

Naegleria fowleri

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Naegleria fowleri is a free-living amoeba (FLA) that is commonly known as the “brain-eating amoeba.” This parasite can invade the central nervous system (CNS), causing an acute and fulminating infection known as primary amoebic meningoencephalitis (PAM). Even though PAM is characterized by low morbidity, it has shown a mortality rate of 98%, usually causing death in less than two weeks after the initial exposure.

amoeba

pathogenesis

neurodegeneration

neuroinflammation

neuropathology

treatment

1. Introduction

Several pathogens, including different bacteria, viruses, fungi, and parasites, have shown the ability to infect the human central nervous system (CNS) ^[1]. They have various molecular mechanisms that allow them to disseminate through the blood–brain barrier (BBB) or the blood-cerebrospinal fluid barrier (BCSFB) and take the brain and spinal cord as their primary target of infection ^[2].

Of those parasites capable of neuroinfecting, a group of opportunistic protozoa known as free-living amoebas (FLA) cause severe health problems. FLA are mitochondriate and eukaryotic microorganisms that can complete their life cycle as parasites or inhabiting natural environments as free-living amoeba. For this reason, FLA are known as amphizoic organisms ^{[3][4]}. These amoebas are ubiquitous and have been found in the air, soil, and water. However, they have also been identified in everyday objects like flower pots, humidifiers, sewers, swimming pools, water pipes, water parks, and even hospital environments ^[3].

Naegleria fowleri is one of the few FLA capable of causing fatal infections in humans. This parasite causes primary amoebic meningoencephalitis (PAM), an acute and fulminating infection that can lead to death 7 to 10 days after the amoeba enters the body. PAM is commonly observed in immunocompetent children and young adults, especially after having contact with amoeba-contaminated water. Even though the disease is extremely rare, it has a mortality rate of 98% ^{[5][6]}. Studies have suggested that an early diagnosis is critical for a patient to survive PAM ^[3]. However, due to its low morbidity, there is a lack of awareness and knowledge of the pathogenesis of the infection. This last issue makes it difficult to develop successful treatment and effective diagnostics tools ^[3]. Additionally, due to its non-specific symptoms, PAM is commonly unreported, unrecognized, or mistaken for bacterial or viral infections ^[4].

Naegleria is a genus of FLA that belongs to the family Vahlkampfiidae, order Schizopyrenida, and class Heterolobosea [7]. The genus consists of 47 species, which can be identified by variations in their genome, specifically their internal transcribed spacers (ITS) and their 5.8S rDNA. Out of all the *Naegleria* species, only *N. australiensis*, *N. italica*, and *N. fowleri* have been described as pathogenic. *N. australiensis* and *N. italica* only affect laboratory animals. However, *N. fowleri* is the only pathogen known to cause the fatal human disease primary amoebic meningoencephalitis (PAM) [8][9]. *N. fowleri* is most closely related to *N. lovaniensis*, although the latter is considered to be non-pathogenic [8].

N. fowleri is a thermophilic and ubiquitous amoeba that can be found in the air, soil, and warm waters [10]. Its natural habitats include hot springs, ponds, rivers, and freshwater lakes. However, it has also been identified in drinking water distribution systems, untreated swimming pools, fountains, hospitals, thermal waters, untreated drinking water, and water parks [11]. *N. fowleri* is a widely distributed parasite, since it has been identified in almost every continent, except Antarctica [10].

N. fowleri occurs in three different forms. When conditions become too hostile, the amoeba transforms into a metabolically inactive cyst, described as a spherical structure that measures from 7 to 12 μm in diameter, with a thick endocyst, a thin ectocyst, and some mucoid-plugged pores [12][13]. The cyst is incredibly resistant and can survive a variety of physical and chemical conditions, including temperatures as low as 4 $^{\circ}\text{C}$; therefore, this amoeba can remain dormant during the cold winter months and reproduce during the summer [10].

When the amoeba faces non-nutritive conditions but is in the presence of water, it transforms into a transitory flagellate. This form has a pear-shaped appearance measuring from 10 to 16 μm and has two flagella of approximately the same length. They have a nucleus, a nucleolus, vacuoles, cytoplasmic inclusions, mitochondria, and a rough endoplasmic reticulum [12]. *N. fowleri* flagellate thrive at 27–37 $^{\circ}\text{C}$, so they are usually present in warm waters or during the summer months [11][14].

Under favorable conditions, the amoeba can be found as a reproductively active trophozoite described as a long and slender structure that measures approximately 22 μm long and 7 μm wide. These trophozoites have a large nucleus with a nucleolus, many mitochondria, food vacuoles, a single contractile vacuole, an endoplasmic reticulum, ribosomes, and membrane-bound cytoplasmic organelles [10][12]. These also exhibit food-cup structures (amoebastomes) that have been related to their feeding [15]. The trophozoites are the only form of *N. fowleri* that can reproduce, feed, encyst, and cause infection in other organisms. Their primary food source comes from Gram-positive and Gram-negative bacteria, but trophozoites can also consume algae and yeast. They divide by binary fission and, being thermophilic, grow better at 35–46 $^{\circ}\text{C}$ [11].

2. Neuroinflammation and its Association to PAM Pathogenesis

N. fowleri may promote further host injury through robust induced immune response [16]. In vivo studies have demonstrated that trophozoites reach the olfactory bulb just 72 h post-infection (PI), although the first sign of tissue

destruction and inflammation are seen after 96 h PI [16][17]. Studies have shown that *N. fowleri* trophozoites induce the production of reactive oxygen species (ROS), which in turn activate the epidermal growth factor receptor (EGFR) pathway and induce the expression of MUC5AC (mucin), and the pro-inflammatory cytokine, interleukin 8 (IL-8), which is one of the most powerful neutrophil chemoattractants. ROS also induce the expression of the pro-inflammatory cytokine IL-1 β through an EGFR-independent mechanism [18]. An in vitro study suggested that ROS stimulate the formation of the NLRP3 inflammasome, a multiprotein complex capable of activating caspase-1 and secreting an active IL-1 β [19]. In addition, an in vitro study demonstrated that *N. fowleri* activates ROS-dependent programmed necrotic cell death (necroptosis) in Jurkat cells. The authors suggest that necroptosis may be a defense mechanism against the amoeba; however, representing a high cost, cell destruction [20].

Following 102 h PI, a few eosinophils and neutrophils begin to surround the trophozoites in the olfactory bulb. Eosinophils can produce various pro-inflammatory cytokines and chemokines such as tumor necrosis factor alpha (TNF- α), IL-6, IL-8, and eotaxin. Nevertheless, eosinophils seem to be unable to remove the parasite in vivo, which may increase leukocyte recruitment and inflammation during the later stages of the infection. During 108–120 h PI, the number of eosinophils seems to decrease while neutrophils and macrophages increase [16]. Neutrophils can eliminate various pathogens through different mechanisms, including degranulation, proteolytic enzymes, antimicrobial peptides, ROS, and reactive nitrogen species (RNS). An in vitro study recently demonstrated that *N. fowleri* induces the production of neutrophil extracellular traps (NETs), which consist of nuclear or mitochondrial DNA combined with histones and protein components of cytoplasmic granules. However, this study also showed that the amoeba was efficiently killed by neutrophils when it was opsonized by immunoglobulin A and G (IgA and IgG) but evaded killing when it was unopsonized [21]. Additionally, it has been suggested that neutrophils require the presence of TNF- α to destroy *N. fowleri*, although evidence has demonstrated that this pro-inflammatory cytokine has no effect on the amoeba [22]. Extensive tissue damage distinguished by lytic necrotic areas, hemorrhages, and cellular debris has been detected in infected tissues. However, the damage has been associated with the presence of inflammatory cells [16].

It has been suggested that *N. fowleri* releases cysteine proteases to cross through the BBB; however, the damage caused to this structure also facilitates the passage of immune cells into the brain. The trophozoites have demonstrated the ability to induce extensive production of leukocyte adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1). These, as well as the disruption of the BBB, have been associated with a high number of inflammatory cells entering the CNS. Besides its NO production, *N. fowleri* can induce the production of this compound by the cerebrovascular endothelium, perhaps through an interaction between cytokine receptors with the endothelium's toll-like receptors (TLR), specifically TLR4, and activating the endothelial NOS (eNOS) as well as the inducible NOS (iNOS). The NO released may further alter the permeability of the BBB, allowing more leukocytes to enter the CNS [23]. Macrophages have also been shown to release TNF- α , IL-1, and NO as a mechanism to destroy different pathogens, although research has demonstrated that *N. fowleri* presents a high tolerance for NO toxicity [22][24]. Even though NO is a potent antimicrobial agent, it also has the potential to damage the host since its reduced form can produce peroxynitrite, a strong neurotoxin [25]. Actually, it has not yet been determined whether NO truly contributes to tissue damage in *N. fowleri* infections [24].

The brain tissue is composed of different types of cells, and microglia is one of them. These cells are the first line of defense within the CNS and can perform antigen-presenting and pro-inflammatory functions when activated by injury or infection [26]. In vitro, *N. fowleri* trophozoites activate microglial cells inducing the production of pro-inflammatory cytokines such as IL-1 β , IL-6, TNF- α , ROS, and RNS [16][27]. It has been suggested that this activation occurs through the interaction of the microglia's TLRs with the amoeba's pathogen-associated molecular patterns (PAMPs) as well as the danger-associated molecular patterns (DAMPs) released by tissue damage [27][28]. In fact, microglial activation may cause severe damage to the CNS when it releases high doses of cytokines, ROS, and RNS, as these molecules can be highly neurotoxic [27]. Furthermore, high ROS levels have also been related to causing cell destruction through lipid peroxidation [29].

The amoeba also promotes the activation of astrocytes, CNS cells involved in maintaining the homeostatic environment and regulating the immune system within the brain. *N. fowleri* lysates can induce the expression of IL-1 β and IL-6 in primary rat astrocytes through the activation of the activator protein 1 (AP-1) transcription factor. The expression of these cytokines was also dependent on the activation of the extracellular-signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPKs) pathways [30]. It has been proposed that the production of these pro-inflammatory cytokines in response to *N. fowleri* are not beneficial and may contribute to brain tissue destruction through an immunopathological process. Indeed, the release of pro-inflammatory cytokines stimulates the further breakdown of the BBB and induces the hyper inflammation of the brain with immune cells from non-neural sites [22]. Most of the tissue damage in human autopsies can be seen in the frontal areas of the brain, where inflammation is more intense. On the other hand, the posterior parts of the brain show no inflammation or tissue destruction despite revealing the presence of trophozoites. This suggests that inflammation plays a vital role in brain tissue damage in patients suffering from PAM [16].

3. Conclusions

N. fowleri is one of the few FLA that is pathogenic towards humans, causing a necrotizing and hemorrhaging meningoencephalitis called PAM. Even though the amoeba causes severe tissue damage through contact-dependent and contact-independent mechanisms, it also induces a robust immune response that further injures the host. PAM is characterized by an extremely high mortality rate and causes death less than two weeks after the initial exposure. It has been suggested that an early diagnosis is crucial for a patient's survival; however, because of lack of awareness and the clinical resemblance the infection has with viral or bacterial meningitis, most cases have been diagnosed post-mortem. Additionally, developing a safe and effective treatment has also been challenging, mainly because the infection is so rare and progresses rapidly. The current treatment regime for PAM involves AmB, combined with other drugs, but it is seldom successful and causes adverse effects. There have been different in vitro and in vivo studies determined to find a safer and more effective drug to treat PAM, some of which have shown potent amoebicidal activity. In fact, anti-inflammatory molecules may help reduce the immunopathological response that has been linked with severe tissue damage. Furthermore, the conjugation of these drugs with nanoparticles has been demonstrated to increase bioavailability, amoebicidal activity, and reduce

cell toxicity in PAM infections. However, further studies are needed to confirm the true potential of these drugs. Until a more effective treatment is developed, prevention strategies are the best way to avoid PAM.

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