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A Review of Clinical and Biological Aspects of Cerebral Amoebiasis

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Abstract

Entamoeba histolytica and free-living amoebae (Naegleria fowleri, Balamuthia mandrillaris, and Acanthamoeba sp.) can cause serious and often fatal infections of the human Central Nervous System (CNS), which is a major cause of concern for the Medical Sciences. While E. histolytica can provoke abscesses in the brain, N. fowleri causes acute, necrotizing, and hemorrhagic meningoencephalitis, and Acanthamoeba and B. mandrillaris provoke granulomatous inflammatory lesions that are typically chronic. Despite being relatively rare, these pathologies are almost invariably fatal, a problem exacerbated by the lack of diagnostic based on clinical symptoms. The review presented here summarizes the current knowledge on the amoebic infections of the human CNS, focusing on their pathogenesis, clinical manifestations, diagnosis, and treatment, based on a systematic revision of the global literature. An overview of the published data reveals the fundamental difficulties of both diagnosing patients and treating them adequately, even in developed countries where healthcare systems tend to lack personnel and resources for the reliable diagnosis of infections, resulting in a very poor prognosis. The review also found that the difficulties of diagnosing amoebic diseases the CNS tends to delay treatment, which tends to accentuate mortality rates, given

the rapid progression of the disease. Overall, the findings of the review highlight the urgent need for more research, and the development of more reliable diagnostic procedures and treatments.

Keywords: Amoebae infections; Cerebral Amebiasis; Granulomatous Amoebic Encephalitis; Primary Amoebic Meningoencephalitis.

1 – INTRODUCTION

Ever since the first amoeba (*Entamoeba gingivalis*) known to occur in humans was described by Gros in 1849, these organisms have aroused interest from researchers around the world [1]. Amoebae can be free-living, commensal, or parasitic, although, even today, the only species generally accepted to be an invasive parasite is *Entamoeba histolytica* [2]. Free-living amoebae (FLA), in turn, have evolved an ability to develop part of their life cycle in host animals, and some are pathogenic, with potentially important implications for human health. In fact, some FLAs, as well as *E. histolytica*, can induce severe pathology in humans, including fatal infections of the Central Nervous System (CNS) [3].

While *E. histolytica* affects the CNS with lesions that produce brain abscesses, known as Cerebral Entamoebiasis (CE) [4,5], FLAs may cause two types of lesions, Primary Amoebic Meningoencephalitis (PAM), and Granulomatous Amoebic Encephalitis (GAE). While PAM is a hemorrhagic-necrotizing meningoencephalitis caused by *Naegleria fowleri*, which affects immunocompetent individuals following an infection [6,7], GAE is a rare infection of the CNS that results in a granulomatous inflammation, which affects both immunocompromised and immunocompetent individuals infected by the FLAs *Acanthamoeba* spp. and *Balamuthia mandrillaris* [8,9].

Cerebral Entamoebiasis is common in developing countries because it is transmitted as an enteropathogen found in contaminated food and water, which tend to be under more effective controls in developing countries [10,11]. By contrast, PAM and GAE are common in both developed and developing countries, and they are related to swimming in pools or contact with water sources contaminated by FLAs [12].

The present review describes the pathogenesis, clinical manifestations, and immune responses of the CNS to infection by pathogenic amoebae. Through an overview of the data, it also provides new perspectives for the more reliable diagnosis of these disorders and the development of more effective therapeutic approaches.

2 – TAXONOMY AND ECOLOGY OF *AMOEBAE*

Amoebae are among the most widely-distributed eukaryotes and are found in all types of environment around the world, including anthropogenic habitats [13–15]. The taxonomic classification of the amoebae is subject to constant review and modification. The traditional classification of the Amoebozoa is based on cell morphology, although this may vary considerably, depending on a wide range of factors, which hampers the reliable identification of homologies [16].

In the present day, molecular taxonomies are the most widely accepted for most amoeba species (Figure 1). In the case of *N. fowleri*, for example, the current

classification is based on the sequences of internally transcribed spacers (ITS1) and 5.8SrDNA gene, with at least eight different genotypes being identified in *N. fowleri* [17,18], four of which (types I, II, III and V) have been identified in PAM patients [19]. For *Acanthamoeba* sp., the most reliable current classification is based on the sequences of the 18S rRNA gene [20,21], for which 22 distinct genotypes (T1–T22) have been described up to now [22,23], of which, the T4 genotype is the most prevalent in environmental and clinical samples [24], while genotypes T1, T2, T4, T5, T10, and T12 are associated with GAE [25,26].

The ability of amoebae to adapt to the conditions found in both natural and artificial environments, and in humans and other animals, is related directly to their physiological versatility that refers primarily to the nutritional capacity of the amoebae and their tolerance of the varying physicochemical conditions they face in different environments. The cystic form is able to tolerate a wide range of osmolarity, temperature, salinity, and pH, which allows the amoebae to survive in substrates ranging from distilled water to cell culture or bodily fluids, and at temperatures of over 37ºC [27,28].

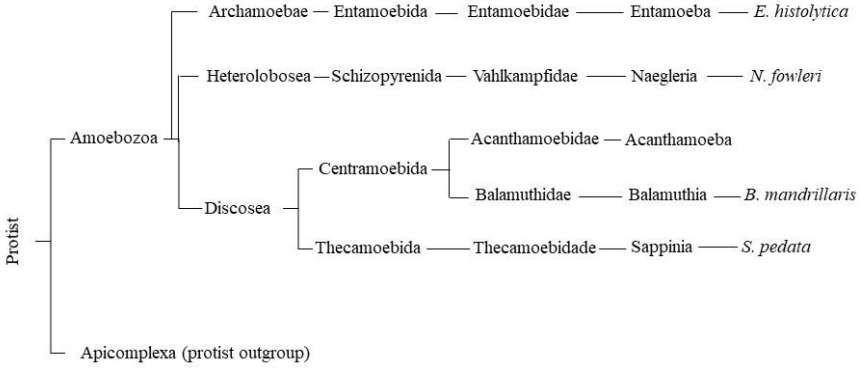

Fig. 1 Phylogenetic relationships of the Amoebozoa based on molecular data [29,30,31]. The dendrogram represents the non-dimensional phylogenetic relationships among the amoebic taxa that were the focus of the present study

3 – CEREBRAL ENTAMOEBIASIS (CE)

Pathogenesis and Clinical Features

Infection by *Entamoeba histolytica* occurs through the ingestion of water or food contaminated with parasitic cysts and is most common in populations from developing countries. Once in the small intestine, the cysts release trophozoites which move to the large intestine, where they feed on bacteria and cell debris, causing an asymptomatic infection in 90% of the cases [32].

However, a combination of factors may interfere with the infection process, including the genetic profiles of the human host and the amoeba, as well as its ability to produce proteolytic enzymes and resist complement-mediated lysis, and environmental factors, such as nutrient availability. This variation results in a diversity of clinical

manifestations, including diarrhea and both invasive and disseminated amoebiasis [33,34]. Depending on the exact combination of these factors, the *E. histolytica* trophozoites may invade the intestinal mucosa, reach the circulatory system, and affect other organs, such as the liver, lungs, and brain (figure 2), which may result in fatal infections associated with damage to the host tissue [35,36].

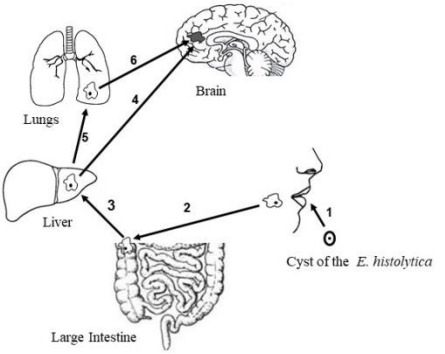

Fig. 2 Diagram of the entry route and access of *E. histolytica* to the CNS in the context of Cerebral Entamoebiasis (CE). The entry route is the ingestion of the amoebae (1), which pass through the large intestine (2) and the liver (3). From the liver, the trophozoites are transported in the blood, either directly to the CNS (4) or via the lungs (5, 6)

Once trophozoites invade the CNS via the bloodstream, *E. histolytica* causes CE [37], a rare complication of amoebiasis whose hallmark clinical feature is the formation of one or more abscesses (Figure 3) [38], primarily in the right cerebral hemisphere [39,40]. These abscesses may have purulent discharge [41] and mimic bacterial meningitis [42]. The clinical features also include high fever, fatigue, severe headache, lethargy, delusions, left hemiplegia, and coma [43,44]. Cerebral amoebiasis results from the spread of *E. histolytica* through the body usually from the liver or the lungs [45,46].

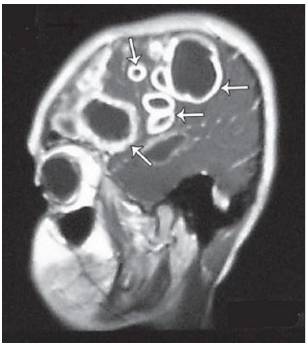

Fig. 3 Magnetic resonance scan showing amoebic abscesses (arrows) in the right front temporoparietal region [5]

Immune Response and Evasion

During infection, both innate and adaptive systems combat the invasion by *E. histolytica*. More specific mechanisms at the onset of amoebic infection include the binding of enterocytes with galactose lectin and N-acetyl-d-galactosamine (Gal/GalNAc lectin) to the surface of the trophozoite cells via the Toll-like receptor, which activates the NFκB and leads to the production of inflammatory cytokines, including IFN-γ, which is associated with the control of infection, and IL-4 and TNF-α, cytokines associated with the progression of the disease [47-48].

When spreading through the blood, the amoebae must survive in the blood vessels and spleen, escaping the defense cells, humoral factors, high oxygen concentrations, the complement system, and oxidative and nitrosative attacks from proteases and glycosidases [49]. The *E. histolytica* trophozoites release cysteine proteases into blood vessels, which cleave and inactivate the components of the C5a and C3acomplementsystem, the principal mechanism that mediates the destruction of the amoebae circulating in the blood [50,51]. In addition to evading the complement system, then, the trophozoites must prevent the action of mast cell histamine, leukocyte lysosomal enzymes, and macrophage cytokines, as well as minimizing their detection by the human immune system [52,53].

Amoebae also can concentrate the antibodies that attack them in specific regions of their plasma membrane called uroids, from which the antibodies are eliminated. This enables them to avoid antibody-dependent cytotoxicity [54]. *Entamoeba histolytica* also uses surface coating to mask itself from the immune system by antigenic surface molecules that have already been recognized [55].

Once they reach the brain, usually after causing liver damage, the trophozoites may suppress macrophage function, given that, in this location, these defense cells may refract the activation of IFN-γ and lipopolysaccharides [56,57], unlike other macrophages, more distant from the lesion site, which will remain functional in immunological terms [58].

Several studies indicate that the invasion of tissue by *E. histolytica* and the formation of abscesses are also linked to the regulation of the immune response. The trophozoites regulate IFN-γ production by immunomodulation, restricting it to levels that permit the survival of the amoebae, even though the population is reduced [59,60]. The amoeba may also evade the immune system by deactivating and killing immune cells. Amoebic trophozoites can kill neutrophils, macrophages, and T lymphocytes through a number of different cytotoxic mechanisms, and then phagocytize these cells, further reducing the pro-inflammatory responses of the infected individual [61,62]. The formation of brain abscesses and the strategies used by the parasite to evade the immune system thus both include immunomodulation, which suppresses the production IFN-γ, eliminates immune cells and soluble immune mediators, and modifies the metabolism of reactive oxygen and nitrogen species, to impede the action of the immune system [63,64].

Diagnosis of CE

The diagnosis of CE is hampered by several factors [65]. Headaches and sensory disturbances are the most common initial symptoms, although gastrointestinal, hepatic, or respiratory symptoms may also occur, in addition to symptoms typical of bacterial

meningitis, which hampers a reliable diagnosis [66]. Diagnosis by computed tomography is also impeded because images of brain abscesses caused by *E. histolytica* are indistinguishable from those provoked by other disorders, eliminating any possibility of a reliable etiology of amoebic infection [67]. The lack of any evidence of amoebic infection in the fluid of the abscess or other parts of the body further hampers diagnosis because it may lead to the mistaken assumption that the symptoms are caused by bacterial infection, with potentially serious implications for therapeutic intervention.

At the present time, the diagnosis of cerebral amoebiasis is based on a combination of tomographic or magnetic resonance imaging with serological or molecular (Polymerase Chain Reaction – PCR) methods. Diagnosis by PCR is important when the parasite is not identified by microscopy [68,69]. When trophozoites are observed microscopically, they are typical. The presence of ingested red blood cells is also considered to be pathognomonic of *E. histolytica* [70].

Treatment and Prophylaxis

Invasive amoebiasis, including the formation of abscesses, is treated with synthetic chemical compounds, in particular, nitroimidazoles. Metronidazole is the most commonly used compound [71,72]. However, there is some disputed evidence that metronidazole may have mutagenic effects and induce neural toxicity in the patient [73].

Recent *in vitro* trials have assessed the use of natural compounds from plant extracts for the treatment of amoebiasis [74,75] through the modification of its DNA replication, which would cause a loss of virulence in the parasite [76]. However, no *in vivo* studies have yet confirmed these findings [77]. The abscesses can also be treated using surgical decompression by drainage or aspiration with needles or catheters [78].

The prophylaxis of CE can be based on the treatment of individuals infected with *E. histolytica*. It is much more effective, however, to implement educational measures that ensure the adoption of adequate living conditions, in particular sanitation and hygiene.

4- PRIMARY AMOEBIC MENINGOENCEPHALITIS

Pathogenesis and Clinical Features

Primary Amoebic Meningoencephalitis (PAM) is an acute, and often fatal type of meningoencephalitis caused by the trophozoites of *Naegleria fowleri* (also known as the "brain-eating amoeba"), which penetrate the olfactory mucosa and neuroepithelium of healthy young individuals during contact with water contaminated by the parasite, typically when bathing in rivers, ponds or pools, especially in the summer months [79]. Once they have penetrated the cribiform plate (which is more porous in children and adolescents), the trophozoites reach the olfactory bulb of the CNS, where they cause an inflammatory reaction in the parenchyma of the frontal lobes of the brain, in which they replicate (Figure 4) [80,81].

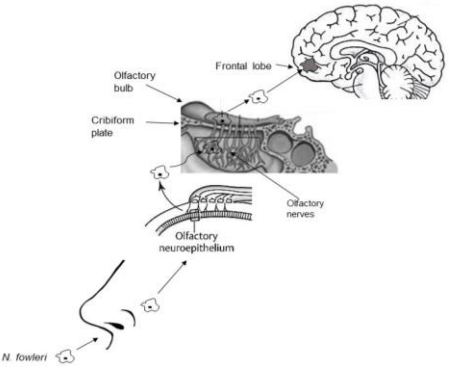

Fig. 4 Diagram illustrating the trajectory of *Naegleria fowleri* from its entry via the nasal cavity, through the olfactory neuroepithelium to the olfactory nerves, where it passes through the cribriform plate to reach the olfactory bulb, and then infects the cerebral parenchyma.

The pathogenicity of the trophozoites and the invasion of the brain by the amoebae depend on a number of different factors such as the abundance of amoebae, soluble factors released by the nerve endings of the cribriform plate, and the release of proteases and other cytolytic molecules, such as phospholipases, by the amoebae [82,83].The combined influence of these factors, together with the immune response of the host, determines the extent of the damage to the nerves and CNS, although it almost invariably results in the death of the patient [84]. Approximately16 people are estimated to die from PAM annually in the United States [85].

The factors that determine the virulence and pathogenicity of *N. fowleri* are still poorly understood, although cytopathological effects, adhesion, direct contact with the target cells, and the release of cytolytic molecules are all considered to be important components of the invasion process in this amoeba [86] .Like the A and B naegleriapores (proteins that are cytotoxic to humans and pore-forming amoeba), proteases and phospholipases may act together to facilitate the invasion of the host and cause cell destruction [87].

Clinically, PAM is a fulminant hemorrhagic meningoencephalitis that provokes cell destruction and cerebral edema, ands causes death within 10 days of the onset of the symptoms [88,89]. The initial symptoms of PAM occur after an incubation period of 3–7 days, although they are clinically indistinguishable from bacterial meningitis, including high fever, headache, neck stiffness, nausea, vomiting, irritability, restlessness, mental confusion, seizures, and lethargy [90,91]. Purulent exudate, extensive damage of the brain parenchyma and meninges are also typical of this disease [92,93].

Immune Response and Evasion

Infection by *N. fowleri* induces an initial inflammatory reaction in the nasal cavity, through which the amoeba can escape the immune system and adhere to the neuro-olfactory epithelium [94]. By passing through the olfactory bulb to reach the brain, the trophozoites provoke an increase in this inflammatory reaction and attract neutrophils

– the primary cellular defense against the parasite. This indicates that the host's inflammatory response and polymorphonuclear cell lysis contribute in a major way to the injury of the CNS [95].

As death occurs shortly after the onset of the PAM infection, there is usually not enough time for the patient to mount a specific detectable humoral immune response to *N. fowleri* [96]. The IgA and IgM antibodies present in the mucous secretions may play an important role in preventing infection by blocking the adhesion of the trophozoites to the olfactory mucosal epithelium [97]. The effectiveness of cell-mediated immunity against *N. fowleri* has been studied using the hypersensitivity response and macrophage migration assay, which have presented contradictory results in terms of the protective role of cell-mediated immunity against this amoeba [98].

It is possible to identify the Damage Associated Molecular Patterns (DAMPs) provoked by *N. fowleri* by the presence of TLRs in the macrophages and NK cells [99]. Intracellular signaling by the TLRs, coordinated by adapter molecules such as MyD88, is capable of activating kinases, translocating the NFkB, and inducing the expression of pro-inflammatory cytokines, mucins, and other antimicrobial products [100].

However, *N. fowleri* can bind to the nasal mucosa, destroy target cells, evade the immune system, and resist the action of cytolytic molecules, such as TNF-α, IL-1, and those of the complement system [101]. Given this, the protective role of both the humoral and cell-mediated immunity is still poorly understood [102].

Diagnosis of PAM

As PAM is an acute, rapid, and lethal infection, rapid diagnosis and treatment are essential for the recovery of the patient. As the results of laboratory tests (Cerebrospinal Fluid, CSF) for PAM and bacterial meningoencephalitis [103] are indistinguishable, misdiagnosis is common, although a history of bathing in pools or other bodies of water is suggestive of PAM [104].

Frequent optic microscopy of the CSF, when conducted immediately after sampling, is the method of choice in the diagnosis of PAM through the identification of mobile trophozoites, which may have flagella [105], together with antibody screening for the identification of *N. fowleri* tissue, while CSF sections may also be used [106].

Diagnostic imaging methods, such as computed tomography or magnetic resonance imaging, are inconclusive because they reveal nonspecific lesions [107], while the usefulness of blood serology with antibody enhancement testing is also limited, given that, in addition to the rapid progression of the disease, healthy individuals may also test positive for *N. fowleri* [108].

Immunofluorescence of biopsied brain tissue can make the trophozoites visible [109], while inverted light microscopy of cultures of infected CSF or brain tissue [110] may also be used. Molecular methods, such as PCR, enable a more rapid diagnosis of PAM, but are not generally available in clinical laboratories [111, 112].

Treatment and Prophylaxis

Although almost all recorded cases of PAM have resulted in death [113], some therapeutic regimens, associated with an early diagnosis and adequate clinical support, have been successful in the treatment of the disease [114,115]. Even so, it is important

to note that this success has been due, at least in part, to infection by *N. fowleri* strains of reduced virulence [116].

The few survivors of the disease, some of which escaped neurological impairment, had been treated with dexamethasone and/or miltefosine, although treatment with these drugs does not always guarantee full recovery [117]. These drugs are normally administered in combination with amphotericin B and other drugs, such as rifampicin and fluconazole. Intravenous amphotericin B is also used as a primary drug to treat PAM [118], although this has severe and systemic adverse effects, which reduces the penetration of the drug into the CNS [119], thus requiring high dosages to obtain the desired effect.

In addition to drugs, physical procedures such as CSF drainage, hyperosmolar therapy, moderate hyperventilation, and hypothermia have been employed to treat PAM [120]. Even when treated, the level of consciousness of the patient tends to decrease progressively, and the prognosis deteriorates rapidly [92]. Preventive measures include the prohibition of bathing in environments known or suspected to be contaminated with the amoeba, blowing the nose after bathing to dislodge any amoebae that may have been inhaled, and wearing nose-plugs when diving into freshwater environments [121].

5- GRANULOMATOUS AMOEBIC ENCEPHALITIS (GAE)

Pathogenesis and Clinical Features

The CNS infection pathways of *Acanthamoeba* sp. and *B. mandrillaris* are still poorly defined, although the available studies indicate two principal routes of infection in the transmission of GAE: the nasal inhalation of amoebae, which pass through the olfactory neuroepithelium (Figure 5) or lungs [122], and the invasion of cutaneous or mucosal lesions [123]. The transplantation of infected organs has also been reported as a potential source of infection [124,125].

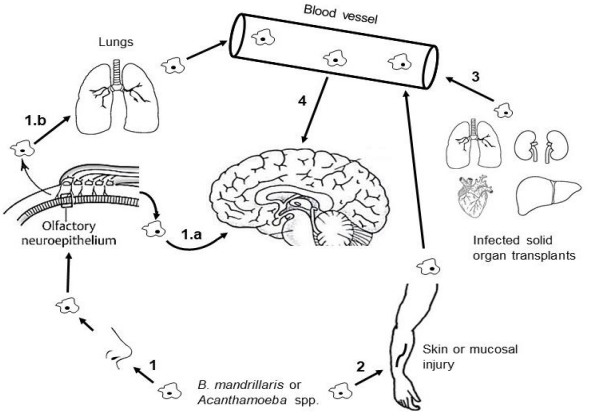

Fig. 5 Diagram showing the potential entry routes and CNS pathways of the amoebae that cause GAE. Three routes of entry are known: (1) nasal inhalation of amoebae that pass directly through the olfactory neuroepithelium (1.a) or lungs (1.b), (2) infection through cutaneous or mucosal lesions, and (3) transplantation of infected organs. The amoebae typically reach the CNS via a hematogenous pathway (4)

The amoebae found in the mucous membranes, skin, lungs or other organs, whether transplanted or not, are disseminated directly through the olfactory neuroepithelium or the hematogenous route [126,127]. It is not clear how the circulating amoebae penetrate the CNS to cause inflammation, although the breakdown of the blood-brain barrier (BBB) must occur through the human cerebral microvascular endothelial cells, using the paracellular pathway or the transcellular pathway (Figure 6) [128].

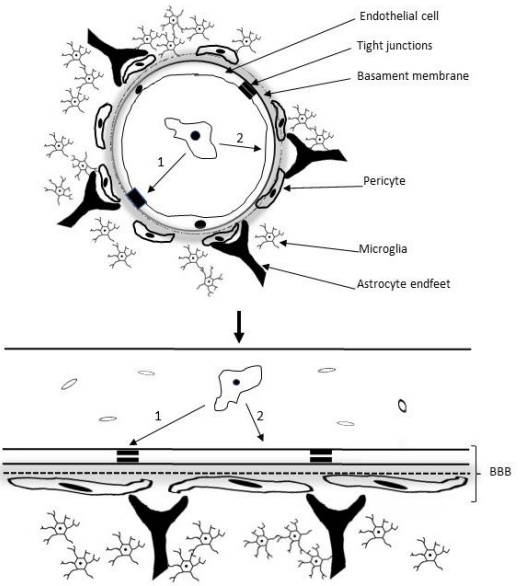

Fig. 6 Diagram of a neurovascular unit showing the elements of the BBB and the pathways used by *B. mandrillaris* and *Acanthamoeba* sp. to reach the brain parenchyma: 1- Paracellular Pathway: Tight junction elements, such as occludins and ZO-1, are degraded by Acanthamoeba proteases, independently of contact (Note that serine proteases, metalloproteases, and Ecto-ATPases facilitate the transmigration and passage of the amoebae to deeper regions of the brain). 2- Trans-cellular pathway: the galactose binding protein (GBP – in *B. mandrillaris*) or mannose binding protein (MBP – in *Acanthamoeba* sp.) bind to the microvascular endothelial cell receptors of the brain, altering the cycle and causing death, though a contact-dependent mechanism.

The principal virulence factors of the amoebae responsible for GAE are adhesins, phagocytosis, and plasminogen activation, as well as enzymes, such as Ecto-ATPases, neuraminidases, superoxide dismutase, elastases, phospholipases, proteases, and glycosidases [129]. A fundamental step in the pathogenesis of GAE is the adhesion of the amoebae to the surface of the host tissue, which results from the expression of a transmembrane protein known as the mannose binding protein (MBP), which binds to the mannose-expressing cells [130,131]. In its extracellular portion, MBP also contains cysteine-rich repeats, which can cause in blood-brain barrier perturbations [132]. It is important to note here that only the trophozoites express MBP, which is lacking in the cysts, and thus cannot form agglomerations or bind to the host cells [133]. After binding to the host tissue, the MBP activates a signaling cascade in which the amoebae release proteases that degrade the principal components of the extracellular matrix. This leads

to increased cell permeability and plays a key role in the breakdown of the cell [134]. The amoebae invade the brain tissue slowly, given that this process is contact-dependent and causes characteristic parenchymal lesions of the CNS, which results in chronic granulomatous encephalitis [135].

Post-mortem microscopic examination has revealed cysts and trophozoites, primarily in the perivascular spaces in the cerebral parenchyma. A necrotic phase is commonly observed. This phase is caused actively by the trophozoites when the amoebae feed or by the inflammatory processes [136].

The mechanisms associated with the pathogenesis of GAE are still poorly defined, although the amoebae are known to provoke a massive influx of extracellular Ca^{+2}, which causes the death of the nerve cells due to the greatly increased levels of intracellular calcium [137]. It is important to note here, however, that much of the damage to the nerve tissue is caused by components of the amplified immune response of the host [138].

Clinically, GAE is a subacute chronic infection with an insidious onset and initial manifestations that may include lethargy, headache, fever, and a stiff neck [139]. Shifts in mental status, neurological seizures, palsy of the cranial nerves, a loss of vision, anorexia, aphasia, ataxia, and coma may arise as the disease progresses [140]. In immunocompetent individuals, the infection progresses to a granulomatous inflammatory lesion in 2–3 weeks if the individual survives to this stage of the disease (Figure 7) [141]. Death usually occurs at between seven and 120 days after the onset of the infection [142].

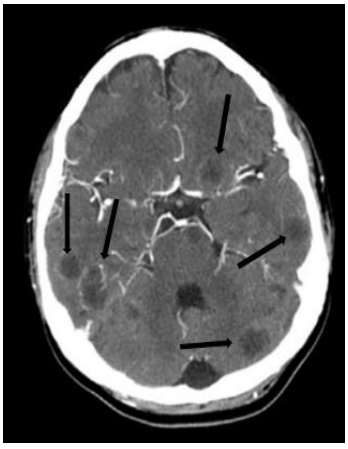

Fig. 7 Computed tomography showing multiple hypo-dense lesions (black arrows) in both cerebral hemispheres [143]

Immune Response and Evasion

Although *Acanthamoeba* sp. and *B. mandrillaris* have a worldwide geographical distribution, the incidence of CNS infections is very low [144], given that most of the human population has antibodies against these amoebae [145,146]. As the amoebae reach the CNS through hematogenous dissemination, the immune response of the host,

including both the innate and adaptive immune responses, must play a fundamental role in the progression of GAE.

As with any infection, the innate immune response represents the first and most important line of defense of the infected individual [147]. While the neutrophils and macrophages are the first cells recruited to fight infection, the macrophages may play a more critical role in the death of the pathogen, given that they can damage the amoebae and are the major cellular component of GAE [148]. Studies of animal models have also indicated the action of natural killer (NK) cells [149]. These cells, in addition to acting as effectors of the innate response, would also regulate part of the adaptive immune response [150].

As they reach the CNS via the hematogenous pathway, the amoebae also activate the complement system, which represents an additional weapon of the innate immune system, by activating the opsonization of pathogens and phagocytosis [151]. The activation of the complement, together with the action of the neutrophils, macrophages and antibodies, form a potent front against the infection by the amoebae [152].

In addition to the complement, neutrophils, macrophages, and NK cells, the TLRs are another important component of the innate immune system, providing recognition of the molecular patterns associated with several different pathogen groups (PAMPs). The bindings of the TLRs to the PAMPs has a number of different consequences, such as the induction of phagocytosis, the production of pro-inflammatory cytokines, the recruitment of neutrophils, the regulation of co-stimulatory molecules in cells with antigens, and the maturation of naïve dendritic cells, which together represent a fundamental step in the activation of the adaptive immune system [153].

The presence of non-phagocytosed amoebae in the CNS tends to produce a type IV Hypersensitivity Reaction (HSR-IV). In immunocompetent hosts, this progresses to a granulomatous inflammatory lesion within 2–3 weeks [141]. This immunological response causes the isolation of the trophozoites and macrophages at the site, which allows the epithelioid cells to secrete their lytic enzymes to fight the confined pathogen, resulting in the extensive destruction of host tissue [138]. In particular, T-cell immunity, which is a pre-requisite for GAE in immunocompetent hosts, contributes intensively to the occurrence of HSR-IV and the granulomatous inflammatory response [141].

The role of T lymphocytes in infections by *Acanthamoeba* sp. is still unclear. Immunocompetent individuals tend to have a good T $CD4^+$ lymphocyte response, in contrast with immunocompromised individuals, and experiments in guinea pigs have highlighted the importance of this response to combat *B. mandrillaris* [154,155]. The role of antibodies in the progression of GAE requires further clarification, given that *Acanthamoeba* sp. can evade the human immune system by degrading immunoglobulins through the proteolytic activity of serine proteases [156,157].

The known cases of survival of GAE may be accounted for, at least in part, by the action of microglia (CNS-resident immune cells), which release IFN-γ that stimulates, in turn, the release of specific cytokines, including TNF-α, IL-6, IL -1β, and IL-1α. These cytokines may initiate the immune response in the CNS [133]. Together with the alternative complement system pathway, IFN-γ and TNF-α are known to

activate pathogen disruption [158]. On the other hand, the B lymphocytes produce immunoglobulins, which stimulate the release of nitric oxide, a compound that is abundant in the formation of the typical GAE granulomas [159].

Diagnosis

Early diagnosis of GAE is difficult, but it is essential to ensure any chance of adequate treatment of this almost invariably fatal infection [160]. In most cases, the definitive diagnosis of GAE is based on the microscopic observation of trophozoites and cysts in histopathological specimens obtained from brain biopsy or cerebrospinal fluid, stained with hematoxylin-eosin or immunofluorescence [161]. *Acanthamoeba* sp. can also be diagnosed by the culture of agar plates containing bacteria or by molecular methods, such as PCR [162].

Serological tests that use monoclonal antibodies are also available, although further testing Is required to determine their sensitivity and specificity [163].However, as the disease usually lasts for weeks or even months, the detection of antibodies may be the most preferable approach, given that it is a noninvasive procedure and is effective for early detection. Several different methods, including IFI, ELISA, NMR spectroscopy, and flow cytometry, have been employed to detect antibodies [164,165].

Imaging has limited diagnostic value in the case of GAE because the physical signs can easily be confused with those of infections such as tuberculosis, toxoplasmosis, neurocysticercosis, and viral encephalitis, which hampers the accurate and reliable diagnosis of GAE [166].

Treatment and Prophylaxis

A few different therapeutic regimens have been proposed for the treatment of GAE, most of which combine drugs, such as trimethoprim-sulfamethoxazole, chlorpromazine, fluconazole, amphotericin B, ketoconazole, and rifampicin, which have been tested clinically [167–169], or prochlorperazine, loperamide, apomorphine, and procyclidine, which have not been tested [170].

Some patients have been treated successfully with pentionidine, isethionate, fluconazole, and sulfadiazine combined with azithromycin or clarithromycin [171,172]. Miltefosine, which is used to treat leishmaniasis, has also been used successfully to treat GAE, in combination with other anti-amoebic drugs [173,174].In addition to its effects on the amoebae, miltefosine may have other immunomodulatory effects that favor the wellbeing and recuperation of the patient [175].

As all these drugs act primarily on the trophozoites, the application of cellulases for the destruction of the cysts has been suggested as an alternative treatment for GAE, although up to now, with no conclusive evidence of any effective action [176]. Phenobarbital has also been administered as an anti-convulsive prophylactic [177]. It is still unclear what effective measures can be implemented to prevent GAE, although the avoidance of bathing in potentially contaminated bodies of water is likely to be the most efficient preventive measure against this disease.

The pathogenic and clinical aspects of the three types of amoebiasis reviewed in the present study are summarized in Table 1.

TABLE 1 Summary of the pathogeny, clinical features, and other aspects of the three principal types of Cerebral Amebiasis, reviewed in the present study

	CE	PAM	GAE
Transmission and Dissemination	Ingestion of cysts and hematogenous dissemination through the invasion of the intestine	Inhalation of trophozoites, followed by dissemination to the CNS through the olfactory bulb	Inhalation, absorption through the skin and mucosa or in infected organs used for transplantation, with dissemination through the neuroepithelium or the hematogenous pathway
Clinical features in the CNS	Brain abscesses, which may have purulent secretion	Fulminant hemorrhagic meningoencephalitis leading to death within 10 days	Chronic granulomatous inflammatory lesions in immunocompetent hosts
Immune Response and Evasion	Amoebic trophozoites kill and phagocyte neutrophils, macrophages and T lymphocytes, decreasing the pro-inflammatory response of the host.	The amoebae destroy target cells, evade SI, and resist the action of cytolytic molecules such as TNF –α and IL-1, as well as the complement system. There is no time for a specific detectable humoral immune response.	Microglia can release IFN-γ, which stimulates the release of specific cytokines that initiate the immune response of the CNS.
Treatment	Metronidazole.	Treatment based on Dexamethasone and/or miltefosine + amphotericin B, as well as other drugs, such as rifampicin and fluconazole.	Treatment based on Trimethoprim-sulfamethoxazole, chlorpromazine or miltefosine + amphotericin B, as well as other drugs, such as rifampicin and fluconazole.
Prophylaxis	Educational measures and investment in basic sanitation.	The avoidance of bathing in freshwater environments potentially associated with the disease, blowing the nose after bathing, and using nose plugs when diving.	The avoidance of bathing in freshwater environments potentially associated with the disease, blowing the nose after bathing.

7 – FINAL CONSIDERATIONS

The amoebae *E. histolytica*, *N. fowleri*, *B. mandrillaris*, and *Acanthamoeba* sp. can cause irreversible brain damage. A fatal outcome may be caused by several different factors the rarity of cases, symptoms that mimic bacterial meningitis and other pathological conditions, inconsistent imaging, a lack of experience in the healthcare personnel, which delays diagnosis and hampers management, a poor immune response, and ineffective antimicrobial therapy [178,179].

Thus, despite efforts to improve diagnosis and treatment, the prognosis of cerebral amoebiasis (CE, PAM, and GAE) remains very poor. Reported cure rates have been very low for more than 50 years, highlighting the need for advances and earlier diagnosis and intervention, to improve this prognosis [180].

The ample geographic distribution of the pathogens that cause cerebral amoebiasis demands that healthcare systems be aware of this type of infection and are prepared to recognize cases. To achieve this, it will be necessary not only to increase the awareness and monitoring of the pathogens, but also to conduct further, more detailed research.

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