Finally, Acanthamoeba cross the blood-brain barrier and enter into the central nervous system (CNS) to produce disease. It is noteworthy that Acanthamoeba may bypass the lower respiratory tract and directly enter into the bloodstream via skin lesions. The olfactory neuroepithelium may provide an alternative route of entry into the CNS [27].
cerebrospinal fluid is continuous with the extracellular fluid of the brain parenchyma and flows unidirectionally from ventricles down to lumbar region but amoebae are generally not found in the cerebrospinal fluid following spinal taps of patients [15], suggesting that amoebae traversal across the blood-brain barrier occurs at the cerebral capillary endothelium.

Neuropathogens breach the blood-brain barrier via the transcellular route, paracellular route, leukocyte-facilitated entry using the Trojan horse mechanisms or by inducing injury to the brain microvascular endothelial cells (BMEC), which constitute the blood-brain barrier [16]. Given the size of Acanthamoeba, i.e., 12 – 35 µm, it is likely that Acanthamoeba traverse the cerebral endothelium at the blood-brain barrier by producing endothelial cell damage or via paracellular route by targeting tight junctions.

**Acanthamoeba Traversal of the Blood-brain Barrier**

The blood-brain barrier crossing is a multifactorial process involving parasite determinants (adhesins, proteases, phospholipases) or host immune responses (interleukin-beta, interleukin-alpha, tumor necrosis factor-alpha, interferon-gamma, host cell apoptosis) (Figure 4). The overall outcome is either increased permeability and/or apoptosis of brain endothelial cells, which promote blood-brain barrier disruption leading to Acanthamoeba entry into the brain. The primary events include Acanthamoeba binding to the cerebral endothelium, mediated by mannose-binding protein expressed on the surface of the parasite (Figure 4) [17,18]. This induces phagocytic properties in Acanthamoeba to engulf and/or produce host cell damage, in actin polymerisation-dependent manner [19]. The primary parasite-host cell interactions result in the activation of Rho-associated intracellular signalling cascades in the host cells. Rho-associated pathways could disturb the function of tight junctions, thus leading to increased blood-brain barrier permeability [20]. With further incubations, Acanthamoeba induces host cell cycle arrest in a protein retinoblastoma-dependent manner [21], and finally leading to host cell death in a phosphatidylinositol 3-kinase-dependent manner [22]. Overall, these pathways result in BMEC dysfunction, which assist amoebae translocation of the blood-brain barrier (Figure 4). A complete understanding of the molecular mechanisms involved in Acanthamoeba-mediated BMEC cell cycle arrest and apoptosis need further investigation, which will identify potential targets for therapeutic interventions.

In addition, Acanthamoeba target tight junction proteins and induce BMEC permeability by secreting serine proteases [23]. The extracellular

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**Figure 4:** Schematic illustration of Acanthamoeba interactions with the brain microvascular endothelial cells at the molecular-level. Acanthamoeba binds to host cells via mannose-binding protein (MBP), and possibly laminin-binding protein (LBP), which leads to cytoskeletal rearrangements involving Rho-associated pathways and host cell cycle arrest/apoptosis. Acanthamoeba secrete serine proteases that target tight junction proteins (zonula occludens and occludin) in a Rho kinase-dependent manner. Both parasite and immune cell factors contribute to brain microvascular endothelial cell damage leading to Acanthamoeba entry into the brain.
serine proteases of Acanthamoeba are shown to degrade occludin and ZO-1 proteins in a Rho kinase-dependent manner as well as types I, III and IV collagen, elastin, fibronectin, fibrinogen, IgG, IgA, albumin, plasminogen and haemoglobin, highlighting the role of proteases in facilitating migration of amoebae from systemic circulation into the brain (reviewed in 4). Notably, proteases induce BMEC permeability but do not produce cytopathogenicity [23], suggesting that paracellular is a probable route of amoeba entry into the brain.

Being an extracellular pathogen, the immune response is likely to play an active role in protection against this parasite, as well as contributes to blood-brain barrier perturbations and disease development. The localization of immune cells in the brain suggests the involvement of pro-inflammatory cytokines. Interferon-gamma through pro-inflammatory network, upregulates the release of specific cytokines including tumor necrosis factor-alpha, interleukin-6, interleukin-beta and interleukin-1-alpha [24,25]. The activated microglia exhibit antimicrobial properties most likely through nitric oxide and production of pro-inflammatory and anti-inflammatory cytokines. But over-production of nitric oxide induces toxic effects and may contribute to pathological complications leading to the blood-brain barrier dysfunction. Our understanding of the imbalance in cytokine levels resulting in neurological manifestations is crucial to devise strategies against this often fatal infection.

Conclusions

It is widely accepted that the vast number of Acanthamoeba encephalitis cases are undetected due to difficulty in diagnosis, lack of awareness and problematic treatment. For example, there is little to report in this infection in Africa, despite millions of AIDS patients, who are susceptible hosts to this pathogen and have exposure to warm climate/environment and subordinate sanitation. Notably, previous studies estimated that 1 in 10,000 AIDS patients died of Acanthamoeba encephalitis in the USA [26]. To establish infection, parasite crossing of the blood-brain barrier is a key step that most likely occurs at the sites of the cerebral capillary endothelium. Acanthamoeba translocation of the blood-brain barrier involves binding, phagocytosis, cell cycle arrest and BMEC damage as well as tight junction protein degradation by secreting proteases. The inflammatory cytokines secreted by astrocytes and microglia may play a significant role in the blood-brain barrier leakage leading to trafficking of immune cells and Acanthamoeba across the barrier. Thus the pathogenesis of Acanthamoeba encephalitis involves both parasite and immune cell entry of the CNS, which in tandem contribute to the neuropathology. The study of blood-brain barrier function in the context of natural infection will provide essential information for the development of novel therapeutic strategies to control CNS infection and inflammation.

Future Research

Acanthamoeba encephalitis almost always results in death. This is due to the unavailability of effective and/or recommended drugs, and the inability of drugs to traverse the blood-brain barrier to gain entry into the brain to kill parasites. Thus, there is an urgent need to initiate both drug discovery and drug delivery programmes. Notably, lipidization of small molecules to enhance transport across the blood-brain barrier, carrier-mediated transport of drugs, or administration of non-viral plasmid DNA encoding antisense RNA against the virulence genes of Acanthamoeba will hold promise. Alternatively, trans-nasal drug delivery to the brain using lipid-soluble small molecules (instilled nasally and cross the nasal mucosa and the arachnoid membrane and enter olfactory CSF) may be a valuable approach. In addition, a complete understanding of the pathogenesis and pathophysiology will help develop preventative and therapeutic approaches against this fatal infection. To this end, describing the relative contributions of each virulence factor and host immune response to it in more depth, including aspects concerning pathogen-host interactions in a natural context and in a quantitative manner, holds the key to a more profound understanding of how these protists cross the blood–brain barrier. Specific Acanthamoeba ligand/BMEC receptor interactions involved in the adhesion and invasion process are required for parasite penetration of the blood-brain barrier. Finding answers to all these issues (and many others) will provide important information essential for the development of new therapeutic strategies in clinical situations.

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Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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