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Neuroimmunology in Africa

Edited by
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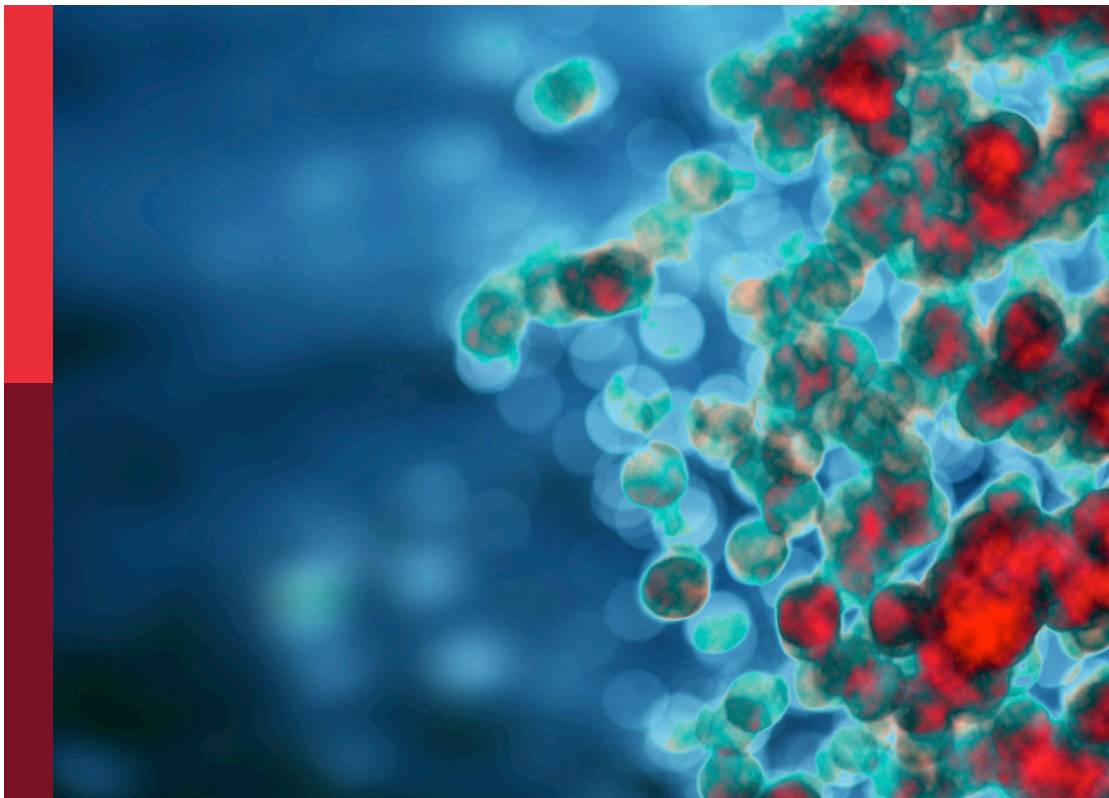

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Neuroimmunology in Africa

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Editorial: Neuroimmunology in Africa

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Editorial on the Research Topic
Neuroimmunology in Africa

Neuroscience in Africa

The history of brain knowledge in our world dates back to the Pharaonic civilization of ancient Egypt. In the 17th century BC, the Edwin Smith Papyrus gave the early reference to the brain by naming it "Marrow of the skull" (1). In this papyrus, the "Marrow of the skull" is recognized as composed of two hemispheres with circumvolutions (beginning of neuroanatomy) and wrapped in membranes; it is likely to spread a liquid (premises of immunology/neuroimmunology). From this period up to Galen, Egypt and then North Africa, were the center of knowledge about the human and animal brain (2, 3).

In the 1970s and 1980s, neuroscience research appeared sporadically in North and South Africa in the form of isolated publications from European-trained academics who joined universities in their home countries. Research was mostly clinical, developed on understanding diseases and developing treatments for neurological disorders such as epilepsy, motor disorders, but also infectious diseases: leprosy, tetanus, meningitis, encephalitis, which will later all come under the field of immunology and neuroimmunology. In some countries, research began to be structured around national associations or societies such as the Moroccan Association of Neuroscience (AMN; 1987), followed by the Southern African Society of Neurosciences (SANS; 1988), the Neuroscience Society of Nigeria (NSN; 1990) and Kenya Society for Neurosciences (KSN; 1992-1993). The Society of African Neuroscientists (SONA) was founded at the end of a scientific conference organized in 1993 in Kenya, federating the existing African Neuroscience Associations and Societies which were 4 at the time, and any individual researcher agreeing to be a member. The International Brain Research Organization (IBRO) has regularly supported all the activities of the SONA, which organizes a biannual congress. Other international organizations have also been involved in promoting SONA under various policies, the International Society for Neurochemistry (ISN) being a special example.

The need to take ownership of neuroscience training was felt and IBRO understood that it was necessary to sponsor neuroscience education in Africa for sustainable development. The first African school in neuroscience was organized in 2000 in South Africa. For more than 20 years now, IBRO has been generously supporting neuroscience schools and training workshops in more than 14 African countries. In addition to schools, two regular workshops were organized in conjunction with SONA conferences: Writing Papers workshop and Teaching Tools workshop. ISN has also contributed significantly to the funding of these activities. In 2015, IBRO created two regularly funded advanced neuroscience training centers (IBRO African Centers for Advanced Training in Neuroscience: ACATN): the first in Cape Town in 2015 and the second in Rabat in 2016. These centers were to organize two to three advanced training courses every year on value-added themes for Africa, with at least one regular school per center. The Cape Town center specialized in Computational neuroscience and the Rabat center on Basal Ganglia and Movement Disorders. In addition, the Rabat center hosted two neuroimmunology schools, in 2017 and 2019, supported also by the International Society of Neuroimmunology (ISNI) through the African School of Neuroimmunology. From these came the idea of this collection "Neuroimmunology in Africa". This collection follows the path of previous collections focusing on neuroscience in Africa. A first collection appeared in Frontiers in Neuroanatomy on "Neuroscience in Africa" in 2019 (4), followed by another collection published as a Special edition of IBRO neuroscience reports titled "Neuroscience in Africa" in 2023 (5).

The momentum that neuroscience in Africa has gained, boosted by these investments in education, can be appreciated in Figure 1, showing that the number of publications in this area co-authored by researchers with African institutional affiliations has been almost doubling every five years, starting from 1995.

This impressive growth was especially driven by nations like Algeria, Cameroon, Egypt, Ethiopia, Ghana, Kenya, Morocco, Nigeria, South Africa, although a steady increase, with a similar slope, can be appreciated also in nations with a more limited output in terms of neuroscience publications.

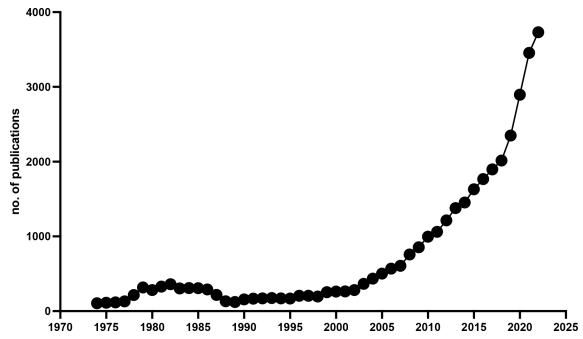

FIGURE 1
Number of neuroscience publications by authors with African institutional affiliations. Data extracted from Scopus using as keywords neuroscience, neurology, nervous system, brain, spinal cord, and African institutional affiliations.

Overview of the collection

The 14 reviews and one original research article in this Research Topic focus on the neuroimmunology of endemic diseases or leading causes of disease burden in Africa and can be divided into four main categories – general neuroimmunology, infectious diseases, neurological consequences and biomarkers of infectious diseases, and non-communicable diseases.

General neuroimmunology

Mapunda et al. review how the immune cells cross various barriers such as the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier and get into the brain, during multiple sclerosis (MS). The article also explores the influence of genetic and environmental factors on how immune cells enter the CNS during neuroinflammation, with special emphasis on Africa. The role of different T helper cells, such as Th1, Th17, GM-CSF-producing Th cells, and cytokines in neuroinflammation and neurodegeneration are covered by Krishnarajah and Becher. Olude et al. review astrocytes and microglia including their physiology, crosstalk between them and the role they play in health and disease, emphasizing the African perspective in the context of stressors such as malnutrition, developmental stress, and environmental pollutions.

Infectious diseases

The total disease burden in Africa is still dominated by communicable diseases (6). Six reviews cover neuroinfections caused by viruses, bacteria, fungi, and parasites. Human immunodeficiency virus (HIV) infections disproportionally affect Africa. Meyer et al. describe the neuroimmunology of HIV CNS infection. CNS injury is caused by the virus, opportunistic infections, and local immune inflammatory reactions. Immune cells and cytokines from the periphery also cause CNS neuroinflammation. Klein reviews the neuropathogenesis of specific endemic mosquito-borne viruses (arboviruses) of the *Flaviviridae* family (such as West Nile virus and Zika virus) and *Togaviridae* family (such as chikungunya virus and Sindbis virus). Neurotropic arboviruses enter the CNS through retrograde transport of virus along axon microtubules of peripheral neurons, infection of olfactory sensory neurons or through the BBB. Scott and Nel describe rabies lyssavirus (RABV) endemic in Africa, that cause the fatal encephalitic disease rabies. Pathogenic RABV strains inhibit innate immune signaling, induce cellular apoptosis and use viral protein to facilitate retrograde axonal transport of the virus to the CNS. Idro et al. review parasites that infect the CNS such as *Plasmodium falciparum*, *Toxoplasma gondii*, *Trypanosoma brucei*

spp., and *Taenia solium* species. The article explains the role of the immune system in neuroinvasion, control and neuropathogenesis of parasites. Mohamed et al. cover fungal CNS infections in Africa, with special emphasis on the neuroimmunology of cryptococcal meningitis, which is the leading cause of CNS fungal infections in humans. Barichello et al. describe bacterial meningitis in Africa, the common bacteria that cause it, how the bacteria get to the brain, interactions of the bacteria with neurons, and the role of microglia and cytokines play in the neuroinflammation associated with bacterial meningitis.

Neurological consequences and biomarkers of infectious diseases

Ngarka et al. sum up the interplay between neuroinfections, the immune system and neurological disorders with a special emphasis on neurological diseases common in Africa as a sequelae of neuroinfections. Neurological disorders associated with HIV infection such as HIV-associated neurocognitive disorders, motor disorders, chronic headaches, and peripheral neuropathy are high in the sub-Saharan region because of high prevalence of HIV. The immune system deregulation in addition to the virus and antiretroviral drugs contribute to these neurological disorders. Infections such as toxoplasmosis, neurocysticercosis, onchocerciasis, malaria, bacterial meningitis, tuberculosis, and the immune reactions they elicit contribute to the high prevalence of epilepsy on the continent. Other neurological disorders attributable to neuroinfections and the neuroimmune response they trigger include sleep disorders, secondary headaches, dementia, motor neuron diseases. Ihunwo et al. explain how some viruses can get to the brain and affect neurogenesis. Zika virus can infect fetal brain and affect neural stem cells, neurogenesis, synaptogenesis, and cause cell death, with severe consequences such as microcephaly and decreased brain tissue. Severe acute respiratory syndrome coronavirus 2 can infect the olfactory bulb and travel to the CNS by retrograde axonal transport along olfactory sensory neurons, target neurons, astrocytes, and microglia and result in neurological symptoms observed in coronavirus disease 2019 (COVID-19) patients. Ndondo et al. review post-infectious autoimmunity in the CNS and peripheral nervous systems, pointing out the peculiarities in Africa. They cover the various conditions that occur after viral infections such as acute necrotizing encephalopathy, measles-associated encephalopathies, HIV neuroimmune disorders, and difficulties associated with classical post-infectious autoimmune disorders such as the Guillain-Barré syndrome in the context of HIV and other infections. NMDA-R encephalitis and myasthenia gravis, as the classic antibody-mediated disease, are also covered. Teunissen et al. summarize research in the use of biomarkers in tuberculous meningitis and pediatric HIV. They explain the possible diagnostic and prognostic values of some inflammatory molecules, such as cytokines and chemokines, and brain injury molecules, such as S100, neuron specific enolase and glial fibrillary acidic protein, when detected in the CSF. The only original research article in the theme by Bertran-Cobo et al., conducted in South Africa, found that myo-inositol, a marker for glial reactivity and inflammation, was elevated in children who are HIV-exposed and uninfected, which points to ongoing neuroinflammatory processes that may contribute to developmental risk in these children.

Non-communicable diseases

The prevalence of non-communicable diseases is increasing on the African continent (6). Ballerini et al. review non-communicable neurological disorders and neuroinflammation, focusing on traumatic brain injury (TBI), stroke, and neurodegenerative diseases such as dementias because they represent a major cause of morbidity and mortality in Africa. Neuroinflammation, encompassing glial cell activation and cytokine secretion, is a major factor in the pathobiology of TBI and stroke. In Alzheimer's disease, neuroinflammation is both a reaction against and a contribution to the neurodegenerative pathology.

Conclusions

All these articles emphasize the importance of the subject of neuroimmunology in Africa, in some cases because of the peculiarities the continent has in terms of infectious diseases but also for its importance to healthcare including diagnosis, treatment and understanding the neurological disorders that occur as sequalae of infectious diseases as well as non-communicable neurological disorders. Various knowledge gaps are highlighted that necessitates further research in these various disorders. This research will not only benefit the African continent but the world at large in understanding the CNS, neuroimmunology and neuroinflammation. For example, trypan dyes developed by Paul Ehrlich in search of drugs to kill African trypanosomes aided Edwin E. Goldmann to discover the BBB (7).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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MINI REVIEW
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Lyssaviruses and the Fatal Encephalitic Disease Rabies

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Lyssaviruses cause the disease rabies, which is a fatal encephalitic disease resulting in approximately 59,000 human deaths annually. The prototype species, rabies lyssavirus, is the most prevalent of all lyssaviruses and poses the greatest public health threat. In Africa, six confirmed and one putative species of lyssavirus have been identified. Rabies lyssavirus remains endemic throughout mainland Africa, where the domestic dog is the primary reservoir – resulting in the highest per capita death rate from rabies globally. Rabies is typically transmitted through the injection of virus-laden saliva through a bite or scratch from an infected animal. Due to the inhibition of specific immune responses by multifunctional viral proteins, the virus usually replicates at low levels in the muscle tissue and subsequently enters the peripheral nervous system at the neuromuscular junction. Pathogenic rabies lyssavirus strains inhibit innate immune signaling and induce cellular apoptosis as the virus progresses to the central nervous system and brain using viral protein facilitated retrograde axonal transport. Rabies manifests in two different forms - the encephalitic and the paralytic form - with differing clinical manifestations and survival times. Disease symptoms are thought to be due mitochondrial dysfunction, rather than neuronal apoptosis. While much is known about rabies, there remain many gaps in knowledge about the neuropathology of the disease. It should be emphasized however, that rabies is vaccine preventable and dog-mediated human rabies has been eliminated in various countries. The global elimination of dog-mediated human rabies in the foreseeable future is therefore an entirely feasible goal.

Keywords: Rabies, lyssavirus, encephalitis, zoonosis, immune evasion, pathophysiology

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INTRODUCTION

Lyssaviruses are responsible for rabies, which is arguably the deadliest encephalitic disease known. The prototype, rabies lyssavirus (RABV), is thought to be able to infect all terrestrial mammals. Transmission is through virus-laden saliva, typically through the bite of an infected animal, but sometimes through other means such as scratches and in rare occasions, organ transplants and other means (1, 2). The genus *Lyssavirus* (family *Rhabdoviridae*) is presently composed of 17 viral species and one putative (3). All lyssaviruses are bullet-shaped particles containing negative sense RNA genomes of approximately 11 000 nucleotides in length. The genome encodes 5 structural proteins, namely the nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and the polymerase (5'-N-P-M-G-L-3') with a 5' – 3' transcriptional bias (4, 5). The N protein

encapsidates the viral RNA, and together with the P and L proteins, forms the ribonucleoprotein (RNP) complex, which can initiate viral transcription and replication (6). The M protein condenses the RNP into the characteristic bullet-shape and recruits the RNP to the cellular membrane during replication. The M protein is also essential for the budding of the enveloped virus from the cell and specifically interacts with the G protein – also known as the transmembrane spike protein, which is the primary antigenic determinant (7, 8).

RABV is not only the type species of the genus, but by far poses the most significant public health threat among all the lyssaviruses. The domestic dog is the primary reservoir for RABV in dog-rabies endemic countries, but several other terrestrial mammalian species can maintain transmission – most notably carnivores such as raccoons, skunks, foxes, and jackals.

THE GLOBAL BURDEN OF DOG RABIES

Globally, an estimated 59,000 people die from dog-mediated rabies every year, of which approximately 40% are children under the age of 15 years (9). Rabies affects the poorest and most underserved communities, with the burden being greatest in developing countries of Africa and Asia (10). However, the disease is seriously underreported for a variety of reasons and remains among the most significant diseases of neglect in the world (11).

By continent, Africa has the second highest burden of rabies, with an estimated 23,500 deaths annually, and has the highest per capita death rate (9). RABV is endemic throughout mainland Africa, with only a handful of island nations having never detected rabies in domestic or wildlife species (e.g., La Réunion, Mayotte, Mauritius) (12).

Of the seventeen recognized lyssavirus species, six confirmed and one putative species have been identified in Africa, namely, RABV, Duvenhage virus (DUVV), Lagos bat lyssavirus (LBV), Mokola lyssavirus (MOKV), Ikoma lyssavirus (IKOV), Shimoni Bat Lyssavirus (SHIBV) and the putative Matlo lyssavirus. Of these, only DUVV (n=3), MOKV (n=2) and RABV have been associated with human fatalities (13). While RABV is only associated with non-volant terrestrial mammals in Africa, DUVV and LBV are both associated with bat reservoirs, while IKOV and MOKV have yet unidentified reservoirs (14, 15).

PATHOPHYSIOLOGY

Viral Entry, Spread and Proliferation

The most common method of viral entry is through the injection of virus-containing saliva into the muscle tissue or other peripheral tissue through the bite of an infected animal (**Figure 1**). After inoculation, RABV typically infects muscle cells — thought to be facilitated through the nicotinic acetylcholine receptor — and replicates therein at a low rate (16). The virus remains localized to

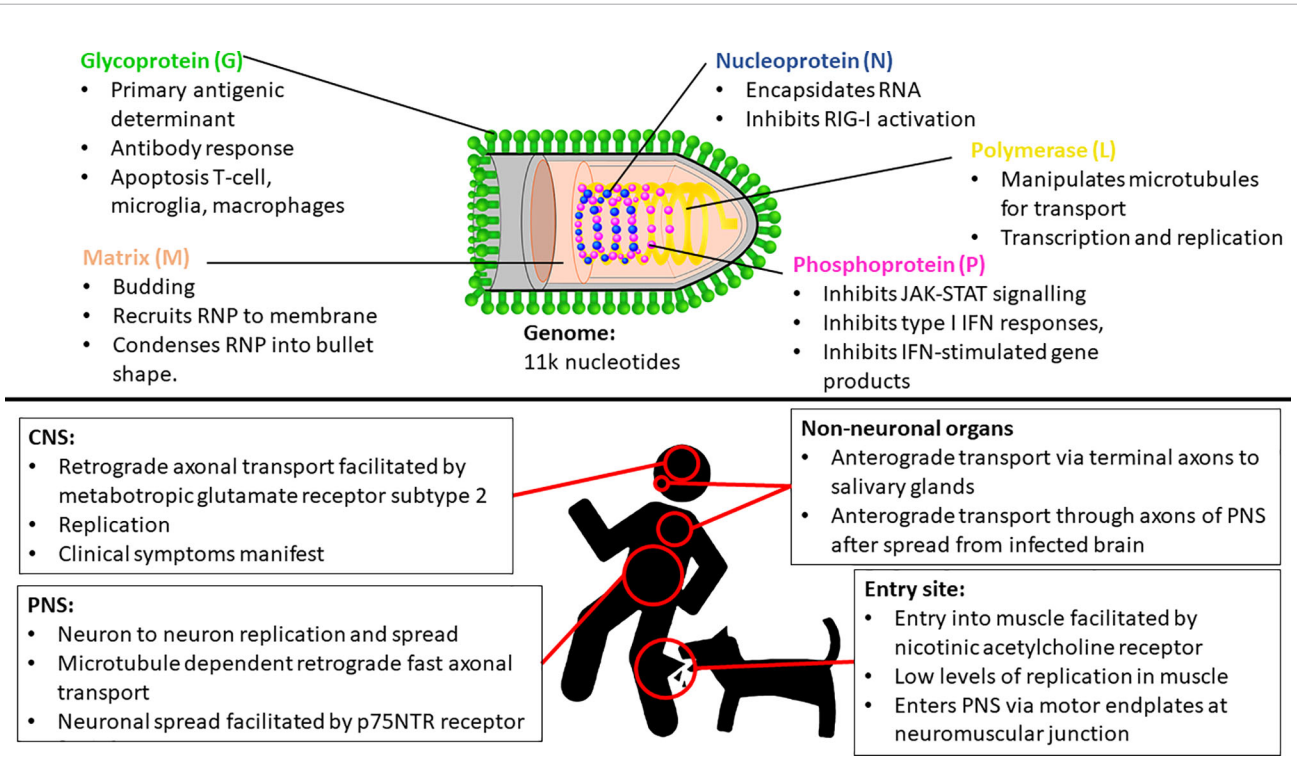

FIGURE 1 | Key insights of Rabies lyssavirus (RABV) entry, spread and proliferation, and some important functionalities of each RABV protein. IFN, Interferon; CNS, Central nervous system; PNS, Peripheral nervous system; RNP, Ribonucleoprotein complex.

the inoculation site for variable periods — which may contribute to the variable incubation period characteristic of rabies (17). In contrast, in the case of higher titers of inoculum, RABV can infect motor endplates without the need for the initial replication in the muscle (18). RABV gains entry into the peripheral nervous system (PNS) *via* motor endplates at the neuromuscular junction, but the exact means of virus internalization remains poorly understood.

RABV travels through the PNS towards the CNS *via* microtubule dependent retrograde fast axonal transport (19, 20). The virus travels from neuron to neuron, replicates, and continues its progression towards the CNS and the brain (21). This neuronal spread is facilitated by the p75NTR receptor, which is non-essential for infection, but facilitates directed and more rapid transport of RABV to the CNS (22). The L protein manipulates microtubules for improved transport efficiency (23), while the M protein facilitates the depolymerization of microtubules resulting in improved viral transcription and replication efficiency (24) (**Figure 1**). While retrograde transport occurs at an approximate rate of 50 – 100mm per day in humans [with species-dependent variation (20, 25)], evidence also suggests that RABV undergoes active, G protein-dependent anterograde transport in peripheral neurons - such as Dorsal Route Ganglion (DRG) neurons — at a rate three times faster than that of retrograde transport (25). However, the significance of this anterograde transport mechanism is unclear, but recent evidence signifies its importance in the spread of RABV through the PNS (including to non-neuronal organs) after centrifugal spread from the CNS (26), contrasting previous evidence that suggested that RABV spreads by both axonal and trans-synaptic transport exclusively in the retrograde direction (21, 27). Once in the CNS, RABV continues to spread *via* retrograde axonal transport thought to be facilitated by metabotropic glutamate receptor subtype 2, which is a cellular entry receptor that is abundant throughout the central nervous system (CNS) (28). The virus reaches the brainstem and subsequently the brain, where it proliferates and clinical symptoms manifest. It spreads to the salivary glands along terminal axons *via* anterograde transport (29) where it continues to proliferate and is subsequently shed in the saliva for transmission to another host. RABV can spread to peripheral, non-neuronal organs anterograde transport, and can be detected in these sites after the onset of clinical symptoms (21, 26).

Symptoms, Disease Progression, Prevention, and Treatment

Rabies presents with a wide variety of clinical manifestations that vary depending on multiple factors, many of which remain unknown. However, the species of lyssavirus or the strain of RABV influences the presentation of differing clinical symptoms. For example, bat RABV infections more commonly present with tremors and involuntary twitching/jerking (myoclonus), while dog strains more frequently present with classical hydrophobia and aerophobia (30). Moreover, the presentation of symptoms localized to the wound were more common in bat rabies exposures than in dog-rabies exposures (30). Two forms of rabies can manifest, namely encephalitic (furious or classical) and paralytic (dumb) rabies. The encephalitic form of rabies is more common and presents in approximately 80% of patients, of which between 50 – 80% present with the classic symptoms such as hydrophobia and aerophobia – symptoms that are unique to rabies (31, 32). However, the remaining symptoms are common to many encephalitic diseases, especially in African countries where diseases such as cerebral malaria are endemic and can result in misdiagnosis of rabies (33). Encephalitic rabies typically progresses to severe flaccid paralysis, coma and death caused by multiple organ failure, in contrast to paralytic rabies which manifests with prominent muscle weakness early in the course of illness (31). While there remains a gap in the understanding of the causes for the manifestation of these two different forms of rabies, it is known that the anatomical site of the exposure is unrelated (34). Initially rabies symptoms were thought to be caused by large-scale neuronal cell death, but neuronal apoptosis is only stimulated during infection with low pathogenicity strains (35, 36). Rather, symptoms are thought to be due to neuronal cell dysfunction (35, 37–41), partly induced by the increased production of Nitric Oxide (NO) *via* inducible nitric oxide synthase (iNOS) in neurons and macrophages (42–44). Elevated levels of NO produced by iNOS leads to mitochondrial dysfunction and as a result, axonal swelling (44, 45) — a pathology that is associated with the onset of symptoms (41, 46), and hypothetically explains the development of encephalitic symptoms (47). Another mechanism behind neurological dysfunction and the onset of neurological symptoms has been demonstrated to be reliant upon a host-derived mechanism that results in the loss of axons and dendrites as a means to prevent the spread of the virus (48).

The survival time for patients manifesting paralytic rabies is approximately 41% longer than that of patients with encephalitic rabies (30, 49), yet the incubation periods for both forms remain similar – ranging from 2 weeks to several months. For most cases, the incubation period is 2 – 3 months in humans, but some exceptional cases have been documented with an incubation period of more than a year and even up to 8 years (50, 51). There is no known accepted treatment for rabies after the onset of clinical symptoms. Palliative care is recommended for rabies patients, which is aimed to reduce suffering and may temporarily prolong survival time, but in all but the most exceptional circumstances, the victim succumbs to the disease (32, 50). However, effective pre- and post-exposure prophylaxis exists for those viruses that fall within lyssavirus phylogroup 1 [RABV, European bat lyssavirus-1 and -2, Bokeloh bat lyssavirus, DUVV, Australian bat lyssavirus, Aravan lyssavirus, Khujand lyssavirus, Irkut lyssavirus, Taiwan bat lyssavirus, Gannoruwa bat lyssavirus (GBLV)]. Experimental evidence suggests that the vaccines are not effective against phylogroup 2 (LBV, MOKV, SHIBV) or phylogroup 3 lyssaviruses (IKOV, West Caucasian bat lyssavirus, Lleida bat lyssavirus) (50, 52–56).

IMMUNE RESPONSE AND IMMUNE EVASION

Upon initial infection, the innate immune response is triggered in the periphery and evidence suggests that this response is

partially effective against even the most pathogenic strains, with some viral particles being eliminated (57). However, further clearance is not achieved as pathogenic strains poorly stimulate and inhibit the activation and maturation of dendritic cells, resulting in a poorer antibody immune response (58–60). This prevention of the maturation of DCs is achieved through the inhibition of the interferon (IFN) autocrine feedback loop that is dependent on JAK-STAT signaling, which is specifically inhibited by the P protein (61).

The ability of lyssaviruses to evade the immune response is directly correlated to its pathogenicity, with pathogenic strains inducing a minimal response and successfully evading immune clearance (18). All the RABV proteins are multifunctional, with roles in viral entry, replication and spread, as well as in the sequestration of the immune system – either directly or indirectly (62). This ability is reliant solely on the immune-suppressive capabilities of viral proteins - primarily being the P, G and N proteins. The P protein is typically involved in sequestering the innate immune response by inhibiting the production of multiple antiviral products such as MxA, OAS1 and IFN-stimulated gene products (62). Furthermore, the P protein inhibits type I IFN responses and subsequent innate and adaptive immune responses through the inhibition of various IFN-related signaling pathways (63–67). The evasion of IFN responses in infected neurons is likely to be essential for the spread of RABV through the PNS, enabling the virus to reach the brainstem and eventually the salivary glands for spread to a new host (57). Similarly, the N is also predominantly involved in the sequestration of the innate response, primarily through the inhibition of RIG-I activation (68–70). Apoptosis in macrophages, T cells (including infiltrating T cells in the CNS) and microglia plays an important role in immune evasion and is stimulated by the G protein of pathogenic strains (71, 72), which appears to assist in the effective infiltration, replication and spread of the virus in the CNS (36, 73, 74).

DISCUSSION

While rabies has arguably been recognized for thousands of years, there remain many gaps in scientific knowledge of the disease and its causal agents. The rapid detection of 10 novel lyssaviruses in the past two decades raises multiple public health concerns, with their broader distribution and possible public health impact being yet unknown (13, 75). While information relating to many of the lyssavirus species remains poor, studies suggest that sustained spillover events from non-RABV lyssaviruses are likely to be rare, as almost all lyssaviruses – except for RABV and ABLV – are restricted to a single host species (76). However, many lyssavirus species have only a single, or few, isolates, including the novel GBLV which has a recent common ancestor with ABLV (56). In addition, host shifts in areas where RABV is endemic are likely to remain undetected due to poor surveillance (76). While host shift events remain rare, their impact can be devastating. North America alone is endemic for multiple terrestrial RABV variants, each being resultant of a host shift event (77). While host shift events may be geographically restricted, the potential for the translocation of the virus through human means remains a distinct possibility and risk (78–81). For example, the largest epizootic in recorded history resulted from the human-mediated translocation of a raccoon from the south-east of the United States to the north-eastern states (82). Further evidence suggests that raccoon rabies was enzootic at low levels for many years before its detection, natural spread, and subsequent human translocation (83). The raccoon RABV variant now accounts for nearly 75% of all terrestrial rabies cases in the USA and resulted in a significant increase in the number of human exposures in those areas where it is endemic (84). Thus, despite the rabies-related viruses not posing a significant health threat at present, continued efforts need to be made to ensure public health safety based on the limited knowledge and surveillance data available.

Despite the availability of an effective prophylactic treatment before the onset of symptoms, there remains no cure once rabies symptoms manifest. In addition, the majority of immunopathological knowledge available pertains to RABV, with limited studies being available for the rabies-related lyssaviruses. Therefore, there is a need for continued investigation into the mechanisms of infection, disease progression, host biology and a better understanding of bat immunology. Over and above, there is a dire need for improved global surveillance for all lyssaviruses. Given the significant public health threat posed by dog-mediated RABV, such surveillance data should play a critical role in the elimination of the disease from those dog populations where it is still rampant due to a failure to effectively break transmission through mass vaccination.

AUTHOR CONTRIBUTIONS

TS: Conception, preparation of first draft, editing and final review. LN: Conception, editing and final review. All authors contributed to the article and approved the submitted version.

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The authors would like to credit the 'Dog and man' image to 'Freepik from Flaticon.com'. The 'Rabies virus' image, adapted under Creative Commons CC BY 4.0 license, is owned by the authors.

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Encephalitic Arboviruses of Africa: Emergence, Clinical Presentation and Neuropathogenesis

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Many mosquito-borne viruses (arboviruses) are endemic in Africa, contributing to systemic and neurological infections in various geographical locations on the continent. While most arboviral infections do not lead to neuroinvasive diseases of the central nervous system, neurologic diseases caused by arboviruses include flaccid paralysis, meningitis, encephalitis, myelitis, encephalomyelitis, neuritis, and post-infectious autoimmune or memory disorders. Here we review endemic members of the *Flaviviridae* and *Togaviridae* families that cause neurologic infections, their neuropathogenesis and host neuroimmunological responses in Africa. We also discuss the potential for neuroimmune responses to aide in the development of new diagnostics and therapeutics, and current knowledge gaps to be addressed by arbovirus research.

Keywords: alphavirus biology, neuroimmunology, Flavivirus, Africa, CNS

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INTRODUCTION

Recent studies indicate that climate changes in Africa may lead to a shift in vector-borne diseases from malaria to arboviruses due to differential effects of warming temperatures on the mosquito species that transmit these pathogens to humans [summarized in (1)]. Thus, neurotropic arboviruses that are transmitted by *Aedes aegypti*, and cycle between wildlife and livestock or humans in west sub-Saharan Africa, are likely to emerge in other areas of Africa where the current climates supports *Anopheles gambiae* transmission of malaria (2, 3). Recent epidemics of yellow fever (YFV) and Rift Valley fever (RVFV) viruses in Nigeria and Uganda (4, 5), respectively, and emergence of West Nile virus (WNV) in the Darfur region (6) are consistent with these predictions. In addition, the United Nations estimates suggest an increase in global population of 37% by 2050 (7), which facilitates transmission of vector-borne diseases through higher population densities and international travel. While the majority of infections with neurotropic arboviruses are asymptomatic, many persons develop flu-like symptoms that progress to neuroinvasive diseases in approximately half of symptomatic patients. In addition, 50-70% of survivors of CNS arboviral infection go on to develop neurocognitive and neuropsychiatric disorders that worsen over time (8). In this subsection, we will review the epidemiology, pathophysiology, and value of neuroimmune changes in diagnostics and therapeutics of medically important, African mosquito-borne neurotropic arboviruses. We will also provide current knowledge gaps and perspectives regarding future research in neurotropic arboviruses.

OVERVIEW OF AFRICAN MOSQUITO-BORNE ARBOVIRUSES THAT INDUCE NEUROINVASIVE DISEASES IN HUMANS

The etiologic agents of arboviral neuroinvasive diseases occur within three virologic genera: *Flaviviridae*, *Togaviridae*, and *Bunyaviridae*. The Phlebovirus RVFV (*Phenuiviridae* family) has been recently and extensively reviewed (9–14). Categorization of these RNA viruses, their key attributes, types of neuroinvasive diseases they cause, in addition to geographic epidemiology, and pathophysiology for medically relevant *Flaviviridae* and *Togaviridae* family members are summarized below (see **Table 1**).

Flaviviridae

Members of the *Flaviviridae* family of viruses are enveloped, with a positive single-strand RNA genome of 9-13 Kb with that replicates as a single open reading frame (ORF) with genes for three structural and seven nonstructural (NS) proteins (15). Structural proteins, which comprise the virion, consist of the viral capsid and the envelope glycoproteins. NS proteins are essential for replication of the viral genome, transcription and translation of viral genes, viral assembly, and may modulate immune function to promote infection and dissemination within humans. Phylogenetic trees indicate that all vector-borne flavirviruses originated in Africa, likely from non-vectored mammalian viruses (16). Medically important, neurotropic flaviviruses that cause CNS disease in Africa are transmitted by *Culex* (West Nile encephalitis viruses; WNV), and *Aedes* (Zika virus; ZIKV, Dengue virus; DENV) mosquito species (17). WNV was first isolated from a febrile patient in the West Nile district of Uganda in 1937, while ZIKV was first identified in a rhesus monkey from the African regions in Kampala, Uganda, in the Zika forest in 1947 (18, 19). A DENV epidemic was first reported in 1823 in the Zanzibar Islands (20). WNV human cases occur in most African countries throughout the continent with the exception of the western Sahara desert, Mauritania, Mali, Burkina Faso, Niger, northern Chad, Libya, and Angola (17) (**Figure 1**). ZIKV outbreaks in humans have occurred in only nine countries: Senegal, Cote D'Ivoire, Burkina Faso, Nigeria, Cameroon, Gabon, Central African Republic, Ethiopia, and Angola (**Figure 1**). DENV, which exists as four closely related but distinct serotypes, is endemic in almost all African countries, with the exception of Morocco, Algeria, Tunesia, Western Sahara, Niger, Chad, Sudan, Gambia, Guinea-Bissa, Guinea, Sierra Leone, Liberya, Ivory Coast, Central African Republic, South Sudan, Congo, Burundi, Botswana, Zimbabwe, Swaziland, and Lesotho (**Figure 1**). Both WNV and ZIKV may also be transmitted *via* transfusion of human blood products, and ZIKV can also be transmitted *via* sexual contact, primarily with males.

WNV and ZIKV are neurotropic viruses that can cause acute flu-like illnesses with fever, headache, rash, pharyngitis, diarrhea, arthralgias, conjunctivitis, and myalgias (21). Most humans infected with WNV or ZIKV are asymptomatic, however 20-25% of cases become symptomatic, and in those infected with WNV, approximately half of these patients will develop neuroinvasive diseases including meningitis, encephalitis, myelitis, and flaccid paralysis. Vertical transmission leading to teratogenic effects of ZIKV during pregnancy is also well documented with approximately 20% of affected fetuses exhibiting morphological abnormalities by ultrasound (e.g., microcephaly or brain calcifications), whereas the vast majority exhibit no overt clinical manifestations at birth (22–24) Diagnostic tests include assessment of serum or CSF virus-specific IgM or PCR detection of viral RNA (21). Reported neuroinvasive diseases in the setting of ZIKV infection include cases of meningitis, encephalitis, and encephalomyelitis. Patients with a concurrent or past history of ZIKV systemic infection may also present with Guillain-Barré syndrome (GBS) and myeloradiculitis, which may respond to intravenous IVIG (25, 26). Neurologic and functional disability associated with these flaviviruses can also continue to cause morbidity in patients after recovery from acute illness. Studies of WNV survivors report that in 50-70% of survivors exhibit symptoms that persist and worsen over time including confusion, muscle weakness,

TABLE 1 | African arboviruses: vectors, geographical distribution, and the illnesses they cause in adults.

Family	Virus	Vector	Geographical distribution	Systemic illnesses	Neurological diseases
Flaviviridae	WNV	Mosquito (*Culex*)	Africa, Mediterranean region, Central Asia, India, Europe, North, Central and South Americas	Flu-like illness	Meningitis, flaccid paralysis, encephalitis, myelitis, memory disorders, Parkinsonism
	ZIKV	Mosquito (*Aedes*), Sexual transmission	Africa, India, Southeast Asia, Carribean islands, Central, North and South Americas	Flu-like illness with arthralgias, conjunctivitis	Meningoencephalitis, ADEM, GBS, memory disorders
	DENV	Mosquito (*Aedes*),	Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific	Fever, headache, pain behind the eyes, muscle pain, fatigue, nausea, vomiting, rash, bleeding hemorrhagic fever/shock	Encephalopathy, encephalitis, Guillain-Barre syndrome, transient muscle dysfunctions, neuro-ophthalmic involvement
Togaviridae	CHIKV	Mosquito (*Aedes*)	Subsaharan Africa	Fever, rash, arthralgias, myalgias	Rare encephalitis, GBS
	SINV	Mosquito (*Culex*)	Northeastern, Central and Southern Africa	Fever, rash, arthralgias, myalgias	Rare encephalitis

WNV, West Nile virus; ZIKV, Zika virus; DENV, dengue virus; CHIKV, Chikungunya virus; SINV, Sindbis virus.

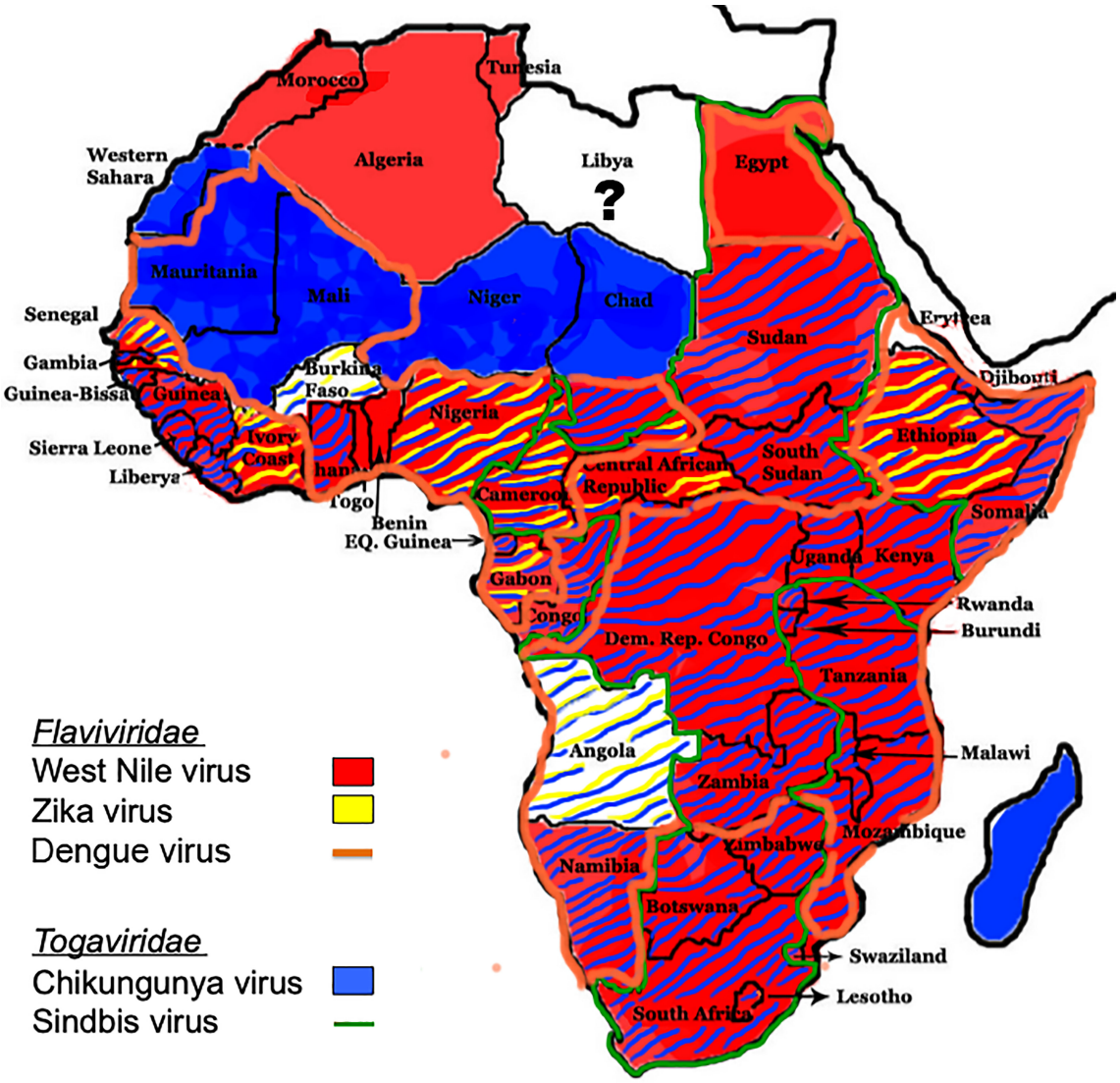

FIGURE 1 | Distribution of flaviviruses and alphaviruses in Africa. The distribution of *Culex*- and *Aedes*-transmitted flaviviruses WNV, ZIKV, and DENV, and *Aedes*- and *Culex*-transmitted alphaviruses CHIKV and SINV, respectively, throughout Africa are shown (17).

concentration difficulties, parkinsonism, and memory impairments, especially in the realm of visuospatial memory (27). Severe cases of ZIKV-induced systemic disease may also lead to neurocognitive deficits, daily headaches, and chronic inflammatory demyelinating polyneuropathies that may persist for years (28–32).

Neurological diseases associated with DENV infection were first reported in 1976 as atypical symptoms of dengue infection, and their incidence rates have varied from 0.5% to 20% (33). Neurological symptoms associated with DENV infection have increasingly been reported in both children and adults, and include encephalopathy due to hepatic failure or metabolic disorders, encephalitis due to direct viral invasion, Guillain-Barre syndrome or transient muscle dysfunctions, and neuro-ophthalmic involvement (34).

Dengue serotypes 2 and 3 are most commonly associated with neurological symptoms (35, 36). Although DENV is not primarily neurotropic, a recent study utilizing genome analysis and characterization of DENV type 2 (DENV-2) strains isolated from cerebrospinal fluid (CSF) and/or serum of patients with dengue encephalitis revealed that the DENV-2 isolates belonged to a new clade of cosmopolitan genotype that are genetically close to strains identified in China, South Korea, Singapore, Malaysia, Thailand, and the Philippines (37). As DENV does not invade the CNS when inoculated peripherally in mice, few studies have determined its route of neuroinvasion or CNS immune responses that exert virologic control.

The pathogenesis of WNV and ZIKV CNS infections in humans is incompletely defined, although excellent mouse models have illuminated mechanisms of immune control in

the periphery and central nervous system (CNS) (38), routes of viral neuroinvasion (39–45), features of virus-induced encephalitis (46, 47), and processes that induce post-infectious neurocognitive sequelae (48–52). Neuroinvasion can occur hematogenously as free virions or within CNS infiltrating immune cells, and *via* retrograde transport along sensory axons from sites of mosquito inoculation in the periphery (53) (**Figure 2**). The brain vasculature exhibits specializations that prevent paracellular and transcellular entry of cells, pathogens, and metabolites. These occur at the post-venular and capillary levels and include tight and adherens junctions (TJ and AJ), low levels of leukocyte adhesion molecules, and low rates of transcellular vesicle trafficking (transcytosis). Rho GTPase signaling pathways that control the assembly and disassembly of endothelial cytoskeletal proteins regulate TJ integrity, which affects BBB permeability. During acute infection with flaviviruses, local expression of BBB destabilizing cytokines activate the RhoA/ROCK/pMLC signaling pathway, which induces stress fiber formation that disrupts TJ and increases paracellular permeability. Increased blood-brain barrier (BBB) permeability during acute infection has also been linked to rising levels of NS1 within the blood, which correlate with severity of disease. NS1 is secreted from virally infected cells and may up-regulate the expression of cathepsin L and endoglycosidase heparanase in brain endothelial cells, leading to the degradation of glycocalyx-like layer (EGL) components with consequent damage to BBB integrity (54, 55). Flavivirus traversal across the BBB is believed to occur *via* paracellular and transcellular routes, the latter of which includes delivery by leukocytes (56). Neuroimaging during the acute setting may be normal or reveal BBB disruption, which is associated with more severe outcome (57).

Once WNV or ZIKV enter the CNS, they infect and injure neurons (or neuroprogenitor cells in the case of ZIKV) through direct (virus infection-induced) and indirect (immune-mediated) mechanisms (58, 59). Microscopic examination of the post-mortem CNS specimens may reveal neuronal cell death, microglial activation, infiltrating macrophages, and accumulation of $CD4^+$ and $CD8^+$ T cells (60, 61). Depending on the flavivirus, these lesions can occur in the brainstem, cerebral cortex, hippocampus, thalamus, cerebellum or spinal cord. While it is well established that both humoral and cell-mediated immune responses critically control viral replication in peripheral tissues, virologic control within the CNS predominantly requires the infiltration of antiviral mononuclear cells (62–64). Viral replication within neurons is detected by the cytoplasmic RNA

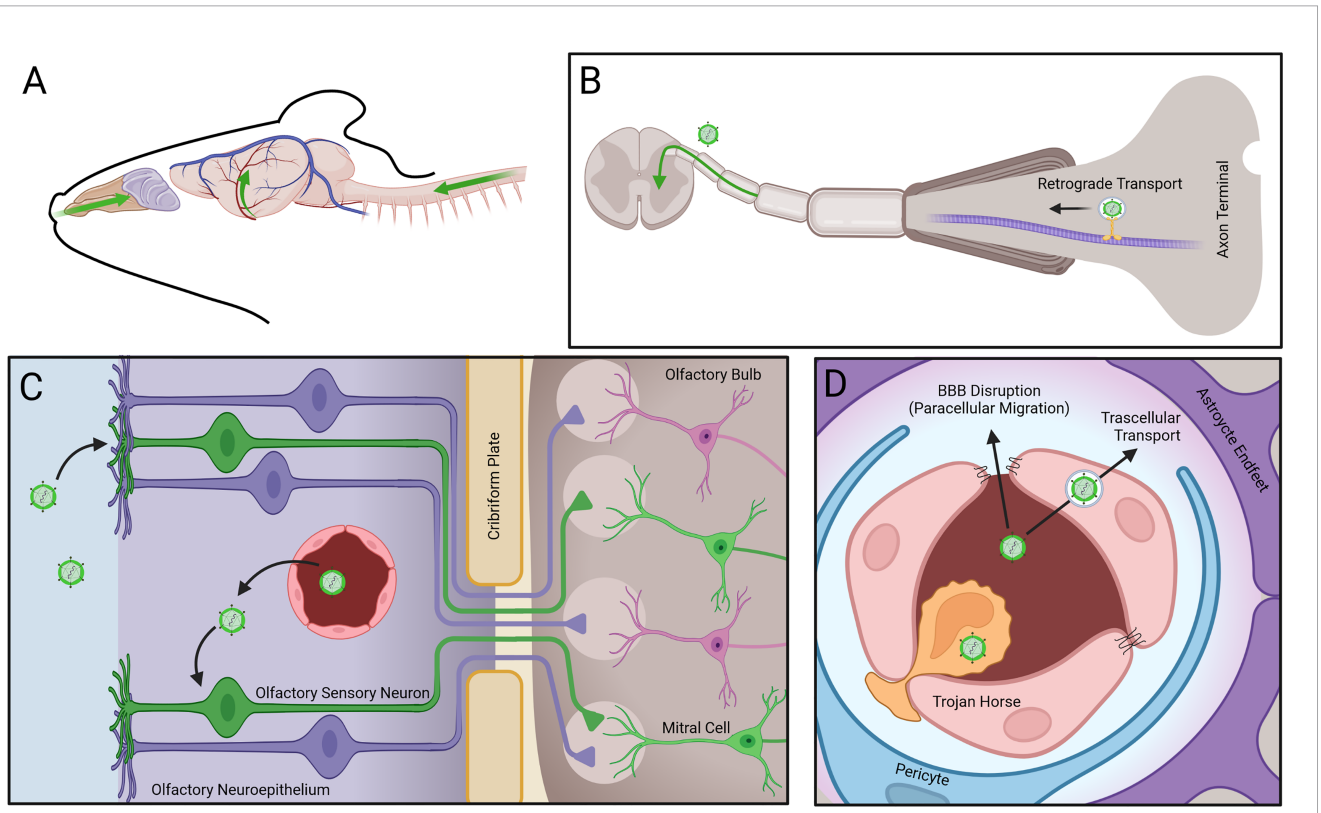

FIGURE 2 | Mechanisms of arbovirus entry into the CNS. Arboviruses enter the CNS via three routes: **(A, B)** Retrograde transport of virus along axon microtubules (MT) of peripheral neurons allows entry into the spinal cord. **(C)** Infection of olfactory sensory neurons (OSNs) in the olfactory neuroepithelium (ONE) following infection from fenestrated capillaries (FC) allows viral intra-axonal migration through the cribiform plate (CP), followed by transynaptic infection of mitral cells (MC) at the glomeruli (G) of the olfactory bulb (OB). **(D)** Virus entry through the blood brain barrier (BBB) occurs via transcellular transport of virions, paracellular migration of virions following disruption of tight junctions (TJs), or via transmigration of infected leukocytes.

helicases RIG-I and MDA5, which signal through the adaptor protein mitochondrial antiviral signaling protein (MAVS) to promote antiviral gene expression and proinflammatory proteins, including T cell chemoattractants in both neurons and activated astrocytes and microglia (38). Antiviral, CD8 T cells recruited to the acutely infected CNS can eliminate virus from neurons *via* non-cytolytic effects of interferon(IFN)γ (65). Subpopulations of effector CD8 T cells persist as resident memory T cells (Trm) that continue to express IFNγ, which maintains microglia activation (49). During acute infection, infected neurons and activated microglia exhibit upregulation of complement proteins (52), which have been implicated in the maintenance or disruption of neural networks (66). Studies in WNV- and ZIKV-infected mice show complement- and microglia-mediated elimination of synapses within the trisynaptic circuit of the hippocampus (52) is associated with defects in spatial and other forms of learning and memory. Studies in humans that succumbed to WNV show similar loss of synapses. Macrophage delivery of interleukin(IL)-1 has been shown to maintain a proinflammatory state *via* direct effects on neural stem cells within the neurogenic niche of the hippocampus, promoting decreased neurogenesis in favor of production of neurotoxic, reactive astrocytes that prevent synapse repair, and persist long-term (67). Future studies are needed to determine whether these processes occur and may be targeted in humans to prevent or treat neurocognitive sequelae after recovery from neurotropic flavivirus infection.

Togaviridae

Members of the *Togaviridae* family of viruses are small, enveloped viruses with single-stranded positive-sense RNA genomes of 10–12 kb that encode five structural and four NS proteins (68). Two thirds of the genome of alphaviruses encodes the non-structural polyprotein(s) in a single ORF immediately after a 5′-non-coding region. Overlapping with the 3′-end of the non-structural ORF, there is a promoter for transcription of a subgenomic mRNA from which the structural polyprotein is translated (69). The genus Alphavirus comprises a large group of medically important mosquito-borne viruses that are transmitted by *Aedes* (Chikungunya virus; CHIKV), and *Culex* (Sindbis virus; SINV) (17). Phylogenetic tree analyses suggest that alphaviruses likely originated from an aquatic habitat, from ancestral strains such as the Southern elephant seal virus and other fish viruses, followed by spread to New and Old World (70). The first reported CHIKV and SINV outbreaks occurred in Tanzania and Egypt, respectively, in 1952 (71). Seroprevalence for CHIKV is found throughout sub-Saharan Africa (**Figure 1**), while SINV occurs in a geographical area that spans from South Africa to Egypt and from Cameroon to Kenya (16) (**Figure 1**).

CHIKV and SINV generally cause febrile syndromes with rashes and joint pain, and are only occasionally associated with neurologic diseases. CHIV infection is asymptomatic in up to 25% of human infections, with symptomatic cases presenting with fever, headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, maculopapular rash and incapacitating bilateral and symmetric polyarthralgia, which may relapse or persist for months to years (72). Rare neurologic complications include seizures, acute flaccid paralysis, Guillain-Barré syndrome, cranial nerve palsies, myelitis, encephalopathy, and meningoencephalitis (73). Persons at risk for CNS disease include neonates exposed intrapartum, older adults (e.g., > 65 years), and persons undergoing immunosuppression for solid organ transplant (74). Case fatality rate for CHIKV encephalitis ranges from 4-28%, with higher rates mostly in older adults. Electroencephalogram in patients with neurologic signs may exhibit slow background activity and generalized epileptiform discharges, while brain MRI may show bilateral white matter hyperintensities and/or focal encephalitis. Postmortem brain examination of a patient who succumbed to CHIKV encephalitis revealed lymphocytic infiltrates with focal necrosis in the hippocampus, frontal lobes and medulla oblongata (75). While many SINV infections are asymptomatic, cases usually present with a maculopapular, pruritic exanthema over the trunk and limbs, mild fever, and arthralgia, particularly in wrists, hips, knees, and ankles, sometimes accompanied by nausea, general malaise, headache, and myalgia (76). Patients can experience persistent joint manifestations that continue for months or years, and in rare cases as a chronic arthritis. SINV is known to cause neurologic disease in horses (77), but human cases are extremely rare.

The mechanisms of CHIKV and SINV neuroinvasion in humans are unknown; however, animal models suggest entry may occur *via* invasion of brain endothelial cells and retrograde axonal transport, respectively (78) (**Figure 2**). Studies examining CHIKV and SINV infection of the CNS in murine models report multiple sites of neuronal and astrocyte infection progressing to cell death *via* caspase-mediated pathways, with microgliosis and perivascular cuffs (75, 79–82). Similar to reports in human cases of CHIKV encephalitis (75), neuronal degeneration in the hippocampus and lymphocytic meningitis is also observed in animals. As with flavivirus encephalitis, CHIKV RNA is detected in the brain by pattern recognition receptors, such as toll-like receptor(TLR)-3, that upregulate innate immune antiviral molecules that can reduce viral replication (83). While increased expression of the T cell cytokine IFNγ has been observed in animal models, mechanisms of T cell trafficking and virologic control within the brain have not been investigated. Likewise, there have been no reports of long-term follow-up in survivors of CHIKV neurologic diseases.

CAN NEUROIMMUNE RESPONSES AIDE IN DIAGNOSTICS AND/OR THERAPEUTICS?

The diagnosis of arboviral neuroinvasive diseases requires virus-specific assays so that novel therapies, such as antibody-based therapeutics, and patient prognoses can be accurately administered. Studies attempting to identify virus-specific innate or adaptive immune pathways *via* genomic approaches in animal models have been instrumental in identifying the critical antiviral pathways that control and clear virus (84, 85), but have failed to support the use of pathway analysis for diagnostic purposes. Knowledge regarding the status of BBB permeability may also be critical for treating acute neuroinvasive diseases. For example, animal studies examining patterns of BBB function throughout the course of flavivirus encephalitis indicate

that induction of interferon responses may promote BBB closure *via* Rac1-mediated effects on TJ integrity (40, 45). Thus, use of anti-viral antibodies for CNS infection may have a limited window of penetration. While there are currently no treatments that limit the replication of specific arboviruses in the CNS, anti-inflammatory treatments, including corticosteroids, have been used in patients with chorioretintis, encephalitis or myelitis (86–88). New anti-inflammatory compounds are also under development (89).

KNOWLEDGE GAPS FOR FUTURE RESEARCH

One of the challenges for limiting arboviral neuroinvasion and dissemination within the CNS is the incomplete knowledge regarding virus-specific entry receptors expressed at the BBB and by neural cells, including those involved in trans-synaptic spread between CNS regions. Entry receptors postulated to be involved in flavivirus entry include $\alpha_v\beta_3$ integrins, C-type lectin receptors (CLR), phosphatidylserine receptors TIM (T-cell immunoglobulin and mucin domain) and TYRO3, AXL and MER (TAM) family of receptor tyrosine kinases (90, 91). Attachment and entry receptors for CHIKV include glycosaminoglycans (GAGs), T-cell immunoglobulin and mucin 1 (TIM-1), and the cell adhesion molecule Mxra8 (92). While many of these receptors are expressed at CNS barriers and within the parenchyma, the demonstration these receptors are required for brain endothelial and neural cell entry is currently lacking. There is also a dire need to identify biomarkers that identify survivors of arboviral neuroinvasive diseases at risk for neurological sequelae, including neurocognitive impairments. Post-infectious neurocognitive sequelae modeled in murine models show benefit from administration of anakinra, a USFDA approved medication that targets the IL-1R for the treatment of rheumatoid arthritis, during acute encephalitis (93). Given the essential role of the IL-1R, in CNS virologic control, it is unclear whether the risk-benefit ratio supports use of this drug in humans with arboviral encephalitis. Future studies are needed to better identify and define safe therapeutic targets to limit the entry and dissemination of neurotropic arboviruses, and to prevent the development of neuroimmune processes that contribute long-term sequelae.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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The Interplay Between Neuroinfections, the Immune System and Neurological Disorders: A Focus on Africa

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Neurological disorders related to neuroinfections are highly prevalent in Sub-Saharan Africa (SSA), constituting a major cause of disability and economic burden for patients and society. These include epilepsy, dementia, motor neuron diseases, headache disorders, sleep disorders, and peripheral neuropathy. The highest prevalence of human immunodeficiency virus (HIV) is in SSA. Consequently, there is a high prevalence of neurological disorders associated with HIV infection such as HIV-associated neurocognitive disorders, motor disorders, chronic headaches, and peripheral neuropathy in the region. The pathogenesis of these neurological disorders involves the direct role of the virus, some antiretroviral treatments, and the dysregulated immune system. Furthermore, the high prevalence of epilepsy in SSA (mainly due to perinatal causes) is exacerbated by infections such as toxoplasmosis, neurocysticercosis, onchocerciasis, malaria, bacterial meningitis, tuberculosis, and the immune reactions they elicit. Sleep disorders are another common problem in the region and have been associated with infectious diseases such as human African trypanosomiasis and HIV and involve the activation of the immune system. While most headache disorders are due to benign primary headaches, some secondary headaches are caused by infections (meningitis, encephalitis, brain abscess). HIV and neurosyphilis, both common in SSA, can trigger long-standing immune activation in the central nervous system (CNS) potentially resulting in dementia. Despite the progress achieved in preventing diseases from the poliovirus and retroviruses, these microbes may cause motor neuron diseases in SSA. The immune mechanisms involved in these neurological disorders include increased cytokine levels, immune cells infiltration into the CNS, and autoantibodies. This review

focuses on the major neurological disorders relevant to Africa and neuroinfections highly prevalent in SSA, describes the interplay between neuroinfections, immune system, neuroinflammation, and neurological disorders, and how understanding this can be exploited for the development of novel diagnostics and therapeutics for improved patient care.

Keywords: neuroinfection, neurological disorder, immune system, neuroinflammation, sub-Saharan Africa, neuropathy, pathogen, central nervous system

1 INTRODUCTION

1.1 The Burden of Neurological Diseases and Neuroinfections in Africa

Neurological disorders represent a major cause of disability for patients and an economic burden globally. In 2016, neurological disorders, comprising 11.6% of global disability-adjusted life-years (DALYs), were ranked as the leading cause of DALYs and the second leading cause of death (16.5% of total global deaths), after cardiovascular diseases (1). Despite being relatively scarce, the available data suggest that the prevalence of neurological diseases in Sub-Saharan Africa (SSA) has been increasing over time (**Figure 1**) (2) but it is considered lower than other parts of the world (3). Nevertheless, according to the World Health Organization (WHO)-World Federation of Neurology joint report, the burden of neurological disease is underestimated by traditional methods of assessment. The burden is further increased in low-income countries, especially in Africa, because of insufficient human and infrastructural resources, coupled with systems unpreparedness to detect and manage these conditions (4). The overall point prevalence of neurological disorders reported in studies carried out in various hospitals in countries of the SSA region was 3.3% in Uganda (3), 4.2% in Nigeria (5), 7.5% in Kenya (6), 8.5% in Tanzania, 2009 (7), and 10% in Zambia (8). However, some retrospective studies reported higher percentages of neurological disorders in patients admitted to some hospitals; 15% in a hospital in Ghana (Sarfo et al., 2016) and 18% and 24.7% in two tertiary hospitals in Ethiopia (9). The most frequently reported neurological disorders were peripheral neuropathy, chronic headaches, epilepsy, pain syndromes, stroke, and tremors/Parkinson's disease (3, 7). In SSA, neuroinfections contribute significantly to the diagnosed neurological disorders (10–12), in some cases constituting 26.7% to 43% (5, 13). These neuroinfections include human immunodeficiency virus (HIV), tuberculosis, meningitis, cerebral malaria, rabies, and tetanus (4–7, 9, 10, 13).

1.2 Inflammation, Neuroinfections and Neurological Disorders

Neuroinfections result in neuroinflammation, which involves immune cell infiltration into the central nervous system (CNS) from the periphery, chronic astrocyte, and microglia activation, increased chemokine, and cytokine expression, to control or eliminate the pathogens but can also be detrimental to the host (14–17). The interplay between infectious pathogens and the immune system in the CNS is covered in more detail in various articles in this Research Theme (Neuroimmunology in Africa). The pathogenesis of neurological disorders is associated with neuroinflammation in general or due to infections (14, 17–20). Neuroinflammation caused by HIV, tuberculosis, cerebral malaria, neurocysticercosis, cerebral toxoplasmosis contributes to the pathogenesis of epilepsy that occurs during or after these infections (21–24). Alteration of the immune system caused by HIV contributes to the pathogenesis of neuropathy (25, 26). Similarly, a possible pathogenic mechanism of sleep disturbances observed in human African trypanosomiasis (HAT) patients is the upregulations of cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) (17, 20, 24).

This review addresses the interplay of the immune system and neuroinfections in the pathogenesis of certain neurological diseases prevalent to the SSA region such as epilepsy, dementia, motor disorders, headache, sleep disorders, and peripheral neuropathy.

2 SPECIFIC NEUROLOGICAL DISTURBANCES RELATED TO NEUROINFLAMMATION AND BRAIN INFECTIONS

2.1 Epilepsy

2.1.1 Introduction

Epilepsy is a chronic disease of the brain that affects an estimated 50 million people worldwide according to the WHO (27). It manifests as repetitive, involuntary epileptic seizures that vary in their clinical presentation (28). The overall lifetime prevalence of epilepsy is estimated at 7.60 per 1,000 population (29). About 80% of the global burden of epilepsy occurs in individuals residing in low and middle-income countries (LMICs) (27). In SSA specifically, a median epilepsy prevalence of 14.2 per 1,000 was documented; over 90% of the patients were aged below 20 (30). Annual epilepsy incidence was also high, reaching 81.7 per 100,000. Mortality was greatest in the 18-24 years age group, suggesting a relatively low life expectancy among persons with epilepsy (PWE) in Africa (30). The main risk factors for epilepsy reported in resource-poor settings include perinatal brain insults, traumatic head injury, and infections of the CNS (11, 30).

2.1.2 Physiopathology of Epilepsy and Common Infectious Etiologies

Epilepsy is characterized by an enduring predisposition to generate epileptic seizures (28). Diverse mechanisms underpin

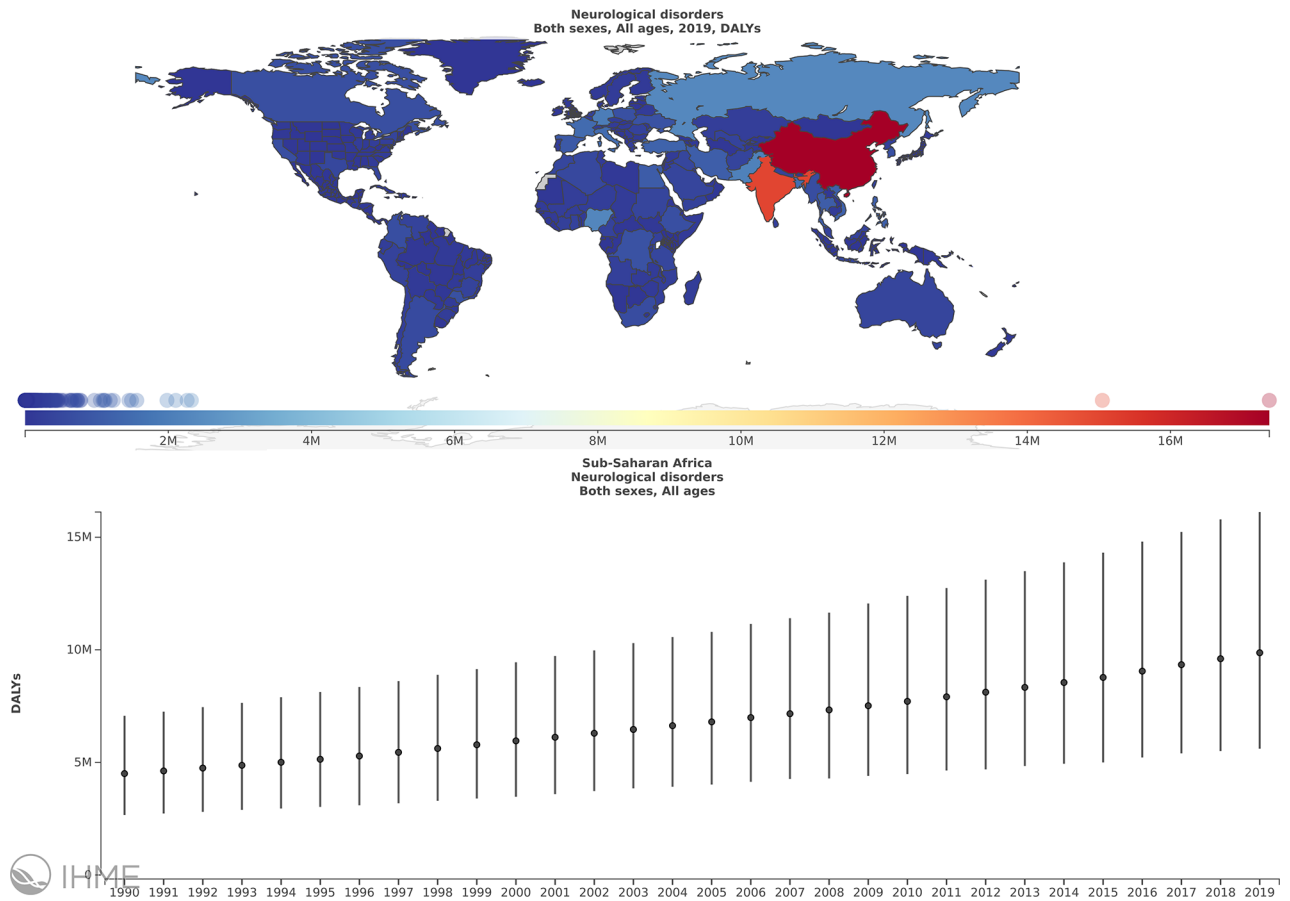

FIGURE 1 | The burden of neurological disorders in sub-Saharan Africa (SSA) over the period of 1990 to 2019. Annual % change in DALYs/100000, Global, Both sexes, All ages, Neurological disorders, Sub Saharan Africa. Obtained from (2) with permission.

epileptogenesis and are often a consequence of brain insults and the resulting inflammation (24). The International League Against Epilepsy (ILAE) recently highlighted six main categories as etiologies for epilepsy: structural, genetic, infectious, metabolic, immune, and unknown etiologies (31). The interplay between brain infections and inflammation, and how these may lead to epilepsy are summarized in **Figure 2**. During an initial brain insult, proinflammatory cytokines (principally IL-Iβ, IL-2, and IL-6) produced by glial cells and neurons may cause cerebral damage (32). Of note, the released cytokines also activate astrocytes and microglia leading to increased production of cytokines by the latter, thus creating a vicious circle. Furthermore, proinflammatory molecules may reach the CNS hematogenously during disseminated systemic inflammation, particularly when the blood-brain barrier (BBB) is compromised (33). Incomplete tissue repair following an initial brain insult could result, after a certain latent period, in a permanent seizure-causing lesion. Between the time of the initial lesion and the development of epilepsy (latent period), several processes occur including brain neuronal hyperexcitability facilitated by both N-methyl-D-aspartate (NMDA) receptor and other glutamate-mediated mechanisms, neuronal loss and gliosis, molecular and structural reorganization, and epigenetic reprogramming; all these processes may ultimately result in recurrent unprovoked epileptic seizures (24).

Although epilepsy can result from non-infectious causes such as traumatic brain injury, hypo-anoxic episodes, or metabolic anomalies, the most common preventable causes of epilepsy in SSA are infections that affect the CNS (22, 30, 34). Epilepsy from an infectious etiology should be understood, as the unprovoked seizures that persist even after the resolution of the acute infection (31). The proportion of epilepsies attributed to infection varies widely from one study to another, ranging from 1% to 47% (30). Infectious etiologies include neurocysticercosis, tuberculosis, HIV, cerebral malaria, subacute sclerosing panencephalitis, cerebral toxoplasmosis, and congenital infections such as Zika virus and cytomegalovirus (31, 35). Recent cohort studies from Cameroon support the addition of onchocerciasis to this list of etiologies of infectious epilepsy, as children with higher *Onchocerca volvulus* parasitic loads had an increased risk of

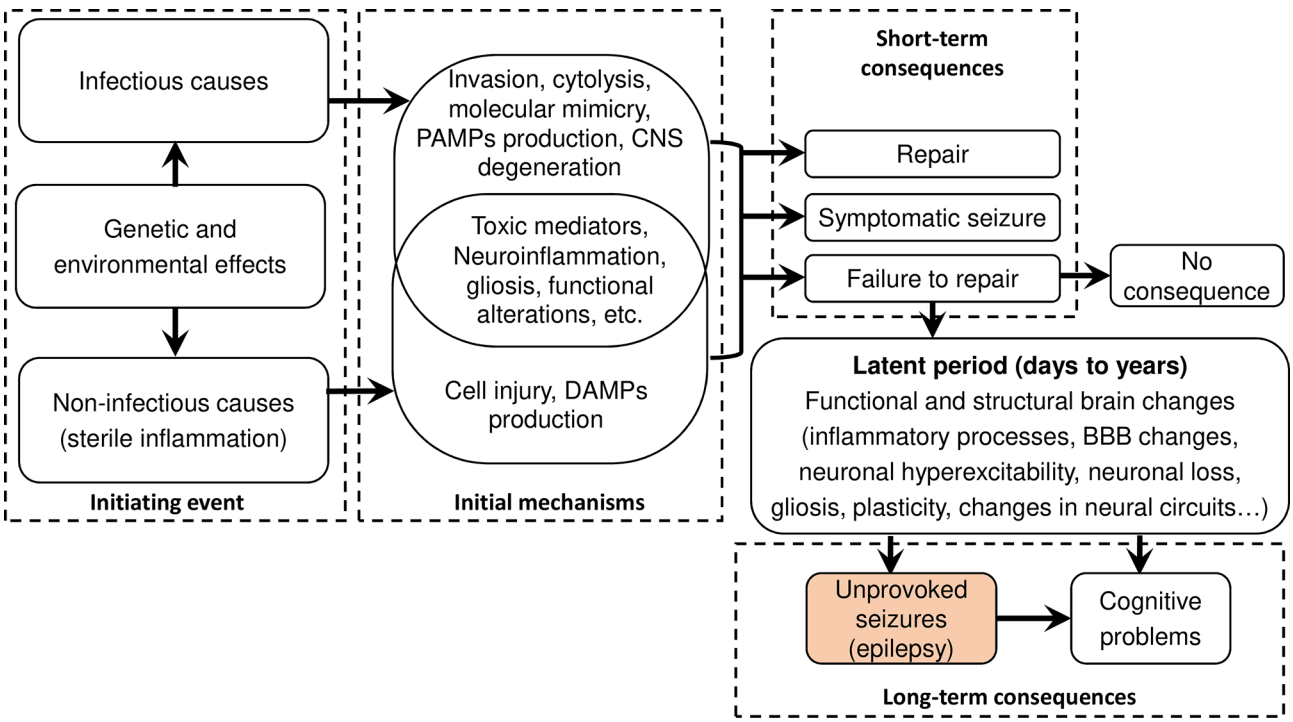

FIGURE 2 | Flow diagram summarizing infectious and sterile epileptogenic mechanisms. After an initial brain insult, a cascade of processes occurs that, under certain circumstances, could create an enduring predisposition to unprovoked seizures. Both damage-associated molecular patterns (DAMPs) in sterile infection and pathogen-associated molecular patterns (PAMPs) activate pattern recognition receptors (PRR) leading to overlapping pathways for inflammation and long-term pathological consequences. Figure adapted from (24).

developing epilepsy later in life (36, 37). These CNS infections can cause a structural cerebral lesion which will act as an organic basis for seizure recurrence even after anti-infectious treatment; therefore substantial overlap exists between infectious and acquired structural causes of epilepsy (38). For instance, neurocysticercosis, perilesional inflammation around the space-occupying lesions may lead to epileptogenesis either by causing gliosis and/or BBB dysfunction (39). Common CNS infections known to cause epilepsy are summarized in **Table 1**.

2.1.3 Diagnostic and Management Approaches for Epilepsy in SSA

The diagnosis of epilepsy is essentially clinical and based on criteria set by the ILAE. Practically, epilepsy can be considered as

TABLE 1 | Infections of the central nervous system implicated in epilepsy (23, 24, 40).

Infectious agents	Mechanism(s)	Clinical consequences
Viruses: arboviruses, coxsackie, enterovirus, rubella, measles, HIV, herpes simplex, cytomegalovirus, flavivirus (Japanese encephalitis), Dengue	- CNS invasion/Inflammation/release of cytotoxic substances/increased neuronal excitability/necrosis - Secondary infections of CNS & metabolic disorders in HIV infection.	Meningitis/encephalitis/ encephalomyelitis, epilepsy
Bacteria: Meningococcus, pneumococcus, *Haemophilus influenzae B* (Hib), *Mycobacterium tuberculosis*	CNS invasion/Inflammation/intracerebral lesions	Meningitis/cerebral abscesses/intracranial empyemas, epilepsy
Parasites: *Taenia solium, Plasmodium falciparum, Naegleria fowleri, Entamoeba histolytica, Trypanosoma spp, Onchocerca volvulus, Toxocara canis, Echinococcus granulosus, Toxoplasma gondii*	- CNS invasion/Inflammation/encephalitis/ intracerebral lesions/autoimmunity? - Combination of parasites increases epilepsy risk (41)	Cerebral abscesses/ cysts/calcifications, epilepsy
Fungi: *Cryptococcus neoformans, C. immitis, H. capsulatum, Candida albicans, A. fumigatus, A. flavus, Mucoraceae* sp., Aspergillus, Blastomyces, Histoplasma	CNS invasion/Inflammation (immunocompromised++)	Meningitis/abscesses, vasculitis/capillary thrombosis, epilepsy

two unprovoked (or reflex) seizures occurring >24 h apart, or one unprovoked seizure and a probability of at least 60% for spontaneous seizure recurrence in the next 10 years (42). Paraclinical investigations to diagnose epilepsy include: electroencephalography (EEG), brain imaging (by computed tomography [CT] scan or magnetic resonance imaging), and blood analysis to exclude metabolic, genetic, or autoimmune causes. Since all paraclinical investigations are not always feasible in some SSA settings, the clinical characteristics of seizures may orientate towards specific etiologies and guide management. For instance, focal seizures in persons with epilepsy could be symptomatic of neurocysticercosis, particularly if accompanied by other focal neurological deficits (43).

The main objective of epilepsy treatment is seizure control. This can be achieved using anti-seizure medications, which must be taken daily. The anti-seizure medications routinely used in LMICs and recommended by the WHO include phenobarbital, carbamazepine, phenytoin, and valproate (44). Regarding preventive management, public health interventions should be instituted to avert epilepsy of infectious origin in SSA. Firstly, institute strategies to prevent any initial insult to the brain and control the incriminated infective agents. The public health actions to fight against neurocysticercosis and onchocerciasis in endemic foci constitute a good example of preventing epilepsy caused by those infections (45, 46). Secondly, treating the cause of an eventual CNS infection can modify the prognosis of the disease. However, epilepsy results from an enduring epileptogenic state, thus treating the underlying infection – for epilepsy of infectious origin – may not completely reverse epileptogenicity. Nonetheless, anti-infectious treatment could reduce the infectious load and improve epilepsy outcomes. The *O. volvulus* parasitic load in persons with onchocerciasis-associated epilepsy correlated positively with seizure frequency and disease severity, and treatment with ivermectin improved seizure outcomes (47, 48). In the same manner, neurocysticercosis cases that received appropriate treatment had higher hippocampal volumes than their untreated counterparts, suggesting more severe brain damage in the latter (49).

2.1.4 Research Gaps and Perspectives

The fact that the very definition of epilepsy depends on seizure recurrence poses a diagnostic challenge. Predicting seizure recurrence after an initial brain insult is still a subject of scientific debate (50). Some progress has already been achieved in this light, as there are now suggested indicators to identify neurocysticercosis patients who are likely to develop epilepsy: those with a strong serologic response (4 bands to *Taenia solium* antigen on neurocysticercosis enzyme-linked immunoelectrotransfer blot (EITB) (39). More research is warranted to understand the infectious threshold and other predisposing conditions that could trigger the development of epilepsy following a CNS infection. Finally, the varied yet understudied epilepsy etiologies in SSA require the establishment of state-of-the-art brain research institutes in the continent and the initiation of North-South collaborations to generate data relevant for the African population.

2.2 Dementia

2.2.1 Introduction

Dementia is a neurodegenerative disorder that leads to a progressive deterioration of cognitive functions. It is characterized by a gradual cognitive decline that interferes with independent daily functioning (51). The *Diagnostic and Statistical Manual of Mental Disorders V* (DSM-V) proposes to replace dementia with the term "major neurocognitive disorder" to encompass the wide spectrum of symptoms experienced by the affected persons (52). According to the WHO, Alzheimer's Disease (AD) is the most common cause of dementia, responsible for 60-70% of all cases (53).

Recent estimates suggest that 0.7% of the world's population has dementia, translating to about 51.6 to 55 million people worldwide (53, 54). Although nearly 60% of dementia patients live in LMICs, Africa has the least burden of dementia compared to other continents possibly due to its relatively younger population (53, 54). Studies conducted in different populations and geographical regions consistently support that advanced age is a major risk factor for developing dementia (55, 56); indeed, the prevalence of dementia is 2% in those aged 65-69 years, much lower than the 20% in those aged 85-89 (55). Other risk factors for dementia include female gender, low education, cigarette smoking, excessive alcohol intake, diabetes, and hyperlipidemia (55). A meta-analysis of dementia studies in SSA estimated a pooled prevalence of 5.0% for all ages and a pooled annual incidence of 2.0% (56). The number of persons with dementia is expected to rise globally, with the highest increase in prevalence projected to occur in eastern SSA by the year 2050 (57).

2.2.2 Physiopathology and Etiologies of Dementia Secondary to Neuroinfections

During neuroinfection, the initial pathogenic invasion of the CNS induces a diffuse inflammatory process that alters neuronal function (58). The activated microglia release cytokines (IL-1, IL-6, and TNF-α) and neurotoxic agents that further exacerbate CNS damage (58, 59). In chronic neuroinflammation lasting weeks to months, as in the case of some subacute or chronic infections, microglia activation can persist for extended periods, releasing quantities of cytokines and neurotoxic molecules that contribute to long-term neurodegeneration (60). Infections of the CNS associated with dementia (or at least, cognitive impairment) in SSA include HIV, neurosyphilis, and meningitis or encephalitis caused by bacteria, viruses, parasites, or fungi (55). Several infections may occur in the same individual (e.g., neurosyphilis in a person infected with HIV), and it is common to have dementia with mixed infectious and non-infectious etiologies potentiating each other. The "seeding" hypothesis describes a possible mechanism of AD where amyloid-β agglutinate into plaques in a bid to trap a microbe (61). Microbes invade the CNS and stimulate microglia to induce an immune reaction that boosts the levels of an enzyme that helps to produce amyloid proteins. The amyloid protein is meant to act as a defense mechanism, engulfing and disabling the microbes. However, failure to clear these amyloid proteins

ramps up inflammation, and eventually amyloid accumulation that constitutes a hallmark for the development of AD (61).

2.2.2.1 Human Immunodeficiency Virus and Dementia

A large proportion of people living with HIV/acquired immunodeficiency syndrome (AIDS) (PLWHA) develop HIV-associated neurocognitive disorders (HAND), independent of opportunistic conditions. A meta-analysis estimated that 45.2% of adult PLWHA in SSA suffer from HAND (62). The pathophysiological mechanisms of HAND are not yet completely understood. Before the widespread introduction of antiretroviral treatment (ART), HIV-associated neuropathology was thought to result from CNS inflammation due to direct penetration and replication of the virus within the microglia and macrophages, as well as neurotoxicity caused by HIV proteins and/or factors secreted from the infected CNS cells (63). However, the persistence of HAND during the post-ART era when several PLWHA have achieved viral suppression warrants additional explanations to the development of neurocognitive symptoms in HIV/AIDS (64). Based on recent research, neurodegeneration in PLWHA most likely involves HIV proteins such as glycoprotein (gp)120, tat, and nef that activate neuroinflammatory and apoptotic pathways, promote oxidative stress, deplete neurotrophic factors, and cause vascular damage (65).

Despite some studies dating back to the 1980s and 90s suggesting that HIV can infect neurons, it appears that the main HIV pathogenic pathway in the CNS is by stimulating infected microglia and macrophages to produce inflammatory factors and reactive oxygen species (ROS) (66). The pathogenesis of HAND entails the following processes (67): (i) CNS tropism by HIV, which causes the viral particles to preferentially invade the brain and spinal cord; (ii) CNS penetration by crossing the BBB mainly *via* adsorptive endocytic mechanisms; (iii) HIV internalization by monocyte/macrophages, which upon crossing the BBB, leads to infection and activation of resident microglial cells by shedding HIV envelope protein gp120; (iv) propagation of infection among microglia accompanied by the release of neurotoxic agents by the latter (TNF-α, IL-1β, glutamate, quinolinic acid) leading to neuronal damage and cognitive dysfunction. Activated microglia also induce astrocyte differentiation and apoptosis and can interfere with normal neurogenesis (68). Interestingly, it has been reported that some HIV proteins can act on the BBB to reduce the entry of antiretroviral drugs (ARVs) (69), thereby making viral suppression difficult in the brain and maintaining the CNS as a potential reservoir for HIV.

Glial cells infected by HIV are involved in inflammatory processes *via* the release of HIV proteins (gp120, Tat, and Vpr) alongside inflammatory cytokines and neurotoxins (66). The released HIV proteins damage neurons and astrocytes (70) and activate virus replication. Secreted cytokines such as IL-1β, TNF-α, and interferon-gamma (IFN-γ) can stimulate viral replication in latently infected glial cells (71), thereby maintaining the CNS infection.

Clinically, HAND patients present with psychomotor slowness, depression, impaired memory, poor visuospatial skills, and impaired executive functions. These symptoms impact the quality of life of the affected individuals. The WHO (72), and the American Academy of Neurology AIDS taskforce (73) clinically classified HAND. The latter classification was recently reviewed (74); the updated version is the most universally used nosology and is considered the gold standard in HIV research; commonly referred to as the *Frascati criteria* for HIV-associated neurocognitive disorders. The Frascati criteria outline three severity levels for HAND: asymptomatic neurocognitive impairment, mild neurocognitive disorder, and the most severe form: HIV-associated dementia (66).

Besides HIV itself, opportunistic neuroinfections in immunocompromised PLWHA can also cause dementia mostly by direct CNS invasion and local brain damage; these include cerebral toxoplasmosis, cryptococcal meningitis, tuberculous meningitis, and cytomegalovirus encephalitis (75).

2.2.2.2 Neurosyphilis and Dementia

CNS invasion by the spirochete *Treponema pallidum*, known as neurosyphilis, is another cause of dementia in Africa. A retrospective study in South Africa found that among 161 patients with neurosyphilis, over half (50.9%) had psychosis/dementia symptoms. Neurosyphilis is responsible for inflammatory processes of the cerebrovascular system and the meninges. Infection with *T. pallidum* may cause chronic meningitis, meningovascular syphilis, or focal gumma in the CNS, which over time may result in dementia (76). The resulting clinical spectrum is wide, ranging from asymptomatic forms to general paralysis which is the most severe presentation of neurosyphilis (also known as dementia paralytica, a condition involving treponemal infection of the brain parenchyma that often presents with cognitive decline and neuropsychiatric symptoms) (75, 77).

2.2.2.3 Other Infectious Causes of Dementia

Encephalitis caused by neurotropic pathogens (herpes viruses; *Borrelia burgdorferi* that causes Lyme disease; hepatitis C; *T. solium* that causes neurocysticercosis may cause cognitive sequelae that could evolve to dementia syndromes in immunocompetent individuals (75, 77). Cerebral malaria is another important cause of cognitive impairment in SSA and a history of it has been associated with long-term mental health disorders and cognitive impairment (78, 79).

2.2.3 Diagnostic and Management Approaches for Dementia in Africa

Diagnosing dementia requires rigorous history taking to document the patient's daily activities (often requiring the corroboration of the anamnesis by a close friend or family member), in addition to a thorough mental status examination by a clinician to investigate impairments in cognitive functions including memory, language, attention, spatial orientation, executive functions, and mood (80). This may be complemented by a standard battery of neurocognitive and/or neuropsychological tests, brain imaging (by magnetic resonance), and/or investigation of the cerebrospinal fluid (CSF) to investigate the etiology of dementia. Typical CSF findings for some dementias caused by

proteinopathies include: reduced amyloid-β, increased tau and P −tau in AD; reduced α−synuclein for Lewy body dementia; real-time quaking-induced conversion, increased 14−3−3 protein, neuron-specific enolase, and tau for Creutzfeldt–Jakob disease (81). Additionally, an infectious workup may be required to rule out common infections associated with dementia as discussed above; this approach seems feasible for resource-limited settings in SSA, where point-of-care tests for HIV and syphilis could be used to raise the index of suspicion regarding a possible infectious etiology when investigating persons with dementia. Persons with dementia of infectious origin who have a positive serology for the infectious disease may also have CSF abnormalities indicating general neuroinflammation (pleocytosis, elevated proteins) or pathogen-specific findings: HIV-ribonucleic acid (RNA), cryptococcal fungi, cytomegalovirus deoxyribonucleic acid (DNA), and a positive Venereal Disease Research Laboratory test for neurosyphilis (75). Finally, genetic testing may be considered in some cases, for instance, those with atypical dementia (81).

Pharmacological treatment of dementia is mainly symptomatic, to improve the patients' quality of life. Adjuvant therapies, such as anti-inflammatory medications, are relevant for dementia of infectious origin as they downplay the associated neuroinflammation. Limited evidence suggests that early treatment of the infectious etiology may reverse the dementia syndrome altogether, or at least preserve cognitive function; examples in the literature include cases with dementia secondary to some viral encephalitis and neurosyphilis (75).

2.2.4 Research Gaps and Perspectives

Although literature currently reports that Africa is the continent least affected by dementia, the prevalence of dementia in it is expected to rise in the future (57). This could be attributed to the rising life expectancy (82) and the persistence of several neurotropic infections in SSA. Therefore, research capacity should be strengthened to improve novel preventive interventions adapted to the African context, and diagnostic and management capacity for dementias. Indeed, several cases of clinically diagnosed dementia remain uninvestigated due to infrastructural and/or technical limitations in these settings. Finally, Central and peripheral inflammatory pathways could become the targets to prevent the development of dementia among at-risk individuals as peripheral inflammation caused by infections or other causes can exacerbate or trigger central inflammation (83).

2.3 Motor Neuron Diseases

2.3.1 Introduction

Motor neuron diseases (MNDs) are a group of neurodegenerative disorders characterized by the selective death of motor neurons. The spectrum of MNDs involves varying degrees of upper and lower motor neuron involvement and is differentiated from neuropathies by the pattern of motor and/or sensory involvement. These disorders range from spinal muscular atrophy (frequent in childhood) to amyotrophic lateral sclerosis (ALS) in adults (84). The most prevalent MND is ALS, which can be inherited or sporadic, and is characterized by mixed upper and lower MND, with sensory sparing (85). The reported all-age global prevalence of MNDs was 4.5 per 100 000 people and all-age incidence was 0.78 per 100,000 person-years, causing 926,090 DALYs and 34,325 deaths in 2016 (86). Africa has an underestimated burden of MNDs as most epidemiological studies were conducted in hospital settings with low prevalence and incidence rates (87, 88). Quansah et al. reported several cases of MNDs in community and hospital settings ranging from 5 to 15/100,000 people and 250 to 750/100,000 people (89). Kengne et al. reported a hospital-based prevalence of ALS of 0.5% in Cameroon (90). Risk factors for MNDs in SSA include severe hypotonia in infants, trauma, family history of MNDs, sensory changes, and spinal anesthesia (82).

2.3.2 Pathophysiological Mechanisms and Infectious Etiologies of MNDs

Motor neuron diseases result from an interplay of genetic, age-related, environmental, and developmental factors (91). The pathophysiological mechanism underlying the etiology, occurrence, and aggravation of MNDs remains not fully elucidated and the center of research. However, recent research using animal models suggests a vital role of glial cells and neuroinflammation in MNDs. Neuroinflammatory processes such as activated microglia, infiltrated T cells, and the subsequent overproduction of proinflammatory cytokines and other neurotoxic or neuroprotective molecules, play a role in the pathophysiology of ALS (92). Several studies propose that the pathophysiology of ALS encompass an exaggerated innate and reduced acquired immunity (93), as well as defective astrocytic clearance of excess glutamate, which results in neuronal excitotoxicity and death (94). These studies provide the basis for further research to understand the role of neuroinflammation in MNDs (95).

Some infections may be able to trigger MNDs, with clinical presentations mimicking ALS. Enteroviruses (a group of positive-stranded RNA viruses including poliovirus, coxsackievirus, echovirus, enterovirus-A71, and enterovirus-D68) have been incriminated in the development of ALS as they can target motor neurons; patients with prior poliomyelitis are at increased risk of developing MNDs (96). Mouse models revealed that infection with enteroviruses induces molecular changes such as defective RNA-processing, impaired nucleocytoplasmic transport, neuroinflammation, compromised protein quality control, and abnormalities of the transactive response DNA binding protein-43 (TDP-43), supporting their involvement in ALS pathogenesis (96). Besides enteroviruses, infection with retroviruses such as HIV has been associated with ALS (97), although further studies are required to firmly establish causality.

2.3.2.1 Motor Neuron Disease Caused by the Poliovirus

The poliovirus is the viral agent responsible for paralytic poliomyelitis, an acute disease of the CNS (specifically the anterior horn of the spinal cord) resulting in flaccid paralysis (98). With the advent of effective vaccines, the number of poliomyelitis cases has been on a steady decline worldwide. The annual incidence of paralytic polio decreased from an estimated 350,000 in 1988 to about 1,000 cases from 2001 to

2004 (99). In addition to the acute disease, the post-polio syndrome is another neuromuscular pathology that affects some poliomyelitis survivors many years after the initial severe disease (100).

The poliovirus is transmitted to man *via* the fecal-oral route. Upon entry into the human host, the poliovirus attaches to host cell surfaces *via* the poliovirus receptor (PVR), a membrane protein (CD155) of the immunoglobulin superfamily. The poliovirus receptor is abundantly expressed in certain tissues such as the nasopharyngeal mucosa, Peyer's patch M cells of small intestines, the anterior horn motor neurons of the spinal cord, and medulla oblongata (101); this distribution of PVR explains the tropism of the poliovirus for these tissues. Infection and replication of poliovirus result in cell death (apoptosis). Suggested mechanisms of virus-induced apoptosis have previously been reviewed (102–104). In case of poliovirus, apoptosis of neuronal cells most likely involves CD155 and caspases (98). Indeed, poliovirus replication in Tg-CD155 mice models induced DNA fragmentation (characteristic of apoptosis) in the three main CNS cell types (neurons, astrocytes, and oligodendrocytes) (105). Paralysis ensues when a certain threshold of local inflammation and motor neuron death is reached; this happens in less than 1% of infected individuals (99). Recovery from paralysis occurs in only 20-30% of affected subjects, but in the majority, the paralysis is permanent and results in muscle atrophy and joint deformities (101).

2.3.2.2 Motor Neuron Disease Caused by Retroviruses

The human retroviruses (both exogenous and endogenous) have recently been considered as a viral etiology for MNDs. In Tanzania, a 12% prevalence of MND was found among HIV-infected persons, as opposed to only 4.7% in the general population (87). Both the Human T-cell leukemia or T-lymphotropic Virus 1 (HTLV-1) and HIV-1 are implicated in MND neuropathology including the development of ALS-like syndromes (106). The association between ALS and retroviruses was further confirmed through state-of-the-art bioinformatics approaches (107). Several pathophysiological pathways incriminate retroviruses in the development of MNDs. Considering HTLV-1 infection, the exact mechanisms for the neurological disease remain unknown. The main hypothesis to explain HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) neuropathogenesis is the so-called "Bystander damage." It suggests that the presence of IFN-γ-secreting HTLV-1-infected $CD4^+$T cells and their recognition by virally specific cytotoxic $CD8^+$T cells in the CNS, induce microglia to secrete cytokines, such as TNF-α, which may be toxic for the myelin of neurons. Clinically, HAM/TSP is a slowly progressive neurological condition that is defined clinically and serologically according to the WHO guidelines (108). Alfahad and Nath reported that at least 35 cases of ALS-like syndrome had been documented in literature since the description of HAM/TSP in the 1980s (106).

Dozens of cases with HIV-associated ALS have been documented in the literature (106) but the mechanism is not yet fully understood. Given that evidence suggest that HIV infects infiltrating macrophages, microglia, and astrocytes but not neurons, it is likely that the latter are affected indirectly. In addition to HIV, a human endogenous retrovirus K (HERV-K) in the brain and cortical neurons, which can be activated by the tat protein of HIV, was reported to be a contributor to MND (109, 110). Expression of HERV-K or its envelope protein in neurons placed in culture (*in vitro)* or in experimental animals (*in vivo)* causes motor neuron degeneration producing a similar phenotype to ALS (109). We surmise that possible interactions between HIV and HERV-K can result in MND in PLWHA, and the fact that symptoms regress with ART further supports the role of HIV in the pathogenesis of the motor neuron symptoms (111). It appears that controlling HIV infection using drugs that cross the BBB would indirectly control HERV-K activation in the neurons and result in clinical improvement.

Clinically, PLWHA with MND present with ALS-like symptoms including asymmetric limb weakness, upper and lower motor neuron signs, fasciculations, brisk muscle jerk reflexes, muscle atrophy, and fatigability. However, compared to ALS in the general population, PLWHA have an earlier age of onset of ALS symptoms, rapid progression, and sometimes a favorable evolution when ART is initiated (112).

2.3.3 Diagnosis and Management of MNDs

The classic form of ALS consists of a mixture of upper and lower motor neuron features. ALS patients complain of asymmetrical limb weakness with difficulty handling objects, and decreased muscle bulk; this weakness progressively migrates to other limbs with possible involvement of respiratory muscles (113, 114). The condition is often diagnosed by applying the El Escorial criteria that take into account both clinical and paraclinical elements (electrophysiology and neuroimaging) (115). Recently, potential biomarkers have been suggested to diagnose ALS; the presence of these biomarkers in the blood (e.g.: percentage of monocytes, immunoglobulin M [IgM], and CD3 lymphocyte counts) or CSF (e.g.: Chitinase-3-like protein 1, Chitinase-3-like protein 2, Alpha-1-antichymotrypsin) raises the index of suspicion in favor of ALS, and may discriminate ALS from other MNDs (116, 117). However, more research is needed to establish an appropriate test for ALS. The lack of a definitive test for ALS can be problematic, especially in contexts whereby patients are seen very early when symptoms are still scanty. In these cases, waiting and observation of the disease progression over the next few weeks and months are needed. In patients with paralytic poliomyelitis, the typical clinical picture is that of an MND with generalized weakness followed by asymmetrical flaccid paralysis and conserved sensory functions (118); therefore polymerase chain reaction (PCR) for detection of poliovirus in stool, throat swabs, blood, and CSF may be indicated when confronted with an MND clinical picture (119).

Management of ALS involves a multidisciplinary team to assess pulmonary function, diet, as well as the use of antioxidants and riluzole. As of now, the medications only slow down the course of the disease; there is no cure (114).

2.3.4 Future Perspectives

There has been a wide range of research to elucidate the course and etiologies of MNDs. Unfortunately, most of this research has been done using experimental animals as well as genetic studies to tackle genetic-MNDs. The challenges that however remain

are: the scarcity of community-based research of MNDs in Africa as a whole (89), the feasibility of translating these rodent studies to human studies, and the absence of effective drugs to treat or potentially reverse the evolution of MNDs. Failure to meet these challenges to date could be a result of inadequate protocols during rodent studies, including the timing of drug administration, small sample sizes, and differences in the mechanisms of MNDs. Further understanding of the molecular pathology of glial cells will contribute to developing therapies to slow the progression of MNDs and reduce incidence and disabilities (120).

2.4 Headache Disorders

2.4.1 Introduction

Headaches are the most prevalent disorders of the nervous system (121). Often underestimated, they usually have an insidious onset. They are divided into primary headaches (such as migraines and tension-type headaches) and secondary headaches, which are a result of an underlying condition (122). Studies have reported a 96% global lifelong prevalence of primary headaches, with females being more affected than males. The active prevalence of tension-type headache worldwide is estimated at 40%, and that of migraine at 10% (123–125). Globally, the prevalence of chronic daily headaches has remained consistent at 3–5% (126), with chronic migraine representing most of it. Headaches are ranked as the second leading cause of years lived with disability (YLD) worldwide with migraine alone accounting for one-third of total YLD in young adults (127, 128). In Africa, recent community-based studies have reported migraine prevalence between 3 to 6.9%, and chronic tension-type headache prevalence at 1.7% (129). In a hospital-based study conducted in Cameroon, headache disorders accounted for about 34% of complaints in out-patient consultation (130).

2.4.2 Immunopathophysiology of Headaches

Since the 1970s, it was suspected that the immune system plays a role in the development of chronic headaches (131). Some key elements in the immune system have been implicated in the pathogenesis of headaches (132). Calcitonin gene-related peptide (CGRP) is an inflammatory neuropeptide that contributes to headache pathophysiology, causes neurogenic inflammation, and activates the peripheral trigeminocervical neuron during the initiation of migraine at the brainstem or cortex level. This induces neurogenic vasodilation, extravasation of plasma proteins, and the influx of mast cells and other proinflammatory cells (133). Based on these initial findings, CGRP-receptor antagonists have been developed to block neurogenic vasodilation in the meninges (134). Other recent studies have revealed that CGRP triggers the secretion of cytokines by stimulating CGRP receptors found on T-cells, resulting in inflammation which might be involved in the pathogenesis of headaches (135).

Plasma levels of both pro- and anti-inflammatory cytokines are enhanced during migraine attacks. The levels of TNF-α increase rapidly and then decrease progressively over time after the onset of a migraine attack (136). Plasma levels of another proinflammatory cytokine, IL-1β, also increase after the initiation of headache. The release of IL-1β is induced by TNF-α and may lead to hyperalgesia. In a small number of patients with new daily persistent headache (NDPH), symptoms may develop after viral infection. In such cases, proinflammatory cytokines such as TNF-α could initiate and maintain CNS inflammation even after the resolution of the infection. Tumor necrosis factor-α is an important component in the pathogenesis of some conditions such as sinusitis and rhinitis, but also in headaches (137). The development of drugs that modulate TNF-α may benefit all these conditions. Adiponectin, which is secreted by the adipose tissue in obesity, is believed to modulate several inflammatory mediators important in migraine. Adiponectin has an anti-inflammatory action through inhibition of IL-6 and TNF-α-induced IL-8 production. Adiponectin also induces the production of cytokine IL-10, which is an anti-inflammatory. Although adiponectin decreases migraine, paradoxically, a sudden increase in its levels may worsen a headache (138). Thus, it is a possible biomarker or therapeutic target for migraine. Another possible immune marker are mast cells; these are granulated immune cells that upon stimulation degranulate and induce a local inflammation. The abundant mast cells in the intracranial dura degranulate their contents into the local milieu, activate the surrounding trigeminal meningeal nociceptors, and promote a prolonged state of excitation (139). The molecules released by mast cells activate the meningeal nociceptors followed by a cascade of neuronal activation mediated by the release of neuropeptides (e.g., CGRP, substance P), which further degranulate residual mast cells and prolong the migraine headache (140).

In secondary headaches caused by infections, the mechanism underpinning the headache symptom is usually non-specific as they largely depend on the causative disease itself and the accompanying inflammation (141). Literature is scarce on the pathogenesis of headaches due to systemic infection; however, the role of fever is debated. It is hypothesized that during systemic infections, there is direct activation of pain-producing mechanisms either by microorganisms or secondary to fever or a combination of both (142), with the subsequent release of proinflammatory substances that play a role in the generation of headache. In local CNS infections such as meningitis and encephalitis, the infective microorganism or its toxins directly invade the meningeal sensory nociceptor terminals causing inflammation and releasing proinflammatory mediators (e.g., bradykinin, prostaglandins [PGDs], and cytokines) (142). The resultant septic meningeal inflammation that causes the headache of meningitis is comparable to the presumed aseptic inflammation of the neurovascular junction of meningeal/dural blood vessels during migraine attacks. Therefore, the phenotypic characteristics of secondary headaches and migraines substantially overlap (142).

2.4.3 Headache in HIV

Human immunodeficiency virus-1 is a neurotropic virus that enters the CNS early and remains latent in glial cells. The infiltration of mononuclear cells triggers the release of

cytokines that activate latently infected astrocytes to express the virus (143). Infected macrophages and glial cells can result in toxicity by releasing cytokines such as TNF-α, and IL-1β (144). The stimulation of this inflammatory cascade is closely similar to the pathogenesis of migraine (143). Another proposed mechanism is plasma membrane alterations by HIV-1 itself with the resultant change in intracellular K^+ and Na^+ concentrations (143). Depolarization is followed by alterations in ionic gradient and a change of membrane permeability causing increased excitatory signals due to excess glutamate release. In acute neuronal infection with HIV-1, viral proteins such as tat and gp120 were associated with increased production of glutamate excitotoxicity through NMDA receptors stimulation and calcium influx-related excitation (145, 146). Another suggested mechanism involves the release of histamine from mast cells primarily because of viral-mediated cell death (147). This is similar to the pathophysiologic mechanism explained in migraine headaches. Evers et al. suggested that central pain processing structures of the trigemino vascular system may be affected by HIV (148). Secondary headaches in PLWHA may be present with non-specific characteristics depending on the opportunistic infection and the level of immunodepression. Opportunistic infections that cause secondary headaches in PLWHA include: cryptococcal meningitis (39%) and CNS toxoplasmosis (16%) (149). Several ARVs such as zidovudine, efavirenz, amprenavir can cause headaches, though the underlying mechanism is not fully elucidated (150–152).

2.4.4 Mechanism of Headache in Malaria

Headache is one of the most common clinical manifestations of malaria (153). Albeit being a non-specific characteristic, headache accounts for up to 75-80% of clinical manifestations in malaria-infected patients (154, 155) with about 30% of cerebral malaria patients reporting headaches (156). The mechanism of headache in acute malaria is not well understood though excessive cytokine release (such as TNF-α and IL-1β) might be an important factor (157). However, the frequency of headaches in non-cerebral and cerebral malaria is not affected by cytokine levels as cytokine plasma concentrations are not correlated to the severity of malaria (158, 159). Hence, the exact mechanistic pathogenesis of malaria-related headaches requires further studies.

Patients recovering from acute malaria manifest some symptoms known as post-malaria neurologic syndrome (PMNS) even when parasites have been cleared (160), and it seems to be an immune-mediated post-infectious syndrome. However, the precise mechanisms underpinning PMNS development after recovery from severe malaria are not well understood. Headache has been reported in about 10% of PMNS, which is often severe and associated with nausea, profound confusion, and impaired memory (161).

2.4.5 Management of Headache and Future Perspectives

When managing a case of headache, the chosen medication should match the patient's needs. The choice of treatment is usually guided by the characteristics of the headache attack, such as severity, frequency, disability, associated symptoms, and time-to-peak. Cognizant of the high prevalence of secondary headaches in SSA, etiological diagnosis of the headache is key in ensuring optimal patient management. Acute headache treatment options include acetaminophen and nonsteroidal anti-inflammatory drugs; both inhibit PGDs synthesis and limit subsequent inflammation in the CNS (162). Patients unresponsive to these treatments may require migraine-specific treatments including triptans (serotonin receptor agonists), which block the release of vasoactive peptides that trigger neurogenic inflammation (162). Corticosteroids also decreased headache recurrence, particularly for migraines whose duration exceeded 72 hours (163). A promising novel strategy focuses on CGRP, a potent vasodilator recently incriminated in the pathogenesis of migraine and cluster headaches attacks. Indeed, a randomized trial established the safety and efficacy of CGRP antibodies for the prevention of frequent episodic migraines (164). For certain specific indications (such as ≥4 headaches a month, ≥8 headache days a month, debilitating headaches, and medication-overuse headaches), preventive therapy may be indicated; this usually consists of propranolol or amitriptyline, among other medications (165). Future research should focus on better understanding the various molecular pathways in headache development, as these are crucial for the development of new treatments with specific targets and few side effects. Given the frequency of headache and its burden of disability, the safety, and efficacy of emerging therapies should be assessed in robust trials to provide evidence-based management options.

2.5 Sleep Disorders

Sleep disorders are a common problem in SSA with some studies reporting a pooled estimate of prevalence ranging from 16.6-55% in sites in some countries such as Ethiopia, Ghana, Kenya, South Africa, Tanzania, and Uganda (166–169). Sleep disorders have been associated with infectious diseases such as HAT and HIV and involve the activation of the immune system (20, 170, 171).

2.5.1 Sleep: Characterization/Stages and Sleep Disorders

Sleep is a complex physiologic, recurring, and reversible state of decreased metabolism, responsiveness to external stimuli, and motor activity regulated by a circadian rhythm (172–174). The neurophysiological stages of sleep can be evaluated using polysomnography (PSG), which incorporates EEG for brain electrical activity, electromyogram (EMGs) measuring muscle tone, and electrooculograms (EOGs) that assess eye movement (20, 175). Sleep normally consists of two broad alternating stages: non-rapid eye movement (NREM) sleep and rapid-eye-movement (REM) sleep (172). The NREM is further divided into three stages N1, N2, and N3. From wakefulness, sleep depth increases from N1, N2, N3 to REM, each with distinct neurophysiological characteristics (20). The N1 and N2 are considered light sleep and N3 is also known as slow-wave sleep. A hypnogram is a graph constructed from wakefulness-sleep staging versus time and includes these different stages of sleep, the number of episodes, and their rhythmicity and duration of sleep (**Figure 3**). Actigraphy is another technique to monitor sleep and wakefulness, which is simpler and less expensive than PSG, but only has a binary function (sleep/

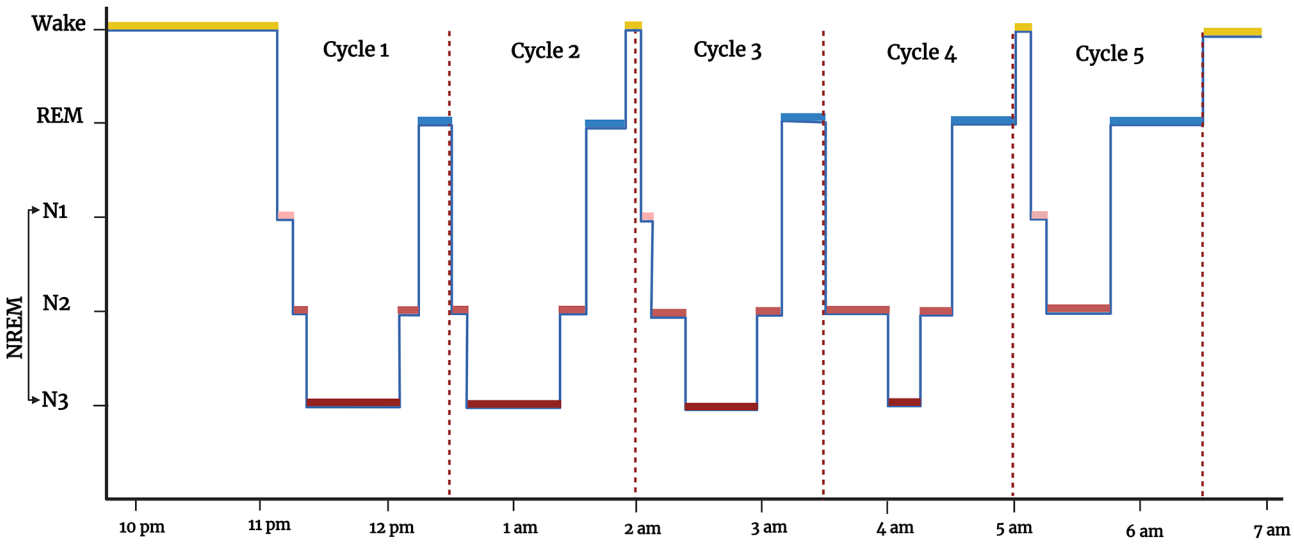

FIGURE 3 | Hypnogram showing the different states of vigilance (wakefulness and sleep) from 10 pm to 7 am: wakefulness (WAKE, in yellow), rapid-eye-movement sleep (REM, in blue), non-rapid eye movement sleep (NREM, in red, which has 3 sub-stages (N1, N2, and N3, in different shades of red). Figure created by EA and WM using BioRender.com.

wake) and does not give details of sleep architecture (20, 176–179).

Chronic disturbances in sleep patterns, poor sleep quality, or sleep-wake disorders are highly prevalent in society and increase with age (180, 181). They adversely affect the quality of life and are associated with significant morbidity and mortality. Sleep disorders can be a symptom of other diseases but can also exacerbate other disorders, especially mental disorders. There are many different types of sleep disorders including insomnia, hypersomnia, parasomnias, narcolepsy, circadian rhythm sleep disorders, sleep apnea, etc. of which insomnia is the most common (180–182).

2.5.2 The Immune System, Systemic Infection, and Sleep

There is a bidirectional relationship between sleep and the immune system (20, 175, 183). Sleep is considered an important restorative and regulatory process for the normal functioning of the immune system (175, 184, 185). Sleep deprivation alters the functioning of immune cells and cytokine expression (175, 185, 186). For example, experimental sleep deprivation reduced natural killer cell activity in humans (187), chronic insomnia decreased the levels of CD3+, CD4+, and CD8+ T cells (188), and sleep deprivation increased the proinflammatory cytokines such as IL-1β, IL-6, and TNF-α (189–191). The immune system also influences sleep. Animal studies have shown that administration of the proinflammatory cytokines IL-1β and TNF-α increase NREM sleep (192), whereas the anti-inflammatory cytokines IL-4 and IL-10 reduce NREM (183). Systemic infections in general cause somnolence as part of sickness behavior, possibly due to the increased levels of inflammatory cytokines and PGDs (20, 175). The increased NREM sleep, reduced REM sleep, and wakefulness during infection are important to preserve energy and support the immune system to fight infections (20, 175, 183).

2.5.3 Human African Trypanosomiasis and Sleep Disorders

Human African trypanosomiasis or sleeping sickness is an endemic disease restricted to Africa where tsetse flies transmit trypanosome parasites to humans. Two subspecies *Trypanosoma brucei (T. b.) gambiense* and *T.b. rhodesiense* cause disease in humans. Another subspecies *T. b. brucei* causes disease in animals and has been used extensively in animal models of the disease. The description of HAT and the parasites that cause it are covered in more detail in the article by Idro et al. in this collection and previous reviews (17).

Human African trypanosomiasis is divided into an early (first) hemolymphatic stage, with general non-specific symptoms of infection, and a late (second) meningoencephalitic stage with neurological and psychiatric manifestations (17, 20). Sleep disturbances are a prominent feature of HAT; thus, it is also known as sleeping sickness (17, 20). They are more pronounced in the late stage of the disease and negatively affect the patient's quality of life. Sleep disturbances occur in 75% of patients with second-stage HAT caused by *T. b. gambiense* and in 85% of patients with second-stage HAT caused by *T. b. rhodesiense* (193, 194).

Sleep disorders that occur in the second stage of HAT are not the increased sleepiness that normally results from systemic infections, but they are disruptions in sleep structure and circadian rhythm or sleep timing (20, 195). Patients with HAT do not sleep more within a 24-hour period but have fragmented sleep, sleep more during the daytime, and sleep less at night (196, 197). Features of HAT are similar to narcolepsy as patients can

fall asleep suddenly (197). Polysomnography studies have shown that HAT patients can move from wakefulness into REM sleep or have very short NREM sleep in between, known as sleep-onset REM periods (SOREM), which is similar to what happens in narcolepsy (197, 198). Actigraphy studies have also shown a disrupted sleep-wake cycle in HAT patients (179) and the actigraphy sleep score has been proposed as a diagnostic and monitoring tool (178).

The sleep disturbances in HAT are caused in part by molecules released from the parasite, such as PGDs, and by the activation of the immune system and inflammation (197). The trypanosomes release PGDs, such as PGD2, and also induce the release of PGD2 from the host, which are somnogenic and can cause disturbances in sleep (197, 199). Activation of the immune system during HAT results in a robust elevation of proinflammatory cytokines and other proinflammatory molecules (17, 200, 201). Cytokines such as TNF-α, IL-1β, and IFN-γ and chemokines such as CXCL10 are upregulated in the brain and CSF of animal models of HAT (17, 201–204) and the CSF of second-stage HAT patients (202, 205). These cytokines such as TNF-α, IL-1β, and IFN-γ alter sleep patterns and circadian rhythm (197, 206). The levels of immune molecules and PSG correlate to the actigraphy findings (178). The levels of cytokines and chemokines have also been suggested as biomarkers for staging HAT (202, 205) and could be possible biomarkers to monitor therapeutic outcomes in HAT patients. More studies are needed to produce diagnostic kits or tools to monitor these molecules to stage the disease and monitor therapeutic outcomes.

2.5.4 Human Immunodeficiency Virus and Sleep Disorders

2.5.4.1 Prevalence of Sleep Disorders and Nature of Sleep Disorders Among PLWHA

A high percentage of PLWHA suffer from poor sleep quality, with an overall prevalence of 58% (207). In some studies done in the SSA region, the prevalence of poor sleep quality in PLWHA ranged from 57% to 61% (171, 208, 209). The sleep disturbances experienced by PLWHA include hypersomnia, insomnia, difficulties in initiating sleep (longer sleep onset latency), fragmented sleep, sleep apnea, and restless leg syndrome, with variable changes in the NREM and REM sleep, as well as circadian rhythm disorders (208, 210–216).

2.5.4.2 Causes of Sleep Disorders Among PLWHA

The pathogenesis of sleep disorders in PLWHA is multifactorial and includes the ARVs, effects of the immune system and viral molecules, disease progression, opportunistic infections, substance abuse, depression, and financial and social concerns (20, 171, 208). Some ARVs especially the non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz cause sleep disturbances such as insomnia, somnolence, and nightmares (189, 208, 217).

Sleep disorders are comorbid with various disorders such as anxiety, depression, and pain in PLWHA (171, 180, 189, 211, 218). There is a bidirectional relationship between sleep disorders and other disorders such as anxiety, depression, and pain. Sleep disorders contribute to the development of anxiety, depression, and pain, and on the other hand anxiety, depression, and pain cause and worsen sleep disorders (20, 219–221).

2.5.4.3 The Immune System and Sleep Disorders Among PLWHA

Given the bidirectional relationship between sleep and the immune system and the alterations in the immune system caused by HIV infection, it is plausible to hypothesize that alterations in the immune system contribute to sleep disturbances in PLWHA and vice versa.

Various studies have investigated the relationship between sleep disturbances and immune system activation in PLWHA with non-conclusive results (171, 222–227). In a study in South Africa on PLWHA taking ART for 4 years, poor sleep quality correlated with both higher current CD4+ cell count and more upregulation of CD4+ cells from baseline (before taking ART) (171). Most of the patients in this cohort started ART late and this has been associated with spontaneous immune activation because of the increase of CD4+ cell count (171, 228), which would lead to an inflammatory state. This is in contrast with some earlier studies that showed a correlation between low CD4 + cell count and poor sleep quality (209, 229, 230). Recently, long sleep hours were associated with low CD4+ cell count and greater severity of the disease (210).

A recent study conducted in the United States of America (USA) did not find a significant association between insomnia and monocyte activation marker soluble CD14 (sCD14) or the proinflammatory cytokine IL-6 (227). In HIV-positive men who have not received ART, high TNF-α concentrations were associated with moderate-to-severe obstructive sleep apnea independent of CD4+ cell count and plasma HIV-RNA concentration (222). Sleep onset insomnia was associated with single nucleotide polymorphisms (SNPs) for IL-1β, IL-6, IL13, and TNF-α in PLWHA classified as having sleep onset insomnia (Gay et al., 2014). However, plasma levels of the proinflammatory cytokines IL-1β, IL-2, IL-6, IL-10, IL-13, and TNF-α did not differ between those with sleep onset insomnia and those without (223). A higher percentage of wake after sleep onset (WASO%) was associated with SNPs of IL1R2 and TNF-α, whereas SNPs of IL-2 were associated with less WASO%. Single nucleotide polymorphisms of IL-1R2 and TNF-α were also associated with short sleep duration (224). Higher levels of c-reactive protein (CRP) and IL-6 in PLWHA were associated with disturbances of various sleep metrics such as later sleep onset, lower total sleep time, and higher WASO (226). Moore et al. reported sex-dependent differences in cytokines and sleep disturbances in PLWHA (225). They observed significant negative correlations between sleep disturbance and the proinflammatory cytokines IFN-γ and TNF-α, but not IL-6, in females, with no significant associations among males.

Studies that have been done in the USA reported an association between inflammatory cytokine levels or polymorphisms and sleep disturbances (222–224, 226). Thus, there is a need for such studies in the SSA region, which has the highest number of PLWHA and has a peculiar situation including PLWHA who started ART later than those in the USA. Further studies are also needed to ascertain whether immune molecules can be used as biomarkers of sleep disorders and to measure the response to

interventions to alleviate sleep disorders and comorbid conditions such as anxiety, depression, and pain. The study by Moore et al. that showed sex-dependent differences in inflammatory molecules and sleep disturbances in PLWHA, suggests that this could be the reason for the variability amongst various studies, hence further studies are needed (225).

2.6 Peripheral Neuropathy and Neuropathic Pain

Peripheral neuropathies (PN) and disorders of the peripheral nervous system are common problems caused by various acquired conditions such as diabetes mellitus, chemotherapy, HIV and other infectious diseases, alcoholism, nutrient deficiencies, or toxic molecules. Furthermore, inherited conditions such as Charcot-Marie-Tooth and Fabry disease represent less frequent etiologies of PN (10, 231, 232). Patients present with predominant sensory symptoms (numbness, tingling, burning, stabbing, or electrical pain), motor symptoms (muscle weakness, wasting, twitching and cramps and paralysis), and autonomic symptoms (orthostatic hypotension, sweat abnormalities, gastroparesis, esophageal dysfunction, bladder dysfunction) (232, 233). Peripheral neuropathies have been reported to affect about 15% of adults in the USA, with diabetics having a higher prevalence of PN (234). In a study conducted in urban and rural Uganda, neurological disorders' overall point prevalence was 3.3%, of which the majority was due to PN, with a crude prevalence of 33.7% (3). In another study conducted in rural Uganda, PN was present in 13% of the cohort and was more common in HIV-positive participants (235).

2.6.1 Peripheral Neuropathy Related to Infectious Diseases

Infectious causes of neuropathy include HIV, hepatitis viruses, varicella-zoster virus, herpes simplex viruses, flaviviruses, rabies virus, human T-cell lymphotrophic virus type-1, *Mycobacterium leprae*, *Borrelia burgdorferi*, *Corynebacterium diphtheria*, *Clostridium botulinum*, and *Trypanosoma cruzi* (10, 236–240). Hepatitis B, C, D are all associated with several forms of neuropathies, and hepatitis A is also associated with a rare form of neuropathy (236). This is of specific concern to the African region as viral hepatitis is considered an endemic public health problem (241). Similarly, the rabies virus has a higher prevalence in Africa and Asia with 95% of rabies-related deaths occurring in these areas (236, 242). Although the prevalence of leprosy, caused by *Mycobacterium leprae*, has been largely decreasing, neuropathy is the main manifestation associated with it (236, 237) and leprosy is the major cause of neuropathy in some endemic countries in the SSA such as Ethiopia (10). Globally the number of new cases of leprosy detected annually is around 200,000, with Africa contributing around 20,000 cases (243). Peripheral neuropathy is an integral part of leprosy and thus is briefly described below. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) 2021, preliminary epidemiological estimates show that 36.7 million people are living with HIV, of which 25.3 (68.9%) millions are in the SSA region (244). Since HIV has the highest prevalence in SSA and has a broad range of associated neuropathies, it is discussed in more detail below.

2.6.2 Leprosy

Leprosy causes irreversible nerve damage, and peripheral neuropathy is present in all forms of leprosy. The sensory neuropathies of leprosy present in various forms including cutaneous nerve damage (resulting in anesthetic or hypo-aesthetic skin lesions), symmetrical pansensory neuropathy, and leprous ganglionitis (245). Both the innate and adaptive immune systems are involved in nerve damage during leprosy (246). *Mycobacterium leprae* invades and/or activates immune cells such as macrophages and T cells as well as Schwann cells, which contribute to the nerve damage that occurs during leprosy (245–247). The bacteria also infect the nerves and cause an inflammatory process that leads to the damage and the thickening of nerves in about 40 to 75% of infected individuals, which is painful most of the time (245). Depending on the type of lesions, there is either Th1 or Th2 immune response plus CD8 cell involvement (245, 246). A Th1 cytokine response (IFN-γ, IL-2, IL-15, TNF-α) is associated with tuberculoid leprosy lesions, while a Th2 cytokine response (IL-4 and IL-10) is associated with lepromatous leprosy lesions (245, 246). Activated T cells attack and kill Schwann cells, which then affects nerve cell function. Infected macrophages cause axonal damage and demyelination through increased production of nitric oxide (NO) and reactive nitrogen species (247).

2.6.3 Human Immunodeficiency Virus-Associated Neuropathy and Neuropathic Pain

Human immunodeficiency virus-associated neuropathy is one of the main causes of neuropathies in SSA due to the high prevalence of HIV in the region (248). The prevalence of PN in PLWHA in several SSA countries ranges from 18-52% (235, 249–251). This neuropathy is caused by both the virus and ART (236, 237). The most common form of neuropathy is distal symmetrical polyneuropathy (DSP) with almost one-third of HIV patients facing this complication (236). Distal symmetrical polyneuropathy is associated with advanced stages of HIV disease and develops as immunosuppression progresses and as HIV viral load increases (252). The DSP caused by some ARVs such as the nucleoside reverse transcriptase inhibitors (NRTIs) is called antiretroviral toxic neuropathies (ATN) and is clinically indistinguishable from HIV-DSP, but they have different pathophysiological mechanisms (236). Other drugs commonly used in HIV-associated infections that may cause DSP include isoniazid, ethambutol, and dapsone (253). Distal symmetrical polyneuropathy may be detected pathologically in nearly all patients dying with AIDS (254). Symptoms of DSP include burning feet, numbness, and paresthesias (255). However, in some patients DSP is asymptomatic. Signs of motor involvement are seen in very few patients until the very late stages of DSP.

Another type of neuropathy associated with HIV that affects African patients is diffuse infiltrative lymphocytosis syndrome (236). In addition, cytomegaloviruses, opportunistic infections, and necrotizing vasculitis during advanced HIV can cause severe mononeuropathies (236). Other forms of neuropathies related to HIV include inflammatory neuropathies and radiculopathies (256).

The prevalence of neuropathic pain in PLWHA is 35%, due to the virus and medications used to treat it (257, 258). Neuropathic pain is defined by the International Association for the Study of

Pain (IASP) as 'Pain caused by a lesion or disease of the somatosensory nervous system' (259). Symptoms include both negative (hypoesthesia, hypoalgesia, numbness, loss of sensation) and positive sensory symptoms (hyperalgesia, evoked pain, spontaneous pain). The pain is progressive, starts in the feet and ascends symmetrically to the hands, and is described as "glove and stocking" distribution (256, 260).

Although HIV-associated neuropathic pain negatively affects the patient's quality of life, to date there are no approved FDA medications to either prevent it or treat it (261, 262). In the case of ATN, substituting the offending drug is the first step in treatment, which may still be challenging in some parts of Africa due to limited access to drugs due to procurement difficulties even though more antiretroviral drug options have become available in recent years. Some drugs used for other types of neuropathic pain such as anticonvulsants, antidepressants, topical agents, as well as non-steroidal anti-inflammatory drugs, and opioids are used and show modest activity (261, 263). However, in clinical trials, antidepressants such as amitriptyline (264, 265), and anticonvulsants such as pregabalin (266) were not effective for the management of HIV-DSP. In a multisite study, PLWHA rated the overall effectiveness of self-care pain management strategies on a scale of 1 to 10 as follows: reflexology (7.53), meditation (7.08), prescribed antiepileptics (6.85) massage (6.84), marijuana (6.82), acupuncture (6.81), feet elevation (6.53) and taking a hot bath (6.45) (267, 268). Those numbers reflect that both medications and self-care management strategies provide inadequate pain management. Thus, there is a need to find new drugs to prevent or alleviate HIV-associated neuropathic pain. Understanding the pathophysiological mechanism of HIV-DSP and the involvement of the immune system may provide new therapeutic targets to manage it.

2.6.4 Human Immunodeficiency Virus-Associated Neuropathy, Neuropathic Pain, and the Immune System

Various mechanisms are involved in the development of HIV-DSP and neuropathic pain (see **Figure 4**). Products of immune activation in response to HIV infection, along with HIV proteins are involved (269). The entry of HIV in macrophages or microglia results in their activation and the release of proinflammatory cytokines, chemokines, glutamate, and viral envelope proteins, including gp120. The viral envelope gp120 that HIV uses to interact with the CD4 receptors and enter the cells, has a direct neuropathic effect on neurons due to activation of chemokine receptors or indirectly through activation of macrophages and Schwann cells (25). In PLWHA, the presence of these proinflammatory cytokines causes infiltration of macrophages and lymphocytes within the peripheral nerve and dorsal root ganglia (DRG) (270–275). The infiltrating macrophages and lymphocytes secrete inflammatory cytokines (TNF-α, IL-1β, IFN-γ, and IL-6) and chemokines and exacerbate nerve degeneration leading to the loss of the small unmyelinated sensory fibers followed by the large myelinated fibers in a dying back pattern of nerve degeneration (260, 276–278). Several chemokine receptors, including C-X-C chemokine receptor type 4 (CXCR4) and C-C chemokine receptor type 5 (CCR5), are expressed widely in the nervous system, for instance in the DRG satellite glial cells. The binding of the viral gp120 with CXCR4 receptors enhances the production of the chemokine C-C motif ligand 5 (CCL5) also known as regulated upon activation, normal T cell expressed and secreted (RANTES) chemokine which then binds to CCR5 receptors and enhances the release of TNF-α, which may induce neurotoxicity and cause axonal degeneration (269, 279, 280). Increased activation of CXCR4 receptors by chemokines, HIV gp120 or NMDA receptors by glutamate, increases calcium influx and stimulates downstream signaling cascades and subsequent production of second messengers particularly kinases, including protein kinase A, protein kinase C, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (281–283).

The increase of calcium levels inside the neuron facilitates nitric oxide synthase (284) to produce NO which further enhances pain *via* the generation of proinflammatory cytokines (285). Mounting evidence has shown that free radicals are involved in causing pain (286–291). In addition, gp120 activates microglia and astrocytes, which upregulate ROS that disrupts mitochondrial transmembrane potential (25, 260, 277, 292). The ROS produced by the interaction of viral gp120 with the receptors present either on microglia or neuron modulates apoptosis through TNF-α and its receptors (293); all these molecules have known neurotoxic properties and may be associated with axon degeneration, neuroinflammation, and hyperalgesia (294, 295).

Mitochondrial toxicity is the main mechanism responsible for ATN (296–299). The increased superoxide levels in patients with ongoing HIV infection damages both neurons and astrocytes and causes neuroinflammation (295, 300). Administration of ddC (a highly neurotoxic out of clinical use NRTI) causes neuropathy *via* several mechanisms including immune system activation. Treatment of rats with ddC, resulted in increased levels of both transcripts and protein levels of TNF-α in the spinal cord and DRG neurons, at a time point when the rats had developed mechanical allodynia, a symptom of neuropathic pain (301, 302). Aging mice treated with ddC had increased neuroinflammation as microglia and astrocytes were activated, and TNF-α, IL-1β, and Wnt5a were upregulated, in the spinal cord (303). Treatment of mice with other NRTIs (zidovudine, lamivudine, stavudine) up-regulated cytokines, including IL-1β, TNF-α, and IL-6 in different brain regions (304). Elevated CCL2 in DRG accompanied by a reduction in intraepidermal nerve fiber density and spinal gliosis have been observed in a model for HIV-sensory neuropathy and ATN using gp120 and ddC (305). In a recent study using female mice, systemic ddC administration induced transcript levels of cytokines (IL-1β, IFN-γ, and TNF-α) in the brain and paw skin, and the phosphorylation levels of the signaling molecule Erk1/2 in the brain, which was associated with the development of mechanical allodynia (306). These effects of NRTIs can augment the effects of HIV, as the virus activates p38 MAPK, Erk1/2 pathways to aid in its replication and proliferation, which is harmful to the host cell as this leads to the release of proinflammatory cytokines and biomarkers

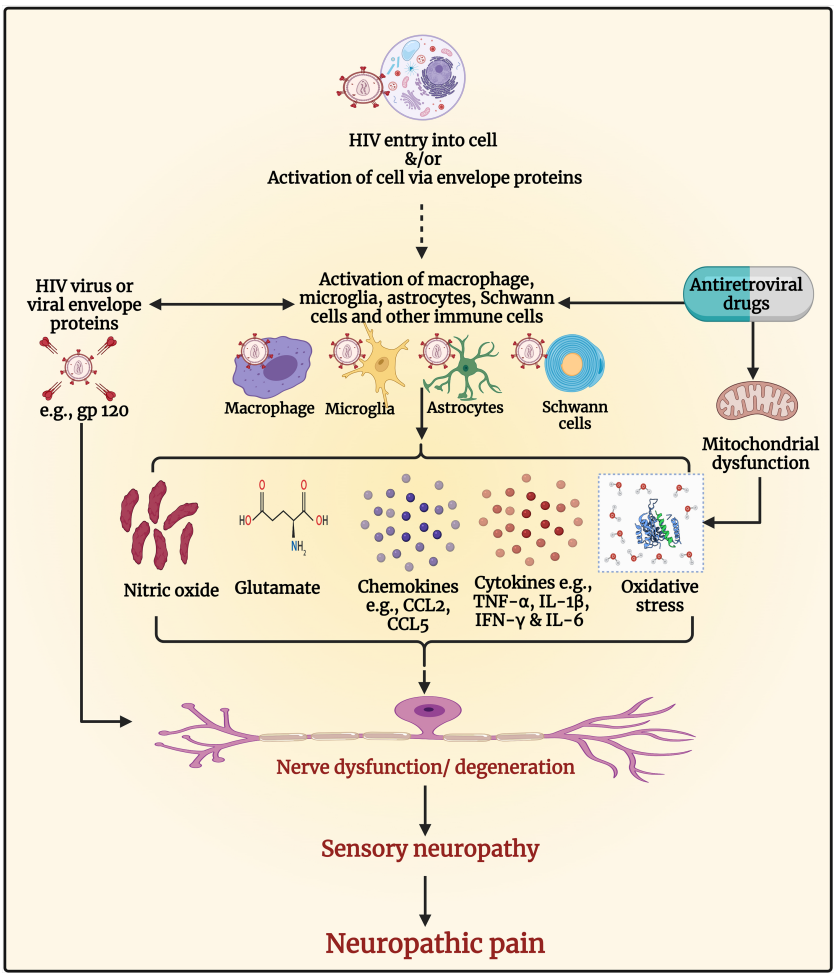

FIGURE 4 | Pathogenesis of human immunodeficiency virus (HIV) associated sensory neuropathy and neuropathic pain. The entry of HIV in macrophages or microglia results in their activation and the release of proinflammatory cytokines, chemokines, glutamate, nitric oxide, and viral envelope proteins, including glycoprotein (gp)120, which can cause nerve dysfunction/neurodegeneration. The viral envelope gp120 has a direct neuropathic effect on neurons due to activation of chemokine receptors resulting in neuronal hyperexcitability and neuropathic pain. It also has indirect neuropathic effects through the activation of macrophages and Schwann cells. The presence of proinflammatory cytokines within the peripheral nerve and dorsal root ganglia causes infiltration of macrophages and lymphocytes, which secrete inflammatory cytokines (e.g., tumor necrosis factor-alpha [TNF-α], interleukin-1 beta [IL-1β], interferon-gamma [IFN-γ], and IL-6), and chemokines (e.g., C-C motif ligand 2 [CCL2] and CCL5) and exacerbate nerve degeneration leading to neuropathy and neuropathic pain. Antiretroviral drugs such as nucleoside reverse transcriptase inhibitors (NRTIs) inhibit deoxyribonucleic acid (DNA) γ- polymerase, the enzyme essential for copying and repair of mitochondrial DNA. This results in the accumulation of mutations of mitochondrial DNA, defective respiratory chain subunits, impaired oxidative phosphorylation, reduced adenosine triphosphate (ATP), and oxidative stress. Oxidative stress causes nerve degeneration. The NRTIs also contribute to neuropathy and neuropathic pain by activating glial and immune cells to release cytokines, chemokines, and molecules that induce neuronal hyperexcitability and neurodegeneration. Figure created by EA and WM using BioRender.com.

that signal apoptosis (307). These studies suggest that proinflammatory cytokines both in the periphery and in the CNS play a role in the pathophysiology of ATN.

Besides NRTIs, other ARVs such as protease inhibitors (PIs) can cause ATN. The PIs such as indinavir, saquinavir, or ritonavir have been reported to cause sensory PN in PLWHA (308). *In vitro*, indinavir caused neuronal atrophy and DRG macrophage cytotoxicity (308). Administration of indinavir induced mechanical allodynia in rats, which was associated with increased expression of phospho-p38 in microglia (309).

2.6.5 Value of Neuroimmune Changes in Therapeutics

Immunomodulators that reduce the expression of the inflammatory cytokines and or inhibit their signaling pathways could be of therapeutic use in the prevention and management of neuropathic pain in PLWHA. B-caryophyllene (BCP), a cannabinoid type 2 receptor (CB2R)-selective phytocannabinoid, prevented the development of and attenuated ddC-induced allodynia and the expression of proinflammatory cytokines and the signaling molecule, Erk1/2 (306). Other immunomodulators

such as minocycline and pentoxifylline also prevented the development of ddC-induced allodynia (302, 306) and alleviated established ddC-induced hyperalgesia and allodynia (310). Administration of IL-10 reduced mechanical allodynia and reversed the upregulation of p-p38 MAPK, TNF-α, SDF-1α, and CXCR4 in a model of gp120 and ddC induced HIV-sensory neuropathy and ATN (311). These animal studies warrant further research to evaluate if they can be translated to therapeutic drugs in PLWHA suffering from neuropathic pain.

3 CONCLUDING REMARKS

Neuroinfections prevalent in the SSA region cause various neurological disorders such as epilepsy, dementia, motor neuron diseases, headache, sleep disorders, and peripheral neuropathy. Infections provide an excellent opportunity to understand the pathophysiology of many primary neurological disorders, since they may give valuable clues about the real reason of the disorder including molecules/pathways or structural damages involved in these disorders. The immune system plays an important role in the pathophysiology of these neurological disorders.

3.1 Epilepsy

Epilepsy hugely affects SSA, with CNS infections as the most frequent preventable cause. Epilepsy often occurs after an initial brain insult followed by a latent phase during which an enduring epileptogenic lesion is established in the patient's brain. Although the pathophysiological mechanisms are not fully understood, CNS infection and neuroinflammation result in the release of pro-inflammatory cytokines by glial cells and neurons. Over time, the inflammatory cascade leads to neuronal loss, gliosis, and NMDA/glutamate-mediated brain hyper-excitability underpinning chronic epileptogenesis. More research is warranted to understand the risk factors, mechanisms, and specific triggers for the development of epilepsy following a CNS infection. This will eventually pave the way for better preventive and therapeutic approaches for epilepsy in SSA.

3.2 Dementia

With the increase of the aging population in SSA, the number of persons with neurodegenerative diseases is expected to rise over time. The pathophysiological processes underpinning the development of dementia include chronic neuroinflammation that activates microglia to release cytokines and neurotoxic substances. The hypothesized development of AD *via* a seeding mechanism is an elegant illustration of how CNS invasion by microbes could increase the risk for non-communicable neurodegenerative conditions. Furthermore, the role of peripheral inflammation in fostering CNS inflammation remains an interesting research direction that could open new therapeutic avenues for dementia and other neurodegenerative conditions.

3.3 Motor Neuron Diseases

Research on MNDs occurrence has identified an interaction between genetic, age-related, environmental, and developmental factors. An underlying neuroinflammatory process consisting of activated microglia, infiltrated T cells, and the subsequent overproduction of pro-inflammatory cytokines constitute a pathological hallmark of MND. These have been documented in individuals infected by viruses (poliovirus, HIV) or activation of endogenous retroviruses such as HERV-K, and often present as ALS-like syndrome. There is currently no cure for ALS. Further understanding of molecular pathology within glial cells will contribute to developing therapeutics that will slow the progression of MNDs and reduce incidence as well as disabilities.

3.4 Headache

Headaches have a huge burden worldwide and neuroimmunology plays a key role in the pathogenesis. The immunopathologic mechanisms underlying headache, both primary and secondary, are non-specific. They involve an interplay of pro- and anti-inflammatory cytokines, stimulating brain dural nociceptors. While there has been considerable advancement in our understanding of neuroimmunology, the mechanisms underlying the genesis of headache during systemic infections are still speculative, ranging from direct (pathogen-related) to indirect (drug-induced and post-infectious) influence. There is a need for further exploration to fill knowledge gaps, including the triggering factors and the exact immune-mediated mechanisms involved in both primary and secondary headaches, to achieve better management strategies in the future.

3.5 Sleep Disorders

The immune system and more specifically proinflammatory cytokines contribute to the pathogenesis of sleep disorders during infectious diseases such as HAT and HIV. How and why the cytokines such as IFN-γ and TNF-α contribute to sleep disturbances in PLWHA in a sex-dependent manner needs to be elucidated as well as the contribution of cytokine polymorphisms to sleep disturbances in PLWHA. Inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ may alter sleep patterns and the circadian rhythm during HAT. More studies on how these molecules contribute to the alteration of sleep patterns during HAT are needed. These cytokines and chemokines such as IFN-γ-induced CXCL10 seem to be useful biomarkers for staging HAT.

3.6 Peripheral Neuropathy and Neuropathic Pain

Both the HIV and the ARVs used to treat the virus cause sensory neuropathy and neuropathic pain. They both activate glial and immune cells to release proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6, and chemokines such CCL2 and CCL5, which cause neuronal hyperexcitability, neurodegeneration, neuropathies including neuropathic pain. Animal studies suggest that immunomodulatory drugs that inhibit the expression or secretion of these proinflammatory cytokines could prevent or alleviate HIV-associated neuropathy and pain. Of interest are the cannabinoids, taking into consideration that some clinical trials have shown that smoked cannabis alleviates neuropathic pain in PLWHA. However, the use of cannabis is limited by its psychoactive side effects, which are CB1R-dependent. Animal

studies showing that the non-psychoactive CB2R agonists alleviate NRTI-induced allodynia and inhibit the expression of proinflammatory cytokines suggest that these molecules could be useful for the management of neuropathic pain in PLWHA with a better side effect profile.

In conclusion, the immune system plays an important role in the pathogenesis of neurological disorders caused by neuroinfections. Further understanding of the role of the immune system in the pathogenesis of these neurological disorders during neuroinfections is vital for the development of therapeutics as well as biomarkers for diagnosis and therapeutic monitoring of these disorders.

AUTHOR CONTRIBUTIONS

WM participated in the conception of the article idea, which was discussed by all authors before writing began. LN, JNSF, EA, WM, AKN participated in the writing different sections of the article. EA put together all the different sections of the article, did the final formatting of the article, and all authors critically reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Neuroimmunology of Common Parasitic Infections in Africa

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Parasitic infections of the central nervous system are an important cause of morbidity and mortality in Africa. The neurological, cognitive, and psychiatric sequelae of these infections result from a complex interplay between the parasites and the host inflammatory response. Here we review some of the diseases caused by selected parasitic organisms known to infect the nervous system including *Plasmodium falciparum*, *Toxoplasma gondii*, *Trypanosoma brucei* spp., and *Taenia solium* species. For each parasite, we describe the geographical distribution, prevalence, life cycle, and typical clinical symptoms of infection and pathogenesis. We pay particular attention to how the parasites infect the brain and the interaction between each organism and the host immune system. We describe how an understanding of these processes may guide optimal diagnostic and therapeutic strategies to treat these disorders. Finally, we highlight current gaps in our understanding of disease pathophysiology and call for increased interrogation of these often-neglected disorders of the nervous system.

Keywords: brain disorders, *Plasmodium falciparum*, *Trypanosoma brucei* spp., *Toxoplasma gondii*, *Taenia solium*, neuro-infections, immune system, glia

INTRODUCTION

Some of the most extraordinary parasites are those that manage to establish infection in the human central nervous system (CNS). Because parasitic infections likely remain more prevalent in Africa than in any other continent, knowledge on their diverse CNS manifestations is of outmost importance. Adding to the death toll caused by cerebral parasitic infections, the neurological, cognitive, or mental health problems affect millions of Africans annually. Despite this, precise estimates of morbidity are lacking, and knowledge remains limited on the pathogenesis of CNS

injury for many of these diseases. Prevention and control of these parasitic CNS infections therefore, remains a global research priority (1).

Parasites as infectious agents are a diverse group of unicellular (e.g., protozoa) and multicellular (e.g., helminths) organisms with complex life cycles and a variety of hosts, including humans. The geographical distribution and transmission dynamics of parasitic infections are often dictated by the ecosystem, with presence of specific insect vectors, zoonotic transmission, and reservoirs. Consequently, they are often locally endemic, and their prevalence depends on a variety of factors, including socio-economic factors (2).

The human brain is protected by several cellular barriers that regulate or restrict passage of molecules and cells, including microorganisms, to the brain parenchyma. Three main barrier systems protect neurons from blood-borne external insults, such as infection: the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier (BCSFB) and the meningeal barriers (3, 4). Inflammation can cause dysfunction of these barrier systems. While brain-resident immune cells and infiltrating leukocytes are central to limit infections by parasites that successfully translocate, the inflammatory responses may also severely damage or alter neuronal function (5). Thus, protective effects and detrimental inflammatory responses need to be balanced to minimize injury (6).

Being eukaryotic organisms, protozoa and helminths represent a particular challenge to the immune system. This is explained partly by their elaborate immune evasion mechanisms. Moreover, parasites undergo complex life cycles comprised sometimes of antigenically distinct extracellular and obligate intracellular stages. As motile organisms, parasites have also developed diverse strategies to migrate or be transported in tissues, resulting in systemic dissemination in the human body. These include mechanisms for translocation across the BBB. The broad array of immune evasion mechanisms and versatility of the host-pathogen interplay is possibly best illustrated by the diverse clinical presentations. While some parasites can cause acute life-threatening neurological damage, their adaptation to the human host also permits chronic, sometimes life-long, CNS infection (7–9).

Thus, to establish an infection in the CNS, a parasite must first breach the normally non-permissive cellular barriers of the brain and then be able to evade the immune responses unique to the CNS. The clinical CNS manifestations are often associated to the processes that result from the specific host-parasite interplay, which remains only partly understood. Here, we outline the current knowledge on host-pathogen interactions and neuro-immunopathogenesis for selected clinically relevant CNS infections by parasites in Africa. The parasites covered in depth, *Plasmodium* spp., *Trypanosoma brucei* spp., *Toxoplasma gondii* and *Taenia solium*, were chosen as examples of parasitic infections implicated in neurological disorders, such as epilepsy, sleeping sickness, headaches and cognitive impairment. Other endemic parasites, amoebas, *Echinococcus* spp., *Onchocerca* spp., *Paragonimus* spp., *Schistosoma* spp., *Sparganosis* spp., and *Toxocara* spp., that present with neurological manifestations are also covered.

NEURO-IMMUNOLOGY OF CEREBRAL MALARIA

Pathogen Description, Prevalence in Africa, Signs, and Symptoms

Burden and Transmission

Malaria is a mosquito borne disease and a leading cause of ill health and death especially among children in sub-Saharan Africa. In 2019, there were an estimated 229 million cases and 409 000 deaths globally. Five African countries - Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%) and Niger (3%) accounted for over 50% of the deaths (10). Human disease is caused by four species of the genus *Plasmodium*: *Plasmodium falciparum, vivax, ovale* and *malariae.* There have also been outbreaks of infections by the monkey parasite – *Plasmodium knowelsi* in Southeast Asia. *Plasmodium falciparum* remains the most prevalent agent. It also causes the most severe infections. In contrast, by 2019, the proportion of clinical cases caused by *Plasmodium vivax* had reduced to 3% from about 7% in the year 2000 (10).

Life Cycle

Malaria parasites are transmitted by female anopheline mosquitoes and although over 100 species can transmit the parasite, in Africa, transmission is largely by *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae* from the Gambiae complex and *Anopheles funestus* from the Funestus subgroup (11).

The parasite's life cycle is made of a vector and human exoerythrocytic (hepatic) and erythrocytic stages. During a blood meal, the female mosquito (vector) bites and injects mature sporozoites from its salivary glands into the host's circulation. These quickly invade the liver hepatocytes and start asexual reproduction and multiplication as in tissue schizogony (exoerythrocytic stage). The tissue schizonts burst the infected hepatocytes releasing thousands of merozoites into the circulation. The tissue merozoites infect the erythrocytes, undergo a series of asexual multiplication cycles (erythrocytic stage), produce new infective merozoites which burst the erythrocytes and a new infective cycle begins. Some merozoites develop into male and female gametocytes. These are taken up when the next mosquito bites an infected person and mature in the mosquito gut. The gametocytes fuse to form an ookinete and the ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect the next vertebrate host. Reviewed in (12, 13).

Clinical Features

In high malaria transmission areas, many individuals, especially older children, and adults, carry asymptomatic parasitemia (14). Symptoms develop 7-10 days after the initial mosquito bite. Clinical disease manifests either as uncomplicated or complicated disease. Patients with uncomplicated malaria have fever, chills, headache, body ache, malaise, and vomiting. Severe or complicated malaria is a life-threatening disease. Patients have severe anemia, prostration, altered consciousness or coma,

respiratory disease or metabolic acidosis, abnormal bleeding, hypoglycemia, repeated seizures, and acute kidney injury (12, 15).

Cerebral malaria is the most severe neurological complication of infection by *Plasmodium falciparum*. In children, coma develops rapidly with seizures following 1-3 days of fever. Status epilepticus is frequent and intracranial hypertension with brain swelling, retinal changes and abnormalities in posture and abnormal respiratory patterns are common. In some however, coma develops slowly with progressive weakness. Systemic complications include anemia, metabolic acidosis, electrolyte imbalance, hypoglycemia, and shock. Mortality is particularly high in patients with deep coma, severe metabolic acidosis, shock, hypoglycemia, and repeated seizures [reviewed in (16)].

On the other hand, cerebral malaria in adults is mostly part of a multi-organ disease. Patients progressively develop generalized weakness, delirium and coma and compared to the disease in African children, seizures, raised intracranial pressure and retinal changes are less common and coma resolution is slower.

Pathophysiology of Cerebral Malaria

a. How the parasites get to the CNS and the interplay between the parasites and the immune system in the CNS

The hallmark of cerebral malaria is intravascular sequestration of circulating parasitized erythrocytes in the cerebral microcirculation (17), **Figure 1**.

Several processes, other than sequestration, are also implicated in the pathogenesis. These include microvascular obstruction by the sequestered erythrocytes, an excessive proinflammatory cytokine response, excitotoxic release, endothelial dysfunction, and dysregulation. The extent of the contribution of the specific mechanisms to disease remain to be elucidated, **Figure 2**.

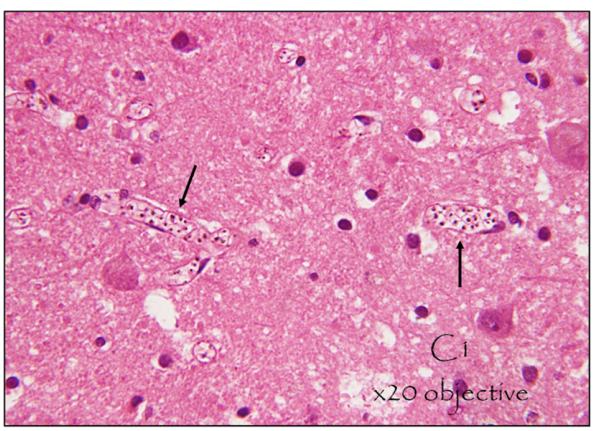

FIGURE 1 | Sequestration of malaria parasites in cerebral micro vessels. This is a hematoxylin and eosin (H&E) stained section of the brain of a middle-aged male who died from cerebral malaria following 5 days of fever, vomiting, and difficulty in breathing, shock, and coma. Appreciate the parasites in the erythrocytes within the blood vessels (seen as black dots) at x20. Photo Courtesy of Dr. Robert Lukande, Makerere University.

Sequestration is due to cytoadherence of infected erythrocytes to the vascular endothelial cells *via* parasite derived proteins on the surfaces of the infected erythrocytes e.g., the *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1). These attach to ligands upregulated on the lining of the microcirculation. The sequestered mass is further increased when adherent cells bind other infected erythrocytes (autoagglutination) or non-infected erythrocytes (rosetting) or use platelets to bind other infected erythrocytes (platelet-mediated clumping). Encoded by up to 60 variant genes, PfEMP1 binds to several host receptors including CD36 and the intercellular adhesion molecule 1 (ICAM1) and binding of infected erythrocytes to ICAM1 is implicated in the pathogenesis of cerebral malaria. Indeed, postmortem studies have demonstrated the upregulation of ICAM1 expression on the cerebral vascular endothelium in cerebral malaria (18).

Sequestration reduces microvascular flow. The presence of parasites inside the erythrocytes further decreases erythrocyte deformability so that erythrocytes have increased difficulty in passing through the cerebral microvasculature (19). Hypoxia and reduced tissue perfusion have therefore been considered important pathophysiological mechanisms. However, significant neuron death is unlikely because with specific antimalarial treatment, coma, especially in children, is rapidly reversible. Despite this, in the presence of increased metabolic demand such as during seizures, the risk of neural injury is higher and may be worse if the patient is hypoglycemic or if blood flow is further compromised by intracranial hypertension [reviewed in (18)].

Cerebral vascular dysfunction is now considered a major process in the pathogenesis of cerebral malaria and a target for the development of adjuvant therapies. Even though the parasites remain largely intravascular, especially in children, they cause some disruption of the BBB function. There is a redistribution of tight junction proteins occludin, vinculin, and zonula occludens 1 (ZO-1), that are central to BBB integrity (20). On immunohistochemistry, BBB impairment is seen in areas of the infected erythrocytes, where they are associated with focal loss of endothelial intercellular junctions (21). Cerebrovascular endothelial cell activation, defined by increased ICAM1 staining and reduction in cell-junction staining, and disruption of junction proteins, particularly in vessels containing infected erythrocytes is observed (20) but such disruption has not been associated with significant leakage of plasma proteins into perivascular areas or in to the cerebrospinal fluid (21). Low levels of nitric oxide bioavailability, high levels of endothelin-1 and dysfunction of the angiopoietin-Tie2 axis are critical (22). In African children, the dysfunctional endothelial function is associated with brain swelling (23, 24) but increased cerebral volume is thought to be the main cause of intracranial hypertension (25).

Activation of the microvascular endothelium is associated with the release of endothelial microparticles. The concentration of microparticles in peripheral blood is a good correlate of the degree of endothelial activation in deep tissues (26). Most proteins associated with the microparticles in cerebral malaria

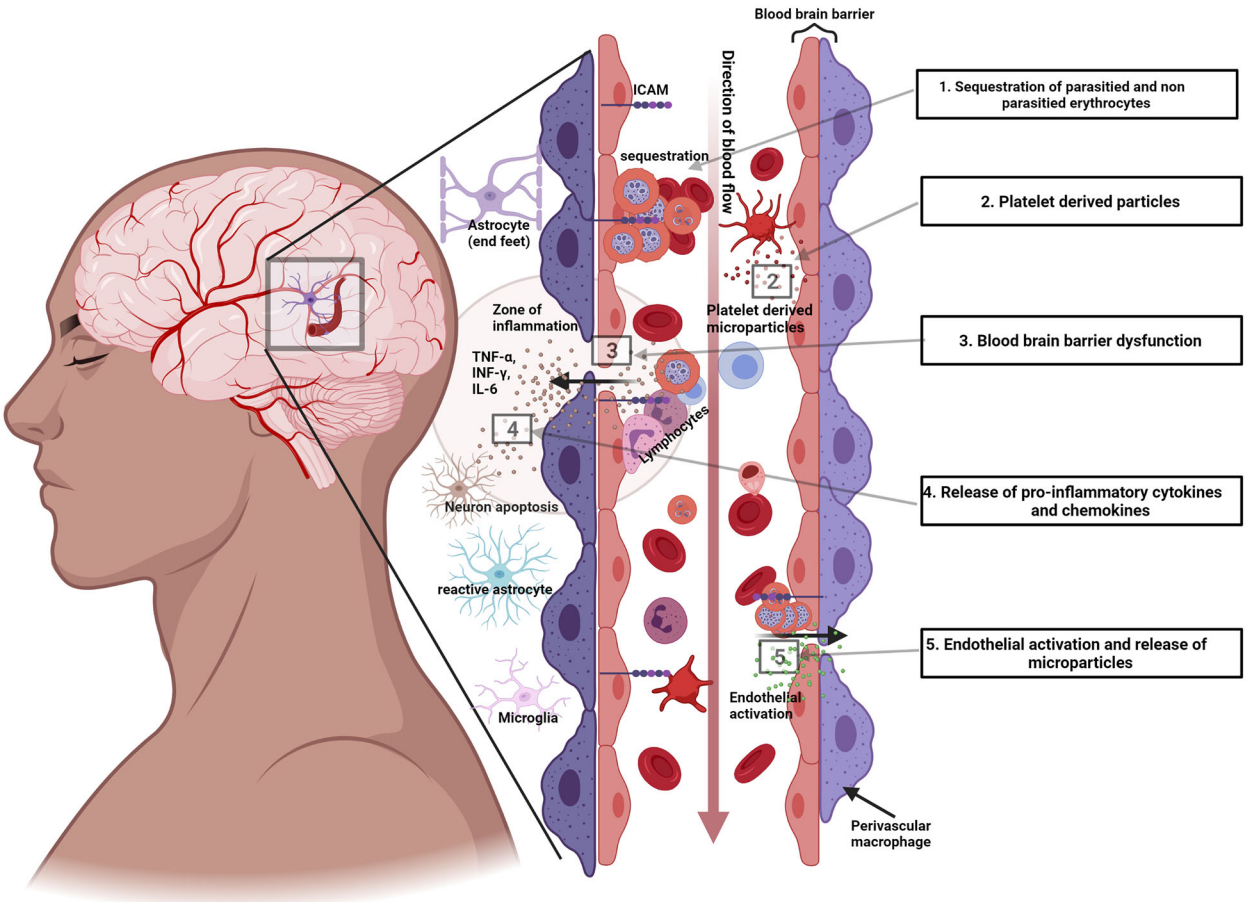

FIGURE 2 | Illustration of some of the pathogenic mechanisms in cerebral malaria. 1) Sequestration of P. falciparum parasitized and non-parasitized erythrocytes. 2) Platelet derived microparticles released into the circulation. 3) Dysfunction of the blood brain barrier. 4) Release of proinflammatory cytokines and chemokines and cytokine and chemokine induced injury. 5) Endothelial activation.

are involved in localization processes and in response to stimuli, with the immune and inflammatory responses (27). The angiopoietins are important in maintaining the integrity of the endothelial lining. Dysregulation of angiopoietin-1 (ANG-1) plays a mechanistic role in the pathogenesis of cerebral malaria while plasma levels of especially ANG-2, positively correlates with disease severity. The ratio of ANG-2/ANG-1 predict fatal cerebral malaria (28).

In the brain, malarial parasites stimulate the production of proinflammatory cytokines which activate the endothelial cells prompting them to produce CXCL10, a chemoattractant for leukocytes. The accumulated platelets in the microvasculature also release CXCL4 from their alpha granules which in turn stimulate the production of tumor necrosis factor (TNF-α) by the local mononuclear leukocytes. Other proinflammatory cytokines, such as lymphotoxin-α (LTα), interferon-γ (IFN-γ), interleukin-1α (IL-1α), and IL-1β, are also upregulated [reviewed in (29)]. All these contribute to the heightened hyper-inflammatory state. *In vitro*, TNF-α induces the release of pro-coagulant and pro-adhesive microparticles from cultured endothelial cells and upregulates ICAM1 expression on endothelial surfaces which further induces the sequestration of parasitized erythrocytes in the cerebral microvasculature. However, although high plasma and CSF levels of TNF-α are associated with poorer outcomes, inhibition of TNF-α by anti-TNF antibodies did not improve outcome (30) but synthetic oleanane triterpenoids reduced plasma levels of IL-10, TNF-α, and IFN-γ (31), thereby enhancing the integrity of the brain blood barrier in experimental cerebral malaria.

Animal studies suggest that Toll-like receptors (TLR) may be involved in promoting cerebral malaria immunopathology; loss of TLR7 conferred partial protection against fatal disease, and loss of TLR signalling dysregulated the cytokine profile towards those with more anti-inflammatory properties (32). Genetics too, may play a role; alternative alleles in one gene may either favor or counteract the development of severe disease (e.g., HMOX1), and different genetic variants within a gene promoter are associated with different severe malaria syndromes (e.g., TNF) suggesting differential gene regulation in context of different inflammatory milieus [reviewed in (33)].

Recent findings suggest a role of CD8+ T cells (killer T cells) in BBB and BCSFB dysfunction (34, 35). In both mouse and human cerebral malaria, it has been noted that CD8+ T cells accumulate within brain vasculature particularly within the cortex compared to other regions (34, 36). Using mouse models particularly, the involvement of CD8+T cells in cerebral malaria pathology has been demonstrated. However, their exact role remains unclear (36). The prevailing hypothesis is CD8+ T cells interact with endothelial cells or epithelial cells *via* the T cell receptor and MHC class I, leading to tight junction disruption in both the BBB and BCSFB (35).

In addition, in both human and mouse cerebral malaria, oxidative stress (reactive oxygen species) is detected in the brain. Hemoglobin degradation by the malaria parasite produces the redox-active by-products, free heme, and H_2O_2, conferring oxidative insult (37, 38).

In summary, five sequential and complex inflammatory process to malarial infection contribute to the development of cerebral malaria. First, the two parasite replication phases, the hepatic and erythrocytic stages, lead to two distinct innate responses that modulate subsequent parasite and host cell interactions. These two are followed by sequestration induced endothelial activation and enhanced chemokine secretion, leukocyte recruitment and eventually permeabilization of the endothelial barrier (39), **Figure 2**.

b. Consequences of this interplay between the parasites and the immune system

The interplay between the parasites and the immune system is manifest in the severity of disease with severe metabolic derangement, deep coma, brain swelling and repeated seizures or status epilepticus. Parenteral artemisinins, artesunate in particular, is the first line specific treatment. Quinine is the alternative first line drug. A range of supportive treatments, including fluid therapy, glucose, blood transfusion, and anticonvulsants are needed to correct the deranged metabolic state and shock, correct anemia, and terminate status epilepticus. Despite treatment, death occurs in up to 20% of children and an even higher proportion of adults. In the long term, 25% of child survivors have long term neurological, cognitive and behavior disorders (16).

Value of Neuroimmune Changes in Diagnostics and Therapeutics

In recent days, several investigative tools and biomarkers have become available for diagnosis and research, in helping to understand pathogenesis and examine emerging therapeutic approaches. The investigative imaging techniques include *in vivo* bioluminescent imaging, a versatile and sensitive tool that is based on the detection of light emission from cells or tissues, real-time *in vivo* imaging, F-fluorodeoxyglucose (FDG) positron emission tomography (PET) and intra-vital microscopy, a recently developed, advanced imaging tool that allows the direct and live visualization of the brain *via* a cranial opening [reviewed in (40)]. The technique can reveal cellular responses over time and space during the course of experimental cerebral malaria (41).

Several diagnostic tools have emerged around the concept of malaria retinopathy. Due to sequestration of the parasitized cells and the vascular changes associated with blood flow obstruction, the retinal microvasculature shows changes comparable to those occurring in the brain, making them an easily observable surrogate marker to assess pathology in cerebral malaria (42, 43). These include optical coherence tomography (an *in vivo* technique that allows optical-signal acquisition by which high-resolution cross-sectional images of the retina), optic nerve-head and the retinal nerve fiber layer are analyzed (44). Others are Teleophthalmology, Fluorescein retinal angiography and the micro-electroencephalogram.

As for biomarkers, three sets – a) screening and diagnostic markers that aid early diagnosis, b) prognostic biomarkers and c) those with potential for future research have emerged [reviewed in (40)].

The data on the coagulation-inflammation interface in cerebral malaria suggest these may be potential therapeutic targets for African children with cerebral malaria. They may include reducing thrombin generation with specific thrombin or prothrombinase antagonists, and augmenting the protein C pathway (45). Also, matrix metalloproteinases, a family of proteolytic enzymes involved in modulating inflammatory responses, disrupting tight junctions, and degrading sub-endothelial basal lamina, represent potential innovative drug targets (46). However, the multifaceted pathogenic mechanisms and absence of therapeutics against the inflammatory responses to date still account for the failure to reduce morbidity and mortality.

Knowledge Gaps

A lot remains to be learnt on the pathogenesis of cerebral malaria. It should be noted that although mice models have been used to study the pathogenesis, the pathology in mice is different; infected erythrocytes do not commonly sequester; instead, monocytes occur in cerebral vessels, and inflammatory cytokines are essential for the pathogenesis.

Despite emerging information about specific parasite subtypes, the intravascular processes leading to cerebral malaria remain to be determined. It has been the understanding that the malaria parasite was not able to penetrate actual brain tissue, but emerging information suggest that malaria parasites can do just that and a recent study mapped the mechanisms they utilize (47). This discovery points to parasites in the brain endothelium as a contributing factor to the pathology of human cerebral malaria. This new line of study urgently needs to be expanded.

The role of both the parasite and host genetics in the development and presentation of disease is poorly understood. For example, in mice models, absence of ApoE, a dominant apolipoprotein in the brain that has been implicated in several neurological disorders, was associated with decreased sequestration of parasites and T cells in the brain (48). Do similar alleles play such roles in humans?

Lastly, other than specific treatment, to date, most adjuvant intervention studies have been disappointing. New approaches are urgently required.

NEUROIMMUNOLOGY OF HUMAN AFRICAN TRYPANOSOMIASIS

Introduction

Pathogen Description

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a disease endemic to Sub Saharan Africa (SSA) caused by two subspecies of a microscopic flagellate protozoan parasite *Trypanosoma brucei (T. b.)*, which are *T. b. gambiense* and *T. b. rhodesiense*. *Trypanosoma brucei* is a unicellular extracellular parasite found in blood or other body fluids of the host such as the lymph and cerebrospinal fluid (CSF) (49). The parasites are transmitted by infected tsetse flies (*Glossina* sp.*)* while feeding on blood. These tsetse flies are found only in the SSA region; thus, transmission can only occur in this region. *T. b. gambiense*, which currently accounts for 98% of HAT (50), is found in large areas of central and western Africa and is considered an anthroponotic disease (51). On the other hand, *T. b. rhodesiense*, which accounts for about 2% of the disease, has limited distribution in eastern and southern Africa is a zoonotic disease, infecting mainly wild animals and livestock (50, 51). Another subspecies *T. b. brucei* is not human pathogenic and thus used extensively in research using animal models of HAT. The three subspecies are morphologically indistinguishable.

Signs and Symptoms

HAT is divided into two clinical stages: an early hemolymphatic phase, also referred as stage 1, and a late meningo-encephalitic phase, also referred to as stage 2. In stage 1 some patients develop a chancre at the bite site of inoculation of the parasite followed by involvement of blood and lymphatic systems with general symptoms of infection including chronic intermittent fever, headache, asthenia, lymphadenopathy, and pruritus. In stage 2 patients have more neurological symptoms such as sleep disorders (described in more detail in the article by Ngarka et al. in this collection), confusion, tremor, general motor disturbances, sensory disturbances, abnormal movements, and speech disorders as well as psychiatric symptoms (52–54). *T. b. gambiense* HAT is more chronic lasting months to several years between infection and death, whereas *T. b. rhodesiense* HAT is more acute lasting several weeks to months, such that in the latter the demarcation between the early and late stages of the disease are less clear (51, 55–57). If untreated the disease leads, in most patients, to cachexia, opportunistic infections, coma and eventually death (53).

Diagnosis

Clinical presentation is non-specific, thus, diagnosis of HAT is confirmed by finding trypomastigotes in blood, lymph (early stage) or CSF (late stage) using microscopy. Serological tests (card agglutination test for trypanosomiasis, CATT) are available for screening for *T. b. gambiense*, whereas there are no serological tests for *T. b. rhodesiense*. The WHO criteria for CNS involvement include the presence of CNS symptoms and finding parasites in the CSF or a WBC count of >5/μl (53, 58). However, some countries use a CSF WBC count of > 20/μl (59, 60). Thus, there is a grey zone where it is not clear what finding WBC counts of >5 & <20/μl mean (54, 61). This has led to a search of biomarkers to better stage the disease, some of which are discussed below.

Treatment

The drugs used for the treatment of HAT have for a long time been divided into drugs for early stage, suramin for *T. b. rhodesiense* and pentamidine for *T. b. gambiense*, and drugs for late stage, melarsoprol for *T. b. rhodesiense*, eflornithine and the nifurtimox-eflornithine combination (NECT) treatment for *T. b. gambiense* (57). These are all administered intravenously except for nifurtimox, which is given orally as part of NECT. However, a new orally administered drug fexinidazole, was recently introduced to treat both early and late stages of *T. b. gambiense* HAT (50, 62, 63). Melarsoprol and NECT penetrate the BBB better and thus they are more effective, however, they are more toxic and have more complex dose regimens than suramin and pentamidine. Acoziborole, another drug administered orally as a single dose is under clinical trials for *T. b. gambiense* HAT with promising results (62).

Prevalence

The number of incident cases of HAT fell to below 1000 in 2018; thus, the WHO aim of elimination of HAT i.e., less than 2,000 reported *T. b. gambiense* HAT cases by 2020 has been met (50, 63, 64). This has been because of a concerted effort on surveillance, medical treatment, and interruption of transmission by the WHO, local governments, many NGOs and public-private partnerships such as that with Sanofi-Aventis and Bayer, the latter donated the necessary drugs to treat HAT (63, 64). However, still there are about 70 million people at some risk of HAT in SSA countries (65). There is a need for continuous surveillance and control programs because there can be a resurgence of HAT. HAT was well controlled during the 1960s but when surveillance was reduced because of disturbances due to wars as well as reduced resources to control HAT the cases went up reaching a peak in the 1990s till interventions were brought about to control it (51, 57, 63, 66).

Trypanosoma brucei spp., Immune System and Neuropathogenesis

Several recent reviews have given a more extensive description on the neuropathogenesis of HAT (54, 67–69). This section will focus on the interplay between the parasites and the immune system in the CNS. Stage 2 of HAT is characterized by CNS involvement in the symptomatology of the disease and neuroinflammation. Trypanosomes have been difficult to find in *post-mortem* studies of brains of HAT patients, possibly because of autolysis, lack of proper antibodies for staining the parasites, or clearance due to drug treatment (54, 70, 71). Trypanosomes were observed in the brain parenchyma during autopsy of a *T. b. rhodesiense* HAT patient who had an acute disease and died before treatment (72). Neuroinflammation is a characteristic feature observed in the brain during *post-mortem* of HAT patients. Perivascular and white matter infiltration by inflammatory cells, predominantly mononuclear lymphocytes,

has been described (54, 68, 70, 73). Morular-shaped plasma cells loaded with immunoglobulins (Mott's cells) are also found in the brain (54, 68). The leukoencephalitis caused by infiltrating cells is also accompanied by microglia and astrocyte activation (68). There are also changes in the monoaminergic neurotransmitters, dopamine, serotonin, and norepinephrine, in the brain during trypanosomiasis, which might contribute to the neuropsychiatric abnormalities observed in HAT (74, 75).

How the Parasites Get to the CNS

Information about how the parasites enter the brain and neuroimmunological changes that occur has been obtained from experiments done principally with rodent models of HAT (54, 66, 68). In rats and mice models of HAT infected with *T. b. brucei*, parasites invade the choroid plexus and circumventricular organs such as the area postrema, pineal gland, and median eminence (76), that lack a BBB, at early stages of the infection. At later stages post-infection, parasites penetrate the BBB and invade the brain parenchyma mainly in the white matter and the septal nuclei than the cerebral cortex, while the tight junction proteins are preserved (77). Double immunohistochemical labeling of parasites and brain endothelial cells (using antibodies against glucose transporter-1 (GLUT-1) in the brains of mice or rats infected with *T. b. brucei* was used to visualize the location of parasites, either inside blood vessels or in the brain parenchyma (77–81).

A study utilizing two different mice strains, C57BL/6 and SV-129/Ev mice, showed that host genetic differences in the expression of immune molecules determine parasite invasion of the CNS (80). C57BL/6 mice infected with *T. b. brucei* had less parasitemia but more T cells and parasites in the brain parenchyma than SV-129/Ev mice. The C57BL/6 mice also had higher IgM in the serum and higher proinflammatory cytokines and adhesion molecules in the brain than SV-129/Ev mice (80).

A series of studies using immunodeficient mice or mice deficient of various cytokines, chemokines, other inflammatory molecules and their signaling molecules elucidated the role of the immune system in the passage of the parasites and T cells across the BBB into the brain parenchyma [see **Table 1** and described in detail in (54)]. In summary, during infection immune cells are activated in a TLR-MyD88 dependent manner and produce cytokines such as TNF-α and IFN-α/β, which are important for control of parasitemia but also possibly for initiation of T cell and parasite invasion of the brain, and for control of parasites in the brain parenchyma (54, 79). TNF-α induces the expression of adhesion molecules, while IFN-α/β-induces limited expression of C-X-C motif chemokine ligand 10 (CXCL10), which facilitates T cell and parasite invasion of the CNS (78, 79). The parasites require T cells and IFN-γ to cross the BBB (81). IFN-γ induces CXCL10 which attracts and/or retains T cells and the parasites in the brain parenchyma (78). IFN-γ possibly induces matrix metalloproteinase-9 (MMP-9) to facilitate T cells and parasites crossing of the parenchymal basement membrane (82). On the other hand, nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) is important for maintaining the integrity of the BBB and prevent unlimited T cell and parasite invasion of the brain (82).

Neuroinflammation: Interplay Between the Parasites and the Immune System in the CNS

Invasion of the CNS is dependent on T cells and accompanied by T cell infiltration of the parenchyma (81). This elicits an inflammatory response in the brain with activation of microglia and astrocytes, which produce cytokine, chemokines, and NO (78, 82, 85–89). Activated astrocytes increase the expression of the chemokine CXCL10, which is important for the recruitment and retention of T cells and parasites (79). There is an increased production of other chemokines such as chemokine (C-C motif) ligand 2 (CCL2), CCL5, CXCL9, CXCL13 (78, 90, 91). There is also a robust upregulation of inflammatory cytokines such as IL-1β, IL-6, IFN-γ, TNF-α (81, 86, 89, 90),. Other inflammatory molecules such as iNOS are also upregulated (82, 92, 93). Although, the chronic inflammation is detrimental to the brain it is also important for suppressing parasite numbers and maintaining the integrity of the BBB. TLR-MyD88 dependent signaling is important for parasite control (79) and NO derived from iNOS is important for maintaining BBB integrity and limiting the invasion of the brain parenchyma by parasites and T cells (82). Some cytokines such as IL-6 and IL-10 have also been shown to reduce systemic IFN-γ and TNF-α, reduce number of trypanosomes in the CNS and to protect against the neuroinflammatory pathology that occur during infection (94) (**Figure 3**).

Consequences of This Interplay Between the Parasites and the Immune System

Microglia activation is concomitant with onset of sleep disorders in mice (85). Microglia and astrocytes together with lymphocytes could cause disturbances through increased expression of cytokines. Cytokines such as IFN-γ and TNF-α have been proposed to contribute to some of the neurological disturbances observed in HAT such as hyperalgesia, sleep disturbances and alteration in circadian rhythm, covered in detail in previous reviews (54, 66).

Value of Neuroimmune Changes in Diagnostics and Therapeutics

There has been great interest in evaluating the cytokine and chemokines upregulated in the CNS during HAT and in animal models as biomarkers for disease staging and monitoring therapeutic outcomes (54, 68, 95). CXCL10 was considered as a candidate marker for late-stage HAT (78), and the sensitivity was increased by combining it with H-FABP and CXCL8 (96), CXCL13 and MMP-9, or CXCL13 and IgM (97). Other immune and inflammation related molecules that have also been evaluated as biomarkers for staging HAT include IgM (98, 99), IL-10 (98–100), CXCL13 (101, 102), MMP9 and ICAM-1 (103).

The new oral drug, fexinidazole, is now available for the treatment of *T. b. gambiense* HAT. However, the treatment of second stage *T. b. rhodesiense* HAT is still reliant on the arsenic compound melarsoprol, which is highly toxic, producing post-treatment reactive encephalopathy (PTRE) in about 10% of the patients, which can be fatal in up to 50% of these cases (104, 105). PTRE exacerbates the neuroinflammation that already exists in HAT such as astrogliosis and the increased presence of immune cells such

TABLE 1 | Immune cells, molecules and their signaling molecules involved in *Trypanosoma brucei brucei* neuroinvasion.

Immune cells or molecules	Immune cells and trypanosome levels in the brain parenchyma of transgenic mice compared to WT mice	Proposed role	Ref.
	Immune cells		
B and T cells	*Rag1*$^{-/-}$ mice, which lack mature T and B cells, had less trypanosomes in the brain parenchyma compared with WT mice. Trypanosomes accumulated in the perivascular compartment, confined between the endothelial and the parenchymal basement membranes, in certain areas of the brains of the transgenic mice	Facilitate parasite crossing of the BBB into the brain parenchyma. Necessary to produce IFN-γ during infection.	(81)
	Chemokines and their receptors		
CXCL10	*Cxcl10*$^{-/-}$ and *Cxcr3*$^{-/-}$ mice had less T cells and trypanosomes in the brain parenchyma compared with WT mice.	Chemoattraction, recruitment or retention of T cells and trypanosomes in the brain parenchyma	(78)
	Cytokines and their receptors		
IFNα/β	*Ifn-α/βr* $^{-/-}$ mice had less T cells and slightly less trypanosomes in the brain parenchyma compared with WT mice.	Facilitate sensitized T cells and a few parasites crossing of the BBB (more of initiation of the process) by inducing a limited release of CXCL10 from astrocytes and endothelial cells	(79)
IFN-γ	*Ifn-γ* $^{-/-}$ and *Ifn-γr* $^{-/-}$ had less T cells and trypanosomes in the brain parenchyma compared with WT mice. Trypanosomes accumulated in the perivascular compartment, confined between the endothelial and the parenchymal basement membranes, in certain areas of the brains of both transgenic mice.	Facilitate T cell and parasite crossing of the BBB in part by inducing the expression of CXCL10. Other mechanisms remain to be elucidated	(81)
IL-12	*Il-12p40*$^{-/-}$ mice had less T cells and trypanosomes in the brain parenchyma compared with WT mice. There was sporadic clustering of trypanosomes around vessels.	Facilitate T cell and parasite crossing of the BBB by inducing the expression of IFN-γ	(81)
TNF-α	*Tnfr1*$^{-/-}$ mice had less T cells and trypanosomes in the brain parenchyma compared with WT mice.	Facilitate T cell and parasite crossing of the BBB by increasing expression of adhesion molecules i.e., ICAM-1	(79)
	Toll-like receptors		
TLR2 and TLR9	*Tlr2-/-* mice had similar T cells but more trypanosomes in the brain parenchyma of the corpus callosum compared with WT mice. *Tlr9-/-* mice had less T cells in the brain parenchyma compared with WT mice. However, they had more trypanosomes in the brain parenchyma of the corpus callosum and less in the septum. *Tlr2/9-/-* mice had less T cells but more trypanosomes in the brain parenchyma compared with WT mice	T cells cross the BBB after they are activated in secondary lymphoid organs in a TLR-dependent manner and might pave way for trypanosomes. However, TLR dependent signaling is essential for parasite control in the brain	(79)
	Intracellular signaling mediators		
MyD88	*Myd88-/-* had less T cells but more trypanosomes in the brain parenchyma compared with WT mice	T cells cross the BBB after they are activated in secondary lymphoid organs in a MyD88-dependent manner and might pave way for trypanosomes. However, MYD88 dependent signaling is essential for parasite control in the brain	(79)
	Nitric oxide		
iNOS	*Inos-/-* mice had more T cells and trypanosomes in the brain parenchyma compared with WT mice	iNOS-generated NO by perivascular macrophages prevents unlimited invasion of the brain parenchyma by T cells and parasite by maintaining the integrity of the BBB	(82)

BBB, blood-brain barrier; CXCL, C-X-C motif chemokine ligand; IFN, Interferon; IL, Interleukin; iNOS, inducible nitric oxide synthase; MyD88, Myeloid differentiation primary response 88; TLR, Toll-like receptor; TNF, Tumor necrosis factor; RAG-1, recombinant activating gene 1; WT, Wild type. Adapted from (83) and (84).

as lymphocytes, macrophages, and plasma cells in the brain white matter (70, 104). Minocycline was found to prevent *T. b. brucei*-induced microglia and astrocyte activation as well as the expression of inflammatory molecules in the brain (87). Immunomodulators such as minocycline warrant to be evaluated for the prevention of PTRE when given in combination with melarsoprol.

Knowledge Gaps

An intermediate stage of HAT, between stage 1 and stage 2 has been suggested (106) and a recent study using CXCL13 as one of the biomarkers supports its existence (101). More studies are needed to characterize this stage, which sometimes respond to stage 1 drugs, in terms of presence or absence of parasites in the brain parenchyma, other possible biomarkers and appropriate treatment regimens.

IFN-γ-induced CXCL10, which is important for T cells and parasites chemoattraction and retention in the brain parenchyma (78), has come out as a strong candidate biomarker for staging HAT. IFN-γ induces other molecules, to facilitate T cells and parasite crossing of the BBB, whose nature is yet to be determined. Finding these molecules induced by IFN-γ could be important both from the pathophysiological point but also to find possible biomarkers for staging HAT.

In conclusion, the immune system plays a role in the role in the neuropathogenesis of HAT and side effects of melarsoprol. Immune related molecules such as the chemokines CXCL10 and CXCL13 are coming out as useful biomarkers for staging HAT. Targeting neuroinflammation with immunomodulators such as minocycline warrant further studies to reduce the incidence and mortality of melarsoprol-induced PTRE.

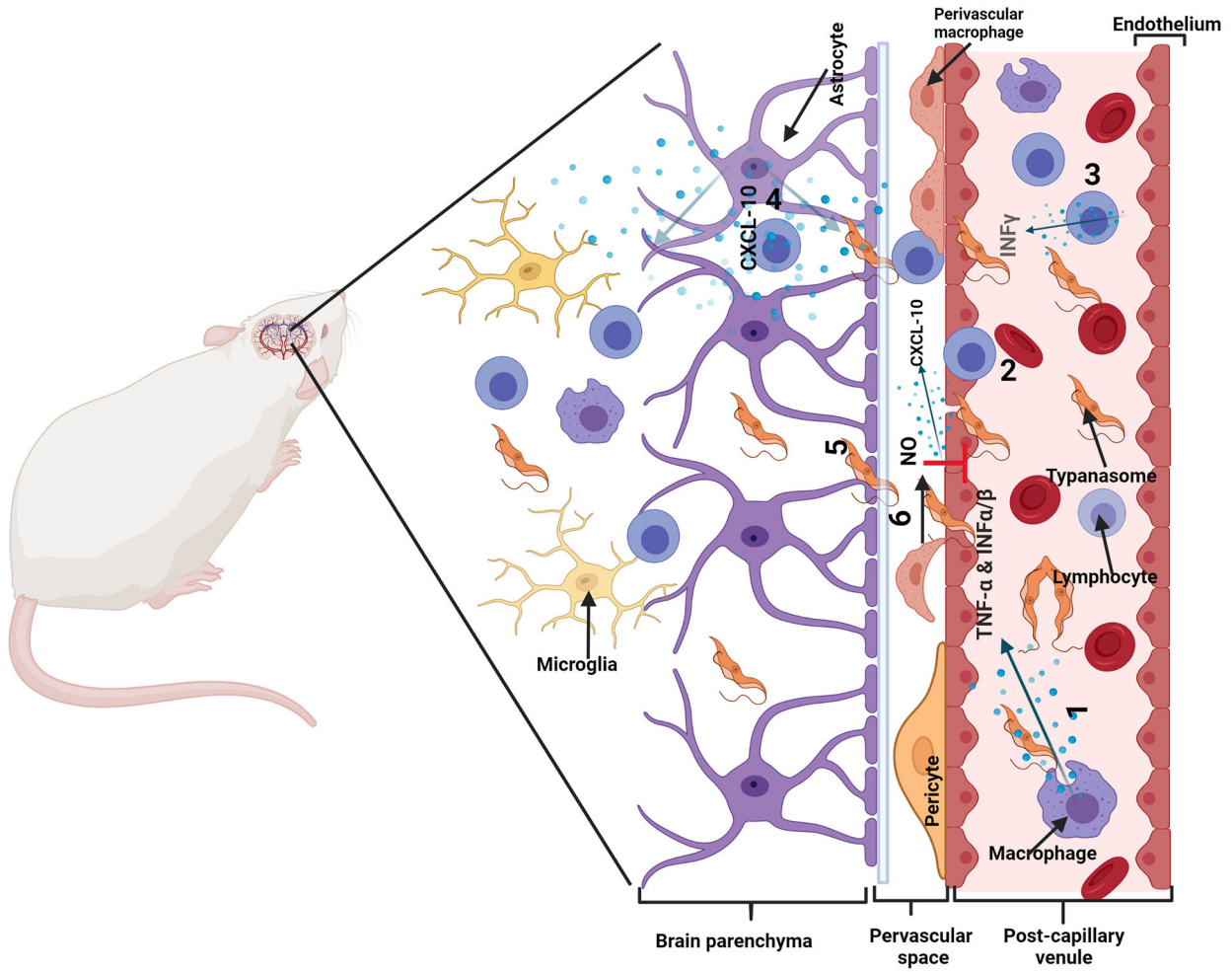

FIGURE 3 | Illustration of neuro-pathogenic mechanisms of trypanosomes: Trypanosoma brucei spp. and T cells cross the blood-brain barrier (BBB), in a multistep manner to invade the brain parenchyma. In infected mice, activated immune cells and astrocytes release molecules that cause T cell and trypanosomes to cross the barriers of endothelial cells linked by tight junctions, the endothelial basement membrane (BM) and the parenchymal BM into the brain parenchyma. 1. Immune cells are activated in a TLR-MyD88 dependent manner and produce cytokines such as TNF-α and IFN-α/β. 2. TNF-α induces the expression of adhesion molecules such as ICAM/VCAM on cerebral endothelial cells which allow attachment of T cells. IFN-α/β induces a limited release of CXCL10 by endothelial cells and/or astrocytes, which is enough for penetration of T cells accompanied by some trypanosomes into the perivascular space. 3. Trypanosome-derived antigens taken up and expressed by macrophages are recognized by sensitized T cells to induce IFN-γ, which amplifies the process through 4. induction of CXCL10 production by astrocytes and 5. molecules that open the parenchymal basement membrane for spread of both T cells and trypanosomes into the brain parenchyma (In the absence of IFN-γ-inducible molecules, the parasites and T cells do not penetrate the parenchymal BM efficiently and they accumulate within the perivascular space as cuffs). 6. Nitric oxide (NO) generated in perivascular macrophages by iNOS can inhibit the effects of TNF-α and prevent unlimited immune cell and parasite invasion of the brain parenchyma. Modified from (54) with permission from Elsevier to reuse.

CEREBRAL TOXOPLASMOSIS IN AFRICA

General Features of *T. gondii* and Toxoplasmosis

Pathogen Description

The single-celled Apicomplexan *Toxoplasma gondii* has felines (cats) as it's definitive hosts (107). However, it was first described in the North African rodent *Ctenodactylus gundi* (108). In humans, ingested oocyst stages (originating from cat feces) or tissue cysts (from meat containing the bradyzoite stage) invade the intestinal epithelium and transform into rapidly replicating tachyzoites. Tachyzoites are obligate intracellular for replication and disseminate rapidly in the organism before differentiating back into bradyzoites, which form persistent intracellular cysts. As chronic infection sets in, tissue cysts form preferentially in the brain and retina of intermediate hosts, for example in humans, rodents and in animals used for meat consumption. At the chronic stage of infection, the immune system plays a critical role in controlling parasite loads but can also contribute to detrimental inflammation (109).

Prevalence of Carriage of *T. gondii* in Africa

Parasite population structure studies have shown that a few major clonal lineages of *T. gondii* predominate worldwide (110). Consistent with this, lineages shared with other global geographical areas are present in Africa and unique African haplogroups (111–113). Studies of seroprevalence indicate that carriage of *T. gondii* is common in the African population (114, 115), with recent reports indicating that seroprevalences vary broadly depending on region and age and range on average between 30-70% (116–121). The seroprevalence levels in humans go together with the reported prevalence in animals used for meat consumption (113, 122).

Clinical Symptoms and Manifestations

In healthy individuals, primary *T. gondii* infection is normally asymptomatic or accompanied by mild flu-like symptomatology, such as fever, malaise and swollen lymph nodes (123). In contrast, upon primary infection in pregnant women, the parasite can transmit across the placenta and cause neurological damage or even be fatal to the developing fetus (124). In conditions associated with immunodeficiency, such as HIV/AIDS or immune-suppressive therapies, reactivation of tissue cysts in the brain can cause life-threatening toxoplasmic encephalitis (TE). In contrast, ocular toxoplasmosis (OT) can manifest as retinochoroiditis in otherwise healthy immune-competent individuals. Following congenital transmission, OT can relapse repeatedly many years later in life (124).

Pathophysiology of Toxoplasmosis

Invasion to the Brain Parenchyma

Strictly, how *T. gondii* enters the human brain is largely unknown and the current paradigms are therefore based on extrapolations from infections in rodents (109). Overall, *T. gondii* infection has a clear predilection for the CNS, including congenital infection, which is likely linked to the fact that cysts chronically persist in the CNS while they are cleared from peripheral organs over time. Thus, *T. gondii* consistently and silently manages to establish chronic infection in the CNS.

To date, molecular mechanisms that define tropism of *T. gondii* for the CNS over other organs have not been identified. Precisely how *T. gondii* gains access to neurons in the brain parenchyma and the mechanisms for chronic persistence within neurons remain enigmatic (125). Following invasion across the intestinal wall and systemic dissemination in the blood, *T. gondii* likely crosses the parenchymal vasculature of the blood-brain barrier (BBB) by different routes (126). A recent study in mice showed that passage occurs preferentially across cortical capillaries, while invasion across post-capillary venules, arterioles, the choroid plexus and meningeal vessels is less frequent (127). Paracellular entry across endothelium implies passing cellular tight junctions (128–131) while transcellular entry may occur after apical parasite invasion of the endothelium and basolateral exit after replication (132–134). Additionally, infected leukocytes, such as parasitized dendritic cells, may mediate transportation into the parenchyma (135–137).

Interplay Between the Parasite and the Immune System in the CNS

The crucial roles that immune surveillance plays in the manifestations of toxoplasmosis is best illustrated by the fact that AIDS patients with low $CD4^+$ T cell count (< 200/μl) and seropositive for *T. gondii* are at risk of developing a reactivated TE due to the loss of T cell-mediated control of brain cysts (124). Similarly, individuals receiving immunosuppressive treatments, for example after organ transplants, are at risk of developing a reactivated toxoplasmosis. Thus, immune-mediated control is closely linked to the pathogenesis for this opportunistic infection.

Control of the Infection by Immune Responses and Immunopathology

Overall, the systemic response to *T. gondii* is characterized by a strong Th1-type immune response that is dominated by production of proinflammatory mediators such as IL-12, IFN-γ, TNF and NO.

As the parasite colonizes the CNS, inflammatory leukocytes are recruited (138). In rodent infections, the inflammatory infiltration is constituted by $CD4^+$/$CD8^+$ T cells, $Ly6C^{high}$ inflammatory monocytes, $F4/80^+$ macrophages and $CD11c^+$ DCs. T cells have a protective effect by secretion of cytokines, principally IFN-γ and TNF. In contrast to responses in mice (8), the initial steps for innate immune sensing in humans remain uncharacterized. However, a role has been recently identified for alarmin S100A11 which is released by *T. gondii*-infected cells and sensed by human monocytes (139). Further, IFN-γ-induced 2, 3 indoleamine dioxygenase (IDO) contributes to parasite control by human astrocytes (140). By counteracting the effects of proinflammatory cytokines, immune-suppressive cytokines likely also play a role in dampening immunopathology. In rodent neuro-toxoplasmosis, monocytic cells, microglia and B/T cells produce IL-10 (141–143). In acute and reactivated human OT, a disbalance between regulatory and proinflammatory T cell populations may account for the immunopathology (144).

Control of Infection by Brain Parenchymal Cells

Knowledge on immune mechanisms leading to the control of intracerebral *T. gondii* in humans is mainly based on extrapolations from reactivated toxoplasmosis in AIDS patients.

In addition to an abundant leukocyte infiltration in the brain parenchyma, astrogliosis and microglial nodules are not unusual findings upon human cerebral toxoplasmosis (145–147). This is indicative of the implication of astrocyte and microglia responses in human TE.

Important roles in the control of TE have been attributed to astrocytes in rodents. These include pro- and anti-inflammatory responses to balance parasite control and intracerebral immune responses to limit neuroinflammation and prevent neuronal damage (148–152). Similarly, microglia exhibit activation by secreting cytokines upregulating MHC class I/II molecules (153–155). Microglia can also suppress the proliferation of parenchymal T cells, and thus may contribute to reducing immunopathology (156).

Finally, the roles of neurons, which are the cells primarily targeted by *T. gondii* and that harbor the tissue cysts (157),

remain unresolved in humans. *In vitro*, neurons respond with cytokine secretion (IL-6, TGF-β_1, CCL3 and CCL4) upon *T. gondii* challenge (156), however, their role in parasite control remains unclear *in vivo*. In mice, it was suggested that parasite cyst-harboring neurons may escape perforin-dependent elimination by $CD8^+$ T cells because neurons may remain MHC class I negative (158). More recent work showed that neuronal MHC I presentation was required for robust control of *T. gondii* in the CNS (159).

It has been proposed that an interplay between neuroinflammation and neurotransmission may underlie cognitive changes associated with chronic toxoplasmosis (160). Indeed, reported neurotransmission alterations during toxoplasmosis in rodents include dysregulations of catecholamines, GABA and glutamate (161–163).

These responses may implicate both neuronal and non-neuronal cells and await further investigations in humans.

Manifestations of Neurological Disease

While primary infection with *T. gondii* is asymptomatic or followed by mild symptoms, reactivation of chronically carried parasites is generally accompanied by neurological and ocular manifestations (123). In mice, Toxoplasma cysts can be sporadically localized in any anatomical cerebral area (164) and it may be assumed this is also the case for humans. Consequently, the clinical neurological manifestations will depend on the anatomical localization of the area of reactivation and on the parasite spread within the CNS. Individuals with severe immunosuppression are at a risk of developing TE and the encephalitic clinical presentation can range from lethargy to coma, incoordination to hemiparesis, memory loss to severe dementia, and focal motor to generalized seizures (165). Main risk groups include individuals with AIDS, but also individuals with organ transplants (166).

TE ranks among the most common neurological infections associated with AIDS. It has been estimated worldwide that 1/3 of AIDS patients seropositive for *T. gondii* and with low T cell count (< 200/µl) develop reactivated TE (124). While data is limited, African studies indicate that TE remains a major problem associated to AIDS, with variability among regions and countries (167–170). The strong association of TE with HIV/AIDS likely depends on failure to control CNS-resident parasites due to compromised antiparasitic T cell responses. Bradyzoite to tachyzoite conversion is accompanied by fast intracellular parasite replication that can result in necrotizing TE. Of note, *T. gondii* persists intracellularly in neurons, which can be MHC negative, and therefore likely not directly targeted by T cells in this respect.

Importantly, primary infection during pregnancy puts the developing fetus at risk of diverse neurological and ocular manifestations due to its immature immune system. Early infection during pregnancy can cause more severe neurological damage in the fetus and eventually abortion, whereas late infections generally cause less severe symptoms (171, 172). Fetal immune responses are largely uncharacterized, while transferred maternal *T. gondii*-specific antibodies likely contribute to protection.

Value of Neuroimmune Changes in Diagnostics and Therapies

Blood serologic tests are broadly used in Africa for general diagnostics (116–120). Further, detection of *T. gondii* DNA by PCR in the cerebrospinal fluid is of high diagnostic value for CNS manifestations (173). Although not broadly applied, radiological methods, for example computer tomography, can provide differential diagnosis with other CNS conditions such as lymphoma, mycobacterial and fungal infections (cryptococcosis). Typically, single, or multiple rim-enhancing lesions with oedema, often in basal ganglia and white and grey matter zones are observed (174). Histopathological examination demonstrating tachyzoites of *T. gondii* or tissue cysts is also of value.

Knowledge Gaps

Diagnostics and Risk Evaluation

The association between the different parasite genotypes and disease manifestation, especially cerebral or ocular disease and congenital transmission, remains unresolved (175). Given the contextuality of the clinical spectrum, the role of human genotypes needs also to be explored, especially in relation to immune surveillance and reactivation. Jointly, the identification of genetic risk factors for reactivated TE in AIDS or for congenital transmission could benefit risk groups and provide health care with tools for risk evaluation (173).

Therapies

Available treatments eliminate acute stage parasites (tachyzoites) (176). To date, drug resistance is not a considerable problem for *T. gondii* infection. Instead, a major problem is that chronic tissue cysts in the CNS are not eliminated by currently existing drug treatments. Therefore, a major advance would be the identification of druggable targets for the bradyzoite cysts (177) because it could potentially eliminate the severe and potentially lethal manifestations of reactivated disease in the CNS. Further, carriage of *T. gondii* has been linked to diverse neuropsychiatric conditions, for example schizophrenia (178), and antiparasitic therapies eliminating cysts may benefit these carriers.

Parasite Control and Vaccines

Strategies aiming at disrupting the parasite´s life cycle by preventing oocyst formation in felines or prevention of tissue cyst formation in intermediate hosts used for meat consumption would be of major benefit. To this end, a further understanding of the life cycle in felines and of immunity in intermediate hosts, including humans, is needed (179).

NEUROCYSTICERCOSIS IN AFRICA

General Features of *T. solium* and Neurocysticercosis

Pathogen Description

Taenia solium is more commonly known as the pig tapeworm. It is a cestode belonging to the class Cestoda along with other flat,

segmented, ribbon-shape worms. The larval stage of *T. solium* are fluid filled cysts with an invaginated scolex known as cysticerci, which typically infect pigs, the intermediate hosts of the parasite. The adult worm of *T. solium* is found in the small intestine of humans, the only known definitive host of the parasite. These worms can produce tens of thousands of oncospheres (eggs) per day, which are then excreted in feces where they contaminate food and water supplies. In the typical lifecycle, these oncospheres are ingested by pigs, where they are activated by digestive enzymes and bile salts. They then migrate through the intestinal wall into the blood supply. At blood vessel terminations within multiple different tissue types (e.g. muscle, subcutaneous or nervous tissue) they develop into vesicular larvae over the course of weeks to months (180). If insufficiently cooked pork meat containing a cysticercus is then ingested by a human, the scolex evaginates in the small intestine and attaches to the intestinal wall where it becomes an adult worm.

Infection of the human nervous system occurs when a human becomes an accidental intermediate host by ingesting oncospheres in contaminated food or water, often due to an adult tapeworm carrier in the household. The oncospheres are then activated in the human digestive tract just as they are in the pig, enabling them to pass into the blood stream and lodge in various tissue types including muscle, subcutaneous tissue, eyes and particularly the central nervous system. When cysticerci are present in the nervous system this is referred to as neurocysticercosis (NCC).

Prevalence of *T. solium* in Africa

T. solium is endemic to almost all sub-Saharan African countries with reports of *T. solium* taeniasis or cysticercosis having been made in at least 29 countries in Africa (181). Prevalence is thought to be minimal or non-existent in North African countries due to a combination of a dry climate, which doesn't favor pig rearing, together with religious and cultural practices which preclude the consumption of pork. The presence of *T. solium* in a region can be ascertained by observing pigs infected with cysticerci (porcine cysticercosis), humans infected with adult tapeworm (taeniasis), or humans infected with cysticerci (cysticercosis and neurocysticercosis). The latter are often hard to diagnose as adults with taeniasis and cysticercosis are typically asymptomatic, whilst neurocysticercosis requires expensive, largely unavailable neuroimaging (e.g., CT scans) for definitive diagnosis (182, 183). As a result, the condition is typically underdiagnosed. Nonetheless, there are regions where *T. solium* is hyperendemic. For example, in the Eastern Cape region of South Africa, approximately 55% of pigs have cysticercosis (184) and up to 10% of people may have taeniasis or cysticercosis (185, 186).

Clinical Symptoms and Manifestations

Seizures are the most common symptom of NCC accounting for between 70 and 90% of all symptomatic NCC cases (187). Other symptoms include headaches, intracranial hypertension, hydrocephalus and meningitis (188). As an indication of the prevalence of NCC in endemic areas, approximately 29% of people with epilepsy have NCC (183). It is estimated that between 20 and 50% of all adults, acquired epilepsy in endemic countries is due to NCC (183, 189, 190). As a result NCC is one of the leading causes of adult-acquired epilepsy globally (191) and one of the most common neurological disorders in Africa (183, 189). Interestingly, in people with NCC, seizures often take months to years to develop following infection. This has led to the intriguing observation that while larvae are alive and viable within the brain, infected individuals are typically asymptomatic (192).

Pathophysiology of Neurocysticercosis

Invasion to the Brain Parenchyma

In pigs, *Taenia solium* cysticerci are more commonly found in muscle tissue than in the brain (193). The opposite appears to be true in humans where cysticerci have a particular tissue tropism for the central nervous system. Why this is the case is not well understood. One possibility is that *Taenia solium* have not evolved to exist in humans as an intermediate host. Therefore, they require the relative immune privilege of the central nervous system to sufficiently evade the host immune response and maintain viability. Whilst *Taenia solium* cysticerci are found within the brain parenchyma, given their size, it is unlikely that the activated ova or cysticerci actively cross the blood brain barrier. Rather it is likely that the nascent cysticerci lodge in terminal arterioles or cerebral capillary beds where they then grow. Over time, and particularly following a host inflammatory response to the cysticerci, the blood brain barrier may break down (194).

Interplay Between the Parasite and the Immune System in the CNS

Following initial infection of the brain parenchyma by *T. solium* cysticerci, there is usually a lengthy period of several months to years in which the host shows minimal to no immune or inflammatory response and experiences no symptoms (195). This phase is termed the vesicular phase as the viable larvae appear as translucent, fluid filled vesicles or cysts. The cysticerci can utilize several mechanisms to evade or downregulate both the humoral and cellular arms of the host immune response (180). Modulation of the humoral immune response occurs in several ways including by taking up host immunoglobulins (IgG, IgM, IgE and IgG) in the cyst tegument to mask parasite antigens (196) and by releasing molecules (such as taeniastatin), which inhibit the complement pathway (197). Taenia cysticerci also modulate cells of the immune system in multiple ways. Firstly, they impede dendritic cell maturation (198), impair classical Toll-like receptor 4 (TLR4) mediated activation of microglia, macrophages and dendritic cells (199) and instead lead to alternative activation and the production of immunosuppressive cytokine such as TGF-ß and IL-10 (200–202). Furthermore, viable cysts can induce regulatory T-cell (T-regs) activity. Broadly speaking, viable, vesicular Taenia larvae are able to shift an initial, transient T-helper type 1

immune response toward a T-helper type 2 response, which is more permissive for chronic infection (199).

At some point, the cysts lose their ability to control the host immune response and begin to degenerate. The cyst wall and fluid become infiltrated by host inflammatory cells and the cysts become opaquer in appearance with turbid vesicular fluid. This is referred to as the colloidal phase and is associated with an intense T-helper type 1 inflammatory response (203). Following this phase, the cyst cavity starts to collapse, and the cyst becomes encompassed by host fibrotic tissue. This is termed the granular-nodular phase. Here the host inflammatory response reflects a more chronic phenotype featuring mixed T-helper type 1 and T-helper type 2 features (204). When imaged using CT scans, *T. solium* cysts in the colloidal or granular-nodular stage are accompanied by two neuroimaging features reflecting the presence of a host inflammatory response: ring-enhancement and visible perilesional oedema (200). Over time the cyst becomes entirely infiltrated by connective tissue, which may include accompanying calcium deposition. This calcific stage (180), is not accompanied by neuroimaging features reflective of a host inflammatory response (200). The colloidal, granular-nodular, and calcific stages of the cysts reflect dying or dead larvae, which are no longer viable.

Manifestations of Neurological Disease

As described above, seizures are the most common manifestation of parenchymal disease. However, when cysts occur in the ventricular and subarachnoid space headaches, intracranial hypertension, hydrocephalus and meningitis may occur (188). There is some correspondence between the likelihood of seizure occurrence and the preponderant stage of cysts in the brain. Seizures are typically infrequent when *T. solium* cysticerci are viable, and are most common when the cyst is dying or degenerating, and somewhat less common when the cysts are in the calcific stage (205). In general, seizures during all cyst stages are usually associated with a detectable inflammatory host immune response surrounding the cyst. This has led to the widely held view that seizures result, at least in part, from the host inflammatory response to the cyst (195, 206).

Value of Neuroimmune Changes in Diagnostics and Therapies

The fact that viable cysts can suppress a host immune response and remain non-symptomatic makes diagnosis difficult in those with viable cysts and latent disease. Even in those with symptomatic NCC, diagnosis is notoriously challenging given the multitude of possible causes of seizures and the fact that expensive neuroimaging (CT or MRI scans) is not typically available in many endemic areas. Serology is certainly of diagnostic assistance with the most sensitive and specific test being the enzyme-linked immuno- electrotransfer blot (EITB) assay, which uses targeted antigens to detect antibodies to *T. solium* in patient serum. On this note (207) established a set of diagnostic criteria for NCC, which combines aspects of clinical history, neuroimaging and immunological evidence, as well as epidemiological factors, to form definitive guidelines for the diagnosis of NCC. This approach allows for a diagnosis to be made when some diagnostic modalities are not available.

An understanding of the interaction between parasite and host immune response, and particularly how this relates to symptom onset (i.e., seizures), is important for optimal management of NCC. Treatment must consider the location, viability, and number of the cysts as well as a characterization of the existing immune response to tailor the management plan to the individual concerned. Given that seizure severity is often correlated with dead or dying cysts and the accompanying immune response, the use of antiparasitic (cysticidal) drugs such as albendazole or praziquantel must be used with caution, particularly when many viable cysts are present. It is possible that mass death of larvae within the CNS could trigger an extensive inflammatory response and worsening of symptoms (208). This is a particularly important issue when cysts are in a sub-arachnoid or ventricular location and treatment could worsen hydrocephalus and/or cause a rapid rise in intracranial pressure. Therefore, when neuroimaging and definitive diagnosis is not available, it may not be sensible to proceed with cysticidal therapy and patients should primarily be managed symptomatically. This should be an especially strong consideration as cysts can often resolve naturally (209). That said, studies have shown that antiparasitic drugs can help reduce symptoms and hasten the resolution of lesions identified by neuroimaging. In addition, both patients and clinicians are understandably hesitant to allow a live parasite to persist untreated in the brain. Clearly the appropriate use of cysticidal agents remains as an area requiring further study and consensus. Steroids are an important component of treatment where they reduce the inflammation, which occurs following the degradation of cysts. As a result, prednisolone or dexamethasone are typically used as adjuncts to cysticidal therapy where they should be administered prior to the cysticidal drugs and continued for approximately a week following the end of antiparasitic treatment (208). Antiepileptic agents are also typically used and are effective in controlling NCC-related seizures. Surgery is rarely needed and only indicated if cysts are in a location that precludes cysticidal treatment and there is an urgent need for intervention (210).

Knowledge Gaps

It is important that we better define the extent of human and porcine cysticercosis and human taeniasis in Africa. Epidemiological studies elucidating the extent of the disease in many parts of Africa are either non-existent or out of date. Improved knowledge on NCC prevalence should then inform government and policy makers to improve sanitation and agricultural practices in the areas concerned. This could include vaccination of pigs. Representative animal model systems on NCC should also be developed and used. These could help elucidate some of the fundamental pathological mechanisms underlying NCC and its associated neurological sequalae (211). Finally, further progress is needed in the development of treatment strategies, particularly for viable parenchymal NCC. An ideal treatment regimen would both

kill cysticerci and safely prevent adverse effects associated with larval death and the associated host immune response.

OTHER PARASITES

Amoebic Encephalitis

Primary amoebic meningo-encephalitis (PAM) is rarely diagnosed in Africa. However, several reports indicate the presence of pathogenic free-living amoebas in water and environment (212–215). Reported cases and seroprevalence studies indicate the occurrence of infections with *Naegleria fowleri* (216, 217), which can enter the CNS *via* the olfactory nerve, *Acanthamoeba* spp. present in water (218) and *Balamuthia* (219). Given the severity of PAM and the lack of effective treatments, more investigations are needed to ascertain the prevalence in African countries.

Schistosoma spp.

Schistosoma spp. such as *S. mansoni*, *S. hematobium* and *S. japonicum* are extracellular helminths that are pathogenic in humans. Schistosomiasis is a neglected tropical disease currently infecting more than 140 million persons, of which 90% of the burden is in the SSA region (220). The prevalence of schistosomiasis in SSA is high in endemic regions of some countries e.g., above 50% amongst school age children in some communities in Ethiopia (221), 40-44.1% in Nigeria (222, 223), 21.1% in Ghana (224), 10.05-26.8% in Zimbabwe (225, 226), while some countries such as Senegal have reported a decrease from 78% to about 11% in school age children over a 12-year schistosomiasis control program (227).

Praziquantel is used for both preventative chemotherapy and treating schistosomiasis. Untreated, chronic schistosomiasis is associated with anemia, stunting, and reduced physical and mental capacity (228).

The lifecycle of *Schistosoma* spp. includes an intermediate host (fresh-water snails) where infective larvae (cercariae) grow and when released into water from the snails attach to and penetrate the skin of the definitive human host and move into the vascular system as schistomula (229). After initially residing in the lungs, they spread into the intrahepatic branches of the portal vein, where they mature into schistosomes. Schistosomes migrate and reside in the mesenteric veins (*S. mansoni* and *S. japonicum*) or pelvic veins (*S. haematobium*), where females lay eggs, which are secreted in feces or urine (229). Inflammatory granulomas form around eggs trapped in tissues and organs, such as the liver, intestinal tissue, and bladder, and result in intestinal, hepato-splenic, or urogenital disease (229)

Cerebral schistosomiasis/neuroeschistosomiasis, although considered rare, can occur when the parasite or its eggs lodge within CNS vessels and elicit an immune reaction (230–233) resulting in neuroinflammation and neurological symptoms such as seizures, encephalopathy with headache, visual impairment, motor deficits, ataxia and paralysis (229). *Schistosoma* eggs may spread to the CNS, through the arterial system as emboli after crossing previously developed pulmonary shunts or anastomosis from veins to arteries or through retrograde venous flow (234). They are deposited in cerebral vessels as emboli. Cerebral disease is mostly produced by *S. japonicum*, because the eggs are smaller and rounder and can reach the brain (233, 235). On the other hand, S. *mansoni* and *S. haematobium* cause mainly a spinal cord disease because the eggs are larger and are mostly retained in vessels at a lower spinal level (233, 235). Adult worms can also migrate *via* vessels to reach meninges and the choroid plexus where they may shed a lot of eggs into the CNS parenchyma, and this is probably the main cause of symptomatic neuroschistosomiasis (230, 232–234).

Schistosoma eggs secrete antigens such as glycans and glycoproteins that elicit an immune response leading to granuloma formation (230, 233, 236). In both human cases with neuroschistosomiasis caused by *S. japonicum* and mice that were injected with *S. japonicum* eggs in the brain microglia/macrophages constituted the major components of the granulomas surrounding the eggs (237). Patients with spinal cord schistosomiasis have increased levels of IL-1beta, IL-4, IL-6 and IL-10 and low concentrations of TNF-α and IFN-γ in both CSF and serum (238). In *S. mansoni* infected mice astrogliosis and microgliosis (228) were observed, as well as elevated IL-10 levels and decreased TNF-α expression (239). Thus, neuroschistosomiasis elicit a Th2 immune response in both humans and animals.

Toxocara spp.

Toxocara spp. such as *T. canis* and *T. cati* are gastrointestinal ascarid nematodes distributed worldwide and found in canids such as including dogs, foxes, wolves, jackals and coyotes, and felids such as domestic cats (definitive hosts) and can also cause infections in humans (considered paratenic hosts) (240, 241). Infected definitive hosts excrete eggs in the feces, which embryonate in the environment and become infective.

Human beings can accidentally ingest eggs containing infective third-stage larvae from contaminated food, soil, and water, and through direct contact with infected pets such as cats and dogs (240, 241). Ingested eggs develop and hatch into larvae in the small intestine, penetrate the intestinal wall and migrate to various tissues through the circulatory system, resulting in immune and inflammatory tissue reaction that can lead to symptoms such as fever, headaches, coughing, and abdominal or limb pains (240, 241). Most infections remain asymptomatic or mild, however the most common clinical manifestations are visceral larva migrans and ocular larva migrans (240–242).

Toxocara larvae can invade the brain, leading to neurotoxocariasis or cerebral toxocariasis, In the brain the larvae can cause eosinophilic meningitis, encephalomyelitis, cerebral vasculitis and epileptic seizures. In experimental animal models, the presence of *Toxocara* larvae in the brain increases the permeability of the blood-brain barrier, the expression of proinflammatory cytokines and iNOS, and astrogliosis leading to neuronal damage (243–245); Disturbances in the profile of neurotransmitters, such as GABA, glutamate, serotonin, dopamine, and noradrenaline, have also been reported (244, 245).

Paragonimus spp.

Paragonimus spp. such as P. westermani, P. africanus, P. heterotremus, P. kellicotti, P. mexicanus, P. siamensis, P. skrjabini, P. skrjabini miyazakii, and P. uterobilateralis, are lung flukes (trematodes) that cause paragonimiasis, a rare zoonotic disease, when they infect humans (definitive hosts) who have eaten undercooked freshwater crayfish or crabs (the intermediate hosts) infected with encysted metacercariae (246, 247). Humans can also get infected after eating raw meat of other animals that are paratenic hosts of the worms (247).

Pulmonary disease is the most common manifestation of the disease. However, beside the lungs the worms can infect other organs including the brain resulting in cerebral paragonimiasis or neuroparagonimiasis, which accounts for less than 1% of symptomatic paragonimiasis (246–248). In the brain worms lay eggs, which elicit an immune reaction and are surrounded by granulomatous lesions that can be cystic or solid. Cerebral paragonimiasis can manifest as headache, dizziness, spastic hemiplegia, hemianopsia, hemiparesis, dysarthria, seizures, mental retardation, visual disturbances, or motor and sensory disturbances (246, 247)

Onchocerca spp.

Onchocerca spp. comprise a group of filarial nematodes transmitted by blackflies of genera *Simulium* and *Culicoides*. They primarily infest hoofed mammals, but canids, felids, and humans are also infected (249). *Onchocerca volvulus* is the human pathogen and causes the disease onchocerciasis commonly referred to as "river blindness (250). It was initially described in 1875 by a British naval surgeon John O'Neill among individuals in West Africa (251). *O. volvulus* is endemic in 31 countries in Africa, Yemen, Venezuela, and Brazil. Globally, approximately, 218 million people are at risk of infection with over 95% of these located in Sub Saharan Africa (252, 253). The primary clinical manifestations of onchocerciasis include varying degrees of onchodermatitis (skin complications) (254) and keratitis (visual impairment) (255) which result from inflammatory responses caused by microfilaria death and/or Wolbachia spp. (endosymbiont bacteria of *O. volvulus*) derived products within the skin and ocular cavities. Other conditions associated with *Onchocerca* infection include lymph node changes, reproductive abnormalities (such as secondary amenorrhea, spontaneous abortion, and infertility). In addition, chronic infection may cause low body weight and diffuse musculoskeletal pain (250).

More recently, neurological manifestations have been proposed as an additional clinical consequence of *Onchocerca* infections (256, 257). Although mechanistic data is lacking, epidemiological evidence suggest a strong association between *O. volvulus* and brain disorders – epilepsy, nodding syndrome and Nakalanga dwarfism (258). These associations were demonstrated in a number of community-based surveys in different African countries, from which a meta-analysis reported a 0.4% increase in the prevalence of epilepsy for each 10% increase in the prevalence of onchocerciasis (259). Furthermore, a study conducted in Cameroon showed a temporal relationship between onchocerciasis and epilepsy highlighting a dose-dependent effect between the density of microfilaria and the risk of developing epilepsy in childhood (260, 261). Similarly, studies from Uganda have shown a decrease in the prevalence of epilepsy with declining *Onchocerca* burdens (262, 263). Epidemiological studies of both nodding syndrome and Nakalanga dwarfism also report consistent associations with *Onchocerca* (264, 265). Based on these data the term Onchocerca associated epilepsy (OAE) was coined to describe this group of disorders (258).

The pathological mechanisms by which *Onchocerca* may led to neurological sequalae remain poorly understood and under investigation (266). Several hypotheses have however been proposed with conflicting results: 1) Direct Invasion of CNS by the parasite or pathogenic proteins/metabolites (267, 268), 2) An *O. volvulus*–induced immune response through an inflammatory process or auto antibodies against neuron surface proteins (269–272), 3) A *Wolbachia* spp., dependent pathway (273), and finally, a tauopathy, manifesting as aggregates of tau protein in the brain (274, 275).

Other Cestodes

Apart from T. solium, other globally distributed cestodes that may infect the brain are also present in Africa (246). Infection occurs via larvae of the genera Spirometra and Sparganum, which cause sparganosis (276) and metacestodes of the genus Echinococcus, which cause echinococcosis also known as hydatid disease (277). Both sparganosis and echinococcosis are neglected food-borne zoonotic diseases caused by the ingestion of contaminated food or water. Humans are accidental intermediate hosts within whom several body tissues may be infested (246). Importantly, brain infection can occur causing neurological disease (278). However, both cerebral sparganosis and echinococcosis are considered rare with the exact epidemiological picture being unclear.

Cerebral sparganosis occurs when plerocercoid larvae (spargana) invade the CNS resulting in tissue damage. The main clinical manifestations include fever, headache, neck stiffness, paresthesia, and seizures. Further, patients may suffer visual and sensory impairment, in addition to motor weakness (279, 280). Additionally, cerebral hemorrhage may manifest (281). The pathological mechanisms of cerebral sparganosis involves the formation of granulomatous lesions or eosinophilic granulomas following worm migration and inflammation (276, 280).

Cerebral echinococcosis is caused particularly by E. granulosus and E. multilocularis, which are forms of Echinococcus able to infect humans. As is the case for T. solium, ingested oncospheres (eggs) migrate through the intestinal wall and pass into the portal system of infected humans. In Echinococcus these are largely entrapped within the liver. However, some occasionally pass from the systemic circulation into the brain parenchyma. Within brain tissue, cerebral hydatid cysts can grow asymptomatically over an extended period to large sizes, this is particularly the case in children. The main clinical features of patients with intracranial

hydatid cysts include raised intracranial pressure, blindness, loss of consciousness, focal neurological deficits and seizures (277, 282).

CONCLUDING REMARKS

Neurological disorders caused by parasites within Africa include epilepsy, sleeping disorders, hyperalgesia, hemiparesis, dementia, long-term neuro-disability, and cognitive impairments. These disorders are because of the parasites or their products such as eggs being within the CNS causing structural damage and/or eliciting an immunological response.

During CM *Plasmodium* parasites sequestered in the CNS within erythrocytes cause the production of proinflammatory cytokines, such as TNF-α, LTα, IFN-γ, IL-1α, and IL-1β, which contribute to the hyperinflammatory state of this neurological disorder. The second stage of HAT, when trypanosomes have invaded the CNS, is accompanied by increased levels of proinflammatory cytokines such TNF-α and IFN-γ, which probably play an important role in hyperalgesia, sleep disturbances and alteration in circadian rhythm, which are prominent neurological features of HAT. The increased levels of proinflammatory cytokines such TNF-α and IFN-γ during cerebral toxoplasmosis serves a role of controlling the parasite in the CNS. Unlike the other CNS parasitic infections mentioned above live *T. solium* parasites are more associated with dampening of the immune system during neurocysticercosis, however when they degenerate a mixed Th1 and Th2 immune reaction is observed, which coincide with the development of seizures.

The immune molecules expressed during CNS infections by parasites can be exploited for therapeutic purposes. Immune molecules such as CXCL8, IFN-γ-induced CXCL10, CXCL13 and IL-10 have come out as strong biomarkers for disease staging of HAT. Finding other molecules including IFN-γ-induced molecules, which facilitate T cells and parasite crossing of the BBB, could be important both to understand the pathophysiology of the individual disease and to find possible biomarkers for staging e.g., HAT. More studies are needed to characterize the intermediate stage of HAT and define what it means in terms of treatment and therapeutic outcomes. In addition, targeting the exacerbated proinflammatory immune reaction that occur during PTRE because of treatment of HAT with melarsoprol could reduce fatalities. Targeting immune molecules such as TNF-α during cerebral malaria have not improved clinical outcomes, suggesting that there is a need to understand more about the role of different immune molecules during CM, to effectively target them for therapeutic purposes.

In conclusion, the immune system plays an important role in the neuropathology and neurological manifestations of CNS parasitic infections. Understanding the neuroimmunology of these parasites is essential not only for understanding the pathophysiology of the diseases they cause but also for the identification of biomarkers and therapeutic modalities to manage these disorders.

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Neurogenesis and Viral Infection

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Neural stem cells (NSCs) are multipotent stem cells that reside in the fetal and adult mammalian brain, which can self-renew and differentiate into neurons and supporting cells. Intrinsic and extrinsic cues, from cells in the local niche and from distant sites, stringently orchestrates the self-renewal and differentiation competence of NSCs. Ample evidence supports the important role of NSCs in neuroplasticity, aging, disease, and repair of the nervous system. Indeed, activation of NSCs or their transplantation into injured areas of the central nervous system can lead to regeneration in animal models. Viral invasion of NSCs can negatively affect neurogenesis and synaptogenesis, with consequent cell death, impairment of cell cycle progression, early differentiation, which cause neural progenitors depletion in the cortical layer of the brain. Herein, we will review the current understanding of Zika virus (ZIKV) infection of the fetal brain and the NSCs, which are the preferential population targeted by ZIKV. Furthermore, the potential neurotropic properties of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which may cause direct neurological damage, will be discussed.

Keywords: neural stem cells, neurogenesis, gliogenesis, ZIKV, SARS-CoV-2

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NEURAL STEM CELLS

Development of Neural Stem Cells

Neural stem cells (NSCs) are multipotent stem cells present in the fetal and adult mammalian brain, which can self-renew and differentiate into the three main components of the central nervous system: neurons, astrocytes, and oligodendrocytes (1) (**Figure 1**).

During the early embryogenesis of mammals, the neural plate and neural tube comprise a single layer of proliferating neuroepithelial cells. Around gestational week (GW) 7-9, neuroepithelial cells line the inner part of the neural tube and, later, of the cerebrospinal fluid (CSF)-filled ventricles, named the subventricular zone (SVZ) (**Figure 1**, left panel). Neuroepithelial cells form a pseudostratified layer of mitotically active cells that rapidly amplify their pool before they differentiate into ventricular radial glial cells (RGCs) (2). RGCs are polarized cells in contact with the monolayer of ventricular ependymal cells on the apical side, and with the meninges and blood vessels on the basolateral side (3, 4). The ependymal cells establish a barrier and a transport system between the brain interstitial fluid and the CSF, which support neurogenesis regulation (5). Unlike the SVZ of other mammals, the human expanding SVZ, between GW 14 to 17, entails of a smaller inner SVZ (iSVZ) and an expanded outer SVZ (oSVZ) separated by a cell-poor region, the inner

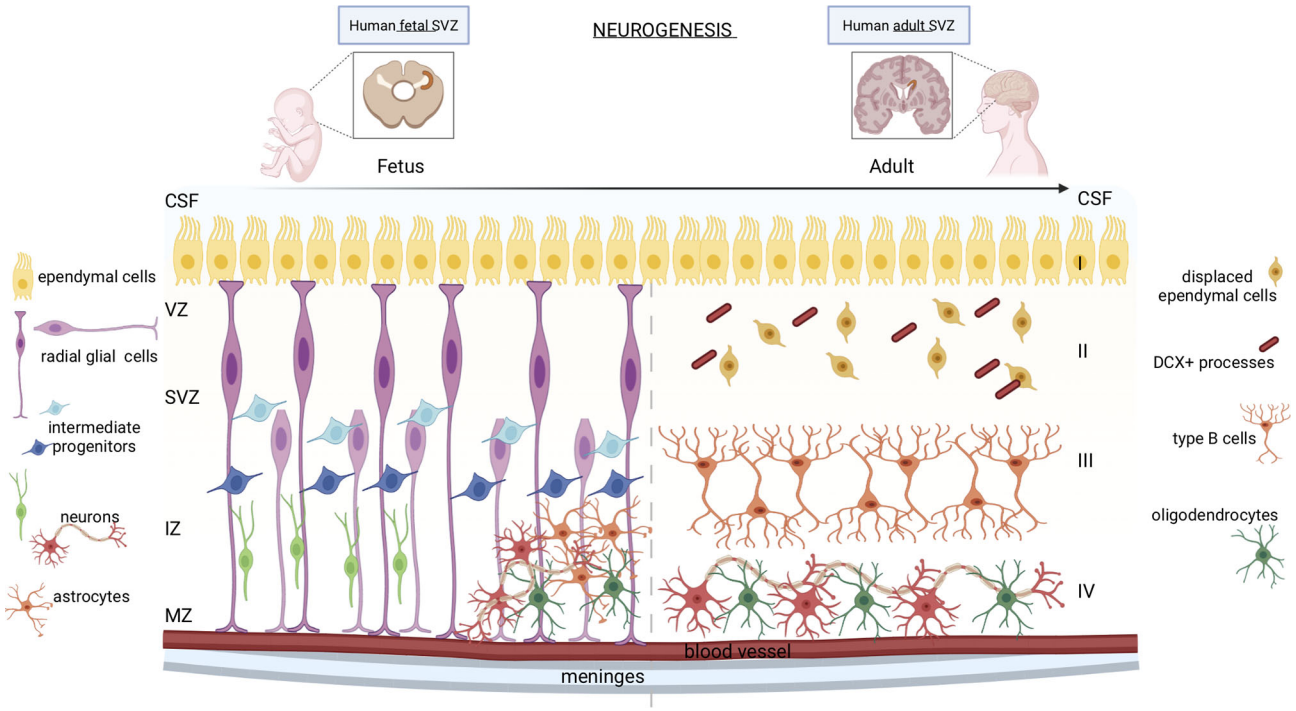

FIGURE 1 | Neurogenesis in human fetal and adult SVZ. In the fetal brain, the neurogenic niche is organized into different strata: the ventricular zone (VZ), the subventricular zone (SVZ), the intermediate zone (IZ), and the marginal zone (MZ). Apical radial glial cells of the SVZ remain in contact with the ependymal cells and with the meninges and blood vessels, while the basal radial glial cells, which constitute the region of the SVZ called outer SVZ (oSVZ), lose contact with ventricles leading to a discontinuous scaffold of radial glial cells (RGCs). Apical and basal RGCs generate neurons through two mechanisms: either directly or indirectly through intermediate progenitor cells, which also originate astrocytes and oligodendrocytes. In the adult brain, the SVZ consists of four distinct stratified areas: layer I formed by ependymal cells in contact with the lumen of ventricles; the hypocellular gap (layer II), mainly formed by displaced ependymal cells and DCX+ astrocyte processes; layer III constituted by type B astrocytic cells; a transitional region to the brain parenchyma (layer IV) formed by mature neurons and oligodendrocytes.

fiber layer (IFL) (6). The human oSVZ contains a new class of actively proliferating progenitors, the basal radial glial cells, that lost contact with ventricles from the apical surface, leading to a discontinuous RG scaffold (7). The basal RGCs initiate asymmetric cell divisions to generate neurons, but then quickly differentiate into intermediate progenitor cells (IPCs), a type of transit-amplifying cell, which further mature into neurons. This mechanism leads to the formation of a highly heterogeneous population of progenitor cells that generate diverse subtypes of differentiated neurons (8). After the neurogenic stages, the human RGCs become gliogenic, generating astrocytes or oligodendrocytes (4). RGCs are often referred to as neural stem cells (NSCs) since they can differentiate into neurons, astrocytes, and oligodendrocytes. The peculiar architecture of the human SVZ sustains the development of several neuronal and glial cell types in the complex cerebral cortex of primates (9).

In the mammalian adult brain (**Figure 1**, right panel), NSCs are present only in two niches, the ventricular-subventricular zone (V-SVZ) of the lateral ventricles, and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus, which are dedicated to the generation of young neurons of the olfactory bulb (OB) and hippocampus, respectively (10). The SVZ organization of the human brain differs from that of well-studied rodents, which allowed the characterization of several functions of NSCs. Indeed, the human SVZ consists of four layers: cell bodies are accumulated in a ribbon (layer III) separated from the ependymal layer (layer I) by a gap that is largely devoid of cells (layer II), originated as a consequence of neuroblast depletion (11). The astrocytic ribbon (layer III) contains cell bodies of large astrocytes, a subset of which proliferate *in vivo* and show *in vitro* multipotency and self-renewal characteristics. Layer IV is a transitional region to the brain parenchyma. During fetal development, the proliferative activity within the SVZ progressively declines (12), but it remains active in neonates, along the wall of the lateral ventricle, generating diverse subtypes of neurons (13, 14).

Little is known about the precise role of neural stem cells in the adult human brain. Although the debate is still open, it has recently been reported that some degree of neurogenesis persists in adulthood, contradicting two decades of history stating that the human brain has no regenerative capabilities (15). In 1998, Eriksson and colleagues detected adult hippocampal neurogenesis in a post-mortem study of brains from neoplastic patients treated with bromodeoxyuridine (BrdU) for tumor-staging purposes. Proliferating cells (BrdU+) have been found in both the SVZ of the lateral ventricle and the subgranular zone

of the dentate gyrus. In SGZ, some of these newly generated cells were observed to be capable to differentiate into neurons (16). Ernst and colleagues reported the presence of neuroblasts not only in SVZ but also in the adjacent striatum, suggesting that neuroblasts and new neurons in the adult human striatum derive from the SVZ (17). Hippocampal cell turnover during adult life was also confirmed by the quantification of integrated radiocarbon into DNA of replicated cells (18). Different studies proved that hippocampal neurogenesis persists throughout adult life (19–21) showing a lower age-associated decline in humans compared to mice (18). The preservation of hippocampal neurogenesis during evolution could be related to human cognitive adaptability. Interestingly, in patients with advanced Alzheimer's disease, hippocampal neurogenesis has been described to drop sharply (19). In a small cohort of patients with amyotrophic lateral sclerosis (ALS), neural progenitor proliferation was increased in the SVZ and decreased in the SGZ (22) Methodological challenges, however, render studies about adult human neurogenesis of difficult interpretation, and contradictory results may depend on the use of diverse technologies (23). The development of new tools such as single-cell RNA sequencing, neuroimaging techniques, and the identification of novel reliable NSC markers will clarify the role that adult human neurogenesis plays in hippocampal function, neuroplasticity, and brain repair.

Neurogenic and Non-Neurogenic Functions

The NSC functions have been extensively studied in mouse models in which, under physiological conditions, they can be divided into neurogenic and non-neurogenic activities.

In the SGZ of the hippocampus, new neurons are generated to regulate and refine the existing neuronal circuits. Indeed, hippocampal NSCs have been shown to have an important role in adult behavior and other learning-related tasks, as the preservation of spatial memory, memory acquisition and maintenance (24). The effects on neurogenesis have been extensively described in animal models. Mice in which the apoptosis-promoting gene *Bax* was conditionally ablated in NSCs to potentiate neurogenesis, showed an increased behavioral performance when tested with a specific cognitive task (25). On the contrary, decreased neurogenesis is associated with a prolonged hippocampus-dependent period of associative fear memory, likely aimed at preserving learning abilities by disposing of old memories (26). In the SVZ, immature neurons and NSC perform different tasks. Immature neurons tangentially migrate to three main areas: the olfactory bulbs (OBs) along the rostral migratory stream (RMS), the human prefrontal cortex along the medial migratory stream (MMS) (13), and the frontal lobe along the arc pathway (14). NSCs residing within the SVZ may contribute to the maintenance and reorganization of the central nervous system, to neurocognitive maturation and plasticity, although their functional role remains controversial (24).

Results from recent studies showed that, besides pure neurogenic functions, NSCs might play a comprehensive range of bystander, non-neurogenic activities to maintain brain homeostasis (27). NSCs produce and secrete an array of mediators that, in turn, regulate complex functions in the brain. For instance, neuroblasts derived both from the SVZ and SGZ can phagocytose apoptotic neuronal progenitors, an essential function in maintaining neurogenesis (28). Moreover, NSCs can curb microglial activation, proliferation, and phagocytosis by secreting factors like the vascular endothelial growth factor. Unchallenged microglia present in the adult SGZ maintain the homeostasis of the neurogenic cascade by removing apoptotic newly born cells by bilateral crosstalk between NSCs and microglia (29, 30). Furthermore, as demonstrated by Snyder and colleagues, neurogenesis-deficient mice mount a more severe response to acute stress, by showing increased food avoidance, behavioral despair in the forced swim test, and anhedonia in the sugar preference test. Thus, SGZ-derived newly generated neuroblasts seem to dynamically regulate stress responses by controlling the hypothalamic-pituitary-adrenal axis (31).

GLIOGENESIS

Glia includes cells of ectodermal origin with diverse and dynamic functions - radial glia, astrocytes, oligodendrocyte progenitor cells (OPCs), oligodendrocytes - which orchestrate fundamental aspects of nervous system development and function (32). During brain development, distinct glia cells accomplish key tasks: neuronal birth, migration, axon specification, synaptogenesis, plasticity, homeostasis, constantly monitoring CNS structure and function (**Figure 2**). Transplantation experiments (33) showed that spinal cord progenitors that are restricted to glial lineage can recover neurogenic potential upon transplantation into the dentate gyrus, but not upon transplantation into the spinal cord or the non-neurogenic CA1 area of the hippocampus. Thus, adult glial progenitor cells are not lineage-restricted but can generate neurons upon exposure to appropriate environmental cues.

Astrocytes produce and secrete molecules that can drive the differentiation of adult neural stem/progenitor cells into neurons (33). Despite the adult hippocampus being composed greatly of neuroglia, which is four times more abundant than neurons (34), the lack of appropriate technical tools has delayed the study of the role of these supporting cells in adult neurogenesis. In recent days, the use of genetic tools and electron microscopy has started revealing that astrocytes and neural stem cells communicate with each other, both in physiological states and disease. It is now clear that astrocytes interact with neurons and other glial cells by secreting soluble mediators that act as gliotransmitters, neuromodulators, trophic factors, and hormones (35). Interestingly, some of these neuroactive molecules can exert either a driving or inhibitory role toward neurogenesis depending on the step in which they act. For instance, ATP, FGF2, and TSP1 have been found to stimulate adult NSCs (aNSC) proliferation (34). Also, neurogenesin-1, IL-1β, IL-6, and WNT3 have been shown to increase neuronal differentiation, while IGFBP6, enkephalin, and decorin reduced it. Neuronal maturation and synaptic integration are also boosted by D-serine. Moreover, Casse et al. (36) reported that

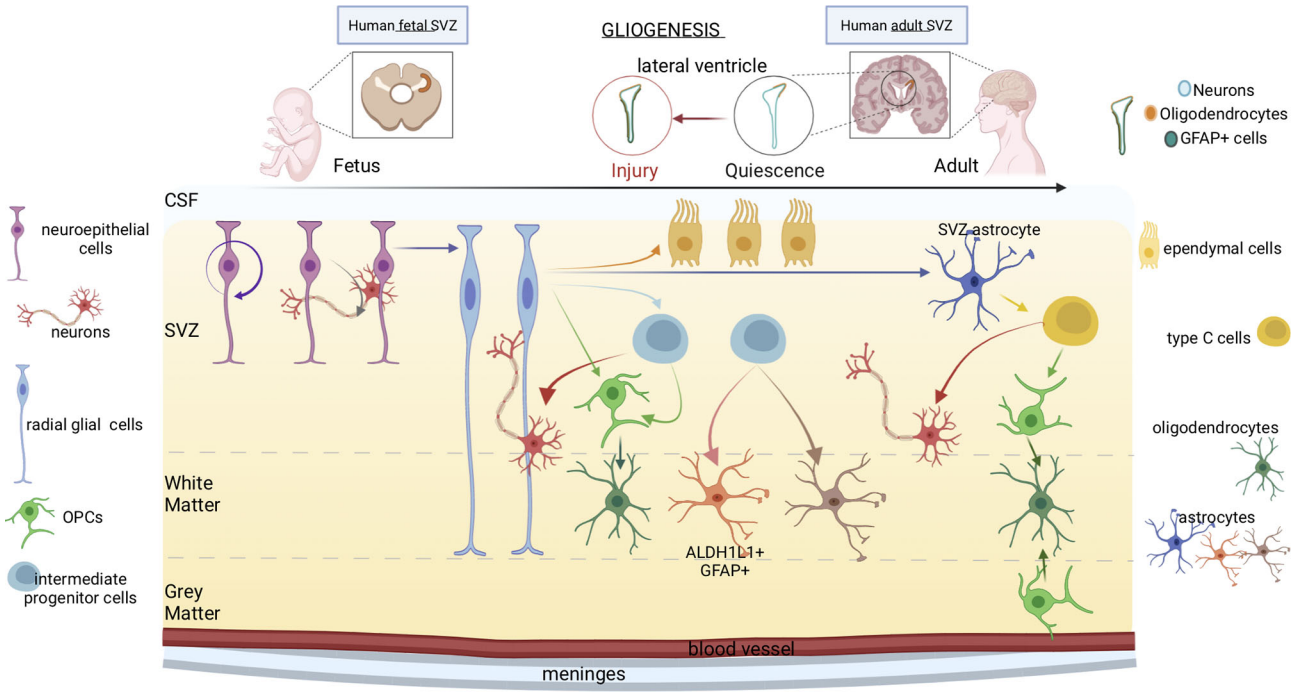

FIGURE 2 | Gliogenesis in human fetal and adult brain. The progression from fetal to adult gliogenesis in the SVZ, which lines the lateral and septal sides of the lateral ventricle (LV), is shown. Arrows indicate cellular self-renewal or differentiation. Neuroepithelial stem cells, which can generate neurons, border the ventricles throughout the neuraxis at the stages of neural tube closure. At the beginning of the neurogenesis, neuroepithelial cells differentiate into radial glial cells. Radial glia produces intermediate progenitor cells and OPCs, which further differentiate respectively into neurons and oligodendrocytes. Radial glial cells have also the capacity to become astrocytes or produce intermediate progenitors. Lastly, intermediate progenitor cells can generate different subtypes of astrocytes. In adults, neural stem cells are neurogenic, yet, under some conditions like in response to injury, gliogenesis generate reactive astrocytes and oligodendrocyte-lineage cells. Oligodendrocytes are produced by either type C cells or OPCs located in the grey matter. Type C cells produce OPCs as well as neurons. The OPCs subsequently differentiate into oligodendrocytes.

astrocytes regulate the synaptic integration of new neurons by reducing connectivity and glutamate reuptake (34). It has also been documented by Toni et al. (37) that maturing neurons depend on pre-existing astrocytes to identify synaptic partners. In the dentate gyrus, the dendritic spines of new granule neurons generate synapses with axon terminals already on site.

Any disease or lesion of the nervous system that induces an immune activation promotes a reactive astrocyte phenotype, with increased expression of the glial fibrillary acidic protein (GFAP). More recently, transcriptomic analyses allowed a sharper distinction between diverse astrocytic subsets in pathological conditions. For instance, during neuroinflammation, the expression of genes involved in synaptic transmission and the release of neurotrophic factors are altered (38). As another example, in both patients and mouse models of Alzheimer's disease, astrocytes rapidly respond to injury by becoming reactive and activating a series of molecular, cellular, and morphological changes (35, 36). Finally, cell surface expression of programmed cell death 1-ligand 1 (PD-L1) driven by the STAT3 pathway in reactive astrocytes is involved in the establishment of an immunosuppressive microenvironment in brain metastases (39).

NSCs also express astrocytic genes in response to the activation of diverse signaling pathways, triggered by morphogenic proteins (BMPs), which signals mainly through SMAD, leukemia inhibitory factor/ciliary neurotrophic factor (LIF/CNTF), which activates the JAK/STAT pathway, and the Notch pathway. *In vitro*, lipopolysaccharide (LPS), the classical inducer of neuroinflammation, stimulates microglia to release a NFκB-dependent secretome that includes interleukin 1 (IL-1), tumor necrosis factor TNF, and complement C1q (37).

Although the generation of astrocytes and their function in the adult brain are not yet well characterized, astroglia remains the predominant cell type of the neurogenic niche in terms of number of cells generated. In support of the important role of astrocytes in adult neurogenesis, Casse et al. (34) described how astrocytes can dysregulate adult neurogenesis leading to cognitive impairment in AD. Thus, a clear link exists between cognitive function and regulations of adult neurogenesis.

The process of differentiation along the oligodendroglial lineage is strictly coordinated by glia-glia and neuron-glia cross-talks at synaptic sites. Furthermore, according to Antel et al. (40), also immune-mediated mechanisms can contribute both positively and negatively to the generation and activation of OPCs. For instance, a subset of B lymphocytes, the B-1a cells, greatly contribute to OPC proliferation. B-1a cells can cross the blood-brain barrier in a CXCL13-CXCR5-dependent manner and are particularly abundant in the neonatal mouse brain. The

fact that B-1a cells promote the proliferation of OPCs has been shown *in vitro* and further confirmed *in vivo* since the depletion of B-1a cells from the developing brain results in a reduction of both OPCs and mature oligodendrocytes. It has been demonstrated that B-1a cells secrete a soluble form of Fcα/μR, the receptor for the Fc region of IgM, which promotes OPCs proliferation and increases the axon myelination in the neonatal mouse brain (38). Altogether, these data demonstrate that B-1a cells infiltrating the brain may contribute to oligodendrogenesis and myelination by promoting OPC proliferation *via* activation of the IgM-Fcα/μR signaling pathway (38, 40).

NEURAL STEM CELLS AS VIRAL TARGET

Congenital Infections Affecting the Developing Fetal Neurodevelopment

TORCH infections are a group of congenital infections that can be transmitted from the mother to the fetus (41). The TORCH acronym refers to pathogens directly involved in the development of the congenital disease: **T**oxoplasma, **R**ubella, **C**ytomegalovirus, **H**erpes simplex 1 and 2, and **O**thers (Chlamydia, HIV, Coxsackievirus, Syphilis, Hepatitis B, Chickenpox, and ZIKV) (39, 40, 42–47). Although viral transmission during the third trimester of pregnancy has a reduced impact on the developing fetus, infection during the first trimester is extremely disruptive, with severe congenital neurological defects in the developing fetus, which include microcephaly, cognitive and intellectual disabilities, sensorineural hearing loss, and blindness. Evidence suggests that NSCs are directly affected by viral infections, which lead to developmental defects in the cerebral cortex mainly by interfering with their differentiation into mature neural cells (41). A summary of the main congenital syndromes associated with viral infections, is presented in **Table 1**.

Here we will focus on two viral outbreaks that created a substantial impact on public health: ZIKV and SARS-CoV-2. While ZIKV infection affects fetal neurodevelopment (48), SARS-COV-2 targets adult endogenous neurogenesis and affects homeostasis of neuronal circuits (49), while data on infected neonates are still scarce.

The Case of ZIKV

ZIKV, a re-emerging arthropod-borne flavivirus, was firstly isolated from the blood of a febrile monkey in 1947 in the Zika forest of Uganda (50, 51). Through the 20th century, few human ZIKV infections were reported, and these were recognized as mild non-life-threatening illnesses (52). Limited seroepidemiology surveys indicated that as many as 80% of infections were asymptomatic or subclinical (52). Therefore, little attention was paid to ZIKV up to the last decade when an outbreak of ZIKV infection occurred firstly in the Yap Island in the Federal State of Micronesia in April 2007 (52) and later in 2013 in French Polynesia when an increased incidence of Guillain-Barrè syndrome was reported to be associated with ZIKV infection (53). However, the rapid spread with millions of cases and the novel association of ZIKV with congenital microcephaly and the Guillain-Barrè syndrome changed the public health landscape such that the World Health Organization declared ZIKV pandemic a Public Health Emergency of International Concern (54) in 2016. Indeed, a seminal pathology study showed the presence of Zika virions and viral RNA in the microcephalic fetal brain with complete agyria and multiple microscopic abnormalities of an aborted fetus due to symptomatic maternal ZIKV infection acquired in Brazil (47). This study was followed up by more investigations, all confirming the pathologic spectrum of brain injury caused by ZIKV and lack of virus-induced cytopathic effects outside of the brain (55) (**Figure 3**, left panel).

TABLE 1 | Main congenital syndromes associated with viral infections.

Pathogen	Genome	Family	Mother transmission route	Congenital syndrome	References
Rubella Virus (RUBV)	single-stranded RNA	Togaviridae	Aerosols	Microcephaly; diffuse and widely distributed calcification at basal ganglia; behavioral disorders; mental retardation.	(39, 42)
Cytomegalovirus (CMV)	double-stranded DNA	Herpesviridae	Blood transfusions, organ transplant and mucus exposure	Punctate and periventricular or cortical calcification; mental retardation; motor disabilities; hearing loss.	(40, 43)
Varicella Zoster Virus (VZV)	double-stranded DNA	Herpesviridae	Aerosols and contact with vesicular fluids	Microcephaly; ventriculomegaly; skin and extremities abnormalities.	(44)
Herpes Simplex Virus (HSV) 1 and 2	double-stranded DNA	Herpesviridae	Sexual contact and ascending infection, perinatal infection	Skin and ocular abnormalities.	(45)
Zika virus (ZIKV)	single-stranded RNA	Flaviviridae	Mosquito bites, sexual	Microcephaly; ventriculomegaly; parenchymal or cerebellar calcification; arthrogryposis.	(46, 47)

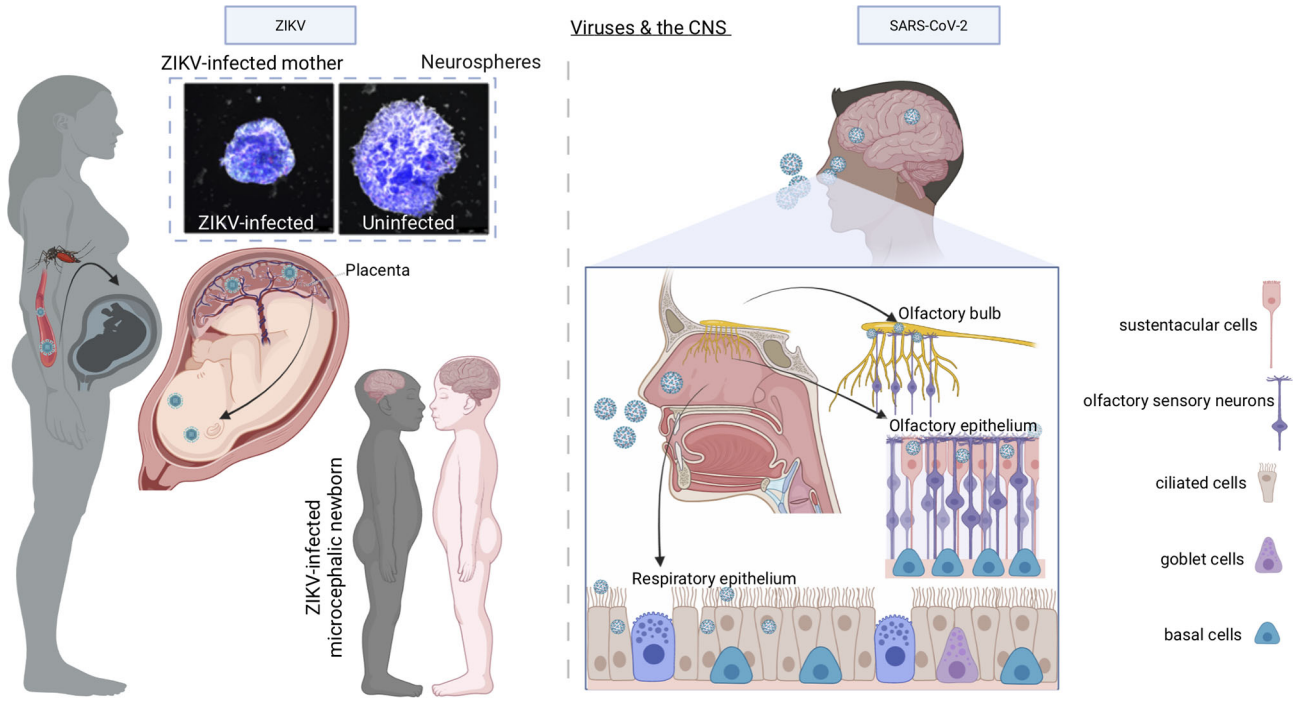

FIGURE 3 | Models of ZIKV and SARS-CoV-2 infection of the CNS. When ZIKV is transmitted to a pregnant woman by bites of infected Aedes mosquitoes, although it causes mild or no symptoms in the mother, it enters the bloodstream, breaches the placental barrier, and infects the developing CNS of the fetus, with devastating consequences including microcephaly, decreased brain tissue, damage to the posterior segment of the eye, hypertonia, and arthrogryposis. *In vitro* experiments with human neurospheres confirm the direct effect of ZIKV on neural cells. Once SARS-CoV-2 is inhaled into the nasal cavity, it infects the sustentacular cells of the olfactory epithelium using ACE2 receptor and multiplies causing infection of the olfactory epithelium, the respiratory epithelium, and olfactory bulb through the numerous ACE2 receptors expressed by the cells of these tissues. From the olfactory bulb, the virus may travel to the CNS by retrograde axonal transport along olfactory sensory neurons.

ZIKV is a single-stranded RNA virus of the *Flaviviridae* family and is closely related to other members of this family, including Dengue, yellow fever, tick-borne encephalitis virus, West Nile, and Japanese encephalitis virus. ZIKV is commonly transmitted to humans by bites of infected *Aedes* mosquitoes (56). Differently from the closely related virus, cases of ZIKV sexual transmission have also been reported (57). Although the members of the *Flaviviridae* family mentioned above are neurotropic viruses that can cause severe illness with a significant possibility of permanent neurological damage or death (58), ZIKV causes a congenital ZIKV syndrome (CZS) only when the infection is acquired during the first and the beginning or whole second trimester of pregnancy (48, 59). Although some features of CZS are in common with other viral infections acquired during pregnancy as cytomegalovirus infection (60) and rubella (61), CZS is peculiar considering the severe microcephaly, decreased brain tissue, damage to the back of the eye, hypertonia, and arthrogryposis (https://www.cdc.gov/pregnancy/zika/testing-follow-up/zika-syndrome-birth-defects.html).

The finding of ZIKV in the amniotic fluid of pregnant women and the brain of microcephalic fetuses suggest a potential trans-placental infection route (47, 62). A potential source of the virus spreading to placental trophoblasts during the very early phases of pregnancy is represented by endometrial stromal cells, especially when decidualized by progesterone stimulation (63). ZIKV can reach and infect decidualized endometrial stromal cells *via* the uterine circulation or by sexual viral transmission.

In Vitro ZIKV Infection

The first evidence of the strong ZIKV tropism in NSCs came by comparing *in vitro* infection of iPSC derived NSCs with immature neurons, the last being less permissive to productive infection than NSCs (64). ZIKV envelope protein was detected in human iPS-derived NSCs 24 h after exposure to ZIKV, and infectious virus was detected in the cell culture supernatant 72 h post-infection, providing evidence of productive infection. Importantly, viral replication induced cell death and dysregulation of the cell cycle. To establish the connection between ZIKV infection and the malformations observed in fetal brains, Garcez et al. analyzed the impact of ZIKV infection in a 3D culture system of neurospheres derived from human iPSC (65). Viral particles were detected on the cell membrane, in mitochondria, and in intracellular vesicles of ZIKV-infected cells in the neurospheres. The presence of apoptotic nuclei, a hallmark of cell death, indicated that ZIKV was cytopathic for human NSCs, thus impairing the proper development of neurospheres.

To investigate how ZIKV infection affects brain development and causes microcephaly, 3D brain organoids derived from human embryonic stem cells can be used to recapitulate fetal brain development during the first trimester of pregnancy (66). Indeed, brain organoids self-organize and show regionalization, cortical differentiation, the presence of neuronal layers, and an outer RGC layer (66). ZIKV infection impaired the growth of human stem cell-derived organoids, with increased apoptosis, reduced proliferation and the ensuing decrease of neuronal cell-layer volume mirroring microcephaly (65, 67).

The analysis of the transcriptomic profile of human embryonic stem cell-derived organoids infected with a prototype strain of ZIKV showed that the innate immune receptor Toll-like-Receptor 3 (TLR3) was upregulated after ZIKV infection (68). Furthermore, TLR3 inhibition decreased the cytopathic effect of ZIKV infection. Pathway analysis of gene expression changes upon TLR3 activation identified several genes associated with neuronal development, indicating that ZIKV affects neurogenesis by interfering with a TLR3-regulated pathway. Thus, ZIKV-mediated activation of TLR3 severely affects neuronal cell fate, leading to an overall reduction of organoid volume mimicking a microcephalic phenotype (68).

Animal Models of ZIKV Pathology

Animal models of ZIKV infection have supported the characterization of ZIKV pathology. In this regard, direct evidence that ZIKV infection can cause microcephaly, with enlarged lateral ventricles and thinner cortical plates as compared to uninfected animals, was provided by Li and colleagues, who investigated ZIKV infection of the embryonic mouse brain, and its effects on brain development (69). Indeed, the Asian ZIKV strain, SZ01 replicates efficiently in embryonic mouse brain by directly targeting different neuronal lineages, including NSCs. ZIKV infected NSCs undergo cell-cycle arrest, apoptosis, and a differentiation blockage, ensuing cortical thinning and microcephaly. Gene expression analysis of infected brains showed the overexpression of flavivirus entry receptors and aberrant expression of genes related to immune responses and apoptosis.

The isolation of ZIKV from the amniotic fluids of pregnant women and the brain of microcephalic fetuses suggests a potential trans-placental infection route (47, 62). Decidualized endometrial stromal cells are a crucial target of ZIKV infection either *via* the uterine vasculature or by sexual transmission, thus likely representing a potential source of the virus spreading to placental trophoblasts during early pregnancy (63). The transplacental infection has been demonstrated in two mouse models of ZIKV infection during pregnancy: female mice lacking type I interferon signaling (*Ifnar1*$^{-/-}$) crossed to wild type (WT) males, and pregnant WT females treated with an anti-ifnar-blocking antibody. In these models, ZIKV infected trophoblasts of the maternal and fetal placenta resulting in an intrauterine growth restriction (70). However, microcephaly, or deficiency of specific brain structures were not detected, possibly due to the different timing of brain development in mouse *vs*. human fetuses, as the development and maturation of the mouse brain includes a significant postnatal phase (71, 72).

In summary, ZIKV is a congenital infection that has serious consequences to the fetus and neonates and NSCs represent its preferred target. After infection, NSCs exit the cell cycle and die. Nevertheless, ZIKV has not been a major public health concern throughout the world since mid-2017 as after an estimation of 4,000 newborns with serious brain damage, the virus has disappeared from the Americas and the Caribbeans. However, an analysis of travelers who visited Cuba in 2017 or 2018 demonstrated ZIKV infection after their return to the United States and Europe (73). These results suggest that even during ZIKV waning infection, outbreaks were undetected until an immunologically naïve population of travelers became in contact with the virus. In the absence of an effective vaccine, travel surveillance is important, particularly for pregnant women.

The Case of SARS-CoV-2

A novel severe respiratory disease emerged at the end of 2019 (coronavirus disease 2019, COVID-19) in Wuhan, China, and caused a still ongoing pandemic with more than 370 million people infected and 5 million deaths worldwide as of January 2022. COVID-19 is caused by a novel coronavirus called severe acute respiratory syndrome (SARS) CoV-2 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it) to distinguish it from SARS-CoV that emerged in the Guangdong province of China in 2003 and caused the severe clinical condition known as SARS (74). Like SARS-CoV, SARS-CoV-2 causes pneumonia with severe inflammation, which can progress to acute respiratory distress syndrome (ARDS) and death (75). COVID-19 can also be a multi-organ disease that may affect the brain (76–78) (**Figure 3**, right panel). Neurological manifestations including loss of smell and taste have been reported in concomitance with COVID-19 in approximately 27% of infected individuals (79) and can persist in subjects who have recovered from COVID-19 (80). However, it is unclear whether the sequela of neurological events depends on the direct infection of the neural tissue, or it is a consequence of the inflammation and activation of the coagulation cascade induced by the virus. In this regard, a recent report has demonstrated the presence of intact virions and SARS-CoV-2 subgenomic RNA (a surrogate of active viral replication) in the olfactory mucosa of a minority of autoptic specimens obtained from individuals who died of COVID-19 (81), suggesting that SARS-CoV-2 can access the central nervous system at the neural-mucosal interface of the olfactory mucosa *via* axonal transport. However, another study in which postmortem bedside collection of olfactory mucosa and whole olfactory bulbs was set up, failed to show the presence of SARS-CoV-2 in sensory neurons (82). These discrepancies might be explained by the difficulties to obtain samples of suitable quality from deceased individuals. Nevertheless, SARS-CoV-2 RNA was detected in the leptomeninges (82) suggesting that virions might have reached the cranial cavity either *via* migration through axonal transport or *via* cerebrospinal fluid and spillover from meningeal blood vessels. The analysis of single nucleus transcriptomes from both the frontal cortex and choroid plexus from autoptic samples of severe COVID-19, has shown

major neuropathological phenotypes (49). SARS-CoV-2 was not detected in the brain although earlier neuroinvasion could not be excluded. These findings indicate that, in COVID-19 patients, cells of the blood-CSF barrier respond to inflammatory signals generated in the periphery by SARS-CoV-2 infection (83), allowing peripheral T cell infiltration (49).

To determine the potential SARS-CoV-2 neurotropism, iPSCs-derived neural cells have been used for *in vitro* infection with SARS-CoV-2 taking advantage of iPSC plasticity to be reprogrammed towards mature neuronal cells both in monolayer cells and structured organoids. To dissect the cellular effects of SARS-CoV-2 infection on the brain, McMahon et al. reported that glial cells and cells of the choroid plexus expressed the entry receptor for SARS-CoV-2 angiotensin-converting enzyme 2 (ACE2) but did not detect viral replication or cell death fragmentation (84). The recent development of cortical organoids containing pericyte-like cells (PLCs), allowed the researchers to demonstrate that PLCs can serve as SARS-CoV-2 'replication hubs', sustaining viral invasion and spread to neighboring cells, including astrocytes (85). Indeed, a neuropathological study of post-mortem brain of COVID-19 patients found that astrocytes are the major site of SARS-CoV-2 infection and replication (86).

Strong evidence from both patients and experimental models indicate that human variants of SARS-CoV-2 could reach the CNS and target neurons, astrocytes, and microglia (87). The crosstalk between astrocytes and microglia plays a relevant role not only in the context of the local CNS inflammation but also in response to peripheral inflammation. In COVID-19 patients, neuroinflammation might arise and progress in response to the strong systemic cytokine storm observed in some patients, but also because of a CNS renin-angiotensin system dysregulation (87). Following SARS-CoV-2 infection of the brain, microglial cells get promptly activated, release an array of pro-inflammatory mediators, reactive oxygen species, and nitric oxide, recruit immune cells from the periphery and activate astrocytes (88–90).

SARS-CoV isolated from human specimens can infect C57/BL6 mice (91). Viral RNA was detected in the brain of infected mice up to 9 days post-intranasal infection while live virus could be isolated at later time point (9 to 15 days post-infection) (91). The virus was mainly localized in the hippocampus (91). Viral infection is associated with a strong neuroinflammatory response, which could either be induced by a direct viral infection of cells in the CNS or by the upregulation of peripheral cytokine levels. The activation of astrocytes and microglia in response to the elevation of peripheral cytokines is associated with a switch into a proinflammatory gene expression program, which could lead to increased blood-brain barrier permeability (87). Even if astrocytes and microglia may not be direct targets of viral infections, they can get activated in response to proinflammatory cues from endothelial cells, macrophages, and/or neurons, thus amplifying neuroinflammation. These data support the hypothesis that astroglia and microglia indeed play a relevant role in the development of the neurological symptoms observed in COVID-19 patients (87). However, the mechanisms by which the infected glia maintains the inflammatory reaction in the CNS remain to be addressed.

SARS-CoV-2 continuously evolves due to mutations that occur during replication of the genome. These mutations result in genetic variations of the circulating variants during the pandemic, which may spread more easily or show immune evasion and resistance to treatments. South Africa has witnessed the rapid emergence of SARS-CoV-2 variants. Some mutations in the C.1.2 lineage, a new lineage of the SARS-CoV-2 virus, have occured in other SARS-CoV-2 variants of concern. More data are being gathered to understand this new variant (National Institute for Communicable Diseases - NICD, 2021. Detection and frequency of the C.1.2 mutated SARS-CoV-2 lineage in South Africa.

https://www.nicd.ac.za/detection-and-frequency-of-the-c-1-2-mutated-sars-cov-2-lineage-in-south-africa/). This variant has not yet been investigated in terms of any effect on the brain and its cell types.

CONCLUSIONS AND FUTURE DIRECTIONS

Use of 3D Models to Study Infection of Neural Progenitor Cells

Human brain organoids derived from iPSCs recapitulate the developmental process of the fetal human brain. They represent a physiologically relevant model to dissect mechanisms of neurodevelopment and study neurological diseases. Indeed, only the use of 3D models has revealed virus-specific and complex immune system strategies, emphasizing the power of brain organoids over 2D systems in modeling viral infections (85, 92).

Congenital viral infections caused by TORCH pathogens are a major cause of fetal brain malformation (93). However, the mechanisms by which distinct TORCH pathogens influence fetal neurodevelopment is still not known. Krenn et al. (92) have shown that brain organoid modeling of ZIKV and herpes simplex virus (HSV-1) infections reveal distinct virus-specific responses causing microcephaly. Both viruses efficiently replicate in early-stage brain organoids and reduce their growth by inducing cell death. However, transcriptional profiling shows that ZIKV and HSV-1 induce specific cellular responses. While HSV-1 activates non-neural developmental programs and impairs neuroepithelial identity, ZIKV infection induces the activation of antiviral and stress-related pathways without affecting the organoid cytoarchitecture. Furthermore, the two viruses display different sensitivities to type I interferons, although they both induce a weaker type I interferon response in 3D compared to 2D models.

SARS-CoV-2 has been linked to a wide variety of neurological conditions (94). The virus can infect the human CNS, either directly or indirectly *via* elusive mechanisms, leading to the inflammation of blood vessels and ensuing clotting, seizures, strokes, and hemorrhages. Recent studies showed that the virus entry receptor ACE2 is poorly expressed in neural cells, but

highly expressed in brain pericytes, specialized cells that wrap around blood vessels and regulate immune cell entry to the CNS (95). Indeed, intranasal infection with SARS-CoV-2 induced a prompt hypoxic/ischemic-like pericyte response in the brain of transgenic mice expressing human ACE2 (95). Likewise, immunostaining of human brains demonstrated the presence of viral dsRNA in the vascular wall, perivascular inflammation, and a restricted loss of blood-brain barrier integrity (96). Since human brain organoids including only neural cells could not be infected with SARS-CoV-2, a human brain 3D model including also pericytes has been developed and shown to support the entry and infection of SARS-CoV-2 (85). This improved 3D model identified ACE2-expressing pericytes as one possible route of virus entry into the brain. Thus, pericytes can serve as a hub for SARS-CoV-2 amplification and spreading to other types of brain cells.

Antiviral Agents Protecting Neural Progenitor Cells

Heparin, a soluble derivative of heparan sulfate widely used as anticoagulant, has potentially attractive features including inhibition of binding and entry of the enveloped viruses, such as herpes simplex (HSV) (97, 98), human immunodeficiency (HIV) (99), SARS coronavirus (100), and influenza (H5N1) (101). The study of heparin effects on ZIKV infection of human NSCs showed that heparin fully prevented ZIKV-induced cell death, while minimally affecting viral replication (102). Moreover, the differentiation potential of NSCs into neuroglia was fully preserved upon heparin-treatment (103).

Indeed, heparin can be exploited as an antiviral agent offering a fast therapeutic option for present and future emerging viruses. In this regard, the activity of heparin against SARS-CoV-2 has been established using a few *in vitro* experimental models (104, 105). Importantly, heparin used in both therapeutic and prophylactic anticoagulant regimes reduced in-hospital mortality compared with untreated patients (106). As COVID-19 is a disease that continues to occur despite highly efficacious vaccines, several drugs, marketed for other therapeutic indications, have been re-purposed to treat COVID-19 patients, and antiviral strategies that include treatment with remdesivir or convalescent plasma have received emergency approval (107, 108). Despite promising results, the use of such treatments is limited, as they can only be delivered intravenously. Additional treatments are therefore required and, indeed, the first orally available antiviral drug against COVID-19, molnupiravir has been approved for use in the UK. There is, therefore, an urgent need to develop additional treatments to curtail morbidity and mortality caused by SARS-CoV-2.

AUTHOR CONTRIBUTIONS

All authors contributed to the preparation and revision of the manuscript. AI mainly contributed to the gliogenesis and viral infection sections. JP prepared the figures. GM mainly contributed to the neurogenesis section. EV mainly contributed to the viral infection of neural stem cells and novel therapies sections. PP-B contributed to the preparation and revision of the manuscript and the figures. All authors contributed to the article and approved the submitted version.

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How Does the Immune System Enter the Brain?

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Multiple Sclerosis (MS) is considered the most frequent inflammatory demyelinating disease of the central nervous system (CNS). It occurs with a variable prevalence across the world. A rich armamentarium of disease modifying therapies selectively targeting specific actions of the immune system is available for the treatment of MS. Understanding how and where immune cells are primed, how they access the CNS in MS and how immunomodulatory treatments affect neuroinflammation requires a proper knowledge on the mechanisms regulating immune cell trafficking and the special anatomy of the CNS. The brain barriers divide the CNS into different compartments that differ with respect to their accessibility to cells of the innate and adaptive immune system. In steady state, the blood-brain barrier (BBB) limits immune cell trafficking to activated T cells, which can reach the cerebrospinal fluid (CSF) filled compartments to ensure CNS immune surveillance. In MS immune cells breach a second barrier, the glia limitans to reach the CNS parenchyma. Here we will summarize the role of the endothelial, epithelial and glial brain barriers in regulating immune cell entry into the CNS and which immunomodulatory treatments for MS target the brain barriers. Finally, we will explore current knowledge on genetic and environmental factors that may influence immune cell entry into the CNS during neuroinflammation in Africa.

Keywords: blood-brain barrier, blood-cerebrospinal fluid barrier, immune cell trafficking, arachnoid barrier, multiple sclerosis

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Abbreviations: aEAE, Active EAE; 2P-IVM, Two-photon intravital microscopy; AJs, Adherens junctions; APC, Antigen presenting cell; AQP4, Aquaporin 4; BBB, Blood-brain barrier; BCSFB, Blood-cerebrospinal-fluid barrier; ChP, Choroid plexus; CLDN, Claudin; CNS, Central nervous system; CSF, Cerebrospinal fluid; E-cadherin, Epithelial cadherin; ICAM-1, Intercellular adhesion molecule 1; JAMs, Junctional adhesion molecules; LFA-1, Lymphocyte function-associated antigen 1; NVU, Neurovascular unit; PECAM-1, Platelet endothelial cell adhesion molecule 1; PSGL, P-selectin glycoprotein ligand; STH, soil-transmitted helminths; TJs, Tight junctions; VCAM-1, Vascular cell adhesion molecule 1; VE-cadherin, Vascular endothelial cadherin; ZO, Zona occludens.

INTRODUCTION

The human immune system has evolved to protect the body from microbial pathogens and trauma and thus ultimately to ensure host survival in a hostile environment (1). The skin as the outer body surface and the gut and respiratory tracts as the inner body surfaces are the most exposed sites for infection and injury. Their epithelial linings form highly specialized antimicrobial barriers towards the outside and are further fortified by site-specific immune defense mechanisms established by cells of the innate and adaptive immune system [summarized in (2)]. Melanization of the skin has been recognized as an essential component of skin innate immunity with melanocytes and melanin exerting antimicrobial functions [summarized in (3)]. Microbial or traumatic injury elicits a rapid stereotypic activation of tissue-resident innate immune mechanisms that allow for the killing of the microbes and the resolution of the inflammatory response. The innate immune response includes activation of tissue-resident dendritic cells (DCs), which will take up and process the antigens and travel *via* the afferent lymphatic vessels to the tissue-draining lymph nodes leading to activation of T and B lymphocytes and thus the adaptive immune response and immune memory against the specific microbes to provide an accelerated and amplified immune responses in case of a further encounter with the same antigen. During their priming, naïve lymphocytes are imprinted with navigation programs (expression of a combination of adhesion and chemoattractant receptors) that ensure their site-specific homing. In this context, DCs in gut and skin draining lymph nodes have been shown to play an essential role as they process food-derived vitamin A and ultraviolet-induced vitamin D3, respectively, to imprint gut homing and skin homing trafficking programs as well as site-specific effector functions in naïve lymphocytes summarized in (4)]. Skin complexion, sunlight exposure and dietary patterns will thus have a direct impact on immune cell priming. These site-specific effector functions i.e., production of cytokines, killing of infected tissue cells, and antibody production, ensure elimination of the injurious agent and reconstitution of tissue function and also establish a site-specific cellular immune memory with tissue-resident memory T (TRM) cells (5). Immune surveillance of a given tissue thus relies on drainage by lymphatic vessels to transport antigens and antigen-presenting DCs to the draining lymph nodes, as well as on blood vessels to allow for efficient immune cell trafficking to the respective tissues.

The anatomical location of the CNS within the skull and vertebral column provides robust protection from injury from the outside. Unless there is a penetrating injury, pathogens are thus unlikely to directly reach the CNS, unless they have escaped the innate and adaptive immune defense mechanisms at the outer surfaces of the body. However, the CNS resides behind blood-brain barriers that restrict pathogen and immune cell entry from the periphery into the CNS parenchyma and lacks lymphatic vessels. The CNS thus has a unique relationship with the immune system that differs from that of peripheral organs and is referred to as CNS immune privilege. The discovery of CNS immune privilege is based on the observation that foreign tissues, when grafted to peripheral sites like the skin, are readily rejected, but when grafted into the brain parenchyma, they survive for prolonged durations (6). These organs, in which experimentally implanted tissue grafts are incapable of provoking immunity leading to graft rejection, have since then been referred to as "immune privileged organs" (summarized in (7). CNS immune privilege also extends to innate immune responses as neither injection of bacterial products (8), nor experimental induction of cell death within the CNS parenchyma (9, 10) elicits a rapid infiltration of myelomonocytic cells as observed during the stereotypic innate immune response to such stimuli in peripheral organs (11).

Based on these observations, CNS immune privilege was originally thought to be based on "immune ignorance" where lack of lymphatic vessels and the endothelial blood-brain barrier (BBB) would inhibit the afferent and efferent arm of CNS immunity, respectively [summarized in (12)]. However, the observations that tissue grafts when transplanted into the cerebral ventricles were readily rejected (13, 14) and that foreign tissue grafts transplanted into the brain parenchyma of animals that had previously rejected a skin tissue graft of the same donor were readily destroyed (6) questioned this concept. Observations demonstrating that activated circulating T cells can cross the BBB in the absence of neuroinflammation [summarized in (15)] and that tracers injected into the cerebrospinal fluid (CSF) drain into the deep cervical lymph nodes (16) finally provided direct evidence for afferent and efferent connections of the CNS with the immune system and asked for revisiting the concept of CNS immune privilege. Recent advancements in the establishment of reporter mouse models combined with epifluorescence, near-infrared (NIR), and two-photon (2P) intravital microscopy (IVM) have led to the rediscovery of lymphatic vessels within the dura mater and their contribution to CSF drainage into the deep cervical lymph nodes and the proposal of a "glymphatic system" ensuring efficient mixing of CNS interstitial fluid (ISF) with the CSF (summarized by (17). These observations have led to questioning the existence of CNS immune privilege.

We have proposed that CNS immune privilege does exist but requires proper consideration of the special anatomy of the CNS and especially of the localization and function of the different brain barriers, which divide the CNS into compartments that differ with respect to their accessibility to mediators and cells of the innate and adaptive immune system (12). In this concept, the CNS parenchyma is immune privileged, allowing it to prioritize the proper function of neurons over eliciting an immune response, while the CNS ventricular spaces and border compartments (subarachnoid and perivascular spaces) are dedicated to CNS immunity and thus lack full CNS immune privilege.

THE BRAIN BARRIERS

Under physiological conditions, the meningeal, endothelial, epithelial, and glial brain barriers maintain CNS homeostasis

by protecting the CNS parenchyma from the constantly changing milieu of the bloodstream (Figure).

The Leptomeningeal Blood-Cerebrospinal Fluid (CSF) Barrier

The meningeal layers are the dura mater, the arachnoid mater, and the pia mater and cover the entire surface of the brain, and spinal cord (**Figure 1A**) and are mainly composed of fibroblasts (18–20). The dura mater is the outermost layer and is directly attached to the skull. Blood vessels in the dura mater lack a BBB and are thus different from those of the CNS proper (12). Along the superior and transversal sagittal sinuses, the dura mater also harbors lymphatic vessels suggested to drain antigens and immune cells from the CNS (21–23). This would require breaching the arachnoid mater below the dura mater, which establishes a bona fide blood– cerebrospinal fluid barrier (BCSFB) between the dura mater and the CSF filled subarachnoid space (SAS). The arachnoid fibroblasts are

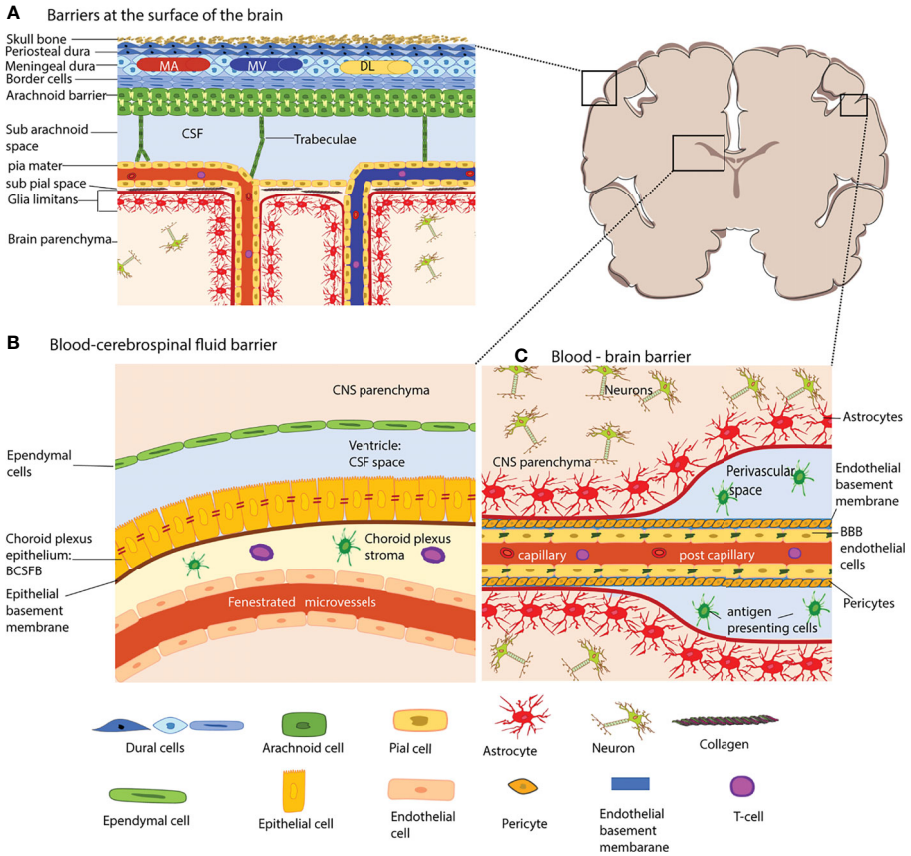

FIGURE 1 | The brain barriers. The schematic coronal brain section depicts the localization of the different brain barriers shown in **(A–C)**. **(A)** Barriers at the surface of the human brain. The meninges are composed of three layers, the dura mater, the arachnoid barrier, and the pia mater. The dura mater is directly connected to the skull bone. In humans, the dura mater is composed of three layers, the periosteal dura, the meningeal dura and the dural border cells. The dura mater has its own network of arteries (MA), veins (MV) and dural lymphatics (DL). The arachnoid barrier is formed by arachnoid fibroblasts which are connected by tight junctions and form a bona fide blood-cerebrospinal fluid barrier (BCSFB) – the arachnoid barrier – between the dura mater and the CSF filled subarachnoid space. Arachnoid trabeculae formed by a collagen core that is ensheathed by arachnoid and pial fibroblasts cross the SAS towards the pia mater and to the leptomeningeal blood vessels. The fibroblasts of the pia mater cover the veins and arteries in the SAS and separate the SAS from the subpial space filled with collagen bundles. The pia mater reflects of the surface where arteries dive into the brain parenchyma and at the same time ensheathes the arteries entering the brain. The glia limitans forms a barrier at all surfaces of the CNS parenchyma, this is the outer surface (*glia limitians superficialis*) and the perivascular surfaces (*glia limitans perivascularis*). **(B)** The blood–CSF barrier of the choroid plexus (ChP). The ChPs are localized in all four ventricles of the brain. The ChP epithelial cells are connected by unique parallel running tight junction stands and establish a BCSFB. The ChP stroma harbors dendritic cells and macrophages and the blood vessels of the ChP are fenestrated. **(C)** The blood–brain barrier (BBB) is formed by highly specialized microvascular endothelial cells connected by complex tight junctions. The endothelial basement membrane harbors a high number of pericytes. At the level of capillaries the endothelial basement membrane and the parenchymal basement membrane of the glia limitans merge. However that the post-capillary venule level they leave a small gap where single antigen-presenting cells can be found. The microvessels are surrounded by the glia limitans, which is composed of the parenchymal basement membrane and astrocyte end-feet. The extravasation of immune cells into the CNS parenchyma occurs at the level of postcapillary venules and thus involves crossing two barriers, the endothelial BBB and after reaching the perivascular space subsequent crossing of the glia limitans. The shapes of the cell types were adapted from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Common Attribution 3.0 Generic License.

connected by tight junctions (24–28) prohibiting free diffusion of solutes and water-soluble molecules across this barrier and also express efflux pumps ensuring transport of toxic metabolites out of the CSF (29).

The arachnoid trabeculae are mainly composed of collagen fibers and fibroblasts that add rigidity to the arachnoid barrier allowing to form a prominent SAS (30). The pia mater is formed by a single layer of flattened fibroblasts covering the surface of the brain and the spinal cord. The cells of the pia mater do not form tight junctions, thus making them permeable to solutes while however still limiting the passage of cellular elements like erythrocytes (31). Additionally, the pia mater sheathes all blood vessels in the SAS and does separate the SAS from the perivascular spaces by reflecting off the surface of the brain (31–33).

The Glia Limitans

The glia limitans envelops the brain and spinal cord parenchyma's entire surface and the perivascular spaces. The glia limitans is composed of a parenchymal basement membrane produced by astrocytes and by astrocyte endfeet (12). In the healthy CNS, the polarized expression of the water channel aquaporin 4 (AQP4) in astrocyte endfeet regulates water transport at this barrier. In addition, astrocyte endfeet are joined together by gap junctions that allow for communication between the astrocytes (34). In the healthy CNS, the glia limitans provides a barrier for immune cells scanning the subarachnoid and perivascular spaces and prohibits their uncontrolled entry into the CNS parenchyma (35, 36).

The Endothelial Blood-Brain Barrier (BBB)

The endothelial BBB forms a barrier between the blood and the CNS. It is established by brain microvascular endothelial cells that are joined together by continuous and complex tight junctions which inhibit free paracellular diffusion of solutes and water-soluble molecules (37, 38) (**Figure 1C**). Combined with the low vesicular activity of BBB endothelial cells that prohibits uncontrolled transcellular diffusion, the BBB establishes a physical barrier for solutes and water-soluble molecules. Expression of specific enzymes, transporters, and efflux pumps make BBB endothelial cells biochemically unique and ensure the transport of nutrients into the CNS and toxic metabolites out of the CNS (38). BBB tight junctions are composed of the transmembrane proteins claudin-5, occludin, and members of the junctional adhesion molecules (JAM). While claudin-5 establishes a diffusion barrier for small molecules (39), occludin regulates calcium movement across the BBB and in addition to TJ stability and barrier function (40). JAM-A, JAM-B and JAM-C have been described in the brain microvascular endothelial cells and have been suggested to play a role in regulating the BBB stability by some but not others (41, 42). Members of the JAM family may however play a role in immune cell migration across the BBB (43, 44). Prerequisites for TJ formation are adherens junctions (AJs), and thus, in addition to their unique TJs, BBB endothelial cells display regular endothelial AJs [summarized in (37)]. VE-cadherin is the main transmembrane protein of the endothelial AJs and keeps neighboring cells attached by homophilic interactions (45). Additional transmembrane proteins localized to BBB cell-to-cell junctions are the platelet endothelial cell adhesion molecule-1 (PECAM-1) that contributes to vascular integrity (46) and CD99 which mediates leukocyte trafficking across the BBB (47).

The unique BBB phenotype in CNS microvascular endothelial cells is not intrinsic to the endothelial cells but relies on the continuous cross-talk with cellular and acellular elements surrounding the CNS microvascular endothelium forming the neurovascular unit (NVU). BBB endothelial cells produce the endothelial basement membrane composed of type IV collagen, α4 and α5 laminins (36). Additionally, a high number of pericytes is embedded in the endothelial basement membrane at the level of capillaries and possibly post-capillary venules, while smooth muscle cells form the mural cell population in arterioles and possibly venules (48). The CNS blood vessels are always separated from the CNS parenchyma proper by the glia limitans. The parenchymal basement membrane, which is secreted by the astrocytes, is with the expression of a1 and a2 laminin molecularly distinct from the endothelial basement membrane (36, 49, 50). At the capillary level, the parenchymal basement membrane fuses with the endothelial basement membrane bringing the astrocyte endfeet in close proximity to capillary pericytes and endothelial cells. At the level of the post-capillary venules, the two basement membranes detach to form a small perivascular space (51).

The Choroid Plexus and the Blood-Cerebrospinal Fluid Barrier (BCSFB)

The choroid plexus (ChP) extends into all four brain ventricles and is surrounded by epithelial cells that form a blood-cerebrospinal fluid barrier (BCSFB) (52) (**Figure 1B**). The ChP produces CSF and ChP epithelial cells are characterized by the expression of a particular combination of transporters (53). Paracellular diffusion across the ChP BCSFB is prohibited by unique tight junctions composed of claudin-1, -2, -3, and -11, occludin, and JAM-A and the scaffolding proteins ZO-1, -2, -3 (54). The capillaries in the choroid plexus stroma are fenestrated and thus allowing for free diffusion of blood-borne molecules into the ChP stroma. The ChP stroma harbors numerous cells of the innate but also the adaptive immune system (55). Furthermore, on the apical side of the ChP epithelial cells, epiplexus or Kolmer cells perform immune surveillance.

THE ROLE OF THE INDIVIDUAL BRAIN BARRIERS IN REGULATING IMMUNE CELL ENTRY INTO THE CNS

Immune Cell Trafficking Across the Endothelial Blood-Brain Barrier (BBB)

CNS immune surveillance has been shown to be ensured by peripherally activated circulating T cells that have the specific ability to cross the BBB to reach perivascular or subarachnoid spaces in the absence of neuroinflammation (12, 56). It should be noted that while immune cells trafficking occurs at the level of

CNS post-capillary venules, transport of nutrients occurs at the level of CNS capillaries (57). This allows immune cells to reach perivascular or subarachnoid space, where they can encounter tissue resident antigen-presenting cells (APCs), like border associated macrophages (BAMs). Recognition of their cognate antigen on these CNS border associated APCs leads to local reactivation of T cells and is the prerequisite for subsequent T-cell migration across the glia limitans into the CNS parenchyma (58, 59).

Leucocyte extravasation is usually a multi-step process where after an initial tether or capture on the endothelium, selectins and their ligands allow immune cells to roll along the endothelium, reducing their speed and next recognize with their G-protein coupled receptors (GPCRs) chemotactic cues on the endothelium leading to their subsequent integrin-mediated arrest and crawling and finally their diapedesis across endothelial barrier (60). The unique barrier characteristics of the BBB extend to its characteristic immune quiescent phenotype. In contrast to peripheral endothelial cells, BBB endothelial cells lack storage of P-selectin protein in their endothelial Weibel Palade bodies [summarized in (61)] and constitutive expression of the atypical chemokine receptor 1 (ACKR1), which transports chemokines from the abluminal to the luminal surface of endothelial cells (62, 63). Thus, immune cell entry into the CNS is very low and limited to activated T cells that do not depend on these trafficking cues. Indeed, activated CD4 T cells were shown to be able to capture *via* α4-integrins on CNS endothelial VCAM-1 (64) and following LFA-1 dependent adhesive interactions to cross the BBB in the absence of neuroinflammation (65–67) (**Figure 2**).

At onset of neuroinflammation, T cells have been shown to cross venules in the subarachnoid space and crawl within the subarachnoid space, where they can also be washed off with the CSF (58).

During neuroinflammation, *de novo* expression of trafficking molecules like P-selectin and ACKR1 allow for increased immune cell entry into the CNS. The interaction between the P-selectin glycoprotein ligand (PSGL)-1 on T cells and E- and P-selectin on the BBB allows for tethering and rolling of activated CD4 T cells along the luminal side of inflamed spinal cord microvessels (68, 69). Rolling on the BBB allows T cells to interact with chemokines displayed on proteoglycans on the luminal surface of the endothelial cells *via* their specific GPCRs or possibly on ACKR1, leading to an inside-out-activation of integrins mediating the firm arrest of the immune cells on the luminal surface of the inflamed BBB endothelial cells (61). The interaction between the integrins LFA-1 and very late antigen-4 (VLA-4, α4β1 integrin) on the T cells and their endothelial ligands, ICAM-1 and VCAM-1, respectively mediates the firm adhesion of T cells to the BBB (70, 71). After their arrest, the T cells polarize and were observed to crawl over extended distances against the direction of the bloodstream on endothelial ICAM-1

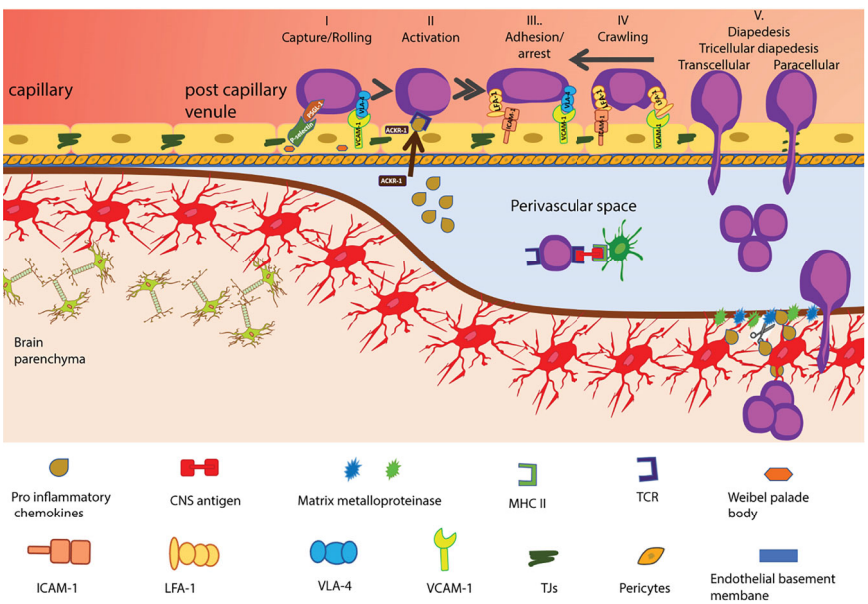

FIGURE 2 | Multi-step T-cell extravasation across the BBB during neuroinflammation. Multi-step T cell extravasation across the BBB occurs at the level of CNS post capillary venules. During inflammation, the rolling of activated T-cells on the BBB endothelial cells is mediated by P-selectin and a4-integrins. After their GPCR-dependent arrest, T cells crawl on the BBB endothelium against the direction of blood flow. High levels of endothelial ICAM-1 and *de novo* expression of ACKR1 that can shuttle CNS chemokines across the BBB promote transcellular diapedesis of T cells while low levels of endothelial ICAM-1 direct T cells mainly to tricellular and bicellular junctions, i.e. paracellular sites of diapedesis. Once T cells have crossed the BBB endothelium they reach the perivascular space. The CNS-antigen-specific T cells may recognize their cognate antigens on perivascular APCs and become reactivated and start to proliferate. The change in local cytokine milieu leads to induction of matrix metalloproteinases -2 and -9 which cleave extracellular matrix receptors on astrocyte endfeet, allowing for T-cell passage across the glia limitans. Once in the CNS parenchyma, T cells induce tissue injury and clinical disease symptoms start to appear. The shapes of the cell types were adapted from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Common Attribution 3.0 Generic License.

and ICAM-2, obviously to find rare tricellular junctions as sites permissive for diapedesis across the BBB endothelium (58, 66, 67). Under neuroinflammatory conditions, high cell surface levels of endothelial ICAM-1 and *de novo* expression of ACKR-1 were shown to reduce T cell crawling distances and increase transcellular T-cell diapedesis across the BBB (62, 66). Importantly, although the BBB junctions become leaky under neuroinflammatory conditions and allow for uncontrolled diffusion of blood-borne molecules across the BBB, this is not accompanied by increased paracellular T cell diapedesis but rather leads to enhanced transcellular T cell diapedesis across the BBB. This underscores that the mechanisms that regulate the junctional integrity of the BBB are distinct from those regulating the cellular pathway of T cell diapedesis across the BBB.

In contrast to CD4 T cells, the molecular mechanisms involved in the multi-step migration of other immune cell subsets across the BBB are less well understood but are distinct from those of CD4 T cells. Although CD8 T cells also rely on α4-integrins to cross the BBB, they show enhanced dependence on LFA-1 to mediate shear resistant arrest and engage in addition endothelial JAM-B (72–76) which is not required for CD4 T cell diapedesis across the BBB (41). Also, although α4-integrins seem to be involved in the migration of most immune cell subsets across the BBB, the precise molecular mechanisms involved in every step of the multi-step extravasation of B cells (77, 78) or innate immune cells such as neutrophils (79), monocytes (75, 80–82) and dendritic cells (83–86) to cross the BBB, are not yet fully understood.

Additionally, several studies have proposed that other molecules such as the activated leukocyte cell adhesion molecule (ALCAM) (80, 87) and the melanoma cell adhesion molecule (MCAM) (88) as well as the nerve injury-induced protein (ninjurin-1) (89) might play a role in the migration of T-cells across the BBB during EAE and MS (76). Future studies to determine the precise role of these molecules in immune cell trafficking across the BBB still need to be done.

Immune Cell Trafficking Across the Glia Limitans

In experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), clinical symptoms start only upon immune cells crossing the glia limitans and reaching the CNS parenchyma (41, 90). This underscores that immune cell entry into the CNS is fundamentally different from that in peripheral tissues and involves two sequential and differentially regulated steps of crossing an outer brain barrier followed by progression across the glia limitans into the CNS parenchyma proper (91).

Under normal physiological conditions, the glia limitans act as a barrier for migrating immune cells by preventing their entry into the CNS parenchyma (52). During neuroinflammation, when BBB integrity is impaired, reactive astrocytes form tight junctions aiming to prohibit the parenchymal entry of humoral and cellular factors from the bloodstream (92). Nevertheless, it has also been observed that under neuroinflammatory conditions, such as during MS or its animal model EAE, immune cells first form a perivascular cuff around post-capillary venules and then can cross the glia limitans and infiltrate the CNS parenchyma initiating an onset of neurological symptoms (91). This process is mediated by local TNF-induced expression and activation of matrix metalloproteinase (MMP)-2 and MMP-9, which allow for cleavage of a-dystroglycan, an extracellular matrix receptor of astrocyte endfeet, and modulation of chemokines, thus enabling T-cell migration across the perivascular glia limitans into the CNS parenchyma (90, 93). *In vivo* imaging studies have provided ample evidence that T- cells can cross the walls of the leptomeningeal veins to reach the SAS (94). If this allows for their subsequent migration across the glia limitans on the surface of the brain and spinal cord into the CNS parenchyma is still a matter of debate.

Immune Cell Trafficking Across the Leptomeningeal Arachnoid Barrier

The role of the arachnoid barrier in regulating immune cell entry into the subarachnoid space is not well investigated. A recent study described the downregulation of claudin-11 in arachnoid barrier cells during EAE and MS. In EAE, the authors detected accumulation of T-cell infiltrates specifically in regions of the spinal cord associated with loss of claudin-11 immunostaining of arachnoid barrier fibroblast (95). This establishes a correlation with impairment of arachnoid barrier fibroblast TJs and CNS immune cell infiltration.

Recent studies have furthermore proposed that the dura mater harbors immune cells dedicated for CNS immune surveillance and directly sourced from nearby bone marrow cavities (96–98). Vice versa, it has been suggested that immune cells can readily reach the dural lymphatics from the subarachnoid space (22, 99).

Furthermore, the accumulation of B-cell follicles observed in the subarachnoid space of post mortem brain samples from progressive MS patients has ignited a discussion on the role played by the meninges in MS pathogenesis (100, 101). These meningeal B cell clusters have originally been described in EAE (102) and recent studies have suggested that these B-cells originate from the dura mater (97, 98) and may or may not migrate from the calvaria to the dura mater through specialized vascular channels traversing the inner skull bone. None of these studies has, however, integrated consideration of the arachnoid barrier which establishes a blood-CSF barrier between the dura mater and the subarachnoid space. Thus, it remains to be shown if the arachnoid barrier is a barrier for immune cell passage into the CNS during immunosurveillance and neuroinflammation.

Immune Cell Trafficking *via* the Choroid Plexus

The ChP has been proposed as an alternative CNS entry site for immune cells reaching the CSF-filled space during immunosurveillance and in neuroinflammation (55). The ChP microvessels do not form a BBB and have a phenotype rather resembling that of peripheral endothelial cells with e.g., constitutive storage of P-selectin in Weibel Palade bodies (103). To reach the CSF, immune cells would need to cross the BCSFB ensheathing the ChP stroma. Adhesion molecules such as ICAM-1 and VCAM-1 are expressed at the luminal surface of ChP epithelial cells, and *in vitro* studies have provided evidence

that T cells can cross the monolayers of ChP epithelial cells from the abluminal to the luminal side with a contribution of epithelial ICAM-1 during the final step of diapedesis and release into the CSF space (104).

CSF from healthy individuals or individuals with non-neuroinflammatory disorders harbors tissue memory CD4+ T helper cells and CD8+ T cells (105, 106). It has been proposed that CSF T cells cross fenestrated capillaries of the ChP in a P-selectin-dependent manner to reach the ChP stroma (103). From there, at least Th17 cells expressing the chemokine receptor CCR6 were suggested to cross the BCSFB expressing the CCR6 ligand CCL20 in a CCR6/CCL20-dependent manner (107). Direct evidence for the migration of T cells from the ChP across the BCSFB *in vivo* awaits application of recently developed advanced imaging methodologies of the ChP (108). It has also been proposed that rather than crossing the BBB, immune cells exit the ChP stroma at the base of the ChP where it folds out from the ventricular wall. The BCSFB basement membrane was proposed to be in direct continuation with the parenchymal basement membrane of the glia limitans superficialis (53), allowing immune cells to crawl along the basement membranes reaching the SAS of the brain. Future studies on the precise anatomy of the base of the ChP are necessary to explore this potential immune cell entry route into the CNS.

Immune Cell Entry Into the CNS in Autoimmune Disease

MS is considered a prototypic organ-specific autoimmune disease targeting the CNS characterized by inflammatory lesions, brain barriers breakdown, demyelination, and axonal damage. The etiology of MS and its pathogenesis is not fully understood, and environmental and genetic factors have been shown to play a vital role in the development of MS. Many MS-associated genetic variants code for molecules related to the proper function of the immune system is consistent with the concept of MS as a T cell-mediated autoimmune disease of the CNS. Further support for a T-cell mediated autoimmunity in MS is derived from its animal model, experimental autoimmune encephalomyelitis (EAE), where neuro-antigen specific autoreactive CD4 T cells infiltrate the CNS and cause CNS pathology resembling that of MS (109).

Histopathologically, active lesions in early MS are characterized by focal white matter demyelination accompanied by perivenular immune cell infiltrates forming a typical perivascular cuff and consisting mainly of CD8 T cells, CD20 B cells, and plasmablasts as well as macrophages (110, 111). Immune cell trafficking to the CNS is thus central to MS pathogenesis and has been recognized as the therapeutic target for the treatment of MS.

MS THERAPIES TARGETING IMMUNE CELL TRAFFICKING TO THE CNS

The options for the treatment of relapsing-remitting MS have significantly grown during the last years. These disease-modifying treatments (DMTs) have in common that they target specific actions of the immune system and come with different side effects. Only few DMTs directly target immune cell trafficking to the CNS. Natalizumab is a humanized function blocking monoclonal antibody binding to the α4-integrin subunit of α4β1-(VLA-4) and α4β1–integrin on the immune cell surface. *In vivo* imaging studies in experimental animals have shown that Natalizumab blocks α4- integrin mediated capture on CNS endothelial VCAM-1 in the absence of neuroinflammation (64) as well as sustained adhesion on inflamed BBB endothelium (112) and thus prohibits T cell migration across the BBB (113). This leads to the reduction of CNS inflammatory lesions with BBB breakdown as well as reduced numbers of CD4 and CD8 T cells detected in the CSF of MS patients (114).

The sphingosine phosphate 1 receptors (S1PR) S1PR1, S1PR3, S1PR4, and S1PR5 are expressed on many cell types including lymphocytes and the BBB and have been shown to be involved in the regulation of many biological processes including lymphocyte trafficking and vascular permeability. Four S1PR modulators, namely fingolimod, siponimod, ozanimod and ponesimod) are currently approved for the treatment of MS (summarized in (115). As S1P signaling is required for the egress of CCR7 expressing lymphocytes from lymph nodes, S1PR modulators trap naïve and central memory cells in lymph nodes while $CCR7^{neg}$ effector memory (T_{em}) and effector memory recently activated T cells (T_{EMRA}) are not affected (116–118). The resulting lymphopenia and change in composition of circulating lymphocytes is thought to reduce immune cell trafficking into the CNS and is considered the main therapeutic effect of the S1PR modulators in MS. At the same time other effects including a direct effect on the BBB remains to be investigated (113).

In addition to their direct effects on immune cell trafficking glucocorticoids have been described to stabilize adherens and tight junctions of the BBB by upregulating expression of VE-cadherin and occludin and claudin-5 in brain endothelial cells (119). Similarly, interferon-beta has been proposed to restore barrier properties of the BBB which will eventually influence immune cell trafficking into the CNS (120).

GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING MS IN AFRICA

MS prevalence is increasing worldwide and shows a heterogeneous distribution globally, with the highest prevalence in Europe and North America (121, 122). In Africa, although MS has not been widely studied, epidemiological reports have shown a diverse distribution of the disease with a higher occurrence in North Africa as compared to Sub-Saharan Africa (122, 123). With the unknown etiology of MS and the complex interplay between genetic and environmental factors involved in disease pathogenesis has been proposed to be necessary for MS development. Most epidemiological studies focusing on the impact of genetic and environmental risk factors on MS development were performed in Caucasian populations with a representation of 85-99% of the population, while with a representation of 56% the African

population is under represented (121). Very little information is available on the African populations, which have great genotypic and phenotypic variability, but several studies have shown that being a member of the African population is itself a risk factor in developing a severe course of the disease. This was proven throughout many studies based on different parameters of severity evaluation of the disease ranging from disability scores, radiological activity or even atrophy (124–127).

GENETIC FACTORS

Genome-wide association studies (GWAS) identified many single nucleotide polymorphisms (SNPs) in genes coding for molecules regulating functions of the immune system (128), which is consistent with the concept of MS being a T-cell mediated autoimmune disease targeting the CNS. There is, however, an overrepresentation of immune cells in the transcriptional, epigenetic and pathway analysis datasets used in the GWAS studies to interpret the relevance of SNPs to MS susceptibility, which naturally favors identification of MS risk factors associated with immune cells. Inclusion of CNS datasets in GWAS is just emerging (129). which may allow to discover additional risk factors outside of the immune system. To this end in Caucasian populations, the most vital genetic link to MS has been found in MHC haplotypes, especially those containing *HLA-DRB1*15.01*, *HLA DQB1*06.02*, and *DQA1*01.02* (130). The few studies in black Africans have revealed a diverse distribution in the *HLA-DRB1 and -DQB1* loci expression. For instance, a study in Morocco showed a positive association between the HLA-DRB1-15 and the genetic predisposition to MS in a Moroccan population of MS patients (131). This gene has been reported to play a role in immunity, a study showed that in HLA-DR1-15 positive patients, Th1 lymphocytes auto-proliferate in an elevated way and leading to the binding and presentation of CNS antigens to T cells (132). African ethnic groups that have a higher distribution of these alleles are protected against parasitic infections like malaria but are at higher risks of developing autoimmune diseases like MS (133–135). Individuals lacking expression of the atypical chemokine receptor 1 (ACKR1), formally referred to as DARC (Duffy blood group antigen receptor for chemokines) are for example resistant to malaria. ACKR1 mediates inflammatory chemokine shuttling across the BBB and enhances transcellular T-cell diapedesis across the BBB during EAE (62, 63). Lack of ACKR1 ameliorates development of EAE and it remains to be shown if individuals lacking functional ACKR1 are protected from MS. Alternatively, also different ACKR1 haplotypes could affect susceptibility to MS. To this end over 900 ACKR1 haplotypes were identified (136). There is in fact evidence that a strong selective pressure for malaria resistance in the Ethiopian population correlates with the development and maintenance of certain *ACKR1* haplotypes (137). A correlation of ACKR1 haplotypes with susceptibility to MS has not yet been investigated.

There is also first studies highlighting polymorphisms in adhesion molecules, e.g. for ICAM4, among African ethnicities (138). It will be interesting to see if polymorphisms in adhesion molecules involved in MS pathogenesis may influence susceptibility to MS in the African population.

Moreover, studies have shown that color tones of the skin influence MS pathogenesis. In two population-based case control studies done in Australia, the researchers assessed the skin phenotype spectrophotometrically by measuring the melanin density of the skin at the upper inner arm and buttock aiming for body sites that are usually not exposed to sunlight. Both studies assessed the association between the skin phenotype and likelihood of developing MS. They concluded that people with a pale skin had a 32.4% increase of developing first demyelinating events. Additionally, low melanin density at the buttock and fair skin were associated with earlier onset of disease. Suggesting that pale-skinned people have a higher risk of developing MS and show earlier MS symptoms as compared to people with black skin (139, 140).

ULTRAVIOLET RADIATION AND VITAMIN D LEVELS

The prevalence of MS increases directly proportional to an increase in distance from the equator. Several studies have confirmed the association between lower sun exposure with lower Vitamin D levels and the increased risk of developing MS. Considering its geographical location, African countries experience more sun exposure during the year compared to other continents (141). Although there are no studies assessing the impact of sun exposure on MS development in African countries, the observed low prevalence of MS in African countries might be due to increased sun exposure.

There is ample epidemiological evidence implicating lack of Vitamin D as a risk for the development of MS. Vitamin D interacts with its specific receptor expressed by all immune cells that influence the transcription rate of Vitamin D responsive genes resulting in strong immunoregulatory effects (142). In addition, in skin-draining lymph nodes DCs metabolize Vitamin D to imprint trafficking and effector programs in naïve T cells (143) (**Figure 3**).

Seasonal differences in MS activity have also been reported. The predicted correlation between sun exposure and increased levels of vitamin D would suggest higher disease activity during low sun exposure seasons such as fall and winter (144). However, recent studies have rather suggested the opposite, namely that disease activity increases during spring and summer (145). Studies from Africa have shown that the majority of the African population have low levels of Vitamin D (146–148). These findings contradict that lower prevalence of MS in Africa is correlated to Vitamin D and rather suggests that immunomodulatory effects of Vitamin D and their potential impact on immune cell trafficking need further investigation (149). Furthermore, many of the reported therapeutic essays of vitamin D supplementation for MS patients still do not prove any efficacy on the EDSS score or annual relapses rate (150). Also studies from us in African MS patients supplemented with high dosage Vitamin D did not now show a significant association between Vitamin D levels and MS status (148).

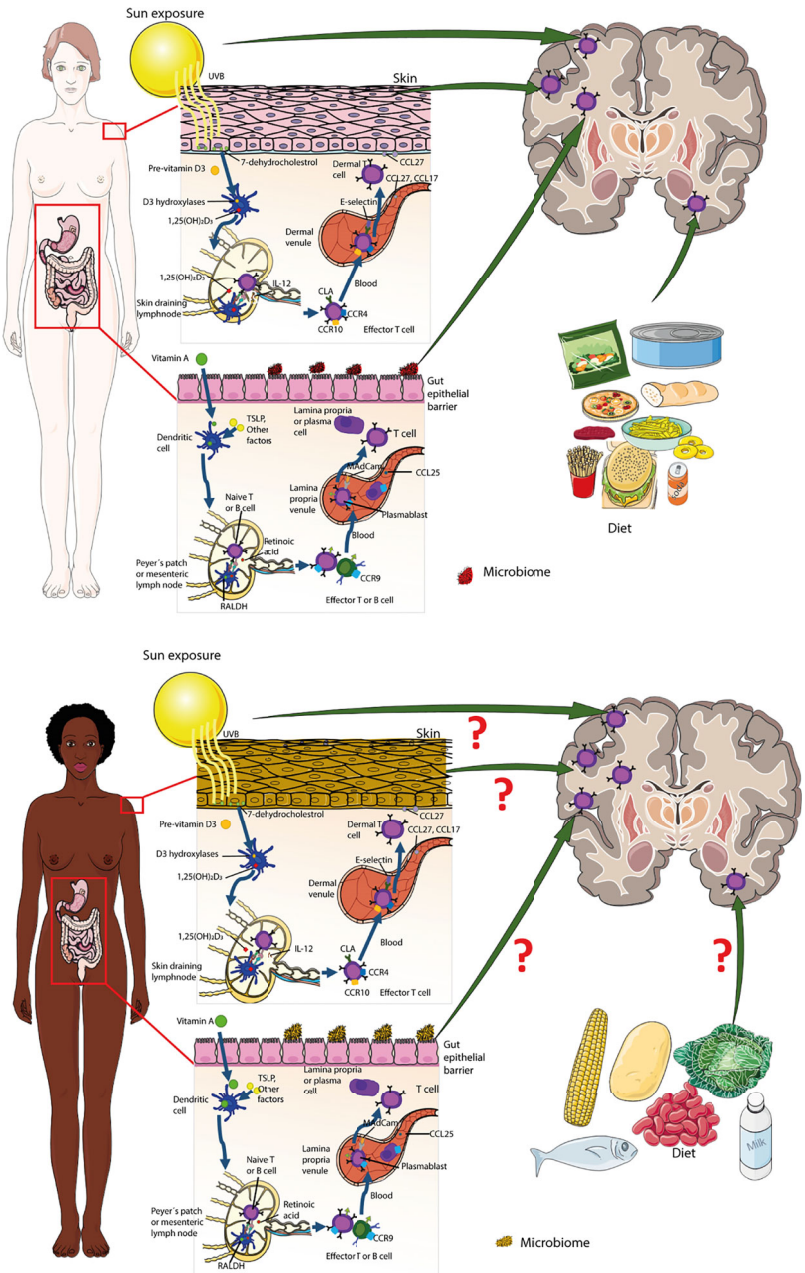

FIGURE 3 | Genetic and environmental factors influencing immune cell entry into the brain. Encounter of microbes takes place at the inner and outer surfaces of the body equipped with special barrier forming epithelia and innate immune cells residing behind these barriers. Priming of T cells in skin and gut-draining lymph nodes imprints their effector function, i.e. expression of trafficking molecules. Pale-skinned people have a higher risk of developing MS as compared to people with black skin. The schematic representation shows imprinting of trafficking properties in T-cells primed in the skin and the gut (adapted from (2), chapter 14). Experimental animal studies have shown that autoagressive T cells primed in skin-draining lymph nodes express CXCR6 and can enter the CNS white and grey matter, while when these T cells are primed in gut-draining lymph odes they express P2rx7 and only infiltrate CNS white matter. How skin color and the gut microbiome of the African population affects T cell priming and their CNS homing properties remains to be shown. The shapes of the cell types were adapted from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Common Attribution 3.0 Generic License.

INFECTIONS

In genetically MS predisposed individuals, studies have shown that microbial infections can act as environmental triggers in inducing or promoting the onset of clinical signs of MS. This has ignited an active debate as to whether infections prevent or precipitate autoimmune diseases [summarized in (151)]. Furthermore, studies conducted in the developed countries

have shown that people who were exposed to a higher level of sanitation during childhood had a higher risk of developing MS in adulthood (152) therefore supporting the hypothesis that infections early in live protect rather than induce or accelerate autoimmune diseases like MS.

Further protective evidence of infections in autoimmune diseases was demonstrated by interventional studies where it was reported that individuals treated with anti-helminth drugs showed an increased MS activity (153).

In most African countries, soil-transmitted helminths (STH) affect primarily the people living in rural areas or urban settings with a lack of clean water and poor sanitation (154). STH is still a considerable burden in children aged 5-14 years in Sub Saharan Africa, although a recent study has shown a vast decline in the prevalence of STH in the last decade (155). Even though there are no studies investigating the correlation between STH infections and the risk of developing MS in Africa, we can speculate that exposure to STH during childhood might contribute to the observed low MS prevalence in Africa. Nevertheless, the question remains if we should consider helminths as beneficial commensals or harmful pathogens. Furthermore, if they are beneficial commensals, will deworming the population with anti-helminth drugs cause an increase in autoimmune disorders?

Moreover, there are some viral infections that have been reported to increase the risk of developing MS later in life. A recent study has suggested a causal role of the *Epstein–Barr virus* (EBV) in MS, where MS patients seropositive to EBV had high levels of HLA1-B*07+ genes. They suggested that these HLA-class I molecules present antigens to T lymphocytes and initiate immune response against viruses, and thus supporting the potential role of EBV in MS pathology (156). Prior exposure to EBV has been shown to increase the risk of developing MS in both white and black individuals (157).

Furthermore, a recent meta-analysis has shown a strong association between infection with human herpes viruses (HHV) and MS, suggesting that infection with HHS increases the risk of developing MS although the precise mechanisms remain unclear (158). In addition, few studies have shown a MS protective role of prior *Cytomegalovirus (CMV)* infection (132).

GUT MICROBIOTA

The gut microbiota plays a vital role in maintaining the host's homeostasis and preventing inflammatory diseases. Diet is considered as the major driving factor in shaping the gut microbiota across the lifetime (159). Mice raised in the germ-free environment are protected from developing clinical EAE (160). These mice developed EAE only when they were exposed to feces from mice that were colonized with gut microbiota, and the subsequent disease was observed to be very mild, suggesting that the gut microbiota participates in the activation of adaptive immune cells (161). This has been further supported by the observation that transplantation of MS twin-derived microbiota to a transgenic mouse model of spontaneous brain autoimmunity induced a significantly higher incidence of disease when compared to transplantation of the healthy twin -derived microbiota (162). These findings provide evidence for pathogenic microbial components in human MS. Considering the geographical and cultural differences between Europe, America, Asia, Australia and Africa, there is a diverse difference in the gut microbiota, which might impact on MS prevalence (51). Studies comparing protective and pathogenic microbial components in MS in different continents will thus be of fundamental importance to understand if the low prevalence of MS in Africa is also due to a specific gut microbiome affecting the priming and trafficking of immune cell subsets.

LIFESTYLE RISK FACTORS

Both active and passive tobacco smoking has been highly associated with MS onset with a clear dose-dependent relationship. The prevalence rate of tobacco smoking in Africa is low as compared to the Americas and Eastern Mediterranean. However, it is currently increasing at a very high speed when compared to other parts of the world (163). In 2010, the Lancet survey published that Mozambique has seen a 220% growth in cigarette consumption over the past 16 years (164). The increase in the number of smokers is yet to determine if it will increase the prevalence of MS in Africa in the coming years.

Lately, there has been much discussion regarding the contribution of dietary intake to MS incidence and severity. As we know, the diet has a significant influence on the gut microbiome, leading to altered immune function. High salt diet food has been described to promote CD4 T-cell differentiation to Th17 cells, thus leading to earlier disease onset with severe clinical manifestations (165). Furthermore, a high-fat diet has been associated with the development of obesity which puts an individual at a high risk of developing MS (166). The available statistics show increasing trends of body mass index and obesity in Africa (167) which heralds an increase in the MS incidence in the coming years.

CONCLUSIONS

CNS autoimmunity is suggested to be either triggered by molecular mimicry where the adaptive immune response is raised against microbial antigens resembling those of the host or by inflammatory cytokine induced bystander activation, where APCs upregulate, co-stimulatory molecules leading to loss of self-tolerance. The initial activation of these autoaggressive immune cells most likely takes place at outer and inner body surfaces, aka the skin and mucosal surfaces, respectively (**Figure 3**). This has relevance to their CNS trafficking properties. In an EAE model autoaggressive T cells primed in skin draining lymph nodes were shown to infiltrate in addition to the CNS white matter also CNS grey matter using CXCR6 (168). In contrast, autoaggressive T cells primed in gut draining lymph nodes solely infiltrated CNS white matter. Thus, the site of immune cell priming will have a significant impact on T cell effector functions that may not be adequately described with the current immune cell classifications. How skin color and the gut microbiome of the African population affects T cell priming and their CNS homing properties remains to be shown. There is thus an

unmet need to compare the specific characteristics of the barrier associated lymphoid tissues in the African population and their impact on immune cell priming during infections to understand the molecular underpinnings of the lower prevalence of MS in Africa and to prevent a future increase of MS in Africa.

AUTHOR CONTRIBUTIONS

JM wrote the first draft and compiled all figures. HT and WG wrote part of the document. BE designed the overall layout and edited the entire document. All authors contributed to the article and approved the submitted version.

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T_H Cells and Cytokines in Encephalitogenic Disorders

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The invasion of immune cells into the central nervous system (CNS) is a hallmark of the process we call neuroinflammation. Diseases such as encephalitides or multiple sclerosis (MS) are characterised by the dramatic influx of T lymphocytes and monocytes. The communication between inflammatory infiltrates and CNS resident cells is primarily mediated through cytokines. Over the years, numerous cytokine networks have been assessed to better understand the development of immunopathology in neuroinflammation. In MS for instance, many studies have shown that $CD4^+$ T cells infiltrate the CNS and subsequently lead to immunopathology. Inflammatory $CD4^+$ T cells, such as T_H1, T_H17, GM-CSF-producing helper T cells are big players in chronic neuroinflammation. Conversely, encephalitogenic or meningeal regulatory T cells (T_{REGs}) and T_H2 cells have been shown to drive a decrease in inflammatory functions in microglial cells and thus promote a neuroprotective microenvironment. Recent studies report overlapping as well as differential roles of these cells in tissue inflammation. Taken together, this suggests a more complex relationship between effector T cell subsets in neuroinflammation than has hitherto been established. In this overview, we review the interplay between helper T cell subsets infiltrating the CNS and how they actively contribute to neuroinflammation and degeneration. Importantly, in this context, we will especially focus on the current knowledge regarding the contribution of various helper cell subsets to neuroinflammation by referring to their helper T cell profile in the context of their target cell.

Keywords: helper T (TH) cells, neuroinflammation, cytokines, multiple sclerosis, EAE (experimental autoimmune encephalitis), GMCSF, granulocyte macrophage colony-stimulating factor

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T CELL POLARISATION: AN OVERVIEW

T cell mediated immunity is reliant on the differentiation of naïve T cells into their effector T cell counterparts. Upon activation, these cells bifurcate into their two major lineages – CD8-expressing cytotoxic T lymphocytes (CTL), and CD4-expressing helper T cells (T_H) (1). $CD4^+$ cells are important in the regulation of the adaptive immune response against a plethora of pathogens. Through differentiation and the secretion of cytokines, these cells help activate antigen-specific B cells to produce antibodies, and hence drive humoral immunity.

About 4 decades ago, it was postulated that CD4 T cells can differentiate into subsets with characteristic effector functions (2). Effector T cells are classified and differentiated based on i) the type of pathogen that

elicited the activation and ii) the subsequent group of cytokines secreted by these cells. The main effector subsets of CD4 T cells were historically described to only bifurcate into two distinct populations, driven by their inflammatory milieu (3). Briefly, type 1 versus type 2 immunity was grossly classified as immune responses towards intracellular pathogens versus extracellular parasites and helminths. However, this historical classification has now been revised to include many further helper T cell subsets extending beyond the scope of the original T_H1 and T_H2 cells.

Further Helper T cell subsets include T follicular helper (T_{FH}) and Regulatory T (T_{REG}) cells. T_{FH} cells work alongside T_H1, T_H2, or T_H17 cells to help B cells generate class-switched immunoglobulins of different isotypes, which are recognised by different innate immune effector cells through cell characteristic expression of cell surface Fc receptors. T_{REG} cells, characterised by their expression of the IL-2 receptor alpha chain CD25 (4) alongside with the transcription factor (TF) FoxP3 (5), have immunoregulatory functions and promote tolerance towards the antigens they recognise, usually self-antigens.

The above-mentioned descriptions of helper T cell subsets fit the historical classification. However, with increasing advances in the field of immunophenotyping, it has become clear that helper T cell nomenclature in the context of a single lead effector cytokine fails to capture the functional diversity of these cells. Thus, we and others propose that T cells should be rather categorised into the kind of help that these cells provide at a site of injury – based on whether their downstream functions affect i) phagocytes (henceforth referred to as type 1 immunity), ii) polymorph-nucleated cells (type 2), or iii) non-immune cells (type 3) (6). This model of naming and classifying T cells is summarised in the form of a schematic as seen in **Figure 1**. Taking this into account, in this review, we describe the role of helper T cells in the context of their target and effector cells in neuroinflammation.

TYPE 1 T_H CELLS AND NEUROINFLAMMATION

T_H1 cells are the most prominent members of the type 1 T_H cell family. T_H1 cells were first characterised by their ability to produce interferon gamma (IFN-γ), a potent cytokine with important immunomodulatory functions. T_H1 cells help

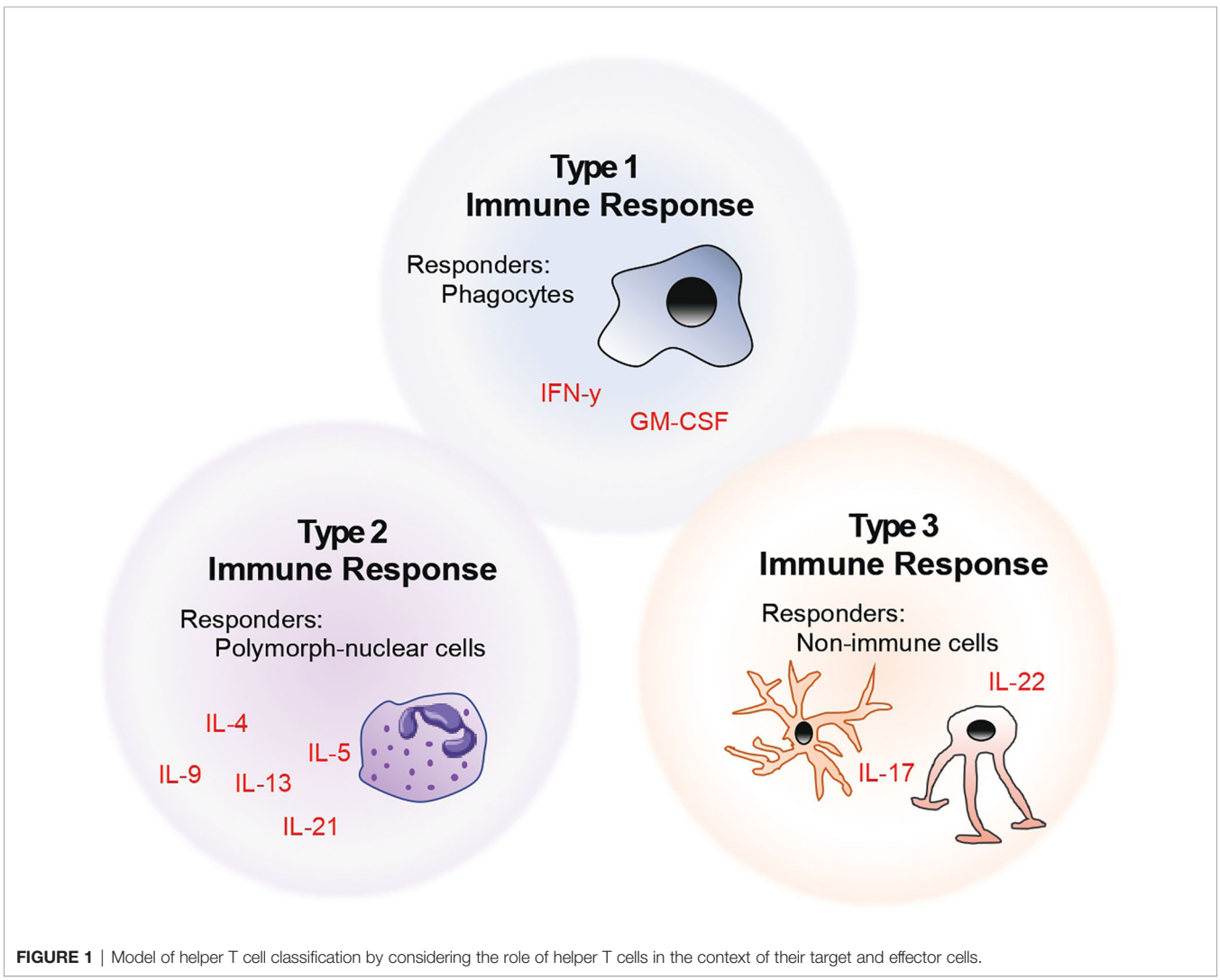

FIGURE 1 | Model of helper T cell classification by considering the role of helper T cells in the context of their target and effector cells.

orchestrate the adaptive immune response against intracellular pathogens (e.g. viruses) through direct activation of phagocytic cells or CTLs. These cells in turn directly kill the pathogen or virus infected or transformed host cell in question and can further promote antibody-dependent cellular cytotoxicity (ADCC) and opsonisation.

In addition to IFN-γ, T_H1 cells can also be recognised by their cell surface expression of the IL-12 receptor (R) β chains (1 and 2) and the chemokine receptor type 3 (CXCR3). Further work from the late 20th century revealed that there are also key TFs which play important roles in T_H1/T_H2 polarisation – and thus T-bet was associated with T_H1, and GATA-3 with T_H2 cells (7–9). The T_H1 signal is self-regulating through a positive feedback loop, as IL-12 and IFN-γ both induce T-bet, which in turn induces IFN-γ and T-bet, too (10).

Early studies in an animal model of multiple sclerosis (MS), termed experimental autoimmune encephalomyelitis (EAE), showed that IFN-γ positive cells were the biggest immune cell population in the diseased brain (11, 12), suggesting that T_H1 cells were potentially very important in the neuro-pathogenesis of the disease. Furthermore, the adoptive transfer of T_H1 cells into naïve animals was shown to drive neuroinflammation, further supporting this notion (13).

The exact role of these brain-infiltrating $CD4^+$ T cells in the context of neuroinflammatory disease is still under investigation. However, a potential downstream target of T_H1 mediated effector functions in the central nervous system (CNS) are the resident macrophages of the brain called microglia. Like most other resident macrophages of the body, several studies have suggested that T_H1 cells secreting their signature cytokine cocktail leads to the activation of microglia into an inflammatory phenotype (14). In the parenchyma of the brain, microglia are the only resident leukocytes, which makes them a solid contender to interact with T cells invading the CNS in neuroinflammatory conditions (15).

The capacity of these cells to present antigens has been shown in several *in vitro* studies (16–19). Subsequently, several follow-up studies suggested that microglial activation is directly linked to immune infiltration of the CNS and the maintenance of encephalitogenicity during the effector phase of EAE (20–22). However, in the non-inflamed brain, most cell types including microglia do not express MHC class II or costimulatory molecules. This makes them unlikely to be responsible for the initial reactivation of encephalitogenic T cells.

Key studies were carried out to investigate the *bona-fide* antigen presentation capabilities of CNS-resident cells, using mouse models where MHC class II expression could be restricted to certain antigen presenting cell (APC) subsets. These experiments revealed that *in vivo*, neither microglia, nor any other parenchymal elements are required to mediate interactions between APCs and helpers T cells (23). Building on these findings, systematic interrogation of each potential APC within the brain revealed that among the conventional dendritic cell (cDC) subsets, cDC2s in particular are powerful APCs in bridging CNS-T cell interactions (24). Whilst microglia may not be the main players in initiating neuroinflammatory pathology, it is however feasible that during the chronic phases of the disease, microglia play a role in chronification and disease perpetuation.

The most likely immune cell target for type 1 cytokines such as IFN-γ is in fact not resident to the CNS, but instead may invade the CNS from the circulation, namely monocytes. In mice and humans, monocytes come in two flavours. One that is patrolling in the blood (in mice, $Ly6C^{low}$) and another capable of reacting to inflammatory stimuli and invading tissues ($Ly6C^{high}$). IFN-γ has been shown to be important for the monocyte to macrophage transition in inflamed sites (25). Nevertheless, the functional consequences of this IFN-γ induced maturation of monocytes remain unclear.

Further studies in animals revealed the extent of the role of T_H1 cells in neuroinflammation. IFN-γ is heavily present in the brain lesions present in EAE mice, and the same holds true for MS patients. Clinical trial data revealed the IFN-γ administration to patients suffering with MS made their symptoms worse, and led to increased relapses (26). In contrast though, mice lacking the IL12R β2 chain (27, 28), or the p35 subunit (29), are susceptible to EAE. The same holds true for animals deficient in IFN-γ (30). Moreover, IL-12 administration to mice suffering from early stages of EAE suppressed the disease – the authors of this study also showed that this was an IFN-γ dependent phenomenon (28). Whilst the majority of historical evidence points towards an overall pathogenic role for IFN-γ producing T_H1 cells (31), many contradictory studies reveal a potential protective role of these same cells in neuroinflammation (32, 33). To date, the mechanisms by which IL-12 and IFN-γ regulate or suppress neuroinflammation remain completely unknown.

TYPE 3 T_H CELLS AND NEUROINFLAMMATION

In the context of autoimmunity, studies revealed that IL-23, a cytokine with a shared p40 subunit with IL-12 (34), is important in driving inflammation in models of multiple sclerosis and psoriasiform inflammation. Additionally, the IL-23R comprises the IL12R β1 chain (35) – and these observations helped to clarify the contradictory data described in the previous section. It was then established that IL-23 is a driver of neuroinflammation by the induction of a subset of helper T cells which secrete IL-17 and therefore also activate a type 1 response (36, 37).

Hence, the way was paved for the coining of T_H17 cells (36). T_H17 cells produce the cytokines IL-17A, IL-17F, IL-21 and IL-22 as lead cytokines (38). The cells are further characterised by the expression of CCR4, CCR6, CD161 as well as IL23R and IL-1R. In addition, these cells express retinoic acid receptor-elated orphan nuclear receptor γτ (RORγτ) intracellularly.

The main reason we call these cells type 3 immune cells is because their primary targets are non-immune cells. Receptors for IL-17 and IL-22 are expressed in various densities throughout the immune as well as stromal compartments. Dysregulation of IL-17 for instance, leads to inflammation of tissues of the body lining, rich in epithelial cells (39). While these mice developed severe skin inflammation, most solid tissues including the CNS

were unaffected. In line with this, dysregulation of any members from this group of cytokines, such as IL-17A/F, IL-21 or IL-22, generally leads to pathologies restricted to barrier tissues, like the skin, lung or gut (40–42).

IL-21 was initially described to play an important role in encephalitogenicity (43) – however, this claim was rebuked by many follow-up studies (44, 45).

Whilst these responses are important to curb off an imminent infection, the flipside of a sustained T_H17 response is tissue inflammation and damage. In neuroinflammation specifically, these cells have been described to be involved in the pathogenesis of EAE and MS. There have been claims that helper T cells which secrete IL-17 are abundant in both the peripheral blood as well as the cerebrospinal fluid (CSF) of MS patients (46). However, overall, there is no evidence of overt dysregulation of IL-17 signalling itself in MS. Even though a clinical trial neutralising IL-17 in MS has shown some early signs of efficacy, it has not been pursued further and approval was never sought for (47).

Even though disease progression and active disease have also been linked with the increased presence of T_H17 cells in patients, the most likely contribution from IL-17 in neuroinflammation may be its effects on the blood brain barrier (BBB). Evidence links IL-17 with barrier function in other organs such as the lung and gut (48, 49), with further experimental data pointing towards IL-17 playing a role in altering of the neurovascular junction being convincing (50, 51). In addition, T_H17 cells from patients in relapsing MS are associated with inflammatory lesions and have increased migratory capacities (52).

Astrocytes are a potential neurological cell type which has been investigated in recent years as an effector cell of T_H17 responses. They are a subtype of glial cells which reside between the BBB and resident brain cells, are characteristically histologically star-shaped (53), and perform a vast range of functions including tissue maintenance, repair, and regulating cerebral flow. Their main function is directly linked to their location within the brain, where they can monitor and regulate the exchange between the CNS and the systemic circulation (54). Increased expression of a functional IL-17 receptor was demonstrated *in vitro* (55), as well as under EAE conditions (56, 57). Disruption of IL-17 signalling in these cells was shown to improve EAE in mice (58). However, the signalling pathway targeted in these studies is by no means IL-17 specific, and thus the contribution of IL-17 *via* astrocytes towards neuroinflammation remains a subject of debate.

Finally, IL-17 also has an effect on a final CNS resident cell type, known as oligodendrocytes. These cells assemble myelin, which is a multi-layered sheath of lipidous membrane around axonal segments. Studies have shown that T_H17 cells interfere and inhibit the maturation cycle as well as the survival rate of oligodendrocytes (59, 60).

HELPER T CELL SUBSETS – HIGHLY PLASTIC?

As discussed previously, recent mounting evidence has led to the belief that helper T cell subsets may not be rigid and cemented in their functional and expression profiles, but that they may adapt according to environmental cues. This is at least true for T_H17 cells. There is a strong propensity for these to differentiate into cells that secrete IFN-γ or play the opposing role by producing non-inflammatory IL-10 (61).

A study by Capone and colleagues demonstrated this principle. In relapsing MS patients, T_H17 cells upregulate the expression of IL-1R and produce higher levels of IL-21,IL-2, and TNF-β (62). Similarly, within the T_H17 compartment of MS patients with active symptoms, another study found elevated expression of IFN-γ and CXCR3 together with reduced expression of IL-10 (63). Conversely, T_{REGs} have been shown to be highly stable (64).

Recent studies have gone a step further and suggested the notion that these subsets may be overlapping in such a manner that their current naming is largely redundant. Cells that secrete both IFN-γ as well as IL-17, hence sitting on the fence between a T_H1 and T_H17 phenotype (65, 66), have been reported on several occasions. These cells express the receptor for IL-23R. In addition, they co-express CXCR3 and T-bet together with CCR6 and RORγτ. Interestingly, they have been described to produce lower amounts of IL-17A compared to classical T_H17 cells but high levels of IFN-γ [reviewed in (67)]. Specifically, in the context of neuroinflammation, cells characterised by the expression of TNF, IFN-γ, IL-2, the CXC chemokine receptor type 4 (CXCR4) and very late antigen 4 (VLA4) were convergent in the blood of patients with MS. These cells were also enriched within the CNS, and were drastically reduced upon therapeutic intervention (68). During acute EAE, cells with a similar mixed helper T cell phenotype can cross the BBB and accumulate in the CNS. Finally, cells with a similar phenotypic profile were also found in brain tissues from MS patients and upregulated in patients during relapse (69, 70).

The observed plasticity across TH cells is clearly beneficial to immunity in the fight against infections. An overly rigid, hard-wired program makes little sense given that the primary role of T_H cells is providing 'help'. This is why we believe that, in the future, a categorisation based on single cytokines or even multiple cytokines will fade in favour of a more nimble and logical description across their specific helper function (6).

GM-CSF: LICENSING OF PHAGOCYTES FOR IMMUNOPATHOLOGY

In line with a categorisation of T_H cells towards their helper function, another prominent cytokine produced by type 1 T_H cells is the granulocyte macrophage colony-stimulating factor (GM-CSF). GM-CSF was originally classified as a growth factor contributing to haematopoiesis upon its discovery, as it was shown to lead to the differentiation of bone marrow progenitors into granulocytes and macrophages *in vitro* (71–73). What makes GM-CSF unique among other CSFs is that lack of either the cytokine, or its receptor, does not lead to any disturbance to myeloid cell development or maintenance in mice (74–76),

despite its receptor being almost exclusively expressed within the myeloid compartment.

In vitro, there are compelling data to suggest that GM-CSF promotes DC differentiation from both human and mouse progenitor cells (73, 77). However, the same could not be readily replicated *in vivo* (78). What was clear is the role of GM-CSF in tissue inflammation, due to evidence pointing to its role in activation and survival of many myeloid cell subtypes such as neutrophils, monocytes and macrophages (79, 80).

GM-CSF expression originates from a plethora of cell types, including haematopoietic cells as well as epithelial or endothelial cells, fibroblasts and stromal cells. Under steady state, healthy physiological conditions, GM-CSF is rarely detected in physiological conditions *in vivo* – rather, its secretion has also been associated with sites of inflammatory injury (81–83). T_H cells secreting GM-CSF were shown to be induced by IL-23 (84), and El-Behi et al. showed that GM-CSF producing cells promote a positive-feedback loop to keep stimulating IL-23 secretion (85). The evidence that GM-CSF is a mandatory cytokine produced by encephalitogenic T cells is overwhelming. IL-1β can further elicit GM-CSF secretion in T_H17 cells *in vitro*, while IL-27, IFN-γ and IL-12 counteracts GM-CSF production (21, 84).

Using a fate-mapping and reporter system for GM-CSF expressing cells, it was shown that secretion of GM-CSF was both IL-23 and IL-1β dependent (86). The specific role of each of these individual cytokines on the expression of GM-CSF is yet to be elucidated. In the same study, cells that formerly secreted GM-CSF were shown to be more likely to express GM-CSF once again in a recall setting as opposed to their GM-CSF naïve counterparts (86). Another study revealed that antigen-independent GM-CSF release by T_H cells, and this cytokine alone, was enough to induce neuroinflammation. Interestingly, whilst GM-CSF lead to severe neurological symptoms, other organs were not affected (87). In this study, the authors showed that GM-CSF-induced infiltration of inflammatory phagocytes was confined to the CNS, liver, and lung. Conversely, the skin, colon, and pancreas were spared. This suggests that the specific tissue microenvironments harbour different cues for the invasion of myeloid cells. In addition, it seems that the microenvironment of the target tissue itself influences the effector function of these cells, since the inflammatory phagocytes found in the CNS had a unique genetic signature when compared to the phagocytes within the other tissues. Microarray analysis of *in vitro*-differentiated cytokine-secreting T_H cells identified a large portfolio of genes that were exclusively expressed in GM-CSF-secreting T_H cells (88). Altogether, these findings support the notion of a distinct T_H subset related to GM-CSF driving neuroinflammation.

CONCLUSIONS

There is no doubt that encephalitogenic T_H cells play an important role of in propagating neuroinflammation. Even though there is a heavy debate as to whether MS is primarily driven by type 1 or type 3 cytokines, if one considers the cellular composition within neuroinflammatory lesions, it should be termed a type 1-driven immune response. However, the ability of type 3 cytokines (e.g. IL-17) to interact with epithelial and endothelial cells, suggests a role of type 3 immunity in BBB dysfunction. The interplay of other factors and the rest of the cytokine network in neuroinflammation remains to be established. Currently ongoing research is targeted towards elucidating these unanswered questions. Among the most pressing questions is the relative role of CNS resident versus invading cells in immunopathology, and how this intertwines with the instruction delivered by CNS invading T_H cells. Equally, among the biggest challenges will be to identify unique molecular patterns of encephalitogenic T_H cells which allows for their targeting and neutralisation without collateral broad immunosuppression.

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SK conceptualised the manuscript and wrote the first draft. Both SK and BB critically reviewed and revised the manuscript.

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REVIEW
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Post-Infectious Autoimmunity in the Central (CNS) and Peripheral (PNS) Nervous Systems: An African Perspective

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The direct impact and sequelae of infections in children and adults result in significant morbidity and mortality especially when they involve the central (CNS) or peripheral nervous system (PNS). The historical understanding of the pathophysiology has been mostly focused on the direct impact of the various pathogens through neural tissue invasion. However, with the better understanding of neuroimmunology, there is a rapidly growing realization of the contribution of the innate and adaptive host immune responses in the pathogenesis of many CNS and PNS diseases.

The balance between the protective and pathologic sequelae of immunity is fragile and can easily be tipped towards harm for the host. The matter of immune privilege and surveillance of the CNS/PNS compartments and the role of the blood-brain barrier (BBB) and blood nerve barrier (BNB) makes this even more complex. Our understanding of the pathogenesis of many post-infectious manifestations of various microbial agents remains elusive, especially in the diverse African setting. Our exploration and better understanding of the neuroimmunology of some of the infectious diseases that we encounter in the continent will go a long way into helping us to improve their management and therefore lessen the burden.

Africa is diverse and uniquely poised because of the mix of the classic, well described, autoimmune disease entities and the specifically "tropical" conditions. This review

explores the current understanding of some of the para- and post-infectious autoimmune manifestations of CNS and PNS diseases in the African context. We highlight the clinical presentations, diagnosis and treatment of these neurological disorders and underscore the knowledge gaps and perspectives for future research using disease models of conditions that we see in the continent, some of which are not uniquely African and, where relevant, include discussion of the proposed mechanisms underlying pathogen-induced autoimmunity. This review covers the following conditions as models and highlight those in which a relationship with COVID-19 infection has been reported: a) Acute Necrotizing Encephalopathy; b) Measles-associated encephalopathies; c) Human Immunodeficiency Virus (HIV) neuroimmune disorders, and particularly the difficulties associated with classical post-infectious autoimmune disorders such as the Guillain-Barré syndrome in the context of HIV and other infections. Finally, we describe NMDA-R encephalitis, which can be post-HSV encephalitis, summarise other antibody-mediated CNS diseases and describe myasthenia gravis as the classic antibody-mediated disease but with special features in Africa.

Keywords: post-infectious, immunity, autoimmunity, neurological disorders, encephalitis, encephalopathy, Africa, peripheral nervous system

1. INTRODUCTION

Africa is a diverse continent, rich with opportunities. It has a predominantly young population demography with varied socioeconomic backgrounds and human potential. Infectious diseases have plagued the continent in the past, continue to do so in the present and will do so into the future. It is a continent that is also bearing the brunt of the resurgence of previous epidemics and pandemics accompanied by new emerging infections (1–3). "Of 25 countries highly exposed to infectious diseases reported by Infectious Disease Vulnerability Index in 2016, 22 were from the African region" (4). Some of these infections could be prevented with more widespread vaccination or treatments.

Measles is a preventable disease that can have devastating neurological sequelae in those that are not vaccinated, with viral persistence resulting in measles inclusion body encephalitis (MIBE) in immunocompromised individuals, or subacute sclerosing encephalitis (SSPE) in those that are infected in infancy (5, 6). According to the World Health Organization (WHO) Africa there are 26 million Africans infected with HIV (2, 4) with limited access to antiretroviral therapy. Even though combined antiretroviral therapy (cART) has reduced mortality and morbidity of acquired immunodeficiency syndrome (AIDS), it is still endemic, and opportunistic infections (OI) and complications associated with long-term HIV infections, including neurological manifestations, have increased (1). Within Africa clinicians are frequently challenged by the layering effect from multiple influences which impact on clinical disease expression and response to interventions. Co-morbid diseases occur: as an example, vertical transmission or even *in-utero* exposure of HIV, followed by infantile infection with measles typically in the setting of an infant born into a poor socioeconomic environment with limited nutrition and stimulation.

The list of infections with devastating neurological consequences includes Influenza virus, Malaria, Ebola virus, other zoonotic viruses, Onchocerciasis with its recently reported Nodding syndrome, Nakalanga syndrome and other neurological sequelae (1, 7). The interplay between these infectious threats and the peculiar challenges faced by many African countries will result in disastrous consequences, with significant morbidity and loss of life. These other challenges include poverty, malnutrition, poor infrastructure, impact of climate change, political conflict and poor health resources and systems (8, 9).

Despite the advances in antimicrobial treatments and prevention through vaccines and other interventions, neuroinfections continue to ravage populations the world over (10–12). New developments in molecular biology, immunology, better understanding of neuroinflammatory responses and advances in neuroimaging have resulted in better insights into the pathophysiology and impact of neuroinflammation. The role of infections as triggers of autoimmunity in both the central and peripheral nervous systems is being unraveled (13–17). To turn the tide of the scourge of infectious diseases requires innovative approaches and research into the investigation and management of these post-infectious autoimmune disorders.

This review explores the current understanding of the post-infectious autoimmune manifestations of CNS and PNS diseases in the African context. We discuss the proposed mechanisms underlying pathogen-induced autoimmunity, highlight the clinical presentation, diagnosis and treatment of these neurological disorders and underscore the knowledge gaps and perspectives for future research using disease models of conditions that we see in the continent. We will cover para- and post-infectious disease models (see **Table 1**), affecting both the central and peripheral nervous systems in children and adults

TABLE 1 | General overview of para- and post-infectious autoimmunity in the central and peripheral nervous systems (see text references).

Disorder	Infectious agent(s)/ trigger(s)	Mechanism/Hypothesis	Clinical + Laboratory	Management
Acute Necrotizing Encephalopathy	Influenza A/B, parainfluenza, COVID-19	Cytokine "storm" Genetic predisposition (RANBP2 mutations)	Diagnostic criteria for ANE are as follows (Proposed by Mizuguchi et al.) (18): (1) acute encephalopathy preceded by viral febrile disease; rapid deterioration in the level of consciousness, convulsion; (2) increased cerebrospinal (CSF) protein without pleocytosis; (3) neuroradiologic findings for symmetric, multifocal brain lesions involving bilateral thalami, cerebral periventricular white matter, internal capsule, putamen, upper brain stem tegmentum, and cerebellar medulla; (4) elevation of serum aminotransferase level (5) exclusion of other resembling diseases	Early Immunomodulation (Intravenous methylprednisolone). Supportive.
Measles-associated Encephalopathies: - APME/ADEM - MIBE - SSPE	Measles Virus	Acute Post-infectious/ Autoimmune – APME/ ADEM Viral Persistence in Immunocompromised host – MIBE Viral persistence/mutation in immunocompetent host	Encephalopathy, multifocal neurological signs and symptoms. Multifocal demyelination (asymmetrical) on MRI. Medically refractory seizures with altered mental status and motor deficits. Usually in immunosuppressed HIV positive patients.	Immunomodulation. Corticosteroids, IVIG Supportive management Antiviral - oral isoprinosine +/- intrathecal interferon
HIV Autoimmune neurological disorders	Human Immunodeficeincy Virus	Attrition and dysfunction of the CD4+ T-lymphocytes, resulting in CD4+ T-lymphocytopaenia. Generation of autoreactive CD8+ T-lymphocytes. Alteration in the balance of regulatory T-lymphocytes and T-helper 17 lymphocytes.	Encephalitis/encephalomyelitis Seizures, encephalopathy, motor paralysis, GBS/polyneuropathy	cART + Immunomodulation (Corticosteroids)
Nodding Syndrome, Nakalanga syndrome and Other Epilepsy	Onchocerca volvulus	Immune-mediated/ Autoimmune (Leiomodin-1); other	Affects children. Epilepsy/atonic seizures with head "drops" + other seizure types. Cognitive impairment with neurological regression.	Possible immunomodulation. Not clear yet.
Acute Disseminated Encephalomyelitis	Viruses (eg, measles, mumps, coxsackie, influenza, COVID-19, etc.), Mycoplasma pneumoniae,	Autoimmune; Molecular mimicry. Role of myelin oligodendrocyte glycoprotein (MOG) antibodies.	Encephalopathy, multifocal neurological signs and symptoms. Multifocal demyelination (asymmetrical) on MRI.	Immunomodulation. Corticosteroids, IVIG
Guillain-Barre Syndrome	Campylobacter jejuni, mycoplasma pneumonia, Haemophilus influenzae, EBV, CMV, COVID-19, etc.	Autoimmune. Molecular mimicry. Axonal damage or demyelination. Anti-ganglioside antibodies are detected in some cases, notably *C. jejuni*-related.	Acute flaccid paralysis, with symmetrical areflexic weakness, neuropathic pain, autonomic disturbances, bulbo-respiratory weakness.	Immunomodulation. IVIG or plasma exchange (PLEX). Supportive care.

from an African perspective. Autoimmune encephalitis and myasthenia gravis, each representing central and peripheral nervous systems, respectively, will be presented as they are well studied models of autoimmunity in the nervous system. Despite the paucity of data these conditions also exist and are likely underreported in the African and other resource-limited settings. Data will be presented where available. Awareness needs to be raised and research gaps must be addressed.

We hope to reach clinicians and scientists working in neurology, including paediatric and adult neurologists, especially the younger African generation. We also aim to inspire new ways of thinking and dealing with the neuroimmune effects of infections given the continental challenges and current state of understanding. The lessons learnt from the past must be used to impart tools and skills to the next generation of neuroscientists and clinicians for dealing with future challenges in the field of neuroinflammation and autoimmunity.

2. PARA- AND POST-INFECTIOUS NEUROLOGICAL DISORDERS

2.1 Neurological Complications of Influenza

Influenza is a single-stranded RNA virus and a member of the Orthomyxoviridae family. Influenza A and B are the major circulating viruses in both adults and children. Influenza epidemics are associated with over 3 million cases of severe illness and about 290 000 – 650 000 deaths, annually (19). The involvement of the nervous system contributes up to 30% of the

mortality from influenza in children (19). Minor genetic variations (antigenic drift) are the cause of seasonal variation and larger reassortments generate new strains (antigenic shift) which can lead to pandemic infections in populations with no pre-existing immunity. Influenza viruses primarily cause respiratory illness in humans.

Two forms of central nervous system involvement associated with influenza virus in children and adults are influenza-associated encephalopathy (IAE) and acute necrotizing encephalopathy (ANE) or acute necrotizing encephalopathy of childhood (ANEC) (19, 20). Although initial reports tended to consider IAE and ANE/C together, the current understanding is that ANE is specific, with a characteristic clinico-radiological signature. The role of influenza infection and vaccination in triggering Guillain-Barre syndrome has been extensively studied worldwide since the 1976 swine flu vaccination programme in the USA; however, the risk is unreported in vaccination programmes in Africa and likely to be small (21).

2.1.1 Acute Necrotizing Encephalopathy

The first case series describing ANE was published in 1995 (18, 22). It is a rare but serious and rapidly progressive condition affecting the brain and causing acute swelling and damage of areas of the brain bilaterally, especially the thalami, symmetrical white matter areas and brainstem. Although it is known to affect adults and children, it is more commonly reported in previously well children. It is usually preceded by influenza A (occasionally influenza B) virus or other viral infections (e.g., parainfluenza, HHV6) associated with a high fever (19, 22–24). It manifests with rapidly evolving alteration of consciousness or coma, seizures, subsequent abnormal movements, and other focal neurological complications. ANE is rare but serves as a good learning model in the understanding of the likely immunological processes, genetic interplay, and the severe indirect impact that a common viral infection can have on the CNS (1, 24, 25).

There are two types of ANE, a sporadic type that is not familial and carries minimal risk of recurrence and ANE1 that occurs in genetically predisposed families carrying Ran Binding Protein-2 (*RANBP2*) mutations (24). There may be other genetic factors explaining reports of familial recurrence in individuals without *RANBP2* mutations. Although ANE is prevalent in the Far East and reported in many other parts of the world (mostly Europe and the Americas) (19, 23, 25–28) there is a paucity of reports in the African continent, where influenza and other viral triggers cause much morbidity and mortality. Current authors JMW and APN have anecdotal experience of two extended families that are managed in our centre, from the Western Cape province of South Africa, one with suspected and another with proven *RANBP2* mutations. These families have not been published yet. It is likely, therefore, that more cases are missed or not reported. Despite the lack of resources, molecular and genetic diagnostic tools in many African countries, the clinical-radiological syndrome is quite striking and should enable clinicians with access to magnetic resonance imaging (MRI) on the continent to be able to diagnose the condition (See **Figure 1**). The understanding and exploration of future treatments for this condition, albeit rare, will arm populations vulnerable to influenza epidemics/pandemics in the fight against the neurological consequences that usually follow.

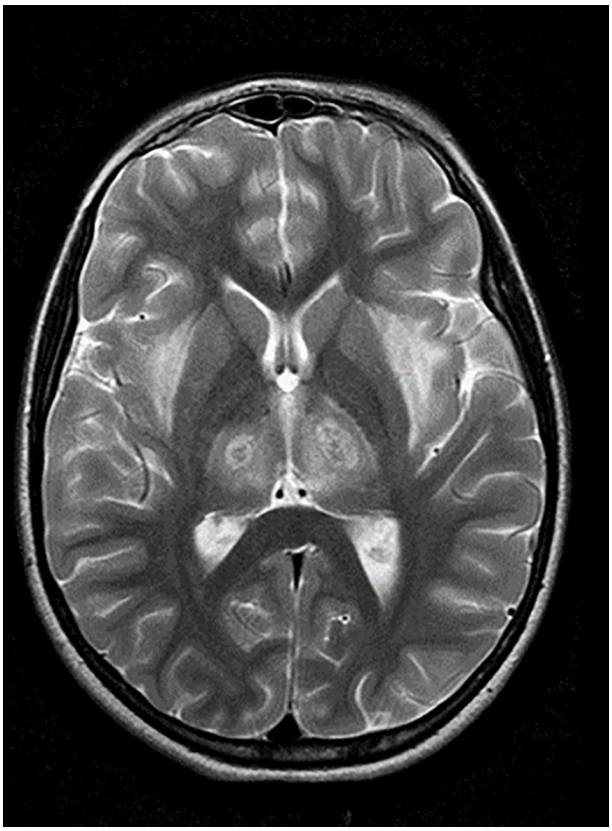

FIGURE 1 | Acute Necrotizing Encephalopathy (Local case). (Personal case of APN and JMW): Axial T-2 weighted MRI of a 9-year-old girl with who presented with classical clinical features of ANE and was admitted to the local paediatric intensive care unit. The MRI shows the classical symmetrical involvement of both thalami (with a target appearance) and symmetrical external capsular white matter affected. She had brainstem involvement (not shown) and was treated with intravenous methylprednisolone early. She survived with mild to moderate neurological sequelae. She was the first in her family to be genetically confirmed as positive for a RANBP2 mutation, with two of her cousins having been previously affected. The genetic result assisted with identification of at-risk family members, counseling and subsequent preventative measures including vaccination and early ANE 'crisis' management.

2.1.1.1 Pathophysiology

The clinical manifestations and the classical neuroimaging findings of ANE are well described, with proposed diagnostic criteria (18). However, there has been no evidence of direct infection and neuronal inflammation in the CSF and neuronal tissues. The current hypothesis is one of a cytokine storm due to abnormal nuclear signalling and possible mitochondrial dysfunction (18, 24). Tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 are cytokines that have been consistently shown to be elevated in the serum and cerebrospinal fluid (CSF) (28). Angiopathy, breakdown of the blood-brain barrier and imbalances between the protective and deleterious effects of these cytokines are thought to play a role in the pathogenesis. The CSF usually

reveals a raised protein but is acellular. There may be abnormal liver enzymes in the serum. The absence of virus and inflammatory cells in the CSF and neuronal parenchyma indicates lack of direct viral invasion. By contrast, abnormal host responses to viral infection are probably important as different viruses result in a similar clinicopathological picture. The intriguing genetic contribution described in association with *RANBP2* mutations raises opportunities for the clarification of the pathogenesis and ultimately future treatment possibilities (22, 24). *RANBP2* is located on the cytoplasmic surface of the nuclear pore that is a channel that allows small molecules to enter and leave the nucleus by passive diffusion. It is involved in the unpacking, modification and recycling of proteins entering or leaving the nucleus. The exact mechanism by which *RANBP2* mutations result in ANE is not clear but may relate to abnormal mitochondrial interactions (22).

2.1.1.2 Treatment

There are currently no evidence-based treatments for ANE. The place of antiviral (e.g., oseltamivir) treatment is not established as there is no evidence of direct viral CNS infection with this condition. There are anecdotal reports of beneficial effects of immunomodulation, especially intravenous corticosteroids (methylprednisolone) given within the first 24-48 hours of illness, and one retrospective study using high dose intravenous corticosteroids (29, 30). Oseltamivir and/or intravenous immunoglobulins are also added to the corticosteroid regimen by some centers, with variable results (30). Supportive management is crucial, especially intensive care as many patients require ventilatory support. ANE is a fulminant encephalopathy with a variable prognosis. Age, early diagnosis, clinical severity, brainstem involvement on neuroimaging and early treatment with corticosteroids are some of the determinants of outcome in case series. A large proportion of cases reported are left with neurological sequelae with variable but significant mortality of up to 30% (24).

2.1.1.3 Covid-19 (SARS-Cov-2) Associated ANE

The ability of other viruses to trigger ANE suggests a host driven pathologic inflammatory response, and the cytokine storm hypothesis is a common thread linking ANE and some COVID-19-associated diseases. There are a handful of cases recently reported with an ANE-like encephalopathy following Covid-19 (31, 32). There are likely many other cases that have not been reported, and this association needs further study. It is not clear how collision of influenza and COVID-19 would impact the nervous system; it could be a real threat to the continent.

2.1.1.4 Gaps in Knowledge

Beyond anecdotal reports there is a paucity of case reports and research relating to ANE from Africa compounded by limited data on influenza neurological complications. The limited access to laboratory services and neuroimaging (MRI) are related challenges which include lack of resources for sedation/anaesthesia for children (33). Up to now, there is still no clarity regarding the pathogenesis and how *RANBP2* promotes the development of ANE. Early biomarkers for ANE are still under investigation, but CSF neopterin is one of those being looked at (34, 35). Treatment evidence is limited to case reports and case series data. Based on the pathophysiology of the disease, there may be a role for targeted therapies, with inhibition of IL-6 or TNF-alpha, but studies are limited. There are currently not enough data to confirm the association of ANE with SARS-Cov-2. Questions remain regarding the risk of vaccination and immunomodulation treatment implications for influenza associated ANE in people carrying *RANBP2* mutations. The answers will guide the approach to potential SARS-Cov-2 associated ANE.

2.1.2 Neurological Complications of Measles (Wild Type) Virus (MeV)

Measles virus is one of the most contagious diseases in the world. Human beings are the only known natural reservoir (5). It belongs to the Morbillivirus genus of the Paramyxoviridae family and is spread *via* respiratory aerosols. Despite it being vaccine preventable, it continues to be a cause of major morbidity and mortality the world over. There is currently a global resurgence of measles in many parts of the world due to reduction in vaccination coverage, the causes of which differ for various continents (10, 36–38). Reported measles cases increased between 2013 and 2018, with 66% of cases being in low and middle income countries (LMICs) and 23% in persons ≥15 years (10). The global eradication of measles is one of the top priorities of the expanded programme on immunization (EPI), with the support of the World Health Organization (WHO) (37–39). This has been further compounded by the disruption in vaccination roll-out programs during the COVID-pandemic with coverage dropping significantly in some regions (40).

It is a single-stranded RNA virus whose genome encodes six structural proteins. Wild type MeV strains use signalling lymphocytic activation molecule 1 (SLAM or CD150) and nectin-4 receptors to infect target cells (5). The H protein binds to the entry receptor on the host cell surface, whilst the F protein undergoes serial conformational changes, following this attachment. This allows the merge of the host and viral membranes creating a fusion pore to effect viral ribonucleocapsid (RNP) delivery into the host cell cytoplasm (5, 6, 41). Therefore, the H and F proteins constitute the viral fusion complex responsible for viral entry into the host cell. The classic clinical presentation includes, fever, morbilliform rash, oral mucosal Koplik spots, coryza, cough and conjunctivitis. Measles infection can result in several devastating complications, such as pneumonia, immunosuppression, gastroenteritis, and malnutrition.

Neurological complications are less common and include primary measles encephalitis, acute post-infectious measles encephalitis (APME), Measles-inclusion body encephalitis (MIBE) in immunocompromised hosts and Subacute Sclerosing Panencephalitis (SSPE) in those infected at a very young age with or without immunocompromised backgrounds (5). In the South African experience following the 2010 measles outbreak, HIV-exposed or infected children were more predisposed to develop SSPE following MeV infection (42). How the virus enters the CNS is not clear, as the known MeV receptors (SLAM and nectin-4) are not expressed (5, 41). The other complication of MeV infection is the secondary immunosuppression that is induced and can persist for up to 6 months following the initial infection.

This immunosuppression will further exacerbate the already negative impact on morbidity and mortality.

2.1.2.1 Acute Post-Infectious Measles Encephalitis (APME/ADEM)

Acute encephalitis occurs within two weeks of initial symptoms and affects about 0.1% of cases. There is no evidence of virus in the brain, and it is thought to be a para- or post-infectious autoimmune disorder similar to acute disseminated encephalomyelitis (ADEM). As in typical ADEM, there is involvement of both white and grey matter with perivenous inflammation and demyelination pathologically (42).

Symptoms include fever, headaches, seizures, focal neurological signs, and encephalopathy. Adults are more likely to suffer from neurological sequelae, and mortality can be as high as 15% (43). Treatment is mostly supportive, but immunomodulation (corticosteroids and intravenous immunoglobulin) has been reported to improve outcomes (43).

2.1.2.2 Measles-Inclusion Body Encephalitis (MIBE)

MIBE occurs in immunosuppressed individuals, usually between 3 weeks and six months following infection with MeV. Unlike APME, MIBE pathology demonstrates evidence of viral entry into the CNS (5, 6, 41). Pathologically, there are intracytoplasmic and intranuclear inclusion bodies (nucleocapsids) in affected neurons, astrocytes, and oligodendrocytes (5, 6). How the virus gains entry without the necessary receptors is a focus of several human and animal studies. Mutations in the HRC domain of the F-protein have been described and are thought to confer an ability for enhanced fusion without the need for H-protein attachment to appropriate receptors (42). These hyperfusogenic MeV mutants demonstrate better viral dissemination without need for H binding (5). Hyperfusogenicity has also been observed in SSPE and therefore MIBE is thought of as a more rapid manifestation of viral persistence in immunocompromised hosts (5, 6).

Clinically, MIBE is a catastrophic form of MeV encephalitis with high mortality and severe neurological morbidity for those that survive. There are medically refractory seizures, altered mental status and associated motor deficits. Up to 75% of those affected succumb following severe seizures and encephalopathy. Status epilepticus is common, often with epilepsia partialis continua (43). In sub-Saharan Africa, this condition has been reported in immunosuppressed patients, mostly adults with HIV infection following MeV epidemics. Neuroimaging is non-specific, may be normal initially and then showing oedema (often along the cortical ribbon) with subsequent cerebral atrophy (44, 45).

2.1.2.3 Subacute Sclerosing Panencephalitis (SSPE)

SSPE affects between 6.5 to 11 cases per 100 000 of immunocompetent patients who contracted the MeV infection in early childhood (5, 43). Almost 100% of those affected will die, usually within 1-3 years of initial SSPE symptoms (5, 46). For those children infected with measles before their first birthday, the incidence can be as high as 1/609 (5). The latency period from infection to symptoms varies from 1 – 15 years. Early signs and symptoms are often non-specific and include mental deterioration, behavioural disturbances, and weakness or impairment of motor function, such as difficulties in walking and frequent falls. Because these features are nonspecific, diagnosis is often delayed, usually years after the initial measles virus infection, especially in resource-limited settings. Later, severe neurologic symptoms such as myoclonic jerks, ataxia, tremors, seizures, and encephalopathy become obvious. The electroencephalogram (EEG) is often abnormal with non-specific slowing initially and followed by the characteristic periodic slow wave complexes (46). Neuroimaging is usually non-specific, ranging from initially normal to cerebral atrophy and white matter hyperintensities later (44, 46). SSPE is characterized by an excessive intrathecal synthesis of MeV specific antibodies in the CSF.

SSPE is almost invariably fatal. In most cases, patients do not survive more than 1–3 years following the appearance of neurologic symptoms. Different drug combinations have been used without much success. There is some anecdotal evidence of longer survival following use of antiviral combination of oral Isoprinosine and intrathecal interferon (5, 46).

2.1.2.4 Gaps

There is still lack of understanding of the factors associated with MeV CNS invasion. It is well understood that an immature immune system before two years of age predisposes to persistent brain infection, but the factors that result in viral persistence are not well known. Viral mutations are thought to play a role in this persistence in the CNS, in both MIBE and SSPE. Regardless of the type of MeV encephalitis, the morbidity and high mortality associated with these complications highlight the need for antiviral treatments against these mutated variants. Vaccination is still the best way to prevent these MeV sequelae, but therapeutic interventions targeting viral entry, CNS dissemination and replication will be crucial for the treatment of CNS infection. International collaborative research into these interventions is urgent in the face of the current global measles resurgence. The coexistence of the HIV epidemic and the global recrudescence of measles in a COVID-19 pandemic melting pot, makes this emergency more acute for Africa.

2.1.3 HIV Infection and Autoimmunity

Southern Africa is the epicentre of the HIV/AIDS pandemic that has ravaged the world since the condition was first recognised clinically in 1981 (47). According to the joint United Nations Programme on HIV/AIDS (UNAIDS), at the end of 2020 the total number of persons living with HIV infection (PLHIV) was 37.6 million, of whom 1.7 million (4.5%) were children less than 15 years of age and 25.3 million (67.3%) were living in sub-Saharan Africa (48).

The clinical progression of HIV infection has been divided into four WHO stages (49). Neurological diseases usually occur in the advanced stages of HIV infection, stages 3 and 4 (50). These diseases can result directly from HIV infection, from opportunistic infections or are thought to be due to autoimmunity. Combination antiretroviral therapy (ART) when administered to HIV-infected individuals will suppress HIV replication, reverse existing HIV-induced immune

dysfunction and prevent clinical and immunological progression. However, ART-mediated immunological reconstitution may inadvertently increase the autoimmune risk (51). At the end of 2020, 27.4 million people living with HIV (PLHIV) were accessing ART, a global coverage of 72.9%, and in sub-Saharan Africa 19.5 million people had access to ART, a coverage of 77% (47).

2.1.3.1 Immune Dysfunction

HIV infection causes progressive immunological dysfunction due to the direct effects of HIV on CD4+ T-lymphocytes (CD4 cells), the consequences of virions or specific viral glycoproteins acting on uninfected cells of the immune system, and chronic immune activation arising from the host response to HIV infection (52–54). The rate of progression of HIV infection varies according to age, being more rapid in infants and young children compared to adolescents and adults.

The immunological hallmark in HIV infection is attrition and dysfunction of the CD4+ T-lymphocytes, resulting in CD4+ T-lymphocytopaenia. HIV-induced caspase-3-mediated apoptosis, and caspase-1-mediated pyroptosis triggered by abortive viral infection and chronic immune activation are the mechanisms responsible for most CD4+ T-lymphocyte attrition (55). The immune dysfunction is not confined to CD4+ T-lymphocytes but extends to other components of adaptive and innate immunity, including CD8+ T-lymphocytes, B-lymphocytes, natural killer cells, monocytes and macrophages, neutrophils, and dendritic cells (52, 56–62).

Autoimmunity, caused by a breakdown of immune tolerance and mis-directed immunological responses to self-antigens, can manifest during HIV infection, particularly in the acute stage when the immune system is relatively intact, or after ART initiation during immunological reconstitution when immune competence is restored (51). Several components of the immune dysfunction in HIV infection may contribute to the autoimmunity risk. HIV infection causes polyclonal B-lymphocyte hyperactivation characterised by hypergammaglobulinaemia, increased circulating immune complexes, spontaneously proliferating B-lymphocytes, and production of an array of autoantibodies (63). The release of protein fragments from dying CD4+ T-lymphocytes during HIV infection leads to disruption of tolerance to self-antigens and induces the generation of autoreactive CD8+ T-lymphocytes (64, 65). One study showed that many epitopes on HIV proteins appear to display high similarity with human proteins, suggesting that the induction of cross-reacting immune effectors may be possible (66). Although regulatory T-lymphocytes play important roles in self-tolerance and control of autoimmune diseases, their role in HIV infection remains inconclusive. However, it has been postulated that dysregulation of this cell subset and/or alteration in the balance of regulatory T-lymphocytes and T-helper 17 lymphocytes, may contribute to the breakdown of immune tolerance in PLHIV with autoimmune diseases (67, 68).

2.1.3.2 Autoimmune Neurological Diseases

In a large cross-sectional study of more than 5000 PLHIV the overall prevalence of all autoimmune diseases was 0.7%, but the prevalence of Guillain-Barre syndrome (GBS) was higher in the study population than in the general population (69). In a larger cohort study of more than 33,000 PLHIV, 1,381 (6%) with autoimmune and inflammatory diseases were identified. The only neurological disease reported in this study was multiple sclerosis (70). Similar prevalence studies have not yet been conducted in African countries. Case reports and case series, including studies from Africa have, however, documented central and peripheral nervous system autoimmune diseases in PLHV.

The main autoimmune mediated polyneuropathies in PLHIV are GBS or acute inflammatory demyelinating polyneuropathy (AIDP), and chronic inflammatory demyelinating polyneuropathy (CIDP). GBS usually develops as an ascending polyradiculopathy. In sub-Saharan Africa HIV infection is recognised as an important antecedent infection (71). In PLHIV, GBS frequently occurs in the presence of relatively preserved immunity. However, GBS can be the initial presenting clinical illness in PLHIV or occur after the interruption of ART during viral rebound, and GBS immune reconstitution inflammatory syndrome (IRIS) may present during the first few months of ART (72–78).

Sub-Saharan African studies have described differences in the manifestations of GBS in HIV-infected and HIV-uninfected individuals. In a Zimbabwean study, 16 of 29 patients (55%) with GBS were HIV-infected. Compared to HIV-uninfected patients, the HIV-infected patients with GBS were more likely to have generalised lymphadenopathy, lymphocyte pleocytosis on cerebrospinal fluid (CSF) analysis and co-existent central nervous system (CNS) disease (79). Eleven of 36 GBS cases (31%) from northern Tanzania were HIV-infected. The HIV-infected patients with GBS experienced more severe disease and a higher mortality rate (80). In an Ethiopian study, 19 of 27 GBS patients with HIV serological results were HIV-infected. The clinical findings of the two patient groups were similar, except for a higher frequency of CSF lymphocyte pleocytosis, ventilatory support and mortality among the HIV-infected patients (81).

AIDP and CIDP may be part of a continuous spectrum. However, CIDP differs from AIDP clinically in that CIDP by definition develops over a longer period (greater than 8 weeks) and may follow either a progressive or relapsing remitting course (82). Acute onset CIDP (A-CIDP) may be indistinguishable from AIDP in the early clinical stage. Two South African case series described CIDP in PLHIV. A prospective study described 23 consecutive patients with CIDP during a two-year period, 10 (43%) of whom were HIV-infected. Although not present in all HIV-infected patients with CIDP, CSF lymphocytic pleocytosis was significantly associated with HIV infection. Most of the HIV-infected patients followed a progressive course, while the majority of HIV uninfected experienced a relapsing remitting course (83). The second study was a retrospective comparative review of 84 patients with CIDP, of whom 39 (47%) were HIV-infected. When compared to the HIV-uninfected patients, significantly more HIV-infected patients experienced a progressive course. Median CSF lymphocyte counts were significantly higher in the HIV-infected patients. Most of the HIV-infected patients responded favourably to corticosteroid therapy, and most were in remission by 6 months. These

observations suggest that in poor-resourced settings, CIDP in HIV-infected patients should be treated with corticosteroids as the more expensive alternatives such as intravenous immunoglobulin and exchange transfusion are not readily available (84).

Acute disseminated encephalomyelitis (ADEM) a rare demyelinating disorder of the CNS has been documented in HIV-infected children and adults (85, 86). It usually follows a monophasic course. However, multiphasic, or recurrent ADEM, as well as atypical neuroimaging manifestations have been documented in PLHIV (87, 88). Other autoimmune neurological diseases have been documented in PLHIV including myasthenia gravis, N-methyl-D-aspartate-receptor antibody encephalitis, HIV-associated opsoclonus-myoclonus-ataxia syndrome, and neuromyelitis optica with or without detectable anti-aquaporin-4 autoantibodies (89–92). Inflammatory neurological diseases caused by unknown mechanisms have also been documented in PLHIV. One such disease is HIV-associated CD8+ T-lymphocyte encephalitis, a rare inflammatory disease that has not yet been reported from Africa but is characterised by the infiltration of the brain by CD8+ T-lymphocytes in the absence of opportunistic infection. Important risk factors include interruption of ART and IRIS after ART initiation. Although it is not known whether autoimmune mechanisms underpin this disease, treatment with corticosteroids improves outcome by reducing mortality (93, 94).

2.1.3.3 Research Priorities

This review identified major knowledge gaps. Studies that utilise advanced immunology and molecular techniques including whole genome sequencing and transcriptomic profiling are needed to advance the pathogenesis of HIV-associated autoimmune diseases. Addressing diagnostic constraints that exist in Africa, including limited neuroimaging facilities, and pathology and immunology support, is required to improve the recognition of neuro-autoimmune diseases in PLHIV. Optimal disease recognition is a prerequisite for undertaking comprehensive epidemiological studies to understand the incidence, autoimmune spectrum, risk factors and autoimmune risk over the course of ART in African settings. Improved diagnosis should also assist in optimising treatment interventions for these diseases through adequately powered, multi-centre, randomised intervention studies.

2.1.4 Onchocerciasis

2.1.4.1 Disease Description

Onchocerciasis is a neglected tropical parasitic disease with an estimated 20.9 million infected people worldwide, more than 99% of whom reside in 31 countries in sub-Saharan Africa (95). Currently in Africa, 218 million people live in areas known to be endemic for onchocerciasis, a disease induced by infection with the filarial nematode *Onchocerca volvulus* (*O.volvulus*) transmitted by Simulium spp. (blackflies) which inject larval stage 3 (L3) into the skin of the host during a blood meal (96, 97). The larvae eventually develop into adult microfilariae (mf) which localize to the subcutaneous nodules where they may exist for up to 15 years (98). The death of these mf provokes an inflammatory immune response, which is the key feature of the clinical manifestations of onchocerciasis infection observed in the eye, skin, and the nervous system (99).

Eye manifestations include features of chronic keratitis and sclerosis leading to ongoing loss of corneal clarity and peripheral vision as well as corneal fibrosis and or opacification that progresses to blindness (100). In addition, the eye features may be complicated with secondary glaucoma of the anterior and posterior segment lesions and optic atrophy (101).

Skin manifestations include an Onchodermatitis which may be acute causing an itchy skin rash of small, sparse popular lesions or closely packed papules of about 1mm radius, while the chronic form manifests with a pruritic, hyper pigmented, flattopped papulomacular rash of about 3mm with or without skin excoriation (102). The chronic form may result in raised, discrete, pruritic hyperpigmented papular nodules termed as 'onchocercoma' which are found around bony prominences such as the iliac crest, ischial tuberosity, elbows, and scapula, If the chronic dermatitis is characterized by hyperpigmented papules and regional lymph nodes enlargement it is referred to as *"Sowda"* (103, 104).

Neurological manifestations include Onchocerciasis-associated epilepsy (OAE). It has been suggested that the clinical presentation of the Nakalanga syndrome and Nodding syndrome form part of the spectrum of OAE (99). The Nakalanga syndrome first described in Uganda in 1950 is characterized by unexplained growth retardation commonly affecting children aged 3-18 years that were previously on the normal growth trajectory (105). Other features include delayed sexual development, intellectual disability, facial, thoracic, and spinal abnormalities with or without epileptic seizures (106–108). Nodding syndrome (NS) is a progressive, epileptic syndrome of undetermined aetiology, affecting previously healthy children with normal growth between the ages of 3 and 18 years (109). Typical features of the syndrome include fleeting episodes of a sudden onset of head nods (atonic seizures) (102). Other seizure types include myoclonic-, absence- and/or generalized tonic–clonic seizures which may commence 1–3 years after the onset of the illness (110). Additional features include deteriorating cognitive and motor function, stunted growth, psychiatric disorders, progressive generalized wasting, and physical deformities (109).

OAE encompasses a large variety of seizures, such as atonic neck seizures (seen in NS), myoclonic neck seizures, absences without nodding, and generalized tonic-clonic seizures. Initially children may manifest with the atonic type of seizures seen in NS and gradually develop generalized tonic-clonic seizures as they advance in age (111). In addition, in some cases impaired cognitive function, malnutrition, dysmorphic features, with arrested sexual debut as seen in Nakalanga syndrome may be associated with OAE (112). A case definition for OAE has been proposed to fulfil at a minimum all the following criteria namely: the age of onset between the ages of 3–18 years old; a history of two unprovoked seizures 24 hours apart; normal psychomotor growth trajectory prior to onset of symptoms; individual from area of high epilepsy prevalence with other siblings affected by epilepsy; and having lived at least three years in an onchocerciasis endemic region (99).

2.1.4.2 Pathophysiology: How the Organisms Induce CNS Disease

The pathophysiological mechanism by which the *O.volvulus* causes disease in the CNS remains poorly understood with no clear consensus on whether or not the mf can cross the blood brain barrier and conflicting reports regarding the presence of *O.volvulus* in the cerebrospinal fluid (113, 114). There is evidence to support two postulated modes of entry which include *via* the eye and the blood stream. Mf have been isolated in the posterior section of the eye suggesting a possible channel of transmission along the inflamed optic nerves which have proximity with the brain (115). Conversely, the presence of microfilariae in the bloodstream and lymphatic system of heavily infected individuals, may cross the blood brain barrier when flowing through the subarachnoid space (116). Recent reports suggest an immune-mediated mechanism rather than direct CNS invasion, as described below.

2.1.4.3 The Interplay Between the Organisms and the Immune System in the CNS, and the Consequences Thereof

There are three proposed mechanisms that illustrate how the *O.volvulus* organisms interact with the immune system in the CNS to cause complications. In the ocular system, there is a cross reaction between the Ov39 antigen of *O.volvulus* and the retinal hr44 antigen which plays a significant role in the development of chorio-retinitis (117). The bacterium *Wolbachia* co-exists with the *O.volvulus* and the other cross reaction also occurs in the ocular system following the stimulation of a Th1-mediated host immune response due to the release of *Wolbachia* surface antigens succeeding the death of the mf. This reaction contributes to the progressive visual impairment seen in Onchocerciasis (118).

More current evidence suggests that OAE, (specifically NS) may be as a result of cross-reacting antibodies between the human protein leiomodin-1(LM1) and the *O. volvulus* surface protein tropomyosin causing an autoimmune reaction (119). LM-1 is a protein present in neurons, muscle tissue and the thyroid gland of healthy individuals and anti-LM1 antibodies were found to be more common in the serum of NS patients compared to controls. In addition, they were also detected in cerebrospinal fluid (CSF) of persons with NS and noted to be neurotoxic *in vitro* (119).

2.1.4.4 Value of Neuroimmune Changes in Diagnostics and Therapeutics

The cell-mediated immune response in the host during early *O. volvulus* infection is markedly increased compared to the chronic infection state where it is diminished for reasons that are still not clear (120). *O. volvulus* infection has been noted to work against the immune responses of the host through molecular mimicry, by impairing T-cell activation and interfering with the processing of antigens (121–123). Furthermore, it has been shown that antigen specific regulatory T-cells (Tr1/Th3) generate anti-inflammatory cytokines, including IL-10 and transforming growth factor-β, which aid in the evasion of host immune responses by O. volvulus. The presence of IL-10 suppresses the Th1-immune response, thereby promoting chronic onchocerciasis (124, 125).

Infection with *O. volvulus* also affects the host's resistance to other diseases, for example increased probability of becoming HIV-positive when exposed to it compared to non-onchocerciasis individuals or a greater susceptible to developing epilepsy, which may all result in a reduced life expectancy of the host (124, 126–129).

Valuable diagnostic tools for *O. volvulus* infection are available which are efficient for individual use, such as skin snips for demonstrating microfilariae/adult worms in nodules excised or detection of *Ov*-specific antibodies, such as the Ov16 serological test (130). On the other hand, use is made of the diethylcarbamazine (DEC) patch test to evaluate the levels of endemicity and to detect recurrence of transmission in previously controlled areas for community-based onchocerciasis control needs (131). Current infection of *O. volvulus* can also be identified *via* DNA polymerase chain reaction or *O. volvulus* antigens *via* immunoblotting or a dipstick assay (132–134).

The approved therapy for mass treatment of onchocerciasis is the drug ivermectin, which enhances immune responses against *O. volvulus* in the treated host (135). The immune response increases the number of circulating CD4 + T-cells resulting in a significant reduction of microfilariae (136). However, repeated cycles of treatment with this drug are warranted in view of its inability to kill the adult worms (137). Doxycycline, an alternative therapy works by significantly reducing the life span of the adult worm through its action on the endosymbiotic Wolbachia bacteria of *O. volvulus*. Caution however should be observed in the simultaneous use of these drugs since the treatment interactions have not been elucidated.

2.1.4.5 Knowledge Gaps

Important gaps in knowledge include understanding the pathophysiological mechanisms that enable the host with prolonged *O. volvulus* infection to have a blunted cellular immune response compared to those with early infection and what determines the *O. volvulus* parasite to trigger development of Nodding syndrome, Nakalanga syndrome or OAE. Information on a more precise estimation of the burden of OAE globally is needed to guide governments and international onchocerciasis elimination programs to set up relevant interventions. Furthermore, to ascertain whether treatment with Ivermectin and doxycycline can modify the clinical presentation of OAE by decreasing its incidence.

2.1.5 Guillain-Barre Syndrome (GBS)

The acute post-infectious paralytic disorder termed GBS occurs worldwide with an annual incidence of 1-2 cases per 100,000 of population (138). Case series and population studies on GBS have been widely reported throughout North and Sub-Saharan Africa that in general follow the clinical and epidemiological patterns to those seen in other parts of the world with similar environmental factors (139). The age distribution of GBS in Africa tends to be younger than that reported in Europe and North America, most likely a reflection of the general population age. The background infections that trigger GBS are very dependent upon climatic region and environmental factors

including epidemic and endemic events and thus likely to have a major influence on the overall pattern and incidence of disease, as seen for example during the Zika virus epidemic (140). The global effort in GBS research has recently accelerated, owing in part to the huge success of the multinational International GBS Outcome Study (IGOS) run from Erasmus University, Rotterdam, that includes input from South Africa (141). A summary of the first 100 years of GBS global research can be found in the freely downloadable book edited for the GBS centenary meeting held in 2016 (142).

2.1.5.1 Differential Diagnosis of GBS

The accurate and confirmatory diagnosis of GBS and its sub-type categorisation is highly dependent upon access to electrodiagnostic testing and CSF examination, procedures whose availability is generally limited to specialist referral centres. Without access to these diagnostic procedures there is considerable diagnostic uncertainly based solely on clinical features, as reflected in levels of certainty described in the widely used Brighton Criteria classification system (143). Since GBS essentially presents as an acute flaccid paralysis in both adults and in children, the extensive differential diagnosis includes a wide range of infectious, inflammatory, metabolic, vascular, and toxic events. Some of these, such as polio and rabies may be highly location- and time-specific; others, such as *Campylobacter jejuni* enteritis and HIV infection (see section 2.3 above) are more widespread (144). In children, where the clinical manifestations may be atypical, the diagnosis of GBS may be particularly difficult to firmly establish. The relationship between SARS-Cov-2 and GBS has yet to be fully clarified in Africa and elsewhere, although cases of GBS have been reported (145). A recent case series reports an increased risk of GBS following SARS-Cov_2 infection and after COVID-19 vaccination. There was a greater risk of complications following SARS-Cov-2 infection compared with the observed vaccination risk (146).

2.1.5.2 Treatment and Management of GBS

The gold standard of current for GBS has recently been summarised in an easy-to-follow 10 steps article taking into account the current evidence-based guidelines (143). This guideline article is currently undergoing translation into languages other than English and includes a simple wallchart in the first figure. In many parts of the world the 2 proven treatments – plasma exchange and intravenous immunoglobulin therapy - are neither available nor affordable and other measures thus need to be considered. Small volume plasma exchange is an alternative approach in resource limited settings that has undergone a recent re-evaluation in Bangladesh (147). Since around 30% of GBS cases require intensive care with mechanical ventilation in order to survive the acute phase of the illness, rapid access to these facilities is required. In addition to specific immunotherapy and intensive therapy support, it is equally important to consider the wide range of early and late complications that arise when managing GBS cases, including aspiration pneumonia and lung injury, cardiac arrhythmia, deep venous thrombosis, limb contractures and pressure sores, and mitigate against these in the management plan. Outcome can also be predicted using a variety of rating scales. The mortality of GBS is clearly predicated upon the level of acute supportive care that can be provided; even in the best clinical settings mortality is around 5%, and 20% of surviving patients have significant long-term residual disability. Long-term clinical monitoring is not usually required for patients who recover well. The recurrence rate is low (<5%). As mentioned above, some patients with A-CIDP may present as GBS and thus will require a different treatment plan.

3 WELL ESTABLISHED POST-INFECTIOUS OR TUMOUR-RELATED AUTOANTIBODY-MEDIATED DISORDERS

3.1 NMDAR-Antibody Encephalitis

Autoimmune encephalitis (AE) mediated by antibodies against neuronal surface antigens (NSA-Ab) represent an expanding spectrum of immune mediated disorders characterized by the subacute onset of complex neurological and psychiatric symptoms usually responsive to immunotherapy (148). Several antibodies have been identified so far, (as shown in **Table 2**); NMDAR, LGI1, CASPR2 and GABAAR antibodies are the most commonly found, although data from within Africa are very limited. Here, we will focus on NMDAR-antibody encephalitis, since it is the only one for which an infectious trigger has been recognised, at least in some cases.

NMDAR-Ab encephalitis (NMDARE) is one of the most common forms of autoimmune encephalitis, with an estimated incidence of 1.5 per million person-year (149). An American cohort found a prevalence of 0.6/100000 people, with a generally higher prevalence of autoimmune encephalitis in African Americans compared to Caucasian subjects (150). No data of the epidemiology of autoimmune encephalitis are available for Africa, and only very limited cases have been reported so far, outlining possible difficulties in achieving the diagnosis (151). NMDAR-Ab encephalitis can affect both children and adults, and although clinical presentations can vary with age (152), it is associated in most cases with a predictable set of symptoms. The multistage characteristic clinical syndrome is usually preceded by prodromic manifestations such as fever, headache, or viral-like illness. This is then followed, within one to three weeks, by psychiatric manifestations, sleep disturbances, memory impairment, seizures, language dysfunction, and in many cases with catatonia, dyskinesias, autonomic instability, decreased level of consciousness, and central hypoventilation.

3.1.1 Triggers and Neuroimmunology

The disease is related to the presence of antibodies directed against the NR1 subunit of the NMDA receptor. These antibodies, mainly IgG1, have been shown to cause NMDAR internalization and reduced expression (similar to that in myasthenia gravis, see below) and have been demonstrated to be pathogenic by *in vitro* (153) and *in vivo* studies (154, 155).

TABLE 2 | Clinical features of main forms of autoimmune encephalitis (AE) associated with known specific antibodies against neuronal surface antigens.

Antigen	Median age (range)	Sex ratio (M:F)	Main clinical syndrome	Other syndromes	Imaging	CSF features	Other features	Associations	Outcome
Antigens with well-known neuronal roles – excitatory or inhibitory									
N-methyl-D-aspartate receptor (NMDAR) (1-3)	21 (2 months-85 years)	1:4	Psychiatric syndrome, sleep disorders, seizures, amnesia followed by movement disorders, catatonia, autonomic instability, hypoventilation	Few cases with purely psychotic features; few with cryptogenic epilepsy	MRI: often normal or transient FLAIR or contrast enhanicng cortical or subcortical lesions. PET: relative frontal and temporal glucose hypermetabolism with occipital hypometabolism	Lymphocytosis in early stages (70%) and OCBs after (>50%); Abs usually present	EEG: frequent slow, disorganized activity (90%). Infrequent epileptic activity (20%). Rarely extreme delta brush pattern.	Ovarian teratoma in about 60%; post-HSV encephalitis (mainly children). Recently a few cases related to SARS-Cov2 infections have been reported.	~50% improve in 4 weeks with first line IT. 80% reach mRS 0–2. 12% relapsed within 2 years ~5% mortality.
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) (4)	55 (14-92)	2:1	LE with prominent seizures	Psychosis	Brain MRI: abnormal in 85% (usually bilateral temporal involvement)	Usually abnormal (75): lymphocytosis, OCBs; abs usually present	EEG: abnormali in 45%	Tumor in 70% cases (lung, thymoma, breast, ovary)	Most patients improve with IT; mortality related to underlying malignancy (15%)
Gamma-aminobutyric acid A receptor (GABAAR) (5)	40 (2 months-88 years)	1:1	LE with prominent seizures/status epilepticus	Psychiatric syndromes and catatonia; various presentation including SPS, opsoclonus, ataxia	Brain MRI: diffuse cortical and subcortical FLAIR signal abnormalities	Abnormal in up to 50% (lymphocytosis +/- OCBs); abs can be absent in the CSF	EEG: usually abnormal (80%) with epileptic activity and encephalopathy	Tumor in 15% cases (mostly thymoma)	Most patients improve with IT; mortality related to status epilepticus (20%)
Gamma-aminobutyric acid B receptor (GABABR) (6)	61 (16-67)	1.5:1	LE with prominent seizures	Ataxia, opsoclonus, status epilepticus	Brain MRI: abnormal in 70%	Common pleyocitosis (80%); rare OCBs. Abs usually present	EEG: usually abnormal (75%) with epileptic activity	Tumor in 50% (mainly lung)	Most patients improve with IT; mortality related to malignancy
Metabotropic glutamate receptor 5 (mGluR5) (7)	29 (6-75)	1.5:1	Encephalitis with psychiatric, cognitive, movement disorders, sleep dysfunction, and seizures	Ophelia syndrome	Brain MRI: abnormal in 50%	Lynphocitosis; abs presence unknown		Tumor (60%) (Hodgkin lymphoma, SCLC)	Response to IT
Glycine receptor (GlyR) (8)	50 (1-75)	1:1	Progressive encephalitis with rigidity and myoclonus or stiff person syndrome	LE, brainstem encephalitis; cryptogenic epilepsy	Brain MRI: mostly normal or non-specific; 5% temporal lobe inflammation. Spinal cord: lesions in 20%.	Pleocytosis in half of the cases, OCBs (20%); Abs can be absent in the CSF	EEG: 70% abnormal (mostly diffuse/ focal slowing, 15% focal epileptic). EMG: continuous motor unit activity, spontaneous or stimulus-induced activity in 60%	Thymoma (15%)	Usually improve with IT.
Antigens that modulate localization or function of potassium channels									
Leucine-rich glioma inactivated 1 (LGI1) (9-10)	60 (30-80) but observed	2:1	LE with or without FBDS and or	Cryptogenic epilepsy; neuromyotonia	MRI: medial temporal lobe hyperintensity (75%)	Usually normal, rare OCBs; abs can be absent	EEG: epileptiform activity in 30% of cases; focal	Tumor in 10% cases (mainly thymoma)	Despite recovery, cognitive deficits persist in many

(Continued)

TABLE 2 | Continued

Antigen	Median age (range)	Sex ratio (M:F)	Main clinical syndrome	Other syndromes	Imaging	CSF features	Other features	Associations	Outcome
	also in children		generalized seizures				slowing in 20%. Frequent hyponatremia (70%).		patients. One-third of patients relapse.
Contactin-associated protein like 2 (CASPR2) (11)	65 (25-77) but observed also in children	9:1	LE, MoS, NMT	Cerebellar ataxia, movement disorders, cryptogenic epilepsies, Guillain-Barre–like syndrome	MRI: medial temporal lobe hyperintensity (30%)	Usually normal (70%); rare OCB, pleocytosis and increased protein; abs can be absent	EEG: epileptiform activity in 40% of cases; focal slowing in 20%. Frequent hyponatremia (70%).	Tumor in 30% cases (mainly thymoma)	Response to immunotherapy. Relapse in 25% of cases.
Dipeptidyl-peptidase-like protein-6 (DPPX) (12)	53 (13-76)	1.5:1	Cognitive impairment, brainstem symptoms and diarrhea	Cerebellar ataxia, PERM	MRI: usually normal or non-specific	Pleocytosis, elevated proteins (30%); Abs usually present	EEG: 70% abnormal (mostly diffuse/ focal slowing)	B cells tumor (10% cases)	Response to immunotherapy (70%)
Antigen with likely cell-cell interaction functions but unclear overall role									
Ig-Like Domain-Containing Protein family member 5 (IgLON5) (13)	64 (46-83)	1:1	NREM sleep disorder, abnormal movement and behaviours with obstructive sleep apnoea and stridor, gait instability and brainstem symptoms	Dementia, movement disorders (chorea); isolated dysphagia	MRI: usually normal or non-specific (80%)	Pleocytosis (30%), increased proteins (50%); Abs usually present		Tauopathy at neuropathology	Up to 50% respond to initial IT but a sustained response is rare.
Neurexin3α (14)	44 (23-57)	1:2	Prodromal fever, headache, or gastrointestinal symptoms, followed by confusion, seizures, and decreased level of consciousness		MRI: abnormal in 20% (mesial temporal involvement)	Pleocytosis in all cases			Elevated mortality (40%)
Antigens normally considered to be associated with demyelinating disease and sometimes associated with encephalitic features									
AQP4 (15-16)	32-41	5-10:1	NMOSD, LETM, ON	Area postrema syndrome, narcolepsy	Brain: frequent over time (85%); mainly medulla, hypothalamus and diencephalon. Spinal cord: usually LE lesions. Optic nerve: extensive, often involving chiasm and tracts.	Abnormal in up to 80% (pleocytosis, elevated protein); rare OCBs (10-15%).		Rare cancer association	Respond to IT but sequelae as well as relapses are frequent.
MOG (17-18)	37 (1-74)	1:1	NMOSD, LETM, ON, ADEM, TM	Encephalitis, brainstem encephalitis, seizures	Brain: abnormal in 75% (WM subcortical lesions +/- brainstem involvement) Spinal cord: abnormal in 50%;	Abnormal in 60% (pleocytosis; rare OCBs).		Can be triggered by infections and vaccinations	Usually respond to corticosteroids (75%) Common relapses.

(Continued)

TABLE 2 | Continued

Antigen	Median age (range)	Sex ratio (M:F)	Main clinical syndrome	Other syndromes	Imaging	CSF features	Other features	Associations	Outcome
					frequent conus medullaris involvement. Optic nerve: extensive, often bilateral lesions; frequent chiasmal involvement.				

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The initial reports of NMDARE described a few cases of young women with psychiatric abnormalities, movement disorders and central hypoventilation in association with ovarian teratoma (156, 157). A subsequent study showed that about 50% of female patients with NMDAR-Ab encephalitis over 18 years bear uni- or bi-lateral ovarian teratomas (158). Compared to teratomas of patients without encephalitis, the teratomas of patients with NMDAR antibodies more often contain lymphoid structures characterized by aggregates of B and T cells, plasma cells and mature dendritic cells (159–163), and abnormal neuroglial tissue (161, 164), that expresses the NMDAR antibody subunit target NR1 (159). Moreover, the infiltrating B cells were shown to produce NMDAR antibodies *in vitro* (159) supporting a primary role of these tumour resident immune cells in the generation of the antibodies and explaining the patients' partial clinical improvement after tumour removal (158).

Besides ovarian teratoma, herpes simplex viral encephalitis (HSVE) is now considered a well-established possible trigger of NMDARE (165). A prospective cohort study showed that 27% of patients with HSVE developed symptoms of AE within 3 months. Patients who developed detectable neuronal antibodies within 3 weeks from onset had higher risk of autoimmune encephalitis. Clinical features and outcome were age dependent, with children 4 years old or younger more likely to develop choreoathetosis, decreased level of consciousness and frequent seizures or infantile spasms, responding less to immunotherapies compared to older children and adults who developed predominant change of behaviour and psychiatric symptoms sometimes accompanied by seizures. Overall, the outcome of post-HSV encephalitis, particularly in younger children, was worse than that of patients with classical NMDAR-antibody encephalitis, although the reason is unclear (165). Several mechanisms, including blood-barrier disruption with increased inflammation and complement deposition, T-cell mediated cytotoxicity, the presence of viral related damage, have been implicated but need confirmation (165).

Similarly, the mechanism underlying post-HSV encephalitis must be clarified. It is possible that molecular mimicry between NMDAR and HSV proteins play a major role. However, the frequent presence of other NSA-Ab (165–168) suggests that other mechanisms might be more likely, such as a secondary release of antigenic proteins from neuronal injury or host inflammatory responses specific to HSV infection. Other infections, including varicella zoster (169), Japanese encephalitis (170), HIV (171) and recently Covid-19 (172–174), have been reported as triggers of NMDAR antibodies as well as other AE, suggesting a model where a viral-induced brain destructive inflammatory process causes the release of neuronal surface proteins and receptors which become secondary targeted antigens of the virus-triggered immune response. The host HLA genetic background could be relevant to whether the HSVE patient develops AE or not.

The diagnosis of NMDARE is confirmed by the detection of IgG antibodies to NR1 in the serum or CSF. The latter is considered highly sensitive and specific for NMDARE (175). CSF analysis can show lymphocytic pleocytosis or oligoclonal bands, although it can show normal finding at onset (158, 175). EEG often shows diffuse slow and disorganized activity, and some epileptic discharges (175). A unique EEG pattern, defined extreme delta waves, can be found in a subgroup of patients and it is considered highly suggestive the diagnosis (176). Brain MRI can be normal or show multiple abnormalities in cortical and subcortical regions in FLAIR with possible contrast enhancement (175). It must be noted that access to brain MRI and antibody testing might be limited in some African situations, hampering the achievement of a correct diagnosis (151, 177).

Since the prognosis of NMDARE is largely time dependent (158), early diagnosis and treatment are pivotal to ensure a good outcome. For this reason, in 2016, a consensus of experts established a set of clinical criteria to help clinician to achieve a diagnosis of probable NMDARE, even without the confirmatory detection of the antibodies which could not be always easily or timely available, although it remains fundamental for the definite diagnosis (178). In a retrospective paediatric cohort, these criteria shown 90% sensitivity and 96% specificity for the diagnosis of probable NMDARE, after a median of 2 weeks from onset (179). Another study, including both children and adults, showed a sensitivity of 49% and a specificity of 98% for the diagnosis of probable NMDARE. Also in this case, the sensitivity increased over time from 16% in the first 2 weeks to reach 87% between 31 and 90 days after onset (180). Differential diagnosis includes mainly viral encephalitis, malignant catatonia, neuroleptic malignant syndrome, and primary psychiatric disorders. Clinical features distinguishing HSVE from non-viral and post-HSV NMDARE are shown in **Table 3**.

Early recognition of post-HSVE NMDARE is relevant to ensure timely initiation of immunosuppressive treatment and better outcomes. This diagnosis should be suspected in patients, and particularly children, with relapsing symptoms post HSVE and confirmed by NMDAR-antibody detection in the CSF, since serum antibodies can occur post HSVE also in patients without encephalitis (165). No brain MRI or CSF features during the acute HSV infection appear to predict the onset of post-HSVE NMDARE. Brain MRI studies at onset of autoimmune encephalitis showed that 82% had contrast enhancement comparable to that found during the viral encephalitis,

TABLE 3 | Clinical features and differential diagnosis between NMDARE, HSVE and relapse and post-HSVE NMDARE.

Clinical features	NMDARE	HSV encephalitis	HSVE relapse	Post-HSVE NMDARE
Prodromes	Headache, fever, diarrhea, flu-like syndrome		Previous HSVE (usually within 3 weeks)	Previous HSVE (within 2-16 weeks)
Main syndrome	Psychiatric syndrome, sleep disorders, seizures, amnesia followed by movement disorders, catatonia, autonomic instability, hypoventilation	Seizures, headache, confusion, fever, personality changes/ psychiatric symptoms	Fever, seizure, altered level of consciousness.	Frequent movement disorders, altered level of consciousness (particularly in children); more frequently seizures and psychiatric disorders in adults.
Brain MRI	Often normal or transient FLAIR or contrast enhancing cortical or subcortical lesions.	Frequent (90%) uni- or bi-lateral temporo-mesial T2/ FLAIR hyperintensities	Frequent uni- or bilateral lesion; frequent new lesions with edema, hemorrhage, and necrosis in the inferomedial temporal lobe.	Contrast enhancement comparable to that found during the viral encephalitis.
CSF	Lymphocytosis in early stages (70%) and OCBs after (>50%)	Pleocytosis, increased protein; frequent red blood cells.	Pleocytosis, increased protein; frequent red blood cells.	Pleocytosis, increased protein.
EEG	Frequent slow, disorganized activity (90%). Infrequent epileptic activity (20%). Rarely extreme delta brush pattern.	Abnormal in 80% (paroxysmal spike and sharp waves). Temporal triphasic waves and PLEDs.	Usually altered; frequent worsening bilateral abnormality with slow wave activity and recurrent periodic complexes.	Can be slow, normal or show epileptic activity
Diagnostic tests	NMDAR antibodies in CSF +/- in serum	HSV PCR in CSF. Possible false negative (early stages; children)	HSV PCR in CSF	NMDAR antibodies in CSF; HSV PCR usually negative
Outcome	~50% improve in 4 weeks with first line IT; 80% reach mRS 0–2; 12% relapsed within 2 years ~5% mortality.	Frequent neurological sequelae, high mortality and morbidity	Frequent neurological sequelae, high mortality and morbidity	Neurological sequelae more frequent and more severe than classical NMDAR encephalitis

similarly to findings observed in patients who did not develop post-HSVE encephalitis. However, patients who developed autoimmune encephalitis were more likely to have necrosis with cystic lesions in MRIs obtained at follow-ups (165). At onset of the post-HSVE NMDAR encephalitis, CSF HSV1–2 PCR is generally negative, showing mild pleocytosis and increased protein levels (165, 167). The limited cases with concomitant CSF detection of HSV by PCR and NMDAR antibodies had clinical phenotypes compatible with the autoimmune disease (181).

3.1.2 Treatment

NMDARE treatment is based on immunosuppression and tumour removal in paraneoplastic cases. Immunotherapy involves an escalation from first-line therapies (steroids, intravenous immunoglobulin, or plasma exchange) towards second-line treatments (rituximab or cyclophosphamide) in non-responders. About 50% of 472 patients who underwent first-line treatment or tumour removal showed an improvement with 4 weeks. Among those who did not improve 57% received a second-line treatment which resulted in a better outcome compared to those who did not receive second-line. Around 10% of patients are refractory to second-line therapies (158). In these cases, bortezomib or tocilizumab have been suggested as third-line therapies (182, 183). Overall, about 75% of patients experience only mild long-term deficits or recover completely, but the remaining 25% have severe sequelae, and mortality due to intensive care complications can be up to 7% (158, 184, 185). Relapses occur in 12% of patients and are more frequent in non-paraneoplastic cases and in patients who did not receive a second-line treatment (158). Again, it must be underlined that access to plasmapheresis and immunoglobulin might be difficult in some African regions (151). Moreover, ICU might not be always available and when it is, mortality risk in ICU is high, mainly in relation to sepsis and tracheostomy requirements (177, 186).

In patients with suspected post-HSVE NMDARE, antiviral therapy should be started, until a relapse of HSV encephalitis is excluded. Once the diagnosis is established, first- and/or second-line immunotherapy should be promptly started. Immune treatment has not been associated with HSV encephalitis relapse (181). It is unclear if early steroid treatment during HSV could decrease the risk of secondary autoimmunity, but a clinical trial is under way (187). Therefore, to date, early steroid and acyclovir combination therapy remains experimental (188).

4 A RECOGNISED AUTOANTIBODY-MEDIATED DISORDER WITHOUT KNOWN RELATIONSHIP TO INFECTION

4.1 Myasthenia Gravis

Myasthenia gravis (MG) is the archetypal autoantibody-mediated disease. It is relatively rare with an estimated annual incidence of 8–10 cases per million person-years (189). It is characterized by fatigable muscle weakness due to loss of acetylcholine receptors (AChRs) at the neuromuscular junction (190). AChR loss is due either to antibodies directly binding the receptor (AChR-Abs) or to antibodies inhibiting the function of muscle specific kinase (MuSK-Abs) which regulates AChR numbers and density. A proportion of patients have thymic hyperplasia or a thymic tumour but otherwise the aetiology is unknown. Other neuromuscular junction disorders are described in **Table 4**.

MG has been widely reported around the world. In South Africa, the incidence figures, age and gender distributions were similar to reports from Europe and North America (191), with a bimodal pattern: mainly females peaking at 30 years at onset and a higher peak at 70–80 years of age with predominantly males. The apparent "increase" in the incidence, compared with a decade previously, was likely due to the greater availability of the AChR-ab testing in addition to better access to specialist healthcare (191, 192). The standardized incidence rate for childhood AChR-Ab MG in a South African study was higher than a report from England (193). Childhood MG is also common in East Asia (191, 194). Most data available in other Sub-Saharan countries comes from small series and case reports (195–197), and MG may be unrecognised and untreated in large parts of Africa.

Clinically MG is characterized by fatigable weakness of ocular, bulbar, and proximal limbs muscle, which is often worse at the end of the day and in milder cases may improve with brief rest periods. Classically, around 15% of MG patients have pure ocular symptoms (double vision and ptosis), and many of the 85% with generalized MG may initially present with ocular symptoms. Fatigable bulbar symptoms include chewing fatigue, swallowing and speech difficulties. Chest muscle and diaphragm involvement can result in insidious, asymptomatic type II respiratory failure with early morning headaches and cor pulmonale. The selective involvement of triceps muscle weakness was described in a small group of African Americans with MG (198) and has also been seen in those with African genetic ancestry of whom 15 of 96 (16%) also had distal finger extensor weakness (Heckmann, unpublished observations). In addition, in South Africa MG patients with African genetic ancestry, both adults with juvenile onset disease and children, are more prone to develop treatment resistant ophthalmoplegia and ptosis (199, 200). Unbiased genome-wide sequencing studies in AChR-ab positive MG patients, with and without the ophthalmoplegic sub-phenotype of MG, have shown association with several muscle-expressed genes known to be involved in muscle atrophy signalling and myosin II function (201, 202). Gene expression studies in the orbital muscles of affected MG patients vs controls, also showed aberrant regulation of muscle atrophy and mitochondrial pathways (203). The importance of these findings for the treating physician is that in MG-induced ocular muscle paralysis, early intervention with immune treatment with the aim of minimizing the duration of ocular muscle paralysis, has shown the best treatment responses (204).

Autoantibodies against the muscle AChR are predominantly IgG1 and IgG3 subclasses and lead to loss of AChRs by two main mechanisms; by complement activation and by cross-linking and

TABLE 4 | Disorders of neuromuscular transmission and differential diagnoses.

	Main clinical features	Basic treatments
Myasthenia gravis		
AChR antibodies Younger females (<50y) and older males (>50y).	Generalised or more localised weakness and fatigue. Increases on repetitive activity. Thymic hyperplasia; must look for thymoma but many older patients have no thymoma or hyperplasia.	Anti-cholinesterase. Steroids and azathioprine. Plasma exchange if available
MuSK antibodies	Often more bulbar and respiratory than generalised weakness.	Anti-cholinesterases can be detrimental. Plasma exchange very effective, steroids and azathioprine not always adequate.
Lambert-Eaton Myasthenic Syndrome		
VGCC antibodies	Weakness that decreases with brief tonic activity. Strongly associated with small cell lung cancer and smoking history, but some patients have no tumour and a purely autoimmune disease.	3,4-di-amino-pyridine helpful but difficult to acquire. Steroids and azathioprine as for MG.
	Often neuromuscular junction effects with weakness in ocular and respiratory muscles.	As per local guidelines
Important Differential Diagnoses		
a. Venoms and Neurotoxins eg. snake bite, botulism, tetanus		
b. Congenital Myasthenic Syndromes	Inheritance mostly autosomal recessive but autosomal dominant in a few. Diverse neuromuscular junction gene mutations in pre and postsynaptic proteins particularly choline acetylase, Collagen Q, AChR, MuSK, DOK7 and others. Not always clinically evident in early life and older onset genetic disorders can be misdiagnosed as autoimmune MG. If suspected, refer to Rodriguez Cruz et al., 2018 for details	Treatment is symptomatic and mutation analysis is helpful in defining treatments for different forms which can respond adversely to the incorrect therapy, eg. anticholinesterase drugs make some conditions worse.

internalization of AChRs (205). Patients can improve rapidly when the antibodies are reduced in concentration by using plasma exchange and steroids. MuSK autoantibodies, by contrast, are predominantly of the IgG4 subtype. They are found in a proportion of patients without AChR antibodies and have a relatively high incidence of bulbar involvement and often respond poorly to immunotherapies (206). In Europe, there is a north-south gradient with MuSK-Ab MG being more common in Mediterranean countries. In patients with African genetic ancestry, either indigenous African (black) or mixed African genetic ancestry, studies from North America (211, A Vincent unpublished) and South Africa (207) have reported a higher proportion of AChR-Ab negative patients with MuSK antibodies. Future studies within the African continent are needed to increase our knowledge of the epidemiology and distribution of MG autoantibodies.

4.1.1 Management and Treatment of MG in Sub-Saharan Africa

MG diagnosis is primarily clinically based although antibody testing can be helpful to confirm the diagnosis and for subgroup classification (208). However, these serological tests are not widely available in many sub-Saharan African countries, and shipping the tests abroad is expensive so most physicians must rely on recognition of the clinical features, neurophysiological studies if available, and reversibility of the symptoms by treatments, to help establish the diagnosis.

The main treatments are cholinesterase inhibitors, that temporarily reverse symptoms, and steroids that reduce the antibody levels. The steroid-sparing agent azathioprine is generally available, as it is included in the WHO list of essential medicines (209), while mycophenolate mofetil, methotrexate, tacrolimus and other immunosuppressive agents can be difficult to find. However, methotrexate as a steroid-sparing agent for newly diagnosed MG, was found to be as safe and effective as azathioprine and is 10-fold cheaper than azathioprine (210). Importantly, methotrexate (and mycophenolate mofetil) must be avoided in potentially child-bearing women, but it is useful in children and older people with MG. The availability of anti-CD20 monoclonal antibody rituximab for patients with refractory AChR-antibody positive MG and MuSK-MG is limited due to its high costs. However, the use of single low doses of rituximab has proven very effective for 6-9 months or longer, in a cohort of refractory cases from South Africa, including myasthenic patients living with HIV (Heckmann, unpublished). It is important to be aware that with limited critical care capacity in low and lower middle-income African countries (211), routine follow-ups and close monitoring of immunosuppressive therapies are crucial to minimize the risk of myasthenic exacerbations. Certain antibiotics may trigger MG crises and should be avoided including tetracyclines, fluoroquinolones (and quinine), and aminoglycosides (https://www.myastheniagravis.org/mg-and-drug-interactions/). Artesunate has been used to successfully treat malaria in an MG patient (212).

MG remains a rare disease and there are numerous challenges to clinical trials, such as poor recruitment of participants (213). Currently, <2% of clinical trials worldwide take place in Africa, mainly in Egypt and South Africa (214). Establishing well-characterised cohorts and registries of MG patients in sub-Saharan Africa could help patients benefit from the development of new therapies, and also advance clinical trials globally for MG.

Other challenges in managing patients with MG in sub-Saharan Africa include concomitant infectious diseases such tuberculosis, Human Immunodeficiency Virus (HIV) infection and hepatitis B/C coinfection, which are prevalent in some African countries. Screening for these infections prior to immunomodulatory treatment are essential and prophylactic treatment for tuberculosis, such as isoniazid with pyridoxine supplementation for 6-9 months, should be considered in MG patients who have evidence of scarring on their chest radiographs when immunosuppression is started (215). The risk of reactivation of latent tuberculosis is highest in the first year of starting immunotherapies, and particularly with higher doses of steroids (216). Overall, the therapeutic approach to MG patients with HIV infections should be similar to those who are uninfected. Worsening of MG within 6 months of starting antiretroviral treatment can be seen as an effect of immune recovery (215). Monitoring MG patients with HIV infection receiving immune therapies, should include 6-monthly HIV viral load estimation to ensure effectiveness of antiretroviral therapy. As with most chronic diseases, monitoring of the patient's disease is useful to direct clinical decision making. The MG-activities of daily living (MG-ADL) is a simple, validated questionnaire which could be used in African settings (217).

5 CONCLUSION

Unravelling the interplay between viral infections, neurological autoimmunity and genetics is work in progress. Viruses need to access the host nucleus to replicate and cause disease. Answers regarding the role of host mutations in RANBP2, a nuclear pore protein, involved in the pathogenesis of recurrent ANE1 makes this condition a very good model for understanding the links between genetics, viral infections and neuroinflammation. Our understanding of the role of viral mutations in the enhanced fusion with CNS target cells and pathogenesis of persistence of MeV in MIBE and SSPE is important for development of future therapies for these devastating MeV CNS complications. There are lots of other unanswered questions regarding recurrence in Guillain-Barre syndrome, the "cytokine storm" target cells and pathogenesis on ANE/NE1, the causal link between Onchocerciasis and associated neurological syndromes, etc. Lessons learnt from well-studied models like myasthenia gravis and autoimmune encephalitis are important in shedding light on the basic immune principles and therapeutic possibilities for both CNS and PNS post-infectious autoimmune diseases.

There are many gaps in knowledge regarding post-infectious autoimmunity in the nervous system. The African continent faces serious challenges in tackling not only the endemic, epidemic and pandemic infections, but the immunological conditions that are sequelae of these infections. Examples of challenges are lack of data, infrastructure, tools, health and scientific research personnel, political stability, etc. Pandemic collision is a real threat that could result in catastrophic human life and economic losses.

There are also things that are relatively easy to do, the proverbial "low-hanging fruit". There is much that Africa can easily achieve with the current limited resources. Some answers are readily available, like the simple evidence that vaccination works. Measles, which still ravages many parts of the continent is preventable (218). The best way to manage the neurological complications of measles is to vaccinate young infants, achieve high vaccine coverage and to promote herd immunity (219). Evidence is also mounting regarding the efficacy and effectiveness of vaccines in preventing Covid-19 morbidity and mortality. Resources must be pooled to bolster vaccine initiatives and expedite roll-out. Education and public campaigns about the importance of vaccination and creation of an atmosphere and infrastructure that enable it are essential. Vaccine hesitancy must be addressed. Political will is required from governments across Africa with continental and intercontinental collaboration. International pressure needs to be mounted to discourage western governments from hoarding resources like vaccines.

There are other silver linings that need to be pursued. A lot of lessons have been learnt in the past when dealing with previous endemics/epidemics like, HIV, malaria, and Ebola. The know-how and health infrastructures built over time to address these scourges in many African countries, must be readapted and used as "tram-tracks" for new programmes to deal with new and emerging pandemics. Data gaps must be addressed, integrated disease surveillance increased, and reporting escalated through multidisciplinary, national, and international collaboration. Investing in the youth of the continent, training young future health scientists armed with modern skills and tools to face future challenges will go a long way.

AUTHOR CONTRIBUTIONS

JW: planning article structure and manuscript review and editing. AK-M: planning article structure, subsection author, and manuscript draft and review. AV: planning article structure, abstract review, subsection author, and manuscript draft/editing. HW: planning article structure, subsection author, and manuscript draft and review. KB: planning article structure, subsection author, and manuscript review. BE: planning article structure, subsection author, manuscript draft, and review and editing. AN: corresponding author, planning article structure, drafting abstract, subsection author, and manuscript draft and editing. JH: co-author of a section and manuscript review and editorial input overall. PC: planning article structure, subsection co-author, and manuscript review. MG: planning article structure, subsection author, and manuscript draft and review.

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A Neurometabolic Pattern of Elevated Myo-Inositol in Children Who Are HIV-Exposed and Uninfected: A South African Birth Cohort Study

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Introduction: Exposure to maternal HIV in pregnancy may be a risk factor for impaired child neurodevelopment during the first years of life. Altered neurometabolites have been associated with HIV exposure in older children and may help explain the mechanisms underlying this risk. For the first time, we explored neurometabolic profiles of children who are HIV-exposed and uninfected (CHEU) compared to children who are HIV-unexposed (CHU) at 2-3 years of age.

Methods: The South African Drakenstein Child Health Study enrolled women during pregnancy and is following mother-child pairs through childhood. MRI scans were acquired on a sub-group of children at 2-3 years. We used single voxel magnetic resonance spectroscopy to measure brain metabolite ratios to total creatine in the parietal grey matter, and left and right parietal white matter of 83 children (36 CHEU; 47 CHU). Using factor analysis, we explored brain metabolite patterns in predefined parietal voxels in these groups using logistic regression models. Differences in relative concentrations of individual metabolites (n-acetyl-aspartate, myo-inositol, total choline, and glutamate) to total creatine between CHEU and CHU groups were also examined.

Results: Factor analysis revealed four different metabolite patterns, each one characterized by covarying ratios of a single metabolite in parietal grey and white matter. The cross-regional pattern dominated by myo-inositol, a marker for glial

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reactivity and inflammation, was associated with HIV exposure status (OR 1.63; 95% CI 1.11–2.50) which held after adjusting for child age, sex, and maternal alcohol use during pregnancy (OR 1.59; 95% CI 1.07 –2.47). Additionally, higher relative concentrations of myo-inositol to total creatine were found in left and right parietal white matter of CHEU compared to CHU (p=0.025 and p=0.001 respectively).

Discussion: Increased ratios of myo-inositol to total creatine in parietal brain regions at age 2-3 years in CHEU are suggestive of early and ongoing neuroinflammatory processes. Altered relative concentrations of neurometabolites were found predominantly in the white matter, which is sensitive to neuroinflammation, and may contribute to developmental risk in this population. Future work on the trajectory of myo-inositol over time in CHEU, alongside markers of neurocognitive development, and the potential for specific neurodevelopmental interventions will be useful.

Keywords: HIV exposure, magnetic resonance spectroscopy, neuroinflammation, brain development, myo-inositol

INTRODUCTION

Human immunodeficiency virus (HIV) infection remains a major public health concern worldwide, with 37.7 million people reported to be living with HIV globally (1). Of these, an estimated 25.3 million people live in sub-Saharan Africa. The widespread roll-out of antiretroviral therapy (ART) and expansion of ART programmes for prevention of mother-to-child transmission (PMTCT) have led to dramatic declines in vertical transmission rates to less than 5% during recent years (2). Globally, the estimated number of new infections in children aged 0 to 14 years has decreased by more than 60% since the year 2000 (3). However, progress in the eradication of paediatric HIV infection has revealed a concern that children who are HIV-exposed and uninfected (CHEU) remain a vulnerable population (2, 4). Approximately 15.4 million children worldwide are CHEU, 13.8 million of whom live in sub-Saharan Africa (1), with the highest number of CHEU residing in South Africa (3). Due to expanding accessibility of both ART and PMTCT programmes this population is increasing in number, however, the implications of HIV and ART exposure as risk factors for long-term child health and development are less well defined (4, 5).

Meta-analyses have found that CHEU are at a greater risk of all-cause mortality and worse developmental outcomes within the early years of life, compared to children who are HIV-unexposed (CHU) (6, 7). In sub-Saharan Africa, recent studies have described HIV exposure to be associated with neurodevelopmental delay (8–11) in children younger than 3 years of age. However, there is inconsistency across studies and settings, and others have reported CHEU having similar outcomes to CHU (12, 13).

There are a number of hypothesised mechanisms by which HIV exposure may impact paediatric brain development. As argued in the two-hit model of early brain damage, inflammatory intrauterine conditions may increase vulnerability of the developing brain to postnatal adverse events (14, 15). Since chronic inflammation can persist in HIV infection despite ART, women living with HIV may have immune dysregulation during pregnancy (16, 17). This may prime the developing brain to trigger exaggerated inflammatory responses against future insults, compromising typical neurobiological development (18–20). Immunological studies suggest the immune system of CHEU is altered compared to that of CHU (17, 21), some revealing proinflammatory immune profiles from birth to 2 years of age (22, 23). Neurobiological development in CHEU may therefore be affected by maternal immune dysregulation during pregnancy, however, studies of early neurometabolic development are lacking.

Exposure to ART has also been associated with potential neurotoxicity (24). Although maternal ART and child prophylaxis are important to prevent HIV transmission, potential metabolic and neurological consequences have been reported (25). Furthermore, environmental stressors are known to influence long term neurodevelopmental outcomes during the period from conception to 2 years of age, and psychosocial risk factors such as maternal antenatal depression and alcohol use in pregnancy may play a key role in child development (26, 27). Overall, there remains a gap in understanding the neurobiological consequences of HIV exposure in the context of high-risk environments.

Neuroimaging studies provide a key opportunity to examine HIV exposure-related neuropathophysiology (28), with reports describing white matter and grey matter differences between newborns who are HEU compared to HU (29, 30) and white matter abnormalities in older children who are HEU (31). Amongst the existing techniques, magnetic resonance spectroscopy (MRS) is a powerful approach, since it provides *in vivo* measurements of neurometabolites in specified brain regions. MRS profiles of the neurotypical brain during childhood are well characterized (32, 33), and this technique has previously been used to describe metabolite alterations in children older than 2 years with perinatal infection or exposure to HIV (34–36). Only one cohort study to date has examined neurometabolic characteristics of CHEU, reporting metabolite alterations in the basal ganglia at age 9 years, and in the frontal grey matter (GM)

and peritrigonal white matter (WM) at age 11 years, compared to CHU (35, 36). MRS data are suitable for dimensionality reduction methods like factor analysis, which groups similar variables into a smaller number of dimensions. Through the combination of metabolite measurements across different brain regions, this method identifies metabolic patterns that underlie latent neurobiological processes. Factor analysis has previously been used in MRS studies to identify metabolic patterns within the context of HIV-related illness (36–38).

The aim of our study was to explore differences in brain metabolites in a well-characterized cohort of CHEU and CHU from similar sociodemographic conditions at 2-3 years of age, using MRS and factor analysis. We hypothesised that CHEU would have altered neurometabolic profiles compared to CHU in GM and WM, related to factors associated with inflammation.

METHODS

Participants

The Drakenstein Child Health Study (DCHS) is a population-based birth cohort study in a peri-urban area of the Western Cape, South Africa, focused on investigating the early-life determinants of child health, development and illness (39–41). The local population is a low socioeconomic community with a high prevalence of several health risk factors including HIV infection.

The DCHS enrolled pregnant women between 2012 and 2015 during their second trimester of gestation and currently follows the mother-child pairs into middle childhood. Inclusion criteria for enrolment were a minimum age of 18 years, gestational period of 20–28 weeks, planned attendance at one of the two clinics and intention to remain in the area. All mothers gave written informed consent.

A subset of children enrolled in the DCHS participated in a longitudinal neuroimaging sub-study. As part of the neuroimaging sub-study, children who had undergone neonatal imaging (41) were invited to be scanned at 2-3 years. In addition, children not imaged at birth were also included selecting for risk factors (maternal HIV and alcohol use during pregnancy) to ensure a representative sample of a high-risk population, along with a randomly selected comparison group. These children were currently active in the study and living in the area. Exclusion criteria applied to children for this sub-study were: medical comorbidities such as congenital abnormality, genetic syndrome, or neurological disorder; low Apgar score (<7 at 5 minutes); neonatal intensive care admission; history of maternal use of illicit drugs during pregnancy; child HIV infection; and MRI contra-indications including cochlear implants (42).

Sociodemographic Data Collection

The HIV status of enrolled mothers was confirmed *via* routine testing during pregnancy and re-checked every 12 weeks, in accordance with the Western Cape PMTCT guidelines (43). Children who were HIV-exposed were tested at age 6 weeks, 9 months, and 18 months using PCR, rapid antibody, or ELISA tests as per guidance. CHEU were confirmed to be negative for HIV at the age of 18 months, or once the mother had stopped breastfeeding if this lasted more than 18 months. CHU were defined as children born to mothers without HIV infection. Mothers living with HIV received ART according to PMTCT guidelines at the time. CHEU were prescribed post-exposure prophylaxis from birth (44). Maternal CD4 cell count and viral load data during pregnancy were abstracted from clinical records and the online National Health Laboratory Service system, collected as part of clinical care protocols. The lowest maternal CD4 cell count within 1 year before child's birth and 3 months after birth was used to maximise numbers.

Sociodemographic and maternal psychosocial data were collected between weeks 28 and 32 of gestation, through interviews and questionnaires adapted from the South African Stress and Health study (39, 40). Infant birthweight and markers of poor nutrition were also collected, in accordance with the World Health Organization (WHO) Z-score guidelines (45). Stunting was defined as low child height-for-age, underweight as low child weight-for-age, and wasting as low child weight-for-length, all calculated as Z-scores lower than -2 of the WHO Child Growth Standards median. Maternal alcohol use during pregnancy was assessed using the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST), and data on moderate-severe alcohol use in pregnancy was retrospectively collected, forming a dichotomous measure (41). Maternal smoking during pregnancy was determined through self-reporting. Maternal depression was assessed with the Edinburgh Postnatal Depression Scale.

Magnetic Resonance Spectroscopy Protocol

Participants in the neuroimaging sub-study underwent a multimodal magnetic resonance imaging (MRI) protocol without sedation, performed between January 2016 and September 2018 at Groote Schuur Hospital, University of Cape Town, on a 3 Tesla Siemens Skyra 70cm diameter bore whole body MRI scanner (Erlangen, Germany) using a 32-channel head coil (42). Once informed consent was acquired from the mother and the child had fallen into deep sleep, children were carried into the scanner, positioned carefully with pillows, blankets, and ear protection. MRS data acquisition was performed during natural sleep, and a trained study staff member remained in the scanner room during the entire session in case the child woke (42).

The MRS protocol was performed by well-trained radiographers who were blinded to the children's HIV exposure status. It consisted of a high-resolution T1-weighted multi-echo magnetisation prepared rapid gradient echo acquisition (MEMPRAGE (46); sagittal orientation, repetition time (TR) 2530 ms, echo times (TE) = 1.69/3.54/5.39/7.24 ms, flip angle 7.0°, voxel size 1.0 x 1.0 x 1.0 mm^3, inversion time (TI) 1100 ms, field of view (FOV) 224 x 224 x 176 mm, 176 slices, scan time 5 min 21 s) and single voxel Point RESolved Spectroscopy (PRESS; TR 2000 ms, TE 30 ms, 128 averages, voxel size 25 x 25 x 25 mm^3, vector size 1024, spectral bandwidth

1200 Hz, scan time 6 min) with Chemical Shift Selective (CHESS) water suppression. A water reference was acquired without using CHESS. Shimming was automatically performed over the voxel volume (with use of the scanner's advanced adjustments) and manually adjusted if necessary, to reduce the spectral linewidths reported by the scanner. Voxel 1 was targeted at the midline parietal GM, voxels 2 and 3 were targeted at left and right parietal WM respectively (**Figure 1**).

Magnetic Resonance Spectroscopy Data Processing

MRS voxels were registered to the T1-weighted structural image with use of MATLAB software (MATLAB. Natick, Massachusetts: The MathWorks Inc.; 2017). Segmentation of the structural image into GM, WM, and cerebrospinal fluid (CSF) was performed using Statistical Parametric Mapping (SPM12) software (www.fil.ion.ucl.ac.uk/spm) to determine tissue composition for each voxel.

LCModel software (version 6.3-1) (47) was run to fit the raw spectral data for quantification, using the appropriate water reference for eddy current correction. Relative concentrations (ratios) to the reference signal, creatine and phosphocreatine (Cr+PCr), were determined for n-acetyl-aspartate (NAA/Cr+PCr), myo-inositol (Ins/Cr+PCr), total choline (glycerophosphocholine and phosphocholine, GPC+PCh/Cr+PCr), and glutamate (Glu/Cr+PCr). Quality of spectra was inspected visually and assessed in terms of full width at half maximum (FWHM) and signal-to-noise ratio (SNR), and Cramér-Rao lower bounds (CRLB) given by LCModel. Spectra with FWHM values greater than 0.08, and SNR values lower than 10 were considered of low quality and therefore excluded.

The four metabolites considered in our study have been characterized in terms of clinical significance in prior studies, from birth through childhood (32, 33). N-acetyl-aspartate is most commonly considered to be a marker for neuronal health or density in the developing brain (32, 33). While we note that

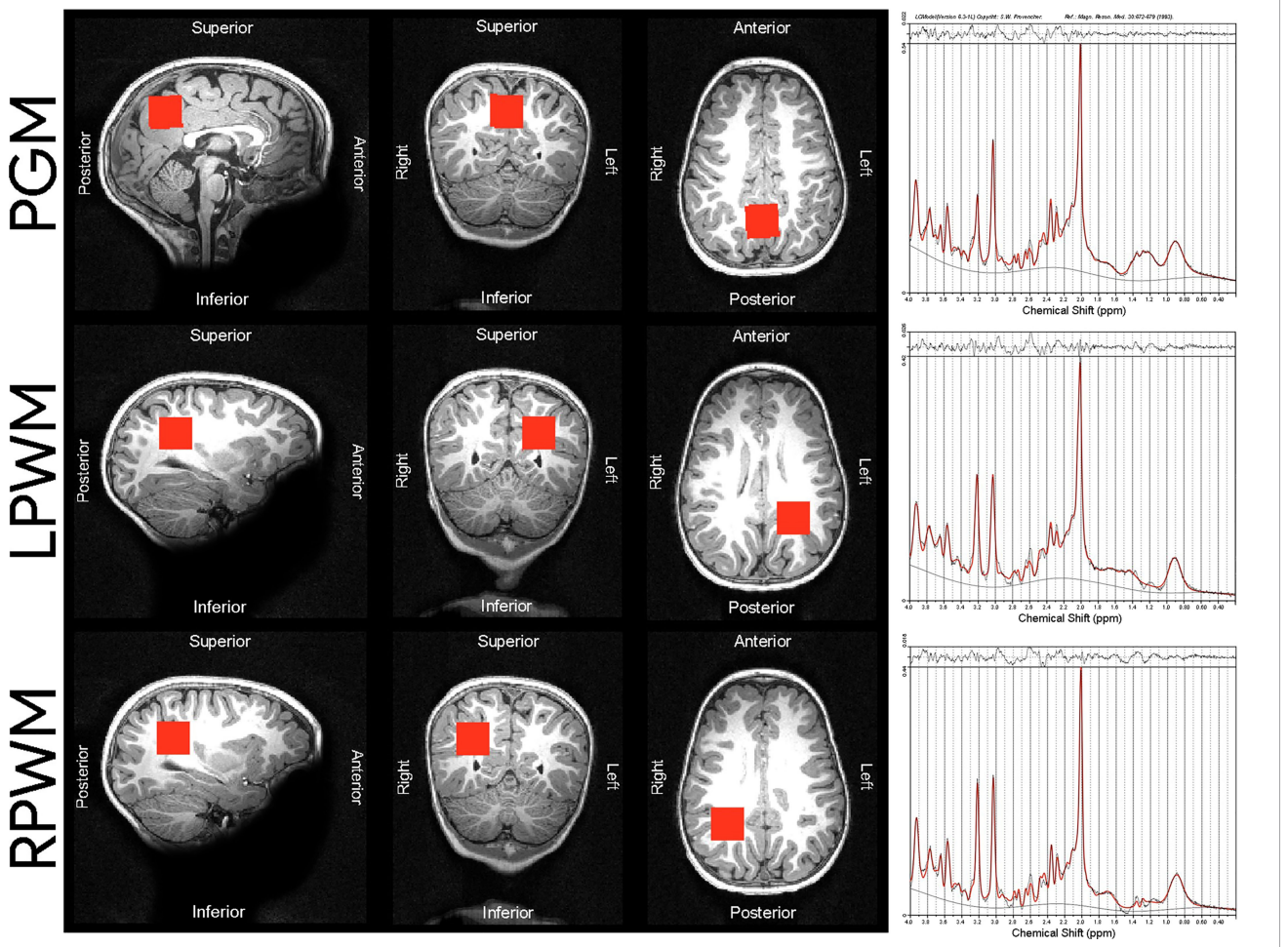

FIGURE 1 | Voxel placements and sample spectra of median SNR and LCModel output from the 3T scanner used in our cohort of children. The following median SNR were obtained for each voxel placement: PGM median SNR = 34, LPWM median SNR = 35, RPWM median SNR = 33. PGM, parietal grey matter; LPWM, left parietal white matter; RPWM, right parietal white matter; SNR, signal-to-noise ratio; ppm, parts per million.

the role of n-acetyl-aspartate in mature brain remains to be fully established and recognise that n-acetyl-aspartate may also play additional roles, such as contributing to myelin synthesis in the mature brain (48), the evidence for this is currently limited. Myo-inositol is considered a marker for glial reactivity, gliosis and neuroinflammation. Total choline is associated with myelination, membrane synthesis and membrane maturation in the WM. Glutamate, the main excitatory neurotransmitter in the brain, is considered a marker for neuronal function involved in many neurobiological and behavioural processes during brain development (32, 33).

Statistical Analysis

Sociodemographic characteristics of the mother-child pairs were reported as mean (± SD) for continuous data, or absolute frequencies (%) for categorical data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using *t*-tests or Wilcoxon tests for normally and non-normally distributed continuous data, respectively, and X^2 tests for categorical data.

Factorability of MRS data was assessed using Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests. Factor analysis was carried out with use of a maximum likelihood approach and varimax rotation, and Root Mean Square Errors of Approximation (RMSEA) of less than 0.05 were considered to indicate statistical goodness of fit of the model. As proposed by Yiannoutsos and colleagues (38), factor scores were constructed for MRS data using a weighted linear combination of all 12 variables (the ratios of 4 metabolites to total creatine in each of the 3 voxels), multiplying each metabolite concentration by its associated factor loading and summing all products to form each of four factor scores (38).

To determine whether the brain metabolic patterns could predict HIV exposure, the factor scores obtained from brain metabolite ratios were included as independent variables in logistic regression models, to estimate odds ratios (OR) and 95% confidence intervals (CI). Both unadjusted and multivariable models were created. Potential confounders were chosen *a priori* due to their reported influence in neurometabolic or neurobehavioral outcomes in children. These included child age (32, 33), child sex (27, 49), and maternal alcohol use during pregnancy (50, 51).

Sensitivity analyses were performed to examine the effect of sociodemographic characteristics that showed significant differences ($p<0.05$) between CHEU and CHU, by additionally adjusting for these variables: maternal age of delivery, and maternal depression during pregnancy. Despite having similar values between groups, infant birthweight was also included in the sensitivity analysis, since its role as confounder or mediator in the causal pathway of maternal HIV infection and child developmental outcomes may vary across settings (52).

Region-specific analyses were run for each metabolite ratio, to explore differences between CHEU and CHU. Comparisons between groups were made using unadjusted and adjusted linear regression analyses with robust standard errors. Child age, child sex, and maternal alcohol use during pregnancy were included as covariates. To account for the presence of GM in voxels targeted at parietal WM, GM percentage was included as a confounder in sensitivity analyses.

Lastly, we planned to examine the association of each child metabolite pattern identified from factor analysis, with maternal immune status during pregnancy and time of maternal ART initiation, using multinomial logistic regression to estimate relative risk ratios. For maternal immune status during pregnancy, a categorical variable was created with the following levels: lowest maternal CD4 cell count during pregnancy ≤500 cells/mm^3 versus >500 cells/mm^3 in CHEU. Similarly, for maternal ART initiation, a categorical variable was created examining maternal ART initiation before pregnancy versus during pregnancy. CHU was used as the reference in both models. A Cramér's V test was run to check for multicollinearity between the categorical variables.

Statistical analyses were performed in R with RStudio software (version 1.2.5033) (53). P values of less than 0.05 (two-tailed) were considered statistically significant.

RESULTS

Cohort and Demographic Characteristics

A total of 1143 mother-child pairs were enrolled in the DCHS. A subset of 156 children had MRS imaging at age 2-3 years. Of these, 143 had a successful MRS acquisition from the parietal grey matter voxel (first voxel in the data acquisition protocol), 134 from the left parietal WM voxel (acquired second), and 92 from the right parietal WM voxel (acquired third and last). A total of 9 participants were excluded from the study after inspection of obtained MRS data due to low quality of spectra in at least one of the three voxels. Our final complete-case cohort included 83 children (36 CHEU, 47 CHU) who had usable metabolite data for all three voxels (i.e., GM, left and right WM) and complete covariate data (**Figure 2**).

Socioeconomic characteristics of the complete-case cohort of children were comparable between groups. Mothers living with and without HIV had similar household incomes, education, employment status, marital status, hospitalization rates and smoking or alcohol use during pregnancy (**Table 1**). However, mothers living with HIV were older at delivery and, among those with available data (N=28 CHEU, N=42 CHU), there were lower rates of depression compared to their uninfected counterparts. Weight at birth was similar for CHEU and CHU. Exclusive breastfeeding duration was comparable between groups, as was the proportion of children with WHO markers for poor nutrition. All mothers living with HIV received first-line three-drug ART regimens, whereas post-exposure prophylaxis for CHEU included nevirapine (77.7%) or nevirapine and zidovudine (22.3%). The complete-case cohort and the original subset of 156 children were similar in terms of sociodemographic characteristics (**Supplementary Table 1**).

Metabolite Patterns of CHEU and CHU

Fractional tissue composition in each of the three voxels of the complete-case cohort did not differ between groups. The percentage of GM in the voxel targeted at parietal GM was

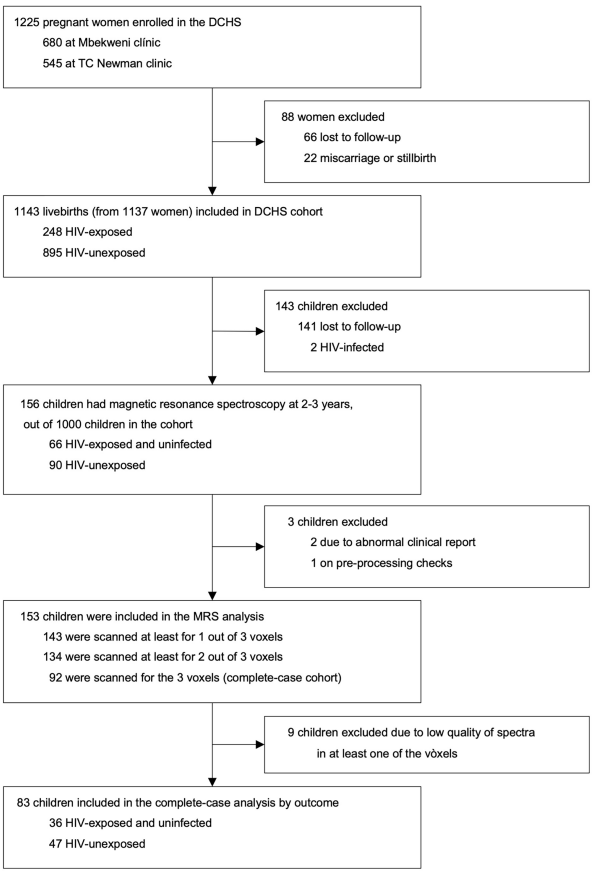

FIGURE 2 | DCHS flowchart for MRS sub-study. Exclusion criteria for the MRS nested sub-study are described in the methodology section.

≈77% for both CHEU and CHU, while the voxels targeted at left and right parietal WM contained ≈52% of WM in both groups (**Table 2**). For all spectral fits the CRLB for NAA/Cr+PCr were ≤7%, for Ins/Cr+PCr ≤6%, for GPC+PCh/Cr+PCr ≤6%, and for Glu/Cr+PCr ≤8%.

Bartlett sphericity and KMO tests confirmed the factorability of our data. Subsequent factor analysis identified four factors (RMSEA < 0.05), which accounted for 69% of data variability (**Table 3**). Each factor is a metabolic pattern composed of loadings associated with each of the metabolite ratios (/Cr+PCr), where a large loading (>0.6) indicates a strong contribution of a certain metabolite ratio to the factor. Factor 1 was composed of large loadings of NAA/Cr+PCr across all three brain regions and a strong contribution of Glu/Cr +PCr in the voxel targeted at parietal GM. Factor 2 was dominated by large loadings of Ins/Cr+PCr across brain regions. Factor 3 was composed of large loadings of GPC+PCh/Cr+PCr in the voxels targeted at left and right parietal WM, and a medium contribution (0.552) of the same metabolite in the voxel targeted at parietal GM. Factor 4 was characterized by large loadings of Glu/Cr+PCr in the voxel targeted at right parietal WM and a medium contribution (0.530) of the same metabolite ratio in the voxel targeted at left parietal WM.

In both unadjusted and adjusted logistic regression models, HIV exposure was significantly predicted by factor 2 (dominated by Ins/Cr+PCr across regions), with an OR estimate of 1.63 (95% CI 1.11 - 2.50) and adjusted OR 1.59 (95% CI 1.07 - 2.47), respectively (**Table 4**). None of the remaining three factors predicted HIV exposure. Sensitivity analyses revealed similar results when separately adjusting for maternal age at delivery, maternal depression during pregnancy and infant birthweight, with HIV exposure being significantly predicted by factor 2 (**Supplementary Table 2**).

Region-Specific Relative Concentrations of Metabolites to Total Creatine in CHEU and CHU

Unadjusted analyses for each individual metabolite relative concentration to total creatine and brain region revealed significantly higher ratios of Ins/Cr+PCr in left (p = 0.025) and right parietal WM (p = 0.001) of CHEU, compared to their unexposed peers. Levels of Glu/Cr+PCr in the right parietal WM of CHEU were also significantly higher than those of CHU (p = 0.034) (**Figure 3** and **Supplementary Table 3**).

The adjusted analyses did not substantially modify the results obtained for Ins/Cr+PCr (p = 0.004) and Glu/Cr+PCr (p = 0.015) in the right parietal WM, while group differences in Ins/Cr+PCr (p = 0.066) in the left parietal WM fell short of our selected threshold for statistical significance. Results remained similar for all metabolite ratios after accounting for the percentage of GM in WM voxels (data not shown).

Association of Maternal Immune Status and ART Initiation With Child Metabolite Patterns

Maternal immune status and ART initiation variables were found to be co-linear in this sub-group (correlation coefficient >0.7, Cramér's V test). Further, given only 72% mothers of CHEU children in this sample had CD4 cell counts taken during pregnancy, we were unable to run multinomial logistic regression using these variables as due to small sample size and missing data we recognized that our ability to draw valid conclusions from this analysis would be limited.

DISCUSSION

Our study is the first to describe the impact of HIV exposure without infection on brain metabolites at 2-3 years of age in a well-characterised cohort of children living in a LMIC setting. By combining MRS data from parietal grey and white matter regions using a factor analysis approach, we demonstrate a neurometabolite pattern of elevated Ins/Cr+PCr in the parietal brain regions of CHEU; this elevation is suggestive of neuroinflammatory processes.

Factor analysis identified four metabolic patterns in the parietal brain regions of our young cohort. Although all factors represent a weighted combination of all metabolite ratios to total creatine in each region, each factor was characterized by large

TABLE 1 | Sociodemographic characteristics of children included in the MRS complete-case analysis, according to HIV exposure.

	CHEU (N = 36)	CHU (N = 47)	p value
	Mean (±SD) or n/N (%)	Mean (SD) or n/N (%)	
Child age at scan (in months)	33.78 (±1.83)	34.15 (±1.75)	0.35
Sex			0.14
Male	25/36 (69.44%)	24/47 (51.06%)	
Female	11/36 (30.55%)	23/47 (48.93%)	
Monthly household income (in ZAR)			0.49
< 1000	12/36 (33.33%)	17/47 (37.17%)	
1000 - 5000	23/36 (63.88%)	26/47 (55.31%)	
> 5000	1/36 (2.77%)	4/47 (8.51%)	
Maternal education			0.82
Primary	3/36 (8.33%)	3/47 (6.38%)	
Some secondary	22/36 (61.11%)	26/47 (55.31%)	
Completed secondary	10/36 (27.77%)	15/47 (31.91%)	
Tertiary	1/36 (2.77%)	3/47 (6.38%)	
Employed mother	9/36 (25%)	9/47 (19.14%)	0.70
Maternal relationship status (partnered)	19/35 (54.28%)	17/47 (36.17%)	0.22
Maternal age at delivery (in years)	29.89 (±4.37)	25.65 (±5.06)	0.0001*
Gestational age at delivery (in weeks)	38.61 (±2.27)	38.85 (±2.86)	0.67
Premature birth (< 37 weeks' gestation)	5/36 (13.88%)	6/47 (12.76%)	1.00
Birthweight (in g)	3030 (±501.76)	3132 (±622.48)	0.40
Nutritional status at 2 years old			
Stunting (height-for-age Z-score < -2)	5/31 (16.13%)	5/41 (12.19%)	0.89
Underweight (weight-for-age Z-score < -2)	2/31 (6.45%)	1/41 (2.44%)	0.80
Wasting (weight-for-length Z-score < -2)	0/31 (0%)	0/41 (0%)	–
Maternal hospitalization during pregnancy	3/36 (8.33%)	4/47 (8.51%)	1.00
Maternal smoking during pregnancy	7/36 (19.44)	11/46 (23.91)	0.67
Maternal alcohol use during pregnancy	3/35 (8.57%)	10/46 (21.74%)	0.20
Maternal depression during pregnancy	1/28 (3.57%)	11/42 (26.19%)	0.032*
Exclusive breastfeeding duration (in months)	1.919 (±2.25)	2.180 (±1.47)	0.54
Maternal HIV diagnosis timepoint			
Before pregnancy	26/36 (72.22%)		
During pregnancy	10/36 (27.77%)		
Maternal lowest CD4 cell count[§] during pregnancy			
≤ 500 cells/mm^3	12/26 (46.15%)		
> 500 cells/mm^3	14/26 (53.85%)		
Highest maternal viral load during pregnancy			
(undetectable) < 40 copies/ml	25/29 (86.20%)		
40 - 1000 copies/ml	2/29 (6.90%)		
>1000 copies/ml	2/29 (6.90%)		
Antiretroviral therapy initiation			
Before pregnancy	20/36 (55.55%)		
During pregnancy	16/36 (44.44%)		
First-line antiretroviral therapy during pregnancy			
Fixed dose combination (Efavirenz+ Emtricitabine + Tenofovir)	33/36 (91.66%)		
Lamivudine + Zidovudine + Nevirapine	2/36 (5.55%)		
Lamivudine + Zidovudine + Efavirenz	1/36 (2.77%)		
Infant prophylaxis			
Nevirapine alone	28/36 (77.77%)		
Nevirapine and zidovudine	8/36 (22.22%)		

*Data are mean (±SD) or n/N (%). *p<0.05. Percentages calculated out of available data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using t-tests or Wilcoxon tests for normally and non-normally distributed continuous data, respectively, and X^2 tests with Yates correction for categorical data. Missing data: maternal relationship status (N = 1 in the CHEU group); nutritional conditions at 2 years old (N = 5 in the CHEU group, N = 6 in the CHU group); maternal smoking during pregnancy (N = 1 in the CHU group); maternal alcohol use during pregnancy (N = 1 in the CHEU group, N = 1 in the CHU group); maternal depression during pregnancy (N = 8 in the CHEU group, 5 in the CHU group); maternal CD4 cell count in pregnancy (N = 10); highest maternal viral load during pregnancy (N = 7). [§]The lowest maternal CD4 cell count within 1 year before birth and 3 months after birth was used to maximise numbers. CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed; ZAR, South African Rand; WHO, World Health Organization.*

contributions from a certain metabolite ratio grouped across brain regions with generally small contributions from the other metabolite ratios. Based on prior studies of paediatric MRS (32, 33), we proposed the following interpretations: Factor 1 was interpreted as a metabolic pattern for neuronal health or integrity, due to high loadings of NAA/Cr+PCr across brain regions. It also contained a strong contribution from Glu/Cr +PCr in parietal grey matter, suggesting that glutamate may

TABLE 2 | Fractional tissue composition in each defined MRS voxel, according to HIV exposure.

Voxel	CHEU (N = 36)			CHU (N = 47)		
	% Grey matter	% White Matter	% CSF	% Grey matter	% White Matter	% CSF
Parietal grey matter	**77.9** (±4.2)	12.9 (±2.8)	9.2 (±3.2)	**77.2** (±4.5)	14.1 (±2.8)	8.7 (±2.9)
Left parietal white matter	45.2 (±8.8)	**52.1** (±8.9)	2.7 (±1.6)	46.8 (±7.0)	**51.1** (±7.5)	2.1 (±1.2)
Right parietal white matter	46.2 (±8.7)	**51.9** (±9.2)	1.9 (±1.3)	46.1 (±6.6)	**52.5** (±7.0)	1.4 (±0.8)

Data is displayed as mean (±SD) percentages. Bold percentages indicate targeted tissue in each voxel. Data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using t-tests or Wilcoxon tests for normally and non-normally distributed data, respectively. All p values were greater than 0.05 (data not shown). CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed; CSF, cerebrospinal fluid.

TABLE 3 | Factor loadings.

Voxel	Metabolite	Factor Loading			
		Factor 1	Factor 2	Factor 3	Factor 4
PGM	**Glu/Cr+PCr**	**0.745**	-0.044	0.036	0.314
	Ins/Cr+PCr	-0.111	**0.767**	0.062	-0.208
	NAA/Cr+PCr	**0.911**	-0.145	-0.052	0.025
	GPC+PCh/Cr+PCr	-0.264	0.211	**0.552**	0.007
LPWM	**Glu/Cr+PCr**	0.439	-0.034	0.100	**0.530**
	Ins/Cr+PCr	-0.182	**0.906**	0.015	0.003
	NAA/Cr+PCr	**0.889**	-0.116	0.053	0.159
	GPC+PCh/Cr+PCr	0.113	-0.086	**0.821**	0.131
RPWM	**Glu/Cr+PCr**	0.151	0.008	-0.043	**0.883**
	Ins/Cr+PCr	-0.111	**0.823**	0.001	0.168
	NAA/Cr+PCr	**0.692**	-0.208	-0.029	0.072
	GPC+PCh/Cr+PCr	0.104	-0.005	**0.862**	-0.115

Bartlett sphericity and Kaiser-Meyer-Olkin tests were performed and confirmed that a factor analysis approach was suitable for our data. Factor analysis identified four main metabolic patterns (RMSEA < 0.05), which accounted for 69% of data variability and are displayed in this table. Factor loadings in bold represent the main components of each metabolic pattern. PGM, parietal grey matter; LPWM, left parietal grey matter; RPWM, right parietal white matter; NAA, n-acetyl-aspartate; Ins, myo-inositol; GPC+PCh, total choline (glycerophosphocholine + phosphocholine); Glu, glutamate;/Cr+PCr, relative to creatine + phosphocreatine.

TABLE 4 | Logistic regression analysis of factor scores as predictors for HIV exposure.

	Mean factor score		Unadjusted logistic regression			Adjusted logistic regression*		
	CHEU(N = 36)	CHU(N = 47)	OR	Confidence interval (95%)	P value	OR	Confidence interval (95%)	P value
Factor 1 (NAA)	-0.182	0.139	0.72	0.45 – 1.12	0.14	0.72	0.44 – 1.50	0.18
Factor 2 (Ins)	**0.368**	**-0.282**	**1.63**	**1.11 – 2.50**	**0.017**	**1.59**	**1.07 – 2.47**	**0.029**
Factor 3 (GPC+PCh)	-0.030	0.023	0.91	0.51 – 1.59	0.80	0.82	0.42 – 1.55	0.54
Factor 4 (Glu)	0.097	-0.074	1.28	0.76 – 2.21	0.35	1.41	0.81 – 2.56	0.23

*Odds ratios (OR) greater than 1 indicate an increased likelihood of association between a certain metabolite pattern and HIV exposure. Bold data represents statistically significant associations. *Adjusted for child age, child sex, and maternal alcohol use during pregnancy.*

NAA, metabolite pattern dominated by n-acetyl-aspartate ratios; Ins, metabolite pattern dominated by myo-inositol ratios; GPC+PCh, metabolite pattern dominated by total choline (glycerophosphocholine + phosphocholine) ratios; Glu, metabolite pattern dominated by glutamate ratios; CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed.

covary with n-acetyl-aspartate in certain regions and therefore with number or density of neurons. Factor 2 (dominated by Ins/Cr+PCr loadings across all regions) was considered an inflammatory pattern for neuroinflammation or gliosis; and Factor 3 (characterized by GPC+PCh/Cr+PCr across brain regions) was interpreted as a pattern for membrane maturation (32, 33). Factor 4 was dominated by Glu/Cr+PCr across WM regions. This made it challenging to assign an interpretation distinct from that of Factor 1. However, given the role of glutamate in neurocognitive processes including memory, sensory and motor processing (see Blüml et al. and references) (33), Factor 4 was broadly interpreted as a pattern for neuronal function.

We found the inflammatory pattern was associated with HIV exposure, both in the unadjusted and adjusted logistic regression models. In the neurotypical brain, levels of the glial marker Ins/

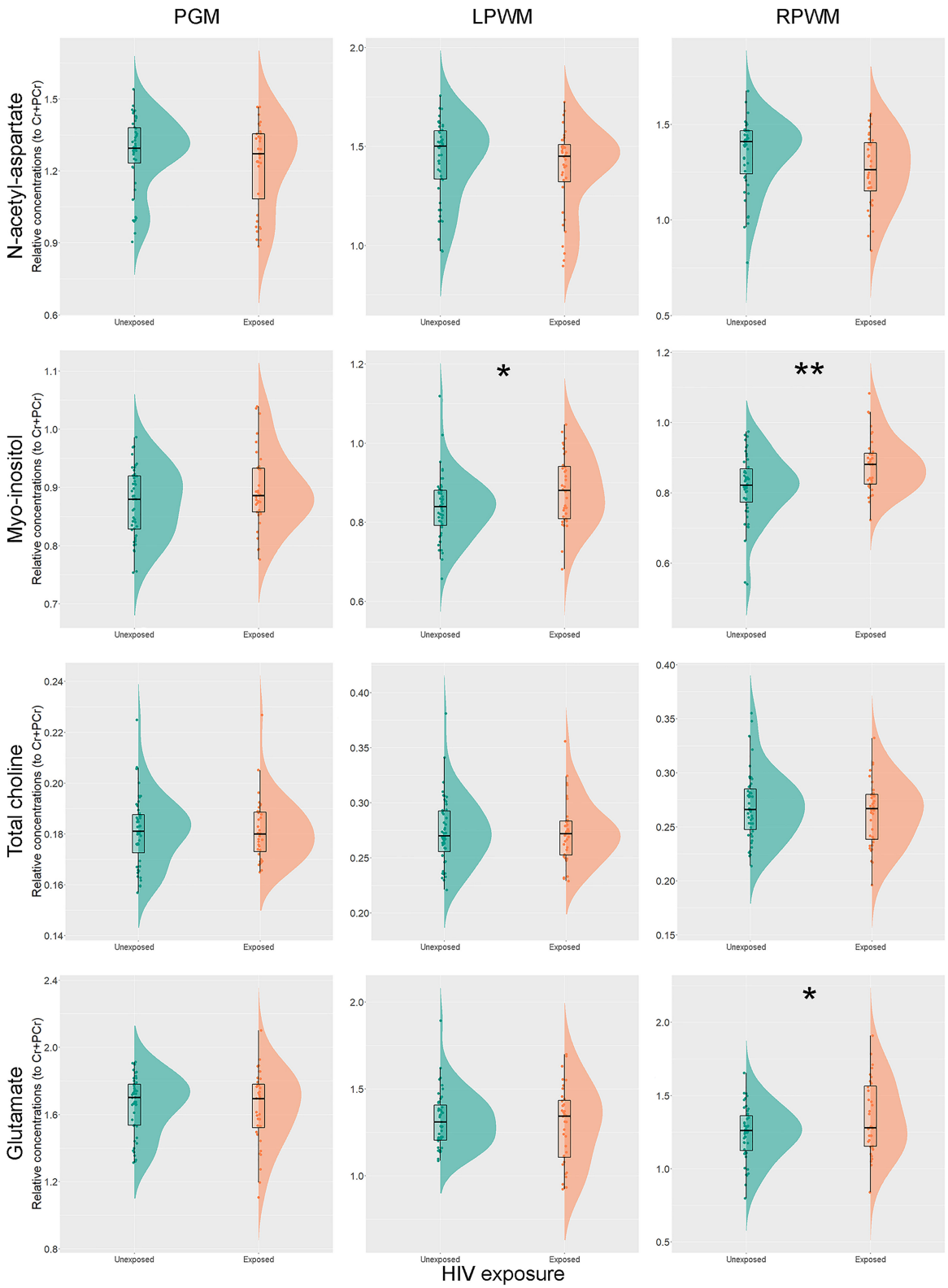

FIGURE 3 | Individual metabolite relative concentrations. Raincloud plots (54) showing individual metabolite relative concentrations to total creatine in the parietal grey matter (PGM), left parietal white matter (LPWM) and right parietal white matter (RPWM) in our complete-case cohort, according to HIV exposure. *p<0.05; **p<0.01.

Cr+PCr reach final, stable values within the first year of life (32). Therefore, a pattern of covarying Ins/Cr+PCr across brain regions at 2-3 years of age suggests neurometabolic development in CHEU may be influenced by underlying neuroinflammatory processes. Of note, maternal alcohol use during pregnancy did not substantially modify the results of the unadjusted analysis, despite its described association with lower glutamate concentrations in the parietal WM in neonates (50).

While there are no previous MRS reports of CHEU at this age, neurometabolic differences in this population have been reported in older children. Low absolute concentrations of creatine and phosphocreatine, n-acetyl-aspartate, total choline, and glutamate were found in the basal ganglia of a South African cohort of CHEU at age 9 years, compared to their unexposed peers, indicating possible neuronal damage (35). A longitudinal analysis of the same cohort found no interactions between age and HIV exposure when exploring neurometabolic development from 5 to 10 years of age (34). Further, at age 11 years, lower absolute concentrations of n-acetyl-aspartate were observed in frontal GM and peritrigonal WM of CHEU, suggesting possible axonal damage (36). Taken together, these results reflect the dynamic nature of neurometabolic development across child ages and brain regions, and the importance of analysing neurometabolites at different ages. However, children at older ages may have been exposed to additional sociodemographic and psychosocial risk factors that may impact their brain development adding a layer of complexity to the interpretation of results. Our study has the advantage of exploring neurometabolic development at a younger age, minimising the influence of socioenvironmental confounders.

Ins/Cr+PCr was significantly higher in left and right parietal WM of CHEU in our unadjusted analysis, and right parietal WM differences remained significant after adjusting for child age, child sex, and maternal alcohol use during pregnancy. WM may therefore be particularly sensitive to neuroinflammation from HIV exposure. Altered WM microstructural development has previously been reported in the right posterior corona radiata and the corticospinal tract of CHEU at age 7 years compared to CHU (31), and in neonates from the DCHS in the middle cerebellar peduncles (29) supporting our findings.

In addition to our main finding of higher parietal Ins/Cr+PCr in CHEU, we found differences in other metabolite ratios between groups. Glu/Cr+PCr levels were higher in the right parietal WM of CHEU in both unadjusted and adjusted analyses, compared to CHU. While covarying levels of Glu/Cr+PCr in WM were considered a pattern for neuronal function in our factor analysis, in the context of HIV exposure and neuroinflammation glial cells are primed and may fail to regulate glutamate. This has been demonstrated in patients with brain injuries or neuropsychiatric disorders, resulting in an unusual increase of this neurotransmitter in the extracellular space (55–57), which may also explain our results here. No results were modified after adjusting for GM percentage in voxels targeted at parietal WM in our sensitivity analyses, despite the presence of this confounder in the composition of such voxels.

Overall, our findings of increased Ins/Cr+PCr in the WM of CHEU add to the literature that HIV exposure may impact on WM development by affecting underlying neuroinflammatory processes. Animal model studies suggest that maternal immune activation induces exaggerated neuroinflammatory processes in offspring (19, 20). One of the main reported effects is microglial priming, where microglial cells become prone to produce an exaggerated response against second hits (18). Therefore, postnatal threats such as infections or environmental stressors, may elicit a neuroinflammatory overreaction in the young brain with long-term consequences (18–20). *In utero* priming of the immune system may take place in CHEU (21–23), and of note, inflammatory metabolite patterns of myo-inositol and total choline have been associated with cognitive impairment in adults (37, 38, 58) and children (35, 59) living with HIV.

Psychosocial variables may also play a key role in the neurometabolic development of CHEU. In LMICs studies, maternal depression and alcohol use during pregnancy have separately been associated with poorer cognitive outcomes in this population (8, 60). A recent US study linked maternal depression with decreased creatine and phosphocreatine, n-acetyl-aspartate, and total choline levels in the developing brain of HIV-unexposed foetuses (61) suggesting maternal immune activation may play a role (62). We found the impact of HIV exposure on Ins/Cr+PCr was independent of maternal depression and alcohol use in pregnancy. However, whether the neurobiological mechanisms underpinning these factors overlap with those derived from HIV exposure needs to be determined in larger samples. Separately, infant birthweight has been associated with maternal HIV infection (63). Although, studies are heterogeneous, suggesting the relationship between maternal HIV status and infant birthweight may vary across settings (51). Given birthweight may be influenced by maternal immune activation during pregnancy (64) and has been reported to impact children's performance in developmental assessments at 2 years of age (8), we examined infant birthweight in sensitivity analyses and found this did not modify our results.

HIV-specific factors have also been found to impact CHEU outcomes, including maternal CD4 and ART. In a sub-study of CHEU from the South African CHER cohort, lower CD4/CD8 ratio in infancy correlated to lower basal ganglia n-acetyl-aspartate and total choline levels at 5 years (65), lower total choline levels at 7 years, and lower myo-inositol levels at 9 years of age (35). The results suggest that an altered immune status in infancy may be associated with poorer neuronal and glial cell density in childhood. Since long-term ART exposure has been linked to mitochondrial toxicity in the brain (24, 66), MRS could also be used in CHEU to measure mitochondrial markers, such as lactate (32, 33). Although we were limited in our ability to examine maternal CD4 and ART in this sample, future studies may examine the relationship between these variables and neurometabolites in CHEU.

The strengths of our study include the use of a robust approach to study the effect of HIV exposure on neurometabolic development at 2-3 years of age, comparing a well-characterized

sample of CHEU to an appropriate control group with similar sociodemographic characteristics from a LMIC setting. Overall, our findings provide novel information about the neurobiological profile of young CHEU in a sub-Saharan African setting. We performed robust sensitivity analyses which did not substantially modify the results obtained in the adjusted logistic regression model. Furthermore, our cohort had a high prevalence of sociodemographic and psychosocial risk factors, comparable to other LMICs, and, all mothers living with HIV in our cohort received first-line triple ART, the majority with a fixed dose combination of efavirenz, emtricitabine, and tenofovir, implying our cohort may have generalisability for other CHEU populations across sub-Saharan Africa.

This study has some limitations to consider in the interpretation of our findings. First, MRS in very young paediatric subjects is technically challenging, since lack of motion is essential for successful data acquisition. As some data were lost due to children motion, the size of our complete-case cohort for analysis was substantially reduced, resulting in potential for underpowering of our analysis. However, sociodemographic characteristics were similar between the complete-case cohort and the full neuroimaging cohort, minimizing the likelihood of selection bias. This reduction in sample size meant we were unable to explore the association of maternal CD4 cell counts during pregnancy with child metabolite patterns, which needs to be investigated in future work. Second, our study design only included voxels placed in the parietal regions of the developing brain, so we are unable to draw conclusions about the presence of an inflammatory pattern in other brain areas of CHEU. Third, since WM is still under maturation in the developing brain (67), the tissue composition of voxels targeted at parietal WM may have included a proportion of GM. Hence, we cannot claim metabolite ratios obtained from these voxels purely belong to WM tissue. To mitigate this limitation, we ran sensitivity analyses for region-specific comparisons of individual metabolite ratios between groups, adjusting for GM percentage in voxels targeted at parietal WM, and found our results held. Fourth, although total creatine is well characterized and stable in the neurotypical brain during the first years of life (32, 33), low levels of this reference have been described in the peritrigonal WM in children living with HIV (36), compared to CHEU and CHU, and in subcortical brain regions in CHEU, compared to CHU (35). In contrast, higher creatine levels have been described in the parietal WM in adult subjects living with HIV, compared to uninfected peers (37). Therefore, although relative concentrations are commonly reported as they have the advantage of being less dependent on correction for relaxation and partial volume effects compared to absolute concentrations, the use of creatine and phosphocreatine as a reference in CHEU studies complicated interpretation as findings may reflect a change in the numerator or denominator. Similarly, the roles of metabolites in the developing brain, particularly n-acetyl-aspartate, remain to be fully established and Factor interpretations should be viewed with some caution. Lastly, we did not correct for multiple comparisons in our analyses, given the exploratory nature of our study and our use of factor analysis as a dimensionality-reduction method to reduce comparisons. Further work will be needed in larger sample sizes to replicate results.

In conclusion, our study presents the first results of the neurometabolic impact of HIV exposure in children from a LMIC setting during their first 2-3 years of life. We report differences in brain metabolite patterns between CHEU and CHU, showing an association of HIV exposure with an inflammatory pattern of elevated Ins/Cr+PCr in parietal brain regions. Our results are suggestive of neuroinflammatory processes in the developing brain of CHEU at this early age, which may be especially relevant in the parietal WM; whether this represents a potential target for specific neurodevelopmental interventions remains to be determined. Future work is needed to assess the longitudinal trajectories of neurometabolites in the population of CHEU, and to investigate associations with neurocognitive development and mechanisms underlying the inflammatory profile.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009; 525/2012 & 044/2017), by Stellenbosch University (N12/02/0002), and by the Western Cape Provincial Health Research committee (2011RP45). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CB-C: methodology, formal analysis and interpretation, visualization, writing – original draft, and review & editing. CW: conceptualization, investigation, data curation, supervision, and writing - review & editing. FR: methodology, formal analysis, supervision, and writing – review & editing. SS: investigation and writing – review & editing. KN: methodology and writing – review & editing. SJ: methodology and writing – review & editing. ARo: investigation and writing – review & editing. NH: project administration and writing – review & editing. ARe: formal analysis and writing – review & editing. HZ: conceptualization, methodology, resources, and writing - review & editing. DS: conceptualization, methodology, resources, and writing – review & editing. KD: conceptualization, methodology, investigation, resources, supervision, and writing – review & editing. All authors approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.800273/full#supplementary-material

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Fungal CNS Infections in Africa: The Neuroimmunology of Cryptococcal Meningitis

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Cryptococcal meningitis (CM) is the leading cause of central nervous system (CNS) fungal infections in humans, with the majority of cases reported from the African continent. This is partly due to the high burden of HIV infection in the region and reduced access to standard-of-care including optimal sterilising antifungal drug treatments. As such, CM is responsible for 10-15% of all HIV-related mortality, with a large proportion being preventable. Immunity to the causative agent of CM, *Cryptococcus neoformans*, is only partially understood. IFNγ producing $CD4^+$ T-cells are required for the activation of myeloid cells, especially macrophages, to enable fungal killing and clearance. However, macrophages may also act as a reservoir of the fungal yeast cells, shielding them from host immune detection thus promoting latent infection or persistent chronic inflammation. In this chapter, we review the epidemiology and pathogenesis of CNS fungal infections in Africa, with a major focus on CM, and the antifungal immune pathways operating to protect against *C. neoformans* infection. We also highlight the areas of research and policy that require prioritisation to help reduce the burden of CNS fungal diseases in Africa.

Keywords: microglia, cryptococcal meningitis, fungal infection, astrocyte, HAART

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INTRODUCTION

Cryptococcal meningitis (CM) is the leading cause of fungal meningitis in humans worldwide, with the largest disease burden reported in Africa (1). The majority of CM cases are caused by members of the *Cryptococcus neoformans* and *C. gattii* species complex (2), encapsulated basidiomycetous yeasts that are prevalent in the environment, growing in soil, some plants (e.g. eucalyptus trees) and pigeon guano (3–5). CM is an AIDS defining illness, responsible for 10-15% of all HIV-related mortality globally, resulting in ~80,000 deaths annually of which nearly three-quarters (73%) occur in Africa, particularly sub-Saharan Africa where up to 60% of people with HIV reside (1). *C. neoformans* produces airborne spores that are acquired by inhalation. In healthy people, host defence mechanisms clear these spores from the alveoli in the lungs preventing symptomatic infection (6), although there is evidence to suggest that these spores may instead become dormant and reactivate during periods of immunosuppression (7). In immunocompromised hosts, these

mechanisms fail allowing proliferation of *C. neoformans* in the lungs and subsequent dissemination to the CNS, causing meningitis/meningoencephalitis (6, 8). In particular, defects in T-cell immunity are highly associated with the development of CNS infection, demonstrating the important role of T-cell-mediated immunity against *C. neoformans*.

Fungal CNS infections, including CM, disproportionally affect patients in low-middle income countries, although their precise prevalence throughout the world is not well established. Global Action Fund for Fungal Infections (GAFFI) has estimated 47 million Africans suffer from fungal diseases each year (9). Across the continent, there is reduced access to gold-standard diagnostic tools and antifungal drugs for the treatment of CM (9). Moreover, it is clear that we currently have limited effective treatments for CM, since approximately one third of HIV-infected patients given antifungal prophylaxis will still go on to develop serious CNS infection (10). These worrying statistics have led to the development of a global initiative to end deaths from CM by 2030 (11), by implementing improved screening and education programs, tackling HIV management and further research into the pathogenesis of CM.

In this chapter, we discuss the epidemiology, clinical features and immunology of fungal CNS infections in Africa (focusing predominantly on CM), highlighting the areas of research that require prioritisation to help reduce the burden of these life-threatening fungal infections in Africa.

EPIDEMIOLOGY OF CNS FUNGAL INFECTIONS IN AFRICA

Human fungal infections of the CNS are an underrepresented group of invasive infections within the African population, occurring as opportunistic infections particularly in individuals living with HIV. The most common CNS infections reported in Africa are CM and histoplasmosis (12, 13). It was estimated in 2017 that ~160,000 people were diagnosed with CM in Africa, with 98% of these cases localised to the sub-Saharan region (1). In particular, most CM cases were reported from South Africa, Nigeria and Mozambique, which averaged 20,000 cases/year/country while North Africa accounted for the least number of CM cases within the continent (1). Although recent years have seen a decrease in the yearly incidence of CM (due to improved access to antifungal and antiretroviral therapy), the mortality rates in Africa still remain high reaching 44% in short-term outcomes in routine care (14, 15) and 73% in long-term follow up studies (16–18). CM cases have been reported infrequently in children (<2% cases) with most cases found in adults living with HIV (19–22). The molecular epidemiology of *Cryptococcus* species causing CM in Africa is still not well understood, despite recent advances in technologies. *C. neoformans* (VNI/AFLP1) has been the major genotype causing CM in Africa, identified in >80% of isolates collected (23–30). Other *C. neoformans* genotypes including AFLP1B/VNII and AFLP1A/VNB have also been isolated from clinical samples and found to cause 5-10% of total CM infections (3, 25, 31–33). Increasing cases of CM as a result of *C. gattii* species complex such as *C. gattii* (VGI/AFLP4), *C. tetragattii* (VGIV/AFLP7) and *C. deuterogattii* (VGII/AFLP6) have been isolated in countries such as Botswana, Ivory Coast, Kenya and Zimbabwe over the past few years (25, 27, 29, 31, 33). Of note, *C. neoformans* (AFLP1A/VNB) and *C. tetragattii* (VGIV/AFLP7) are more common in Southern Africa (3, 31, 34, 35), whilst *C. deuterogattii* (VGII/AFLP6) has so far been only isolated in Ivory Coast (24, 29, 33).

Besides *Cryptococcus*, other human fungal pathogens are capable of invading the brain and causing CNS disease in the setting of immunodeficiency and/or traumatic or inadvertent iatrogenic inoculation into the CNS during neurosurgical procedures. The susceptibility of patients to fungal CNS infection with species other than *C. neoformans* is heavily dependent on specific risk factors, geographic location and environmental exposure. For example, CNS infection with *Candida* species is associated with CARD9 deficiency, a primary immunodeficiency caused by inherited deleterious mutations in *CARD9*. Neutrophil influx into the *Candida*-infected CNS is protective and requires CARD9 expressed by microglia (discussed below) (36, 37). CARD9 deficiency is rare, although several cases have now been reported from Africa, predominantly in Algeria (38). Interestingly, the majority of these CARD9-deficient patients shared the same mutation whereas there was greater diversity in the type of *CARD9* mutations in Asian patients (38), but whether genetic variation at the population level contributes towards the geographical distribution of invasive CNS fungal infections is unknown.

Another fungal CNS infection that has been emerging in Africa is histoplasmosis, caused by the dimorphic fungus *Histoplasma capsulatum* with the var. *duboisii* being characteristically prevalent in Africa (39, 40). This fungus is the most common pathogenic dimorphic fungus causing endemic infections in Central and West Africa and in the island of Madagascar (41). Indeed, the World Health Organisation (WHO) recently recognised histoplasmosis as a neglected tropical disease requiring further attention (9). Common risk factors for histoplasmosis include advanced HIV infection and iatrogenic immune suppression (41). CNS involvement occurs in 5-20% of patients, usually in patients with advanced infection and poor response to therapy (41, 42). Like CM, diagnosis and treatment of histoplasmosis in the African continent in hampered by availability to gold-standard diagnostic testing and antifungal drugs. Therefore, a global effort to reduce drug costs and improve accessibility will not only improve clinical outcomes in CM, but also for other life-threatening invasive fungal infections such as histoplasmosis.

CRYPTOCOCCAL MENINGITIS: DIAGNOSIS, CLINICAL FEATURES AND TREATMENT

CM can be diagnosed by the identification of encapsulated yeast cells in the cerebrospinal fluid (CSF) using India Ink staining (43).

However, this method can often return false negative results and is generally insensitive. Newer tests based on the detection of Cryptococcal antigen (CrAg) are much more sensitive and can allow for a rapid and low cost diagnosis (44), which is critical since many cases of CM are localised to countries with limited resources. The CrAg test works by detecting the *Cryptococcus* polysaccharide capsule antigen in the CSF; the latest versions of which are based on a lateral flow assay using an immunochromatographic dipstick. This technique is much faster and simpler than culture and/or microscopy based diagnostic assays, and can be performed at the patients' bedside (45), and is also superior to other CrAg-based detection assays (e.g. latex agglutination assay) that require specialised laboratory equipment and skilled personnel (46). The World Health Organisation (WHO) recommends CrAg screening is performed in HIV-infected patients with a CD4 count of less than 100-200 cells/μL. A study on the effectiveness of CrAg screening in sub-Saharan Africa showed that mortality was significantly decreased when a CrAg screening program was introduced (47). Moreover, plasma CrAg titters are correlated with mortality and can lead to early identification of patients at risk of developing severe CM and death, even when symptoms are absent (10). However, several countries in Africa have limited access to the CrAg test meaning that these effective screening programs are not fully implemented in areas where they would have the greatest benefit. Therefore, improving access to these diagnostic tests is a critical step to help introduce prophylactic antifungal therapy and reduce mortality.

CM can present in the CNS as meningitis, encephalitis, or meningoencephalitis and can also result in cerebral mass lesions called "cryptococcomas" which are typically found along the perivascular spaces. CM is hard to distinguish from other types of meningitis, as there is a lack of specific clinical symptoms. In general, patients present with headache, fever, confusion and/or neck stiffness (13). Several areas of the brain can be affected by CM, including the basal ganglia, the white matter of the cerebral hemispheres, and the cerebellum (48). Computed tomography scans of CM patients usually reveal non-specific features, with ~40% of patients returning normal scans. In contrast, MRI imaging seems to perform better at assessing dilated perivascular spaces and leptomeningeal enhancement, particularly in immunocompromised patients (48), which are among the most common imaging features observed in CM patients (49).

Treatment of CM remains challenging due to the limited selection of antifungal drugs available. Even with treatment, over 70% of patients surviving CM suffer from neurological and sensory impairment, leading to disability and reduced quality of life (50). The gold standard drug for CM treatment is the combination of Amphotericin B (AMB) with flucytosine (5-FC), however a typical course of AMB and 5-FC treatment costs approximately (US)$800 per patient (50), and is usually only available in countries with well-funded healthcare systems. In Africa, only a small number of countries are registered to provide 5-FC, and even when registered there is little evidence it has been prescribed to patients in some areas. Therefore, improving the affordability of 5-FC and enhancing awareness of the drug's effectiveness is a crucial step towards ending CM deaths (51). In addition, the use of liposomal formulations of AMB is hindered by cost. Thus, because the use of AMB-deoxycholate (AMB-d) requires prolonged hospitalization for parenteral administration and is associated with renal and metabolic adverse effects, many resource-limited settings in Africa do not use AMB for the treatment of CM. Currently, the most commonly prescribed antifungal drug for CM in Africa is fluconazole, which has been shown to be inferior to AMB (52–55). There are now several reports of fluconazole resistance developing in *C. neoformans*, associated with chromosomal changes in the fungus (56), making management of CM especially difficult in settings where alternative options are not available. Thus, novel therapeutic approaches are needed. Adjunctive immune-based therapy with interferon gamma (IFNγ) has showed promising results in recent clinical trials (57, 58). Treatment with recombinant IFNγ combined with antifungal drugs showed that the addition of recombinant IFNγ resulted in improved clearance of fungi from the CSF compared to patients treated with antifungal drugs alone, although these studies were not large enough to determine if this correlated with improved survival (57, 59). Another experimental treatment that has been suggested is the use of corticosteroids to reduce immunopathology-associated neuroinflammation (see next section), such as dexamethasone, which has been shown to reduce mortality in patients with bacterial meningitis (60). However, dexamethasone treatment for CM in HIV-infected patients actually resulted in a higher mortality rate and disability than in the placebo group, and thus these trials were suspended for safety reasons (61). We therefore still require better antifungal treatments to improve clinical outcomes in patients with CM, which will depend on a better understanding of the immunology of CM (discussed below).

CRYPTOCOCCAL MENINGITIS-ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY RESPONSE SYNDROME

HIV-associated CM management is often complicated by immune reconstitution inflammatory syndrome (IRIS) (62). In sub-Saharan Africa, where most CM infections and deaths occur, most individuals with CM have HIV infection with a profound decline in their $CD4^+$ T cell counts. When antiretroviral therapy is initiated in individuals with this kind of severe immunosuppression, they undergo immune restoration albeit at varying rates (63, 64). This immune restoration occurring prior to pathogen clearance rescues pathogen specific immunity (65). These individuals then mount a pro-inflammatory response, a phenomenon termed IRIS. A similar pro-inflammatory response termed post infectious inflammatory response syndrome (PIIRS) occurs in non-HIV-associated cryptococcal meningitis (66, 67).

There are two kinds of HIV-associated CM-IRIS. First, unmasking CM-IRIS, which occurs following initiation of

highly active antiretroviral therapy (HAART) in persons without any prior signs and symptoms of CM. Increased availability of HAART has not been matched by expanded CrAg screening for all individuals with advanced HIV disease, which has meant that unmasking CM-IRIS is on the increase (68). Second, paradoxical CM-IRIS, which occurs following initiation of HAART in persons previously treated for CM with documented microbiological recovery, and clinical resolution continues to decline from 20% - 30% to 3% - 6% as antifungal treatment regimens become more efficacious (69, 70). The median duration from HAART initiation to paradoxical IRIS diagnosis is 110 days (IQR, 73-227 days) (71). The main risk factors for paradoxical CM-IRIS is a high baseline CSF fungal load and a delay in CSF fungal clearance with poorly fungicidal drugs, low CD4 count, a rapid decline in HIV viral load following HAART, and early initiation of antiretroviral therapy following CM diagnosis (54, 62–69, 72, 73).

Diagnosis of CM-IRIS depends on demonstration of a rise in CSF white cell counts and protein levels, as well as evidence of inflammation on brain imaging in the setting of negative CSF fungal cultures. There is currently no definitive treatment for CM-IRIS. The recent IDSA guidelines recommend no specific treatment for minor IRIS presentation. However, for major IRIS complications manifesting with profound CSF pleocytosis and raised intracranial pressure, IDSA guidelines recommend 0.5–1.0 mg/kg per day of prednisone equivalent or higher doses of dexamethasone for severe manifestations tapered over 2 to 6 weeks (74). Although steroids have no role in treatment of active (culture positive) CM infection (see above), their use in HIV-associated IRIS is associated with improved outcomes (61, 75).

The immunopathogenesis of paradoxical CM-IRIS is better understood than unmasking CM-IRIS as summarized here. Type 1 immune responses are driven by Th1 $CD4^+$ T cells secreting IFNγ, which polarizes macrophages to an M1 phenotype associated with production of pro-inflammatory cytokines (TNFα, IL-1β, IL-12, IL-6) and enhanced synthesis of nitric oxide (**Figure 1**). As a result, M1 macrophages are highly fungicidal to phagocytosed *Cryptococcus*. In contrast, type 2 responses are driven by IL-4/13-secreting Th2 $CD4^+$ T cells which polarize macrophages to an M2 phenotype, characterized by secretion of anti-inflammatory cytokines (IL-10 and TGFβ) and arginase expression, which counters nitric oxide synthesis and thus impairs clearance of *Cryptococcus* (76). The protective and non-protective roles for Th1 and Th2, respectively, may be organ-specific however, since enhanced expression of Th1 and Th2-associated cytokines are both correlated with better survival in the CSF of patients with cryptococcal meningitis (72), indicating that while Th2 is strongly associated with promoting fungal infection in the lung (72), this may not be true for the CNS.

Much as the Th1 response is appropriate for enhanced fungal clearance in both humans and murine models (see next section) (72, 77), the timing of this response and the balance with type 2 immunity is critical since dysregulated type 1 immune responses are thought to underlie the pathogenesis of IRIS (78). Current evidence shows that at paradoxical CM-IRIS diagnosis, there is a marked change in the number and phenotype of immune cells in CSF compared to when CM was diagnosed (71). For example, there is a significant increase in the number of T-cells within the CSF at the time of IRIS diagnosis, which exhibit a pro-inflammatory phenotype. Suppressive HAART rescues adaptive

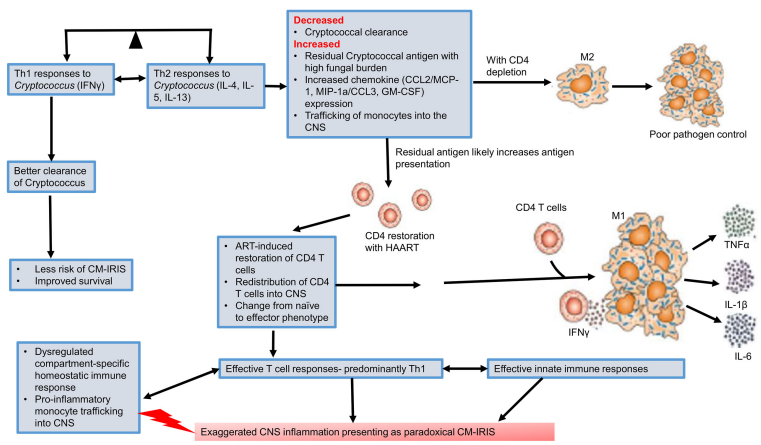

FIGURE 1 | Pathogenesis of cryptococcal meningitis immune reconstitution inflammatory response syndrome (CM-IRIS). Th1 immune responses are required for better clearance of *Cryptococcus*. This reduces the risk of CM-IRIS and results in improved survival. However, there is an imbalance as the far as the Th1-Th2 paradigm is concerned with extreme HIV-associated CD4 depletion. A predominantly Th2 response is associated with M2 macrophages and poor pathogen clearance. Initiation of HAART restores $CD4^+$ T cell counts. In a setting of poor pathogen clearance, the residual cryptococcal antigen induces an expansion of predominantly Th1 $CD4^+$ T cells with secretion of IFNγ. This polarizes macrophages to an M1 phenotype which secretes pro-inflammatory cytokines and chemokines that recruit more innate immune cells. This predominantly Th1 immune response generates a dysregulated and exaggerated CNS inflammation that presents as paradoxical CM-IRIS. CNS, central nervous system; CM-IRIS, Cryptococcal meningitis immune reconstitution inflammatory response syndrome; HAART, highly active antiretroviral therapy.

immune responses from the destructive effects of uncontrolled HIV replication, which had led to a decline in helper T cells. It is therefore conceivable that during paradoxical CM-IRIS, there is an increase in cryptococcal-specific peripheral blood and CSF activated (HLA-DR$^+$) CD4$^+$ and CD8$^+$ T cells compared to when the initial CM diagnosis was made. Moreover, there is enhanced CXCR3/CXCL10 mediated signaling and trafficking of activated T cells into the CNS (79). Once within the CNS, recruited activated T cells secrete cytokines/chemokines (CCL2/MCP-1, MIP-1α/CCL3, GM-CSF) that enhance monocyte trafficking into the CNS and differentiation into inflammatory macrophages (80, 81). The recruited monocyte/macrophages are activated by *Cryptococcus*-specific Th1 cells (82). Indeed, the phenotype of CSF monocytes at the time of IRIS diagnosis has been found to have changed from a highly phagocytic classic (CD14^{++} CD16$^-$) phenotype (observed at the time CM diagnosis), to more pro-inflammatory predominantly intermediate (CD14^{++} CD16$^+$) and non-classical (CD14$^+$ CD16^{++}) phenotypes (71, 82). This shift is accompanied by an aberrant pro-inflammatory state characterized by enhanced production of TNFα, IL-1β, IL-6, and IFNγ (**Figure 1**). This exaggerated pro-inflammatory response results in damage to neurons with a rise in CSF neurofilament light chains during IRIS (83). A murine model for CM-IRIS shows that enhanced Th1 T cell infiltration in the CNS results in upregulation of astrocyte *Aqp4* mRNA, which upregulates aquaporin-4 postulated to enhance brain edema and thus neuronal injury (84).

In the context of 'Test and Treat', where HAART is initiated as soon as individuals have a new HIV diagnosis and in the absence of CrAg screening for those with advanced HIV disease, one area that requires more data is whether persons recently initiated on HAART (<14 days) who present with unmasking cryptococcal IRIS have a higher risk of mortality compared with persons who develop CM after more than six months of HAART (85). Understanding the mechanisms for the immunopathogenesis of unmasking IRIS should be prioritized as well as determining whether interrupting HAART in persons who develop unmasking cryptococcal IRIS could have a survival benefit.

CRYPTOCOCCAL MENINGITIS: NEUROIMMUNOLOGY

Like most invasive fungal infections, CM is largely a disease of immune-compromised patients. By studying the immune defects that promote susceptibility to CM, we are better able to understand how the mammalian immune system fights these fungal infections. This information is critical for understanding patient responses to adjunctive immune-based therapy and developing criteria to assess patient prognosis and clinical outcomes. The predominant risk factor for CM is loss of CD4 T-cells from advanced HIV infection (majority of CM cases) however there are increasing numbers of non-HIV CM being reported (66, 86). Several of these also associate with T cell dysfunction caused by various factors including lymphoma, autoimmune diseases (e.g. lupus, psoriasis, sarcoidosis), immunosuppressive therapy and idiopathic CD4$^+$ lymphocytopenia (66, 87). As introduced above, T cells are essential for the activation of macrophages to kill *C. neoformans* and thus promote fungal clearance. In this section, we outline the mechanisms of fungal entry into the CNS, followed by the immunology of CM focusing on CNS-resident macrophages, astrocytes and brain-infiltrating lymphocytes, and how these different cell types contribute to protection and pathogenesis specifically within the *Cryptococcus*-infected CNS.

C. NEOFORMANS ENTRY TO THE CNS

The mechanisms governing *C. neoformans* entry into the CNS are thought to be largely mediated by two main pathways, the Trojan Horse method and transcellular migration. In this section, we will outline the evidence for each of these invasion mechanisms, although it should be noted that the relative dependence on each *in vivo* for different pathologic conditions (e.g. host immunosuppression, *C. neoformans* vs *C. gattii*), is not well understood.

The Trojan horse approach involves *Cryptococcus* yeast getting access to the CNS by transporting inside phagocytic cells, such as macrophages, monocytes, and neutrophils. In support of this hypothesis, a few research studies have shown that depletion of alveolar macrophages in mice decreased the dissemination of *C. neoformans* to CNS (88, 89). Another study compared dissemination to the CNS when mice were infected with bone marrow-derived monocytes previously infected with *C. neoformans in vitro*, or with free yeast. They found that the fungal burden was higher in the brain with infected bone marrow-derived monocytes compared to free yeast cells, suggesting that infected monocytes were more efficient at disseminating infection to the CNS than free yeast (90). Indeed, depleting circulating monocytes at a later stage of infection in mice reduced infection severity and reduced fungal burden by 40% in spleen, lungs, and brain (90), thus supporting the role of phagocytes in neuroinvasion. Moreover, depleting 99% of circulating monocytes in mice before infection abolished the development of CM and cerebral cryptococcomas and reduced fungal burden in the brain by ~90% (91). Neutrophils have also been shown to potentially promote transmission to the during *Cryptococcus* infection (92). Using intravital imaging, it was shown that neutrophils can expel *C. neoformans* within the brain vasculature, contributing towards brain infection (92), and depleting circulating neutrophils resulted in a reduced number of yeast cells in the perivascular space and reduced brain fungal burden by ~ 64% (91). Finally, the Trojan horse model has been modeled *in vitro*, where cultured brain microendothelial cells were challenged with yeast-containing macrophages. This *in vitro* model showed that *C. neoformans* bound CD44 on brain endothelium *via* hyaluronic acid. Mutant strains that were unable to make hyaluronic acid (*cps1Δ*) had a profound defect in cellular transmigration (discussed below), but could be transported within macrophages indicating that Trojan Horse mediated entry can enable transport of yeast that would otherwise be restricted from the CNS (93).

Transcellular migration across brain endothelium has also been observed to promote *C. neoformans* entry into the CNS (94–96). Intra-vital microscopy experiments in mice showed that free yeast cells were able to cross capillary walls, a process that was dependent on fungal-expressed urease since blocking urease reduced transmigration into the brain (96), although it should be noted that urease also promotes intracellular survival within phagocytes (97) indicating that urease blockade might prevent fungal CNS entry by Trojan Horse as well. Other *C. neoformans* virulence factors that promote CNS entry include the metalloprotease MPR1, hyaluronic acid synthase CPS1 (as mentioned above) and transcription factor HOB1. Mutants deficient in these factors are unable to invade a model blood-brain-barrier (BBB) *in vitro*, and are avirulent in mouse infection models with a reduced capacity to establish brain infection (98) (99). In order for transcellular migration to occur, C. neoformans yeast must first be internalised by endothelial cells. Interactions between CD44 and hyaluronic acid form part of this process, but it was recently demonstrated that endothelial-expressed EphA2-tyrosine kinase receptors also play a key role (100). Inhibiting EphA2 prevented transmigration of *C. neoformans* (100), and a similar dependence on EphA2 has been observed for CNS entry by several other pathogens including *Chlamydia trachomatis*, Epstein-Barr virus, and malaria parasites (101–103), indicating that ephA2 may generally be involved with BBB permeability and pathogen entry (104).

MICROGLIA

The CNS is populated by tissue-resident macrophages that exist in distinct functional subsets and localise within specific anatomical compartments. The most numerous of these CNS-resident macrophages are called microglia, which are found throughout the brain parenchyma and are involved in immune surveillance and brain development (105). Microglia are equipped with an immune arsenal to protect against brain-invading pathogens, including the expression of multiple PRRs such as the C-type lectin recptors (CLRs) and toll-like recptors (TLRs), nitric oxide synthesising enzymes and components needed to process and present antigens to $CD4^+$ T cells **(Figure 2)**. *In vitro* studies showed that stimulating microglia using TLR agonists (e.g. Pam_3 CSK_4, LPS, and CpG) during *C. neoformans* infection drove the production of proinflammatory cytokines such as TNFα, IL-6, and IL-1β, which resulted in enhanced *C. neoformans* phagocytosis and prevented fungal intracellular replication within microglial phagosomes (106). Immortalised microglia have been shown to phagocytose *C. neoformans* leading to increased iNOS expression which is important for limiting fungal growth (107, 108). These antifungal actions are regulated by IFNγ, produced by infiltrating Th1-polarized $CD4^+$ T cells. IFNγ has also been shown to induce the expression of MHC Class II by microglia *in vitro*, potentially allowing their interaction with infiltrating $CD4^+$ T cells **(Figure 2)** (109–111). A study showed that immunomodulation with CD40 (a T-cell co-stimulatory molecule) and the cytokine IL-2 in *C. neoformans*-infected mice reduced the fungal burden in various organs including the brain, which correlated with an IFNγ-dependent increase of MHCII expression on microglia (112). Moreover, IFNγ knockout mice showed the critical role of IFNγ in activating microglia and inducing anti-cryptococcal activity (113, 114). Furthermore, patients with CM who feature neutralising IFNγ autoantibodies tend to have a persistent infection and lower survival rate (115). Despite these clear protective roles for microglia in controlling

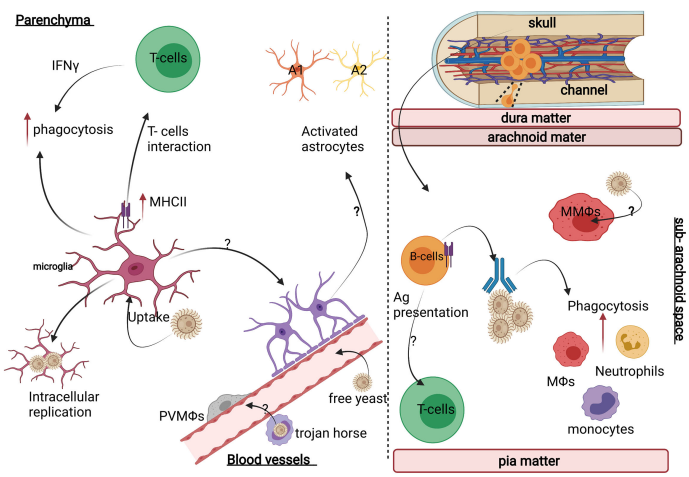

FIGURE 2 | Neuroimmunology of *C. neoformans* infection. (Left panel) In the brain parenchyma, *C. neoformans* mostly interacts with brain-resident microglia and astrocytes, which differentiate into distinct functional states depending on the inflammatory context and infiltrating immune cells (e.g. T-cells). For example, astrocytes can develop into pro-inflammatory A1 or tissue-healing A2 subsets, but whether this occurs during CM is unknown. (Right panel) In the meninges, *C. neoformans* mostly localises to the sub-arachnoid space where it will encounter meningeal macrophages (MMΩs) and a variety of resident lymphocytes including B-cells (deriving from skull bone marrow and connecting channels) and T-cells. Image created with Biorender.com.

C. neoformans infections, some studies have shown that microglia are prone to latent intracellular infection, where *C. neoformans* survives and replicates inside microglial phagosomes **(Figure 2)** (116, 117). Indeed, post-mortem examinations of human brain tissue showed *C. neoformans* polysaccharide capsule is engulfed and localised inside microglia (116). Therefore, although microglia can engulf *Cryptococcus* yeast cells, the killing of yeast cells may not always occur in human microglia even when IFNγ is present (118).

Although we can gain insights into anti-cryptococcal immunity using microglia cell lines and *in vitro* models, *in vivo* studies are needed to analyse the behaviour of microglia in their natural environment since microglia rapidly lose their tissue-resident identity when removed from their microenvironment. *In vivo* studies analysing antifungal activity of microglia are so far limited. In a murine model of CM-PIIRIS, full activation of microglia did not occur until 21 days post-infection, which coincided with a significant influx of infiltrating inflammatory myeloid cells and lymphocytes and a decrease in brain fungal burdens (77). A similar observation was made following acute infection with *C. neoformans* in mice, where microglia numbers expanded >1 week post-infection which coincided with an influx of monocytes and T-cells (91). Interestingly, these effects do not occur with *C. gattii*, which demonstrates a reduced capacity for entry into the CNS compared with *C. neoformans*, with *C. gattii*-infected animals typically succumbing to significant lung disease (91). In contrast, recent *in vivo* studies showed that *C. albicans* CNS infection results in a rapid activation of microglia (within 24h), which quickly initiate protective immunity upon *C. albicans* infection. Microglia highly express CARD9 (caspase recruitment domain-containing protein 9), a signaling adaptor protein downstream of the CLRs (37). Human CARD9 deficiency results in a profound susceptibility to CNS candidiasis, aspergillosis and phaeohyphomycosis but not cryptococcal meningitis (119–121). It was recently shown that CARD9 expression by microglia is required to sense the fungal toxin Candidalysin which is secreted by *C. albicans* (36). This toxin activated the production of IL-1β and CXCL1 from microglia, which in turn recruited CXCR2-expressing neutrophils to the brain to clear the fungus (36). CARD9 deficiency does not appear to promote susceptibility to CM in humans, and deficiency in CARD9-coupled CLRs do not promote susceptibility to CM in experimental mouse models (122, 123). Thus, microglia have an important role in antifungal immunity that is context-dependent. Future studies should focus on how microglia function during CM using the latest technologies, murine models and human samples in a bid to widen our understanding of immune regulation within the *Cryptococcus*-infected CNS and how this promotes fungal clearance, as well as associated inflammatory syndromes (see IRIS section above).

NON-PARENCHYMAL CNS-RESIDENT MACROPHAGES

In addition to microglia, there are other CNS-resident macrophages found in the perivascular spaces (perivascular macrophages, PVMs), within the meninges (meningeal macrophages) and associated with the choroid plexus (choroid plexus macrophages) **(Figure 2)**. Each of these populations are poorly studied in the context of CM, with many insights into the biology of these populations only gained in recent years with the advent of new technologies (e.g. single-cell RNA sequencing) that have allowed us to better define the markers and possible functions for these cells (124–126).

Analysis of human brain autopsy tissue showed that PVMs appear to harbour intracellular *C. neoformans*, indicating that these cells interact with and phagocytose *C. neoformans* (109). Indeed, the location of PVMs would ideally position them next to the main site of infection in CM **(Figure 2)**. However, an extensive analysis of cryptococcal brain infection in mice showed that the main myeloid effector cells in the brain following *C. neoformans* infection were monocytes and neutrophils recruited from the blood, and that infection and inflammation were largely confined to the perivascular spaces where CNS-resident macrophages, including perivascular macrophages and microglia, were rare (91). Meningeal macrophages are also situated in the tissues most commonly involved in human CM. Yet, there is little research done to understand the specific contributions of these cells to fungal clearance and pathogenesis. Lastly, PVMs are the primary site for simian immunodeficiency virus (SIV) infection in the CNS, which affects the function of PVMs (127). This is important in the context of CM since it is not yet known how HIV infection (the predominant risk factor for CM in humans) affects the behaviour and function of CNS-resident macrophages such as PVMs and microglia, and the downstream consequences of this for susceptibility to cryptococcal infection. We therefore require a greater understanding of the interplay between HIV and fungal infection in these macrophage subsets and the impact of this on pathogenesis.

ASTROCYTES

Astrocytes are the most numerous glial cells within the CNS and the majority of studies on astrocyte function to date have focused on their roles in maintaining neuronal health and forming a major component of the blood-brain-barrier (BBB). In recent years, new studies have revealed that astrocytes perform important immune functions and contribute towards CNS pathologies (128). During infection, astrocytes undergo a poorly understood complex process known as 'astrogliosis', where structural and functional changes occur. These changes are controlled by the CNS microenvironment which give rise to functionally-differentiated phenotypes that are optimised for tissue repair or resistance to infection **(Figure 2)** (129–131). Whether fungal CNS infections affect astrocyte phenotype and/or function remains an open question. One study showed that murine astrocytes undergo astrogliosis following intravenous infection with *C. neoformans* **(Figure 2)** (130), confirming that astrocytes could play roles in the pathogenesis of CM. Furthermore, *in vitro* experiments using astrocyte cell lines

found that *C. neoformans* can interact with and infect human astrocytes driving an increase in MHCII expression (132, 133), providing evidence that these cells might be involved in immunity during CM. It will be worth further investigating astrocyte behaviour in CM in future studies particularly as (1) astrocytes appear to become activated during human CM and this is blunted in HIV-infected patients (134–136), and (2) astrocytes regulate traffic through the BBB thus they might have significant role in prevention of *C. neoformans* invasion of the CNS.

T-CELLS

There is growing evidence that T cells are present in the healthy CNS, which have a unique CNS-resident phenotype and are important for CNS homeostasis and animal behaviour (137–140). Mice deficient in adaptive immunity (e.g. *Rag1*$^{-/-}$) have behavioural abnormalities, which was recently linked to their role in promoting microglial maturation in the developing brain (141–145). Moreover, T cells have been shown to promote pathology in several neurodegenerative diseases (146–148), as well as supress astrogliosis during ischaemic stroke (147). These lymphocytes are therefore integral to the outcome of CNS inflammation and important regulators of pathology in this tissue.

The specific functions of CD4^{+} T cells in the *Cryptococcus*-infected CNS remain poorly defined. As outlined above, these lymphocytes are thought to be required to activate antifungal killing pathways in myeloid cells but may also promote tissue pathology (149–151). CD4^{+} T cell recruitment to the cryptococcal-infected brain was recently shown to require CXCR3. Both human and murine T cells significantly upregulated CXCR3 in response to *C. neoformans* infection, and this chemokine receptor was required for Th1 polarization. Interestingly, *Cxcr3*$^{-/-}$ mice were protected from infection-associated CNS inflammation and thus had improved survival, but this did not correlate with reduced fungal burden. These studies therefore show that CXCR3^{+} Th1 T cells are not needed to help control fungal infection in the brain, at least in the context of an IRIS-like syndrome (152). Similarly, knockdown of CCR2 in mice was also shown to improve survival independently of fungal control in the CNS, although CCR2 was not involved in the direct recruitment of Th1 T cells to the CNS but acted indirectly by promoting the initial recruitment of inflammatory monocytes (153). Collectively, these studies indicate that T-cells have a complex role in CM, both for fungal clearance and mediating immunopathology, which is likely context- and time-dependent.

B-CELLS

B-cells produce anti-cryptococcal antibodies that are required for effective opsonisation of the fungus (particularly the capsule) and uptake by phagocytes, including macrophages (154). Patients with X-linked agammaglobulinemia (XLA), an inherited immune-deficiency caused by mutations in the *BTK* gene and characterised by an absence of B cells, have been reported to develop CM (155). Furthermore, reduced production of IgM in HIV+ patients has been correlated with a greater risk for developing CM (156). Treatment with the BTK inhibitor Ibrutinib, a drug used in the treatment of B-cell lymphomas, has been reported to promote CM in a small number of patients, although the exact underlying mechanism (s) and relative incidence of CM in Ibrutinib-treated patients remain unclear (157). Mice with B-cell and/or antibody deficiencies also have increased susceptibility to *C. neoformans* infection, characterized by higher brain fungal burden (158). Thus, B-cells provide critical support to phagocytes in the fight against CM and clearance of yeast cells from infected tissues **(Figure 2)**.

CNS border tissues, such as the meninges, were recently shown to be populated by CNS-resident B cells which infiltrated the CNS from the skull bone marrow *via* a series of bone channels **(Figure 2)**. These channels provide the meninges with a constant supply of CNS-resident B cells, which were shown to have an immunoregulatory phenotype and were optimised at recognising CNS-derived antigens (159–162). Furthermore, meningeal IgA-secreting plasma cells have been shown to curtail *Candida* invasion in the CNS (163), but whether these CNS-resident B-cells proliferate in response to *C. neoformans* infection and/or provide local protection against cryptococcal infection has not yet been determined.

CONCLUDING REMARKS

The majority of deaths from invasive fungal infections in humans occur in Africa, and many of these are preventable. Improving access and reducing cost of 'gold-standard' diagnostics and treatments is urgently needed to reduce the impact of fungal CNS infections on global human health. However, even with access to antifungal drugs, mortality and morbidity from fungal CNS infection remains high. Worryingly, we are also seeing more cases of fungal CNS infections reported particularly amongst non-HIV immunosuppressed populations. It is therefore clear that we require more insights into the pathogenesis of these diseases and adjunctive immune-based therapies that boost the effectiveness of antifungal drugs. Recent advances in neuroimmunology have led to the development of models and technologies leading to novel insights into how immune responses are initiated and regulated within the CNS. Many of these models and approaches have yet to be utilised by the fungal immunology field, but their application holds significant potential in terms of discovery and future therapeutic benefit. In summary, we hope that future studies focusing on CNS antifungal immunity will shed light on how these infections may be better managed and treated, which alongside enhancing public awareness and education on the

impact of fungal CNS infections, may lead to reduced mortality and improved health across Africa.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript, edited the final draft and approved the final submission.

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REVIEW
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Biomarkers of Tuberculous Meningitis and Pediatric Human Immunodeficiency Virus on the African Continent

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Biomarkers in body fluids are helpful objective tools in diagnosis, prognosis and monitoring of (therapeutic) responses of many neurological diseases. Cerebrospinal fluid (CSF) biomarkers are part of the diagnostic toolbox for infectious neurological diseases. Tuberculous meningitis (TBM) and Human immunodeficiency virus (HIV), are important burdens of disease in Africa and can negatively affect brain health. Two thirds of the world's population of people living with HIV reside in sub-Saharan Africa and 25% of the global burden of tuberculosis (TB) is carried by the African continent. Neuroinflammation and damage of specific neuronal cell types are key constituents in the pathophysiology of these central nervous system (CNS) diseases, and important potential sources of circulating biomarkers. In this review, we summarize current research in the use of biomarkers in TBM and pediatric HIV as case demonstrations for high prevalence neurological diseases in Africa. Inflammatory molecules, primarily when detected in CSF, appear to have diagnostic value in these diseases, especially when measured as profiles. Brain injury molecules, such as S100, Neuron specific enolase and glial fibrillary acidic protein may have prognostic value in TBM, but more studies are needed. There is a need for more cost-economic and high sensitivity technologies to drive further biomarker discoveries and translate into healthcare improvements for these important healthcare problems in a globally fair way.

Keywords: biomarkers, tuberculous meningitis, HIV, inflammation, cerebrospinal fluid, blood plasma/serum

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INTRODUCTION

Biomarkers in body fluids are helpful objective tools in diagnosis, prognosis and monitoring of (therapeutic) responses of many neurological diseases. Cerebrospinal fluid (CSF) biomarkers are part of the diagnostic toolbox for chronic neurological diseases such as Alzheimer's disease and Multiple Sclerosis, and for infectious central nervous system (CNS) diseases such as meningitis (1, 2). Biomarkers previously tested exclusively in the CSF compartment in neurological diseases, can nowadays be measured in the systemic blood as well, and blood and CSF neurobiomarkers are progressively being used as useful endpoint measurements in trials targeting CNS diseases

(3, 4). Acute and chronic infections, including tuberculous meningitis (TBM) and Human immunodeficiency virus (HIV), are important burdens of disease in Africa and can have detrimental effects on brain health. Two thirds of the world's population of people living with HIV reside in sub-Saharan Africa and 25% of the global burden of tuberculosis (TB) is carried by the African continent (5, 6). Neuroinflammation and damage of specific neuronal cell types are key constituents in the pathophysiology of these CNS diseases, and important potential sources of circulating biomarkers. Although biomarker research for infectious CNS diseases is not as intensively studied as for some other neurological conditions, there are interesting pilot data from which parallels with widely studied disorders can be drawn, and which highlight the need for further research into the diagnostic and prognostic potential of biomarkers in the African context. In this review, we will summarize current research on biomarkers in TBM and pediatric HIV as case demonstrations for high prevalence neurological disease in Africa and discuss options for biomarker development with consideration for the unique challenges on the continent.

TUBERCULOUS MENINGITIS

In the World Health Organisation's 2020 TB Report TB was remained the deadliest infectious disease globally. In 2019 the international TB incidence was 10 million, with numerous countries within Africa ranking amongst those carrying the largest global burden of TB (6). TBM is estimated to occur in one out of 100 TB cases (7), and is the most fatal and debilitating manifestation of TB, leading to high rates of death and disability in adults and children (8).

Pathogenesis of TBM

Tuberculosis infection occurs after the inhalation of infectious droplets of aerosolised *Mycobacterium tuberculosis* (*Mtb*), which stimulates an innate immune response in the lung tissue that leads to the containment of the bacilli within a granuloma (9). However, in the elderly, immune compromised or very young, the infection may progress to active TB disease associated with destruction of the lung parenchyma and dissemination of the TB bacillus to other organ systems, including the CNS (10). Despite the protective blood brain barrier (BBB), *Mtb* gains access to the brain through numerous postulated mechanisms, including rearrangement of the actin cytoskeleton of brain microvascular endothelial cells (11), bacillary endothelial adhesion (12), or the Trojan Horse whereby *Mtb* is trafficked into the CNS in infected innate immune cells (13). The limited resident CNS immunity facilitates bacillary survival and replication and the development of silent tuberculous lesions, often referred to as the Rich's Focus, which be located on the cortical pia or adjacent to the ventricles and meninges (14). Rupture of these lesions is thought to result in granulomatous inflammation.

Mycobacterium tuberculosis is recognized by the brain's resident immune cells, microglia, through pattern recognition receptors including toll-like receptors. Activation of microglia leads to secretion of a number of pro-inflammatory mediators (discussed below), recruitment of peripheral immune cells and activation of astrocytes which aid in the immune response (15). The cerebral immune response is an important determinant of poor outcome as the formation of thick inflammatory exudate causes cerebral vasculitis and occlusion as well as hydrocephalus and raised intracranial pressure. Consequently, the brain is at high risk of ischaemia and infarcts are seen in almost 70% of patients (16).

A delay in starting treatment is a major determinant of poor outcome, yet timely diagnosis of TBM is challenging due to its non-specific presentation (17). Similarly, clinical tools are limited in accurately predicting patient outcomes making it difficult to triage limited resources to patients at greatest risk. Biomarker studies have, therefore, aimed to identify markers to improve accurate and early diagnosis and prognosis. Biomarkers may also serve as valuable proxy measures of novel treatment efficacy, and to elucidate disease pathophysiology and new intervention strategies.

Inflammatory Biomarkers

Numerous cytokines and chemokines are elevated in the CSF of adult and pediatric TBM patients, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β, IL-10, IL-6, IL-8, IL-2, monocyte chemoattractant protein (MCP)-1 and macrophage Inflammatory Protein (MIP)-1α among others (18–21). Cytokine levels vary across studies, even when the same testing platform has been used, possibly due to variations in the timing of sample collection, the synergistic interplay between pro- and anti-inflammatory cytokines, and variability in the strain of *Mtb* (19). Initial concentrations of pro-inflammatory cytokines like TNF-α and IFN-γ are highest on hospital admission followed by a subsequent decline over several weeks. Levels of intrathecal anti-inflammatory cytokines, such as IL-10, may be low if CSF samples are obtained when the inflammatory cascade is still developing (22–25). The ubiquitous finding across all studies is that CSF cytokine levels are elevated in TBM with some decrease after the initiation of treatment and inflammation continues despite drug administration. The degree of the attenuating influence of treatment, however, varies between cytokines. Combinations of inflammatory biomarkers could thus add value to the diagnosis of TBM. Numerous studies in pediatric TBM have examined the diagnostic accuracy of various combinations of host protein biosignatures in both serum and CSF taken on hospital admission. Protein combinations for CSF that have shown promising area under the curve (AUC), sensitivity and specificity include vascular endothelial growth factor (VEGF), myeloperoxidase (MPO) and IFN-γ (AUC = 0.97, sensitivity = 91.3, specificity = 100), as well as the combination of soluble intracellular adhesion molecule (sICAM)-1, MPO, CXCL-8 and IFN-γ (AUC = 0.97, sensitivity = 87, specificity = 95.8) (26). In serum a modified 7-protein biosignature developed for pulmonary TB [c-reactive protein (CRP), neural cell adhesion molecule (NCAM)-1, IFN-γ, CFH, apolipoprotein (Apo)-AI, IP-10 and serum amyloid A (SAA)] only showed modest sensitivity and specificity for pediatric TBM, but a 3 -protein signature (adipsin, Aβ42 and IL-10) was associated with improved diagnostic performance (27). While the potential of developing a bedside diagnostic tool for

multiplexed proteins is intriguing, study sample sizes remain small and further validation is required in larger studies across the age range.

The association between CSF inflammatory mediators and various indicators of injury severity and outcome has yielded conflicting results. Several studies (19, 21, 22, 24, 28, 29) have found no association between the British Medical Research Council TBM stage (30) and the levels of TNF-α, IL-10, IL-1-β, IL-6 or IL-8. However, other studies show a significant positive correlation between the levels of TNF-α, IL-1β and IFN-γ and TBM stage (31, 32). Similarly, the association between CSF inflammatory biomarkers and outcome also is poor (20, 21, 24). Cumulatively these results indicate that while the cerebral inflammatory response is an important early disease process, biomarkers of inflammation do not necessarily reflect the degree of cerebral tissue injury and the severity of the disease; therefore, biomarkers of brain tissue injury may be important additional tools to predict and monitor disease severity.

Brain-Specific Biomarkers

Brain-specific proteins have become valuable tools for diagnosis and prognostication in other forms of brain injury and infection, such as traumatic brain injury or stroke (33, 34). The cell-specificity may indicate the nature of cellular injury, their concentrations reflect injury severity, and their temporal profile provides insight into recovery or evolving injury (33). Only recently, brain-specific injury biomarkers have been investigated in TBM. A pediatric TBM study found elevated concentrations of CSF brain biomarkers S100B, glial fibrillary acidic protein (GFAP) and neuron specific enolase (NSE) which were associated with infarcts on brain imaging (20). Further, in serial samples over the first 4 weeks of hospitalization inflammatory mediator concentrations decreased in all patients, whereas these brain biomarkers continued to rise in patients who died and their trend over time was a promising prognostic biomarker (20). Similar findings have been reported in adult TBM (35) and a follow-up pediatric TBM study using whole genome transcriptomics in CSF confirmed the upregulation of genes and pathways associated with brain injury, including neuroexcitotoxicity (36). These studies highlight that injury processes initiated by the host inflammatory response are ongoing despite treatment. Further investigation into these mechanisms of injury is crucial to elucidate novel therapies directed at ameliorating brain injury, and brain-derived biomarkers will be an important tool in this quest.

Compartmental Differences in Biomarker Concentrations

Adult and pediatric TBM data indicating that CSF cytokine concentrations are significantly greater than those seen in serum (20, 25) suggest compartmentalisation of the immune response at the site of disease, and a confounding effect of peripheral organs to serum cytokine levels. The detection of brain-derived biomarkers in blood is challenging and may additionally be influenced by their intrathecal concentration, their molecular weight and half-life (37). Brain-derived proteins can diffuse into the blood regardless of BBB breakdown (37, 38), but this is likely augmented when the BBB is compromised (39). Consequently, serum concentrations reflect only a fraction of CSF levels and only transiently. Although serum brain-specific injury biomarkers (such as S100B, GFAP and NSE) work well as diagnostic and prognostic tools in traumatic brain injury, they have been challenging to detect in TBM (20). This could be due to the extent of tissue injury, or the uncertainty around the timing of blood sampling relative to the onset of brain injury, which in TBM is likely to result from lasting injury processes rather than an acute discrete event. However, testing platforms used for TBM studies to date may have lacked adequate sensitivity to detect low quantity brain injury markers in blood. Newly developed assays with improved sensitivity (34) may warrant re-evaluating the role of serum-based brain biomarkers, especially as CSF requires invasive sampling.

Cerebrospinal fluid reflects changes in the brain more robustly than serum, implying that there is compartmentalisation within the CNS. Ventricular CSF, sampled as part of the management of TBM associated hydrocephalus, demonstrates significantly higher brain injury biomarker concentrations than lumbar CSF, while inflammatory biomarkers are greater in the lumbar compartment (20). This is similarly reflected in transcriptomic data, which showed upregulation of pathways associated with brain injury in the ventricular CSF and those associated with inflammation in the lumbar CSF (36). This likely reflects a decrement in brain-derived proteins along the brain-spine axis (37, 40) and the contribution of spinal sub-arachnoid inflammation present in as many as 76% of TBM patients (16). These data suggest that biomarker diagnostic, treatment, and prognostic thresholds must take the CSF compartment source into account.

(NEURO)INFLAMMATORY MARKERS IN PEDIATRIC HIV

Pathogenesis of Pediatric HIV

Infection with HIV can cause a range of brain disorders, of which neurocognitive impairments is the most common phenomenon. HIV infects the CNS *via* transmigration of infected $CD4^+$ cells and monocytes across the BBB (41, 42). Microglial cells and perivascular macrophages are cell types that subsequently become the source of chronic infection in the CNS (43). The pathogenesis of HIV-associated neurodevelopmental impairments in children is not fully understood. An aberrant immune regulation, characterized by chronic low-grade neuroinflammation is accepted to be a key mechanism that contributes to impaired brain functioning in children (44, 45) and adults (46) living with HIV. Viral proteins (e.g. Tat and gp120) that are released from infected cells activate microglial cells and astrocytes to produce pro-inflammatory cytokines and chemokines that impair neuron functioning when exposed over a chronic period.

HIV Exposed Uninfected Children

World-wide and specifically in sub-Saharan Africa, important progress has been made in reducing vertical transmission of HIV to infants through the implementation of effective

and widespread prevention of mother-to-child transmission (PMTCT) programmes (47, 48). While PMTCT success has resulted in the decline in pediatric HIV infection, discussed below, the number of HEU infants, i.e. perinatally exposed but not infected children, has rapidly risen. In 2018, the global population of HEU children was estimated to be 14.8, 13.2 million of whom resided in sub-Saharan Africa (49). Maternal HIV infection during pregnancy may have negative consequences for the development of the HEU child. Although HIV uninfected, the large population of HEU children is at increased risk of morbidity and mortality in general (50–52). Moreover, HEU children are at risk of impaired behavioral and neurocognitive functioning (53, 54). The prevalence of cognitive delay between 1 % and 31% and severe motor delay from 0 to 39% in HEU children was reported in a meta-analysis (55). A recent neuroimaging study showed that cortical surface area and thickness within frontal regions were associated with cognitive development, and in temporal and frontal regions with language development in HEU children (56). Impaired educational performance in HEU children (57) is a growing concern since these children may fail to progress academically and to acquire appropriate skills to sustain employment as adults in low- and middle-income countries (LMICs).

Our understanding of the pathogenesis of neurodevelopmental deficits in HEU children remains limited. This is in part due to the lack of appropriate animal models or post-mortem brain tissues from HEU children for neurobiological research. Evaluations of human systemic immune markers have provided important insights on the involvement of aberrant (neuro)immune regulation on neurocognitive delays in HEU children. In a South African birth cohort, increased granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-10, IL-12p70, IL-1β, IL-2, IL-4, IL-6 and neutrophil gelatinase-associated lipocalin (NGAL) in HEU infants, predicted worse motor functioning at 2-years follow-up (58). Interestingly, in this study maternal HIV infection was associated with lower levels of inflammatory markers in mothers and their children (e.g. IL-1β, IL-2, IL-4 and IFN-γ) compared to HIV uninfected mothers and their children (58), suggesting a suppressed immune profile in HEU children in this South African cohort. Similarly, another study with a Zimbabwean cohort also found decreased IL-6 levels in HEU children compared to HIV unexposed children (59). On the other hand, contradictory findings in European and American populations were reported. Increased circulating levels of IL-8 and IL-1β were detected in HEU infants as compared to unexposed infants in the Netherlands (60). Significantly increased levels of plasma IL-4 were found in Brazilian HEU children aged 6 to 12 years (61). Further, in Brazilian HEU neonates, increased circulating levels of IFN-γ and TNF-α compared to HIV unexposed neonates were reported (62). The conflicting findings between continents may be attributed to differences in HIV subtypes. HIV subtype Clade B is predominantly present in America, Western Europe, Australia and Asia and represents about 12% of the world's HIV infected population (63) whereas HIV subtype Clade C is present in countries of Southern Africa and India (64). Clade C tends to exert immunosuppressive effects as compared to the pro-inflammatory effects exerted by Clade B (52), which may explain the lower levels of inflammatory biomarkers reported in the Southern African cohorts. These studies underscore the importance of research on the involvement of the (neuro)immune system in neurodevelopmental delays in various African populations such as in Southern Africa, considering the expanding numbers of HEU children of mothers with predominantly HIV subtype Clade C, which represents about 50% of all HIV infections (64).

Perinatally HIV Infected Children

Despite successful PMTCT programmes, millions of children are still born with HIV today (49). Children born with HIV (perinatally HIV, PHIV) show neurocognitive impairments as compared to uninfected peers, despite long term HIV suppression by combination antiretroviral therapy (cART). Studies reported a prevalence of severe cognitive delay between 21% to 35% and severe motor delays ranging from 14 to 81% in perinatally HIV infected children (55). The cause of these poorer neurocognitive outcomes as compared to peers is not well defined, but alterations in cerebral volume, white matter (WM) integrity, neurometabolites, and regional perfusion suggest underlying cerebral insults (65–68). HIV encephalopathy, a neurological disorder typical for children born with HIV is characterized by cerebral atrophy, basal ganglia calcifications, and white-matter abnormalities seen on conventional computed tomography or magnetic resonance imaging (MRI) (69). Even without these macrostructural imaging abnormalities, such as WM lesions (WML), microstructural WM injury as demonstrated by changes in diffusion values with diffusion-tensor imaging (DTI) is present in well treated PHIV-infected children (67, 69).

Long term HIV related immune activation may further contribute to this CNS pathology. HIV related systemic immune activation as indicated by systemic inflammation, monocyte and endothelial activation, with raised CRP, MCP-1, soluble CD14 (sCD14), soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1), IL-1, IL-6, IL-8, IL-10, IL-18, TNF-α), and soluble TNF receptor II (sTNF-RII) concentrations is reported in well treated PHIV (70–73). In HIV infected adults, elevated sCD14 levels in CSF were associated with increased levels of CSF neurofilament light-chain (NfL) levels and reduced brain tissue levels of the neurometabolite N-acetylaspartate (NAA) (74, 75).

In general, in children and more specifically in PHIV children, reports on intrathecal markers are scarce. In a recent Dutch cohort study, well treated PHIV children had increased systemic CRP, IFN-γ, IP-10, and MCP-1 as compared to controls, indicative of immune activation and inflammation. These children had suppressed HIV viral load levels in both blood and CSF (76). Intrathecal markers of immune activation and inflammation such as sCD14, and IL-6, and NFH were not elevated in CSF, but relative elevation of these markers within the normal range were associated with poorer cerebral and cognitive outcomes, indicating that immune activation and neurodegeneration may play a role in pediatric HIV related cerebral insults (76).

In addition to associations of immune activation markers and neurodegeneration, an association between HIV related inflammation and neuroretinal thinning (as measured by Optical Coherence Tomography) in a cohort of cART treated perinatally HIV-infected children was detected (77). Ongoing immune activation, inflammation, and neuronal injury could therefore occur simultaneously with retinal thinning in PHIV. Taken together, one may postulate that chronic HIV related immune activation, inflammation and microstructural neuronal injury may precede functional neurocognitive impairments and macrostructural MRI abnormalities.

TB and HIV Co-infection

People living with HIV are 18 times at risk to develop active TB disease as compared to HIV uninfected people (6). Interestingly, HEU children are also at significant risk at TB infection (78, 79). HIV and TB coinfection in children has become an important challenge to diagnose and manage globally. Moreover, TB and HIV coinfection potentially exacerbate each other's negative effects on the CNS. Evidence from a computed tomography imaging study showed that PHIV children with clinically diagnosed TBM presented with higher ventricular enlargement, gyral enhancement and cerebral atrophy as compared to HIV-negative children (80). Even though the effects of TB co-infection on cognitive functioning in PHIV or HEU children is unclear, a study in Zambian adults with HIV showed that co-infection with TB significantly contributed to impaired cognitive function as compared to people with HIV but without TB (81). It is therefore reasonable to hypothesize that TB and HIV co-infection in children will lead to poorer brain health and neurocognitive performance than these infections independently. The immune system may play a pivotal function in the potentiating effects between HIV and TB infections (82), and possibly their effects on the brain. For example, the proportion of peripheral blood $CD14^+CD16^+$ monocytes are higher in TB and HIV coinfected patients as compared to people living with HIV but without TB infection (83). $CD14^+CD16^+$ monocytes that are infected with HIV, migrate across the BBB, which is the primary mechanism by which HIV infects the CNS (84), resulting in cognitive impairment. Hence, TB infection may facilitate neurocognitive disorders in HIV patients by increasing the $CD14^+CD16^+$ monocyte subset. In human post-mortem brain tissues, it was found that patients with TB and HIV co-infection had increased markers of activated microglia and astrocytes in certain brain regions as compared to patients that only had TB or HIV (85). Therefore, TB and HIV co-infection can have an additive effect on neuroinflammatory regulation, which is potentially reflected by peripheral blood (neuro)inflammatory markers. Literature on the associations of biomarkers of neuroinflammation and neuronal injury with impaired brain health in children with TB and HIV coinfection is lacking and an important topic for future studies.

DISCUSSION AND OUTLOOK

The presented literature suggests that TBM and HIV are associated with increased intrathecal immune responses, the temporal profile and extent of increase are likely dependent on the disease mechanisms. The pathogenesis of TBM and pediatric HIV differs. TBM represents a more acute infection while neuro-HIV follows a more chronic infective process. In both cases diagnosing these conditions and determining their impact on the brain is difficult. By discussing these two conditions this review hopes to offers insights into the generalizable use of biomarkers across the spectrum of CNS infection, those which are acute and often short-lived with treatment, as well as those which persist and manifest over the longer term. In addition to the classical increased pro-inflammatory cytokines, TBM is characterized by cytokine changes induced by acute neuronal and vascular damage, whereas pediatric HIV involves a chronic low-grade neuroinflammatory response to products of CNS HIV infection.

Given the relevance of early inflammatory increases in pathologies like TBM, blood-based inflammatory biomarkers are highly needed. However, in view of the current lack of brain-specific inflammatory biomarkers, this is a challenge. A possible solution could be the analysis of inflammatory mediators or their transcripts in brain-derived exosomes in plasma (86, 87), which could confer desirable brain-specificity in blood.

Given the dynamic character of the immune-response and the involvement of several immune-related markers, it is likely that profiles or arrays of different markers should be measured. Novel multiplexing technologies enable such profile analysis, and different platforms are available. While these technologies may differ in sensitivity of detection of low circulating levels of these inflammatory molecules in CSF, they also differ in costs of instrumentation, reagents and level of automation. It is expected that some of the more affordable technologies may even become available in bed-side point of care formats, which is especially relevant in LMICs. Once the wet-analysis is finalized, profile analysis requires statistical tools for interpretation, for which algorithms or Apps could be developed to enable interpretation for the individual patient.

To date, few studies have taken advantage of novel ultrasensitive technologies to measure brain-injury biomarkers in blood, especially Neurofilament Light (NfL). From the studies performed in TBM, it appears that brain injury markers may have prognostic value, which is now robustly being shown for NfL in other chronic and acute diseases, such as SARS-CoV2-related encephalitis (4, 88, 89). With reference to pediatric diseases, blood biomarkers NfL and GFAP are increased in children with acute demyelinating disorders and have potential value for the decision who to treat, and to monitor therapeutic responses (90). Interestingly, levels of these biomarkers are relatively high in healthy newborns and children, which may allow the use of less expensive technologies. For example, Beerepoot et al. showed that levels of blood based neurobiomarkers NfL and GFAP concentrations show a U shape across the lifespan: they are high in newborns, and the lowest levels probably are reached around age 15, after which they increase again (90).

Biomarker studies in any disease requires an infrastructure of biobanking and systematic recording of clinical and other relevant disease characteristics, in addition to sufficient funding to perform such studies. In addition, pre-analytical

aspects may be another challenge. For some biomarkers, samples are ideally processed within a couple of hours after collection (91). Different processing solutions should thus be defined for specific biomarkers for use in a variety of settings. Fortunately, a stringent pre-analytical protocol is not required for some biomarkers, like NfL and GFAP (91).

In conclusion, there is clearly a strong need for and demonstrated value of fluid biomarkers to aid precise biological diagnosis of neuroinfectious disorders highly prevalent on the African continent. With the current technological developments in other disease areas, more technological opportunities become within reach to measure disease relevant proteins in accessible matrices. It is of utmost importance that these technologies are transformed into tools that can be implemented in resource-low conditions to enable access to these healthcare improvements in a globally equitable way that maximizes benefit to patients.

AUTHOR CONTRIBUTIONS

All authors drafted an equal part of the manuscript and all have critically revised the different versions of the manuscript until its final version.

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REVIEW
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Astrocytes and Microglia in Stress-Induced Neuroinflammation: The African Perspective

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Background: Africa is laden with a youthful population, vast mineral resources and rich fauna. However, decades of unfortunate historical, sociocultural and leadership challenges make the continent a hotspot for poverty, indoor and outdoor pollutants with attendant stress factors such as violence, malnutrition, infectious outbreaks and psychological perturbations. The burden of these stressors initiate neuroinflammatory responses but the pattern and mechanisms of glial activation in these scenarios are yet to be properly elucidated. Africa is therefore most vulnerable to neurological stressors when placed against a backdrop of demographics that favor explosive childbearing, a vast population of unemployed youths making up a projected 42% of global youth population by 2030, repressive sociocultural policies towards women, poor access to healthcare, malnutrition, rapid urbanization, climate change and pollution. Early life stress, whether physical or psychological, induces neuroinflammatory response in developing nervous system and consequently leads to the emergence of mental health problems during adulthood. Brain inflammatory response is driven largely by inflammatory mediators released by glial cells; namely astrocytes and microglia. These inflammatory mediators alter the developmental trajectory of fetal and neonatal brain and results in long-lasting maladaptive behaviors and cognitive deficits. This review seeks to highlight the patterns and mechanisms of stressors such as poverty, developmental stress, environmental pollutions as well as malnutrition stress on astrocytes and microglia in neuroinflammation within the African context.

Keywords: astrocytes, microglia, reactive oxygen species, malnutrition, environmental pollution

INTRODUCTION

Astrocytes: From Physiology to Neuroinflammation

Astrocytes are glial cells of the central nervous system (CNS) of neuroectodermal origin. In fact, neurons, oligodendrocytes and astrocytes derive from a common multipotent self-renewable neural stem cell in a process that occurs with precise timing (1). While neurogenesis takes place early during embryonic development and is accomplished at about birth, gliogenesis follows neurogenesis and is finalized in postnatal life (1), with synaptogenesis and neuronal function depending on astrocyte morphology, maturation and regional specification (1).

Astrocytes play key physiological functions in the CNS that, if altered, may lead to or amplify tissue damage and neuroinflammation and hamper relevant brain functions, such as cognition and memory.

First, astrocytes contribute to the complexity of CNS structure. Evolution has led to the relative expansion of astrocytes, especially in the human brain where the number of astrocytes exceeds that of neurons and astrocytes present complex arborisation architectures (2). CNS damage triggers a complex response in astrocytes, which become reactive and undergo transcriptional remodelling, early upregulation of the intermediate filament glial fibrillary acidic protein (GFAP), morphological changes and proliferation (3). This reaction alters physiological tissue topology and may lead to scar formation as observed in traumatic, neuroinflammatory and neurodegenerative CNS disorders (3, 4). By contrast, decreased astrocyte numbers and GFAP signals have been evidenced in mental disorders (5), underlying a distinct pattern of astrocyte pathology. Astrocytes are involved in the control of neuroinflammation, as conventional or inducible GFAP deficient mice display exacerbated expression of Toxoplasma encephalitis, Staphylococcus aureus-induced brain abscess, spinal cord and brain injury (reviewed in 6). In the experimental model of multiple sclerosis, the experimental autoimmune encephalomyelitis (EAE), astrocyte depletion may worsen or attenuate disease depending on whether depletion occurs immediately after EAE induction or during the chronic phase respectively (7, 8), indicating that GFAP positive cells may display protective or detrimental functions at distinct stages of disease.

Second, astrocytes regulate neuronal survival *via* release of and/or response to crucial mediators like neurotrophins, well known growth factors for neurons to which astrocytes may become sensitive under pathological conditions and react with the synthesis of neurotoxic nitric oxide (9, 10). Furthermore, astrocytes sense neuronal activity as their fine extensions take contact with pre- and post-synaptic neurons (forming the so-called tripartite synapse) and bear neurotransmitter receptors (11). They may modulate the concentration of glutamate, the main excitatory neurotransmitter in the brain, present in the synaptic cleft *via* specific transporters called glutamate/aspartate transporter (GLAST) and Astrocytic Glutamate Transporter 1 (GLT1) (11). *In vitro* and *in vivo* evidences indicate that neuroinflammation is characterized by alterations in expression of glutamate transporters and glutamate buffering (12, 13). The accumulation of glutamate in the extracellular space causes neuronal damage by excitotoxicity, a phenomenon observed in neurological and psychiatric disorders (reviewed in 14). Furthermore, astrocytes can provide metabolic precursors of glutamate back to the neurons through monocarboxylate transporters (MCT), thus satisfying neuronal metabolic needs and limiting novel neurotransmitter synthesis (15, 16) Though scarce information is available about astrocyte metabolism in neuroinflammatory mouse models, it is known that respiration-deficient astrocytes may survive as glycolytic cells *in vivo* in the absence of tissue inflammation and damage and that inflammatory cytokines increase glycolytic rates of astrocytes *in vitro* (17, 18), suggesting sustained glycolytic proficiency of the astrocyte in neuroinflammation. Interestingly, disruption of MCT transporters in astrocytes *in vivo* causes amnesia, underlying a key role for astrocyte-neuron metabolic coupling in long term memory formation (19).

Third, neuronal activity strongly depends on continuous supply of oxygen and glucose through the cerebral blood flow (15). Astrocytes cover most of the cerebral vasculature and create neurovascular units which link synaptic activity to vessel tone, thus regulating microcirculation (15). Further, astrocytes are key constituents of the blood-brain barrier (BBB) and their interaction with endothelial cells regulates BBB development and function (20). On the one hand, astrocyte factors control formation of tight junctions, blood flow, microvascular permeability, cell matrix and angiogenesis; on the other hand, endothelial signals regulate astrocyte maturation and expression of receptor proteins and ion channels on the glial membrane (20). Thus, for example Aquaporin-4 (AQP4) expression at astrocytic endfeet in contact with the vasculature together with the inward rectifying K+ channel Kir 4.1 provides local control of water and ion homeostasis (20). Autoantibodies directed to AQP4 are at the basis of the pathogenesis of neuromyelitis optica (NMO), an inflammatory CNS disorder characterized by astrocyte loss, axonal damage and demyelination (21–23), while the occurrence of anti-Kir 4.1 antibodies in MS is controversial (24). Further, Kir 4.1 has been reported as downregulated in ALS and epilepsy (25, 26), while upregulated in animal models of depression (27). Notably, mice lacking astrocyte Kir 4.1 display ataxia and seizures and die prematurely (28) while animals overexpressing astrocytic Kir4.1 develop a depression-like phenotype (27).

Fourth, astrocytes can exert and control immune reactions in the CNS (29–31). Similarly, to microglia, they bear a repertoire of pattern recognition receptors (PRR), which allow recognition of genome, proteins, and glycolipids of microbial origin (aka pathogen-associated molecular patterns, PAMP) (29). PRR include toll-like receptors, scavenger receptors and complement factors and have been initially identified as tools of the innate immune system to fight infections (29). However, PRR also recognize danger signals, that are endogenous molecules released or activated during stress or damage under sterile conditions (and collectively called DAMP, damage-

associated molecular patterns) (32). DAMP include molecules from the extracellular matrix (e.g. biglycan and fibrinogen), cytosol (e.g. S100 proteins and heat shock proteins), and nucleus (e.g. histones) (reviewed in 32) Activation of innate immune pathways has been demonstrated in infectious, autoimmune, neurodegenerative disorders (29, for review). PRR engagement on glia cells activates pro-inflammatory responses required to eliminate or, at least, to contain infectious agents or damage. Critical is the activation of the transcription factor NF-kB, which controls gene expression of inflammatory cytokines, chemokines, nitric oxide synthase, apoptosis regulators (6, 29). In fact, *in vivo* inhibition of NFkB signalling in astrocytes protects from spinal cord and brain injury, EAE and toxic demyelination (33–36).

While rare in physiology, under pathological settings reactive astrocytes may modulate adaptive immunity in the CNS in several ways. Reactive astrocytes may release chemokines, such as CXCL10 and CCL2, which attract T cells from the circulation into the CNS parenchyma (37, 38) and *in vivo* deletion of astrocyte CCL2 and CXCL10 protects from EAE (39, 40). Next, T cell-derived factors such as IFN γ stimulate the expression of MHC class II molecules on astrocyte membrane, so that these glia cells become efficient in presentation of CNS antigens (e.g. myelin proteins) to T cells (41, 42), thus potentially sustaining local autoreactive adaptive immune responses. On the other hand, IFN γ can be released also by regulatory T cells and IFN γ signalling has been shown to be protective *in vivo* in EAE (43–45). Astrocytes are also great producers of TGFbeta, a known immunosuppressive mediator, and blockade of TGFbeta synthesis in astrocytes enhances tissue pathology in stroke and infectious CNS models (46). Further, astrocytes have been shown to express CTLA4, CD39 and CD73 (47, 48), which limit T cell activation, and FasL and TRAIL which trigger T cell deletion (31, 49). Regarding the interaction with B lymphocytes, astrocytes may release CXCL12, which promotes B cell recruitment to the CNS (50) and BAFF, a mediator important for B cell development, survival and function (51). Overall, these observations indicate a key role for astrocytes in regulating adaptive immune reactions. Activated astrocytes support B cell survival and activation, in turn, activated B cells induce a better T cell proliferation (52).

Microglia in Health and Disease

Microglia, a set of small glial cells within the CNS, were first described by del Río Hortega (53). Several decades passed before the importance of microglial functions in the CNS were appreciated. In 1920s, del Río Hortega had provided histological evidence that these cells derive from the mesoderm and not the ectoderm; the source of all other neural cells (oligodendrocytes, neurons and astrocytes) (53–55). It is now accepted that these glial cells originate in the yolk sac during fetal development and emerge at an earlier stage than tissue macrophages (56–58). Under basal condition, microglia display a multitude of physiological effects in such cellular processes as neurogenesis, cerebral angiogenesis, synaptic pruning, and oligodendrogenesis during brain development in both rodents and primates (59–61). Indeed, microglia contribute to neurogenesis and olidendrogenesis during prenatal and neonatal period (59, 62, 63). They emerge concomitantly with newly-born neurons and heavily invade neurogenic niches such as the ventricular and subventricular zones. This spatiotemporal co-existence between microglia and newly-born neural cells (neurons and oligodendrocytes) indicate the potential role of microglia in the regulation of neurogenesis and oligodendrogenesis (62, 63). For example, a subgroup of microglia expressing CD11c play a major role in the initial phase of myelination in developing brain (61). Through their phagocytic activity, microglia contribute to the removal of cell debris of dying neural cells and create optimal environment for neuronal connectivity.

Microglia affect cell survival/death programs of neural cells and remodel synaptic connection between developing neurons by secreting a variety of pro- and anti-inflammatory cytokines (Tumor Necrosis Factor (TNFα), Interleukin 1β (IL-1β), IL-6 and IL-10) and growth factors such as insulin growth factor 1 (IGF1) and brain-derived neurotrophic factor (BDNF) (61, 64–67). Thus, any alteration to these developmental effects of microglial can have long-lasting impact on brain structure and function (65, 67–69).

In addition to their homeostatic effects, microglia play a major role in the immune response to a variety of insults including pathogens (viral, bacterial or parasitic), trauma, stroke, and neurodegenerative diseases (70). For their immune-related function, microglia are referred to as the immune-competent cells of the CNS. Under basal condition, microglia form a network of cells characterized with small perikarya and long thin processes. These processes dynamically "sniff" their environment for signs of tissue damage such as high extracellular concentrations of calcium ions or adenosine triphosphate (ATP) (71). A damage to neural tissues triggers a cascade of cellular events within microglia. These cells send long processes towards the site of damage and adopt morphological changes whereby their cell bodies become enlarged and adopt an amoeboid shape. Their processes become short and thick. The genetic program of activated microglia is shifted towards cell division and phagocytosis and is characterized by the synthesis and release of a myriad of inflammatory cytokines and trophic factors (71). These cellular and molecular responses appear to be beneficial during the acute phase of insult. However, a prolonged activation of microglia can become deleterious (72, 73). While microglial cellular response appears to be non-specific, many line of evidence suggest that these cells exert a strong pro-inflammatory response during the initial phase of insult followed later by a regulatory response that consist mainly in the production of anti-inflammatory cytokines (IL-4 and IL10) and trophic factors (73, 74). The secondary wave of anti-inflammatory cytokines and trophic factors contributes to the recovery process of CNS tissue from the injury.

Astrocytes and Microglia Cross-Talk

Cell-cell interactions control CNS physiology and pathology (6, 75–84). Astrocyte-microglia interactions, for example, play important roles in CNS development, health and disease (31, 85, 86). In 2012 Ben Barres and colleagues reported that LPS

induces a neurotoxic phenotype in astrocytes (87). Follow up mechanistic studies stablished that LPS induces the production of IL-1α, TNF-α, and complement component 1q (C1q) by microglia, which act on astrocytes to induce neurotoxic activity mediated by a lipid and additional as-yet unidentified neurotoxic factor (79, 88). In addition, these microglia-induced neurotoxic astrocytes display decreased phagocytic activity, and the reduced expression of neurotrophic factors (79). Finally, the analysis of patient samples suggest that these neurotoxic astrocytes contribute to the pathology of multiple neurologic diseases, including Huntington's disease, Alzheimer's disease, and multiple sclerosis (MS), among others (79). Collectively, these findings opened new avenues about the microglial regulation of astrocyte responses, and its contribution to CNS pathology. For instance, microglia can likely produce both positive and negative regulators of astrocyte pathogenic responses (see 80). Several molecules have been found to be involved in astrocyte-microglia communication, and the control of these cell-cell interactions by the commensal flora in specific diseases such as MS (89–96). For instance, VEGF-B was identified as a microglial product that boosts disease-promoting astrocyte responses. The transcription factor aryl hydrocarbon receptor (AHR) in microglia boosts TGFα while repressing the production of VEGF-B. Furthermore, AHR can also be activated in the CNS by metabolites produced by the commensal gut flora (which as a result of their chemical structure cross the BBB) and induced by environmental chemicals (83, 97–99). These thus contribute to regulation of astrocyte-microglia communication and CNS pathology (see 100).

Astrocytes can control microglia responses (100). Although multiple mechanisms likely mediate the control of microglial responses by astrocytes, some candidate pathways have already been identified. For example, fate-mapping and other studies established that astrocytes produce GM-CSF (100), 101), a known regulator of microglial activation (84, 102, 103). Astrocytes have been shown to modulate microglial responses *via* the production of GM-CSF (8, 104). Similar observations have been made for IL-6 (105–109). The above-mentioned findings exemplify the important role astrocyte-microglia interactions in CNS physiology. The recently developed RABID-seq (**Ra**bies **B**arcoding **I**n **D**roplets) which uses a library of genetically barcoded rabies virus in combination with single-cell RNA-seq to study CNS cell-cell interactions *in vivo* (110), identifying interacting cells, the mechanisms involved, and the biologic consequences of those interactions, has helped to highlight an important role for microglial-astrocyte interactions mediated by EphrinB3 and EphB3 in the promotion of CNS pathology (110) by inducing proinflammatory gene expression in the CNS (111), potentially *via* the activation of MAPK and the NLRP3 inflammasome (112, 113). In addition, EphB3 signaling in astrocytes induces the production of D-serine (114), which promotes synaptic damage *via* NMDA receptors (115). We also found that EphB3 in astrocytes is activated by its membrane-bound ligand EphrinB3 expressed by microglia. Interestingly, EphrinB3 harbors an intracellular domain that can trigger specific signaling pathways. Indeed, reverse signaling *via* EphrinB3 boosts the expression of NF-kB-driven transcriptional programs in microglia that promote inflammation and neurodegeneration (see 104, 110).

Early-Life Immune Challenge

In Africa, vast populations are exposed to stressors across all age groups with early life exposures carrying the greatest neurological burdens. These early life challenges alter the developmental trajectory of the CNS and consequently result in neurodevelopmental disorders (116). Epidemiological studies have shown a correlation between early life immune challenge and brain related diseases such as schizophrenia (117, 118), autism spectrum disorder (119, 120) and attention deficit hyperactivity disorder (121). It was suggested that the emergence of these brain related diseases are linked to altered early life function of microglia, as these cells play a pivotal role in synaptic pruning, neuronal connectivity and removal of dying neurons during brain development (122). Furthermore, depletion of microglia during early life induces persistent changes in social behavior such as reduced anxiety-like behavior and impaired working memory (123, 124). These effects were absent when microglial activity was inhibited during adulthood (125).

Experimental studies have shown that early life exposure to pathogens such as bacteria or viruses alters brain development trajectory and consequently leads to persistent cognitive deficits and behavioral dysfunctions. Indeed prenatal or neonatal exposures to either viral mimetics (polyinosinic:polycytidylic acid: PolyI:C) or bacterial active ingredient (Lipopolysaccharide: LPS) reprograms the hypothalamic-pituitary adrenal axis and affects brain development and plasticity that lasts into adulthood (126–129). These long-lasting effects are not due to the pathogens *per se*, but are triggered by maternal immune response to these pathogens (130, 131). We and others have shown that maternal immune activation alters adult brain plasticity and cognitive functions *via* maternally borne mediators such as interleukin-6 (IL-6) (132–134) and transforming growth factor-β (TGF-β) (135).

In addition to pathogens, non-infectious agents such as stress (136–138) or exposure to air pollution (diesel exhaust particles) can also activate maternal immune system and consequently alters fetal brain development (139). Indeed, exposure to these non-infectious agents induces microgliosis in the fetal brain and leads to an enhanced reactivity of microglia later in life, which is accompanied with cognitive dysfunction such as learning and memory deficits (140).

Long-Lasting Impact of Maternal Infection in Africa

The major cause of deaths in sub-Saharan Africa is infectious diseases (69%). A significant percentage of these deaths is associated with infection during pregnancy because pregnancy is characterized by an immune tolerant state to prevent rejection of the fetus (141–143). A relatively large epidemiological study has shown that the frequency of maternal infection and its resulting complication was higher in African low-income

countries (15 African countries), when compared to high-income non-African countries. Indeed, obstetric infection led to maternal mortality of about 10.7% in low-income countries when compared to that seen in high income countries (about 4.7%). These infections include urinary tract infections, chorioamnionitis and abortion related infections (144, 145). While pregnancy-associated maternal death had received much attention, data concerning the impact of maternal infection on the brain development of children born to surviving mothers is scarce. As discussed above, experimental evidence strongly suggest that maternal infection can alter the developmental trajectory of fetal brain mainly by microglia. Few epidemiological studies have focused on pediatric patients in Africa (Gambia, Nigeria). In a cohort of 128 children in Gambia, a sizable fraction of these pediatric patients showed brain related delay such as learning difficulties (55%) and speech disorder (42%) (146). A similar set of studies in Nigeria show that children showed signs of epilepsy (60%), intellectual disability (7.2%) (147) and cerebral palsy (16.2%) likely due to early life events such as birth asphyxia and infection (148). These correlative studies strongly suggest that the prevalence of adult behavioral dysfunction and cognitive deficits in this African population is due, at least in part, to early life exposure to infectious pathogens.

Despite the prevalence of maternal infection, and its potential role in the emergence of such diseases as schizophrenia and autism spectrum disorder, few epidemiological and clinical trials have addressed these developmental diseases in Africa (149, 150). A recent study has shown that schizophrenic patients from South Africa (Xhosa ethnic group) carry damaging mutations in genes involved in synaptic function, such as receptors for glutamate and γ-amino-butyric acid (GABA) as well as postsynaptic proteins, scaffold proteins, and cell adhesion molecules (151). These mutations are comparable in nature to those observed in schizophrenic patients in Sweden (152). The mutation of these genes has been associated with intellectual disability, schizophrenia and autism spectrum disorders (153). It appears that the prevalence of schizophrenia is related to early life challenges such as childhood trauma (154). Similarly, maternal malaria has been associated with altered placental-fetal barrier by macrophage inflammatory mediators and complement factors (C5a), which can lead to altered fetal brain development (155).

The long term consequences of maternal infection on fetal brain development and function in sub-Saharan Africa has received little attention despite the overwhelming prevalence of infection during perinatal period. There is a need for studies that focus on the mechanistic link between perinatal infections and adult brain plasticity and function in Africa. These studies should take into consideration that subsaharan African mothers frequently experience multiple infections (parasitic, viral, bacterial) throughout pregnancy, which could be compounded with such factors as stress and malnutrition (156).

Malnutrition and Neuroinflammation

Besides the lack or shortage of food, several sociocultural factors e.g. poverty, poor social infrastructure, food security, uncontrolled population explosion, land and crop degradation, and lack of access to health services, contribute to the rising levels of malnutrition in Africa (157, 158). Others factors include famine, limited knowledge about safe hygiene practices, pediatric environmental enteropathy (PEE), natural disasters as well as internal population displacements as a result of civil (religious or ethnic) unrest leading to children staying in unhygienic camps amongst others (159, 160). In particular, a strong link has been established between nutrition, inflammation and neurodevelopment from foetal life to adolescence on the continent (161). Malnutrition can be generally defined as the intake of insufficient, excess or disproportionate amount of energy and/or nutrients (162, 163). In all its manifestations, malnutrition presents as either (i) undernutrition, (ii) micronutrient imbalance, (iii) overnutrition and, (iv) diet-related non-communicable diseases (e.g. cardiovascular disease, stroke, diabetes etc.) (157, 163).

Scope of the Problem

The statistics of numbers and people group affected by malnutrition creates a wide scope of problems in Africa. As a major global public health burden, the greatest concern is among infants, children, adolescents and women (particularly pregnant women) representing the most vulnerable category at greater risk of malnutrition (164–166). Globally in 2014, about 462 million adults worldwide were underweight, while 1.9 billion were either overweight or obese. An epidemiological study performed in 2016 showed that approximately about 155 million children under the age of 5 years were suffering from stunting, whereas 41 million were overweight or obese. In 2020, 40% of 149 million (59.6 million) stunted children under 5, 27% of 45 million (12.2 million) estimated to be wasted, and 24% out of 38.9 million estimated to be overweight or obese were from Africa (167; 163). The complicating fact however is that while there is a global decline in malnutrition, Africa has continued to record an increase in all forms of malnutrition, and for the most part, cases of undernutrition (168). This trend remains a serious concern as one of the leading cause of early child morbidity and mortality (157, 164).

The most common form of malnutrition recorded in developing countries most especially in Africa is undernutrition. Key indicators of undernutrition are wasting (low weight-for-height), stunting (low height-for-age) and underweight (low weight-for-age) (169, 170). Children under the age of five are the most severely affected of these vulnerable groups, with an estimated 45% of deaths attributed to undernutrition in this age group, mostly in low- and middle-income countries (170–172). Malnutrition is also responsible for significant abnormalities in physical and mental development with undernourished children usually having cognitive performance deficits and serious learning challenges (167, 173).

The continuous exposure of children and vulnerable groups to infectious agents under poor sanitary and unhygienic environment is of particular interest and has been shown to permanently weaken the immune systems and also cause a chronic inflammation of the intestine referred to as pediatric environmental enteropathy (PEE) in children (174–179). This gut disorder is as a result of both structural and functional changes in the intestinal mucosa characterized by intestinal villi

atrophy, malabsorption, disruption of the intestinal gut barrier and an increased permeability (180, 181). This then makes it easier for microbes to translocate through the altered intestinal barrier. Over 75% of children in developing countries have been reported to affected by PEE (182).

Maternal Malnutrition

Gressens et al. (183) noted a reduction on cortical astrogenesis in mice pups fed with low protein diet during the first fourteen days of gestation. Although the effect of malnutrition on the permeability of the blood-barrier (BBB) is not yet fully understood, alterations in astrocyte development might affect BBB formation (184, 185). Malnutrition during pregnancy causes a reduction on GFAP expression on rat hypothalamus (186) and hippocampus (187), and mice cerebellum (188). While several studies have reported a reduction on synaptic contacts after a period of malnutrition (189), recent data suggests that microglia dysfunction in their ability to respond to environmental stimuli during gestation and lactation affects synaptic plasticity *via* epigenetic regulatory mechanisms (67). In the adult brain, synaptic plasticity and basal neurotransmission has been found to be affected by certain soluble factors (e.g. BNDF) released by microglia (185).

In general, early-life malnutrition in the form of overnutrition or undernutrition can have a lasting impact on astrocytes. Abbink et al. (190) posited that both overnutrition and undernutrition present with a very similar phenotype, specifically increased GFAP expression and glucose transporters. It is worthy of note that in the case of overnutrition, although energy levels remain high, a lack in nutrients might still occur. This could suggest that the observed changes are associated with alterations or shortages in circulating nutrients, changes in the metabolic profile, or just general energy imbalance, rather than it being a specific effect of either a lack or excess of energy. In a recent study conducted by Kogel et al. (191) on the effect of long-term semi-starvation on primary cortical rat astrocytes using an undernutrition model, authors provided morphological and genetic evidence for pro-inflammatory astrocyte subtype-induction suggesting that inflammatory processes are a relevant factor in undernutrition. This response is characterized by elevated pro-inflammatory cytokines and genes associated with starvation. Furthermore, a shift toward the pro-inflammatory A1-like phenotype and an altered morphology suggest an increased astrocytic reactivity.

Crosstalk Between Malnutrition, Maternal Immune Activation and Neuroinflammation

Maternal immune activation (MIA) occurs when the measured levels of inflammatory markers in the dam exceeds normal range (192). It is usually a result of triggering of the maternal immune system by either infectious or non-infectious (malnutrition in this context) stimuli (193). This often leads to the release of inflammatory cytokines and immunologic alterations, and their transmission *via* innate placental immune activation to the developing foetus leading to adverse phenotypes particularly in the central nervous system (193–195).

There are strong emerging data from both animal and human studies that malnutrition-induced MIA results in foetal brain programming and modifications of their immune and metabolic genes through inflammatory and epigenetic mechanisms during critical periods of CNS astrocytes, microglial and immune system development (190, 194–197). Indeed, malnutrition during *in utero* and early life, notably due to undernutrition in the mothers, can affect the children's growth, metabolism, immune function, brain, and cognitive development (198–200). Interestingly, neuroinflammation has recently been revealed as one of the key underlying mechanism responsible for deleterious consequences of diet-induced MIA on offspring neurodevelopment.

Microglial priming has been proposed as a major consequence of MIA, representing a vital connexion in a causal chain that leads to the wide spectrum of neuronal dysfunctions and behavioural phenotypes observed in the juvenile, adult or aged offspring. (201). In a study conducted by Ozaki et al. (202), authors observed maternal immune activation in mid-pregnancy led to an increase in IL-6 expression in embryonic microglia, but did not cause any marked changes in their morphologies either at E18 or after birth. However, they observed a sustained alteration in the microglial process motility pattern and deficits in behaviour when MIA was induced earlier (at E12).

These observations further strengthen the notion of the existence of a connecting link between maternal immune activation during pregnancy, and neuroinflammation and neurodevelopment disorders in the offspring. A significantly programmed imbalance in the expression of inflammatory mediators such as interleukin 6 (IL-6), IL-1 α, IL-10, tumor necrosis factor-α (TNFα), C-reactive protein or the complement system has been insinuated to play a role (118, 131, 134, 203–205).

Together, malnutrition-induced MIA induces the release of damage-associated molecular patterns (DAMPs), which then activates Toll-like receptors on maternal innate immune system and placental cells to produce pro-inflammatory cytokines (206–208). Following this, placental innate immune activation occurs and by means of passive transport as well as active placental production, cytokines across the placenta barrier with resultant interaction and activation of transplacental metabolic, hypothalamic–pituitary–adrenal (stress) and neuroendocrine signaling pathways (209). This consequently leads to foetal microglial priming, activation and neuroinflammation in the developing brain and also, the induction of immunological memory on the foetal microglia and the peripheral immune cells (194, 197). The resultant outcome is the occurrence of a dynamic crosstalk between the CNS immune cells (microglia) and peripheral immune cells (monocytes) (210). Second "hits" or wave of stress after birth (for instance by malnutrition) usually results in exaggerated responses and chronic inflammation in both the brain and periphery, manifesting as lifelong neurobehavioural deficits and may perpetuate a continuous cycle (194, 201, 211; **Figure 1**).

The Vicious Tripartite Cycle of Malnutrition, Poverty and Neuroinflammation

The relationships between nutrition, inflammation and neurodevelopment has been noted to be reciprocal; this further supports the concept of the vicious cycle posed by malnutrition (161, 163, 196). Poverty amplifies the risk of, and risks from, malnutrition. People who are poor are more likely to be affected

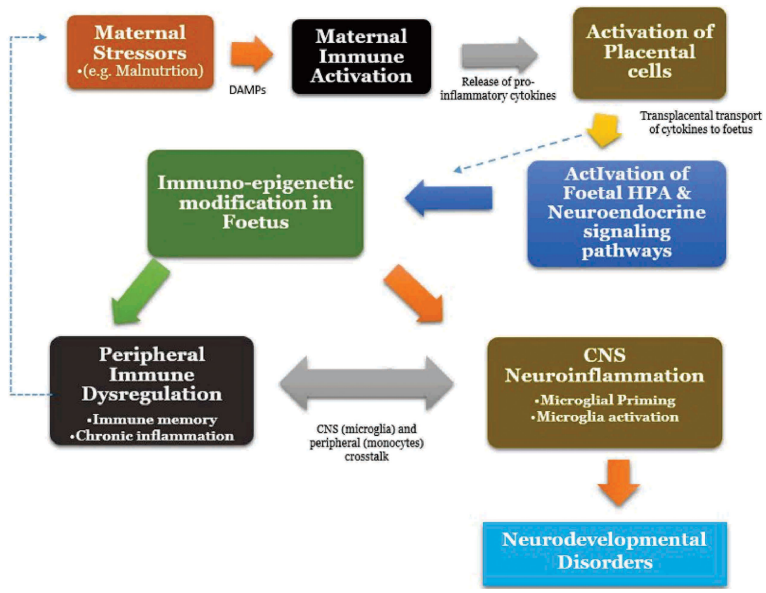

FIGURE 1 | Malnutrition and Maternal immune activation, Neuroinflammation Crosstalk. HPA, Hypothalamic-Pituitary-Adrenal Axis.

by different forms of malnutrition. Also, malnutrition increases health care costs, reduces productivity, and slows economic growth, which can perpetuate a cycle of poverty and ill-health (161, 212; **Figure 2**). This portends significant risk for the African population viz neuroinflammation.

Pollution-Induced Neuroinflammation in Africa

Africa is home to major stressors of the CNS that are known to alter the microglia-astrocyte physiology. This section reviews existing knowledge on glia interaction in the face of pollutants (metals, pesticides and contaminated air. Other than infectious diseases which are not fully addressed in the review, these pollutants pose as predisposing factors to CNS disorders in Africa, owing to the immense exponential rise in use and impact of chemicals in health, economic growth and sustainability especially in sectors of agriculture, mining, education and several other industrial processes. This has come with grave complications on communities of both users and non-users when exposed to these pollutants with bio-accumulation in the soil, water and in the air (213, 214). In a twist of tales, Africa is neither a major producer nor a consumer of chemicals in global

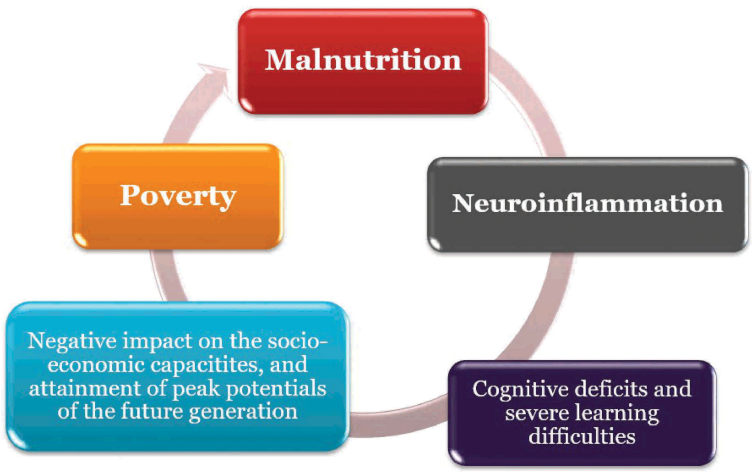

FIGURE 2 | Vicious cycle of Malnutrition, Poverty and Neuroinflammation.

terms, but has the highest levels of pollution because of non-existent or poorly implemented government environmental laws and waste disposal policies, poorly regulated mining sector, fossil fuel burning and wrong agriculture practices in the pharmaceutical, beverage, and food industries (215–217 and 214). From the lead (Pb) and cadmium (Cd) rich electronic dumpsite of Agbobloshie in Ghana, vanadium (V) rich crude oil and gas flares in Nigeria's Niger-Delta, illegal mining in Congo and several other African countries, communities release multiple neurotoxic factors daily with the young and women being most vulnerable (218). Since early life stress produces altered neurobehavioural deficits in adult life (128). Many of these stress factors directly or indirectly easily cross the placenta, and the blood–brain barrier (which is not fully developed in in humans until about 6 months post-partum) and may lead to congenital malformations and risk of neonatal neurotoxicity (219–221). These pollutants exist in the form of several sulfides, sulfates, hydroxides, phosphates, silicates oxides, and organic compounds (222, 223) and may also cause acute or chronic effects on the CNS in the general population (216, 224, 225).

Neuroinflammatory Mechanisms of Pollutants

Increasing evidence shows that astrocytes-microglia interplay may determine the phenotypic outcomes of the innate immune cells in disease conditions of the CNS. Glial activation can either aggravate tissue injury or promotes brain repair, most likely due to the nature of stress factors like the pollutant, dose and time course of exposure, and precise interplay of signals from the environment (226). Chemical pollutants include metals such as selenium, cadmium, arsenic, nickel, mercury, chromium, lead, zinc, and cobalt which are of paramount attention due to their potential role in toxicity when in trace amounts as well as other toxic pollutants such vanadium, tin oxide, copper etc. (227, 228). Other chemical pollutants include pesticides and air pollutants.

In metal pollution, microglia and astrocytes are known to express endogenous pattern recognition receptors (PRRs) in response to signals released by necrotic neurons or other pathologic products produced during disease including oxidized proteins and lipids (229), messenger ribonucleic acid (mRNA), fibronectin, hyaluronic acid, heat shock proteins, amyloid-beta, neuromelanin, and alpha-synuclein (230, 231). These are capable of responding to a variety of damage-associated molecular patterns (DAMPs) and in turn activate inflammation and neurodegeneration promoting molecular signaling events (232). The production of inflammatory mediators is further increased by activated glia, leading to a feed-forward cycle of inflammation and further release of neurotoxic mediators of tissue injury. The activated glia release diverse inflammatory factors including cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO) that are toxic to neurons (233). Cytokines such as tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) are often upregulated very quickly in activated glial cells and can directly amplify inflammation through recruitment of both innate and adaptive immune cells, leading to neuronal apoptosis (231).

When exposed to pollutants, microglia and astrocytes typically increase the production and release of inflammatory cytokines which enhance (ROS) generation, impede antioxidant activity, and result in neuronal injury or neuronal loss in the brain or other parts of the CNS (234). While the precise mechanisms are not yet fully understood, microglia have been shown as the first line of action and they respond in a dose-dependent fashion, while astrocytes are known to accumulate more toxic elements and express cytokines much later. Exposure to metals such Lead (Pb), Methyl mercury (MeHg), Vanadium (V), Tin Oxide (TO) results in gliosis by activating Toll-like receptor 4 (TLR4) -myeloid differentiation primary response 88 (MyD88) -nuclear factor (NF)-κB signaling cascade, increasing receptor phosphorylation and the activation of Mitogen-activated protein kinase (MAPK) cascades with subsequent initiation of signal transduction some of which are responsible for the production of pro-inflammatory cytokines (218, 234–236). This exposure is associated with upregulated activation of nuclear factor erythroid 2–related factor 2 (Nrf2) which acts against electrophiles and oxidants in the detoxification of ROS to maintain homeostasis (237). When exposed to ROS, Nrf2 acts by separating from the cytoplasmic repressor protein - Kelch-like ECH-associated protein 1 (Keap1), transferring to the nucleus, and activating the expression of antioxidant response elements (ARE)-dependent genes, including the phase II detoxifying/antioxidant enzyme HO-1 and NQO1 (238). The activation of the apoptotic caspase-3 pathway, which results in neuronal damage neurons is also suggested (239). This mechanism induces primary microglial toxicity and may be the pathological basis of metal pollution induced neurological dysfunction.

Mercury

MeHg pollution inhibits the astrocytic uptake of cysteine; an essential precursor for glutathione (GSH) synthesis. Implying that MeHg pollution induces neuronal oxidative damage (240). Part of the inhibitory mechanisms of MeHg include astrocytic glutamate uptake inhibition and glutamate efflux (241). This results in excessive glutamate in the synaptic cleft and, consequently leads to neuronal excitotoxicity. *In vivo*, mercury has been shown to induce microglial production and secretion of lysosomal proteases, leading to neuronal toxicity while astrocytes, when co-cultured with neurons, increase neuronal resistance to the damaging effect of MeHg (242, 243). Astrocytes and microglia therefore mediate protective effects against MeHg-induced neuronal toxicity. Microglia increase interleukin-6 (IL-6) production and release (244).

In organotins however, *In-vitro* studies have shown increased expression of IL-1β, tumor necrosis factor (TNF-α), IL-6, and nitric oxide synthase (iNOS) in the cultured astrocytes and microglia (245, 246).

Manganese (Mn)

Molecular mechanisms involved in Mn-induced neurotoxicity involve direct damage to the substantia nigra, globus pallidus, basal ganglia, striatum, and various other cellular components of the nervous system. Mn accumulates in the mitochondria of various cellular components in the brain, causing F_0/F_1 synthase and succinate dehydrogenase abnormality, leading to reduced ATP production (247). Diminishing ATP levels increase

intracellular calcium levels and induce severe oxidative stress, forming ROS. Manganese was also shown to oxidize dopamine into reactive quinone species and disruption of antioxidant enzymes *via* binding to their thiol and hydroxyl groups (248). Glia activation has however been shown to occur in astrocytes and microglia in manganism (249) as it potentiates the effects of LPS and cytokines on activation of both microglia and astrocytes leading to increased production of TNFα, IL-1β, ROS, and NOS2 expression that can cause neuronal injury (250).

Manganese activates NF-κB and mitogen-activated protein kinase (MAPK) in microglia resulting in inflammatory gene expression and production of inflammatory mediators (251). The inflammatory effects are tightly regulated both at the level of IKK activation as well as by nuclear proteins that modulate transcriptional activity of inflammatory genes- NR4A1 (Nurr1) (252). Microglia then release neuroinflammatory mediators and pro-inflammatory cytokines, as well as reactive oxygen and nitrogen species (ROS and RNS), all of which can act on astrocytes to amplify inflammatory responses in the CNS (250).

In astrocytes, higher levels of accumulation of Mn occur than in neurons. This makes them target cells for transport of Mn into the brain as well as for initiating inflammatory signaling during neuronal stress and injury. Since astrocytes are a heterogeneous population of cells with different morphological and physiological characteristics depending on their location with the brain (253), they invariably serve as the major homeostatic regulator and storage site for Mn in the brain and a prominent contributor to Mn-stimulated nitric oxide (NO) production through NOS2 (254, 255). The regulation of astrocyte activation is under the control of many factors including cytokines IL-6, IFNγ, tumor necrosis factor-alpha (TNFα), toll-like receptor activators, neurotransmitters, ATP, reactive oxygen species, hypoxia, glucose deprivation, ammonia, and protein aggregates (256). Frequently, these activators are by-products of already injured neurons or factors released by activated microglia which indicate that astrocyte activation is often later in disease progression (257).

Cell culture models of glia cross talk in managanism indicate that removal of microglia or use of antioxidants has shown to reduce neuronal loss indicating microglial activation may serve as a critical step in mediating neuronal injury during Mn exposure and that microglia also likely directly promote activation of astrocytes that then amplify neuronal damage (258). However, astrogliosis is often more persistent than microgliosis and is believed to be important in amplifying inflammatory processes and thereby inducing greater damage (259).

Pesticides

Over 45% of neurotoxic chemicals are pesticides. Exposure to toxic doses of these chemicals activates the CNS immune system by reducing Nrf2 activation, activating the NF-kB pathway, or the opening of voltage gated calcium channels in neurons. These lead to increased oxidative stress, neuroinflammation, neuronal apoptosis, activation of p38MAPks, nucleotide-binding domain, leucine-rich repeat (NLR) family pyrin domain containing 3 (NLRP3) inflammasome, and reduced serotonin. Examples include the organophosphates which primarily cause accumulation of acetylcholine at cholinergic synapses, resulting in muscarinic and nicotinic receptor over-stimulation leading to oxidative stress, lipid peroxidation (260). Organophosphates can also alter the cyclic-AMP-protein kinase A signaling pathway of which affects the expression and function of several nuclear transcription factors such as c-fos, p53, AP-1, Sp1 and CREB (Ca2+/cAMP response element binding protein) involved in the switch from proliferation to differentiation of neural cells (260).

Dieldrin is an organochlorine extensively used as pesticides for corn, cotton, and citrus crops has been reported to induce severe alteration in the function of dopaminergic neurons and $GABA_A$ receptor (261) with evidence of significant oxidative stress, mitochondrial dysfunctions, and generation of pro-apoptotic proteins such as caspase-3 and Bcl-2 in the dopaminergic neurons (262). Endosulfan is an off-patent organochlorine insecticide and acaricide. It has been used globally as a pesticide since the 1950s to control a variety of insects including whiteflies, aphids, leafhoppers, Colorado potato beetles, and cabbage worms applied extensively to coffee, tea, and cotton crops, among others (263). It induces severe oxidative stress, induces the expression of pro-apoptotic proteins and inflammatory cytokines, and activation of glial cells (264). Pyrethroids are synthetic insecticides, which are used for the controlling insect pests in agriculture, public health, and animal health. They mediate prolongation of the kinetics of voltage-gated sodium channels, which are responsible for generation of the inward sodium current that produces the action potential in excitable cells leading to a hyperexcitable state, damage BBB and cause induction of severe endoplasmic reticulum stress, neuronal apoptosis, microglial activation, and neuroinflammation (265, 266).

Rotenone and pyridaben are two mitochondrial complex I inhibitors and are highly lipophilic. They easily cross BBB and produce ROS, Ca^{2+}-mediated hyperexcitation, nuclear translocation of NF-kB, activation of p38 MAPKS, the formation of NLRP3 inflammasome, and mitochondrial dysfunctions (267–270).

Traffic Related Air Pollutants

TRAP exposure induces oxidative stress products, such as malondialdehydes (MDA), thiobarbituric acid reactive substances (TBARs) as well as ROS such as H_2O_2. (Nrf2), superoxide dismutase (SOD), glutathione (GSH), heme oxygenase 1 (HO-1), and catalase (CAT) are commonly elevated in the central nervous system, indicating need for detoxification. This induction activates glia response with astrocytic activation usually occurring either concomitantly with, or immediately after microglia stimulation, thus contributing to the release of oxidant species and pro-inflammatory cytokines (222, 271, 272).

Diesel Exhaust (DE): has been shown to induce oxidative stress, to activate microglia and to enhance levels of several pro-inflammatory cytokines (IL-1a, IL-1b, IL-3, IL-6, TNF-α) in the olfactory bulb and the hippocampus and microglia activation resulting in decreased adult neurogenesis in the hippocampal subgranular zone (SGZ) and the subventricular zone (SVZ) [Reviewed in (222, 273)].

CONCLUSION

In summary, multiple factors not limited to those discussed in this review may modulate neuroinflammation within the African context. These stressors assault the CNS through several cellular and molecular pathways to modulate neuroinflammatory responses that can be traced back to early development, with possible persistence into adult life and risk of mortality (274, 275). The most usually implicated pathways have oxidative stress, cerebral vascular damage, neurodegeneration and infiltrating systemic inflammation or nanoparticles as major route to damage. Microglia and astroglia respond to these stressors *via* multiple mechanisms that are still a subject of intense investigations. With the continent being home to over 1.3 billion people with myriads of stressors, the increasing burden of neurological disorders may be a ticking time bomb for neurological disorders (276). Therefore, further research in collaboration with Africans on epidemiological and mechanistic studies into the association of stressors and neuroinflammation will go a long way in understanding pathways that may be beneficial in treating or managing cases. Such studies will help to determine the neurological disease burden and to what extent these stressors contribute to neurological disease progression, co-morbidities with other neurodegenerative diseases and mortalities, by looking at the genetic and molecular adaptations and or vulnerabilities that exist in the African space compare to their Western cohorts.

AUTHOR CONTRIBUTIONS

MO, AM, CF and FQ contributed to concept note development. MO wrote the abstract and conclusion, AM wrote section on microglia, CF wrote on astrocytes, FQ contributed the astrocyte and microglia cross talk section, OM wrote on malnutrition, MO and JO wrote on environmental pollutants, JO supervised all contributions of MO. OM worked on references with the assistance of MO. All authors contributed equally to manuscript proof reading and editing.

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Non-Communicable Neurological Disorders and Neuroinflammation

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Traumatic brain injury, stroke, and neurodegenerative diseases represent a major cause of morbidity and mortality in Africa, as in the rest of the world. Traumatic brain and spinal cord injuries specifically represent a leading cause of disability in the younger population. Stroke and neurodegenerative disorders predominantly target the elderly and are a major concern in Africa, since their rate of increase among the ageing is the fastest in the world. Neuroimmunology is usually not associated with non-communicable neurological disorders, as the role of neuroinflammation is not often considered when evaluating their cause and pathogenesis. However, substantial evidence indicates that neuroinflammation is extremely relevant in determining the consequences of non-communicable neurological disorders, both for its protective abilities as well as for its destructive capacity. We review here current knowledge on the contribution of neuroinflammation and neuroimmunology to the pathogenesis of traumatic injuries, stroke and neurodegenerative diseases, with a particular focus on problems that are already a major issue in Africa, like traumatic brain injury, and on emerging disorders such as dementias.

Keywords: neuroinflammation, traumatic brain injury, stroke, alzheimer's disease, spinal cord injury

INTRODUCTION

The real contribution of neuroinflammation to the pathogenesis of non-communicable diseases such as Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, traumatic and spinal cord brain injury, has long been under debate, while considering both the protective and destructive effects. (1). A large number of experimental and clinical trials modulated

neuroinflammation in these diseases as possible treatments, but to date we continue to only use steroids to treat brain edema after CNS injury. This is probably due to the fact that neuroinflammation is extremely heterogeneous, in terms of cells involved and their phenotype, but also heterogeneous in different brain regions (2). Despite the recent re-discovery of the significance of astrocytes, there is no doubt that microglia have been the most studied immune cell type of the CNS, and best exemplifies this diversity of functions (**Figure 1**) (1, 2). Our pharmacological tools to modulate inflammation have poor penetrance in the central nervous system (CNS) and are not able to perform the fine tuning required to re-establish the correct homeostasis of neuroinflammatory mechanisms. On the other hand, a huge knowledge gap exists concerning why neuroinflammation loses its protective functions in some pathological circumstances.

At a first glance, infectious diseases in Africa are such a relevant issue that non communicable diseases may seem a secondary problem. Africa, however, is the continent with the highest prevalence of traumatic injuries of the CNS (3), and also a region where the mean age of the population is more rapidly increasing, posing the urgent problem of treating non communicable diseases of the elderly, such as stroke and neurodegenerative disorders. We briefly review here the current knowledge on the role of neuroinflammation in these diseases, trying to focus these concepts toward the African context.

TRAUMATIC BRAIN AND SPINAL CORD INJURIES AND NEUROINFLAMMATION

Traumatic brain (TBI) and spinal cord injuries (SCI) are significantly increased in low-medium income countries of Africa, as compared to the rest of the globe (3, 4). The mean incidence is twice as high, approximatively 300/100,000 for TBI and 130 cases per million for SCI (3, 4). However, incidence distribution is not homogenous, some African nations being more affected than others (3). The major cause of TBI and SCI in

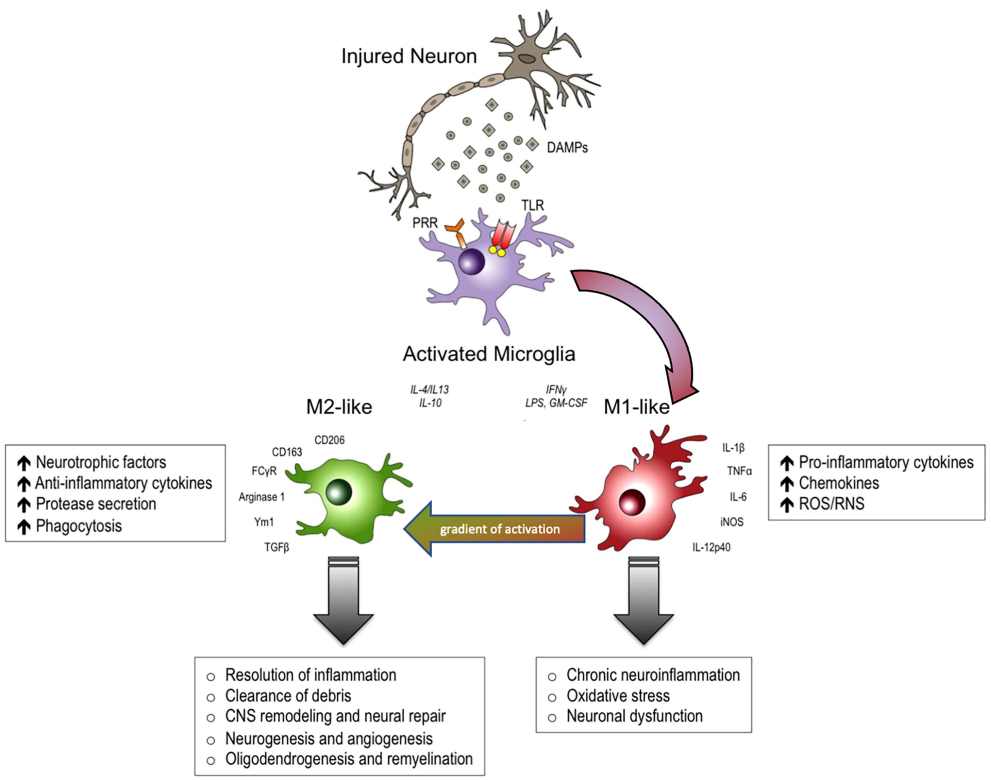

FIGURE 1 | In response to danger-associated molecular patterns (DAMPs), and other extracellular signals released by injured neurons, microglia can become polarized towards pro-inflammatory M1-like and anti-inflammatory M2-like activation states that can have distinct roles in neurodegeneration and tissue repair. M1-like microglia release pro-inflammatory cytokines, chemokines and free radicals that impair brain repair and contribute to chronic neuroinflammation, oxidative stress and long-term neurological impairments. M2-like microglia release anti-inflammatory cytokines, neurotrophic factors and proteases, and they have increased phagocytic activity. M2-like microglia promote immunosuppression and resolution of M1mediated neuroinflammation, and participate in CNS remodeling and repair by modulating neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and remyelineation. However, there is much overlap between and M1 and M2 like cellular responses. DAMPs, danger-associated molecular patterns; PRR, pathogen recognition receptors; TLR, toll-like receptors. Modified from (1). Reproduced with permission.

Africa are road traffic accidents, accounting for 30-50% of cases, but assault and falls are also common causes (3–6). Young males are the most affected, but TBI affects all age groups, including pediatric patients, and both sexes (3). Social and ethnic disparities have also been reported (3). On the whole, despite the significant increase of publications in this field (6), reports on TBI and SCI epidemiology in African countries, are still limited in numbers, exposing a huge knowledge gap, which calls for more systematic studies.

Pathogenesis of Traumatic Brain Injury

TBI can be classified according to different clinical scales but, based on the pathogenesis, a primary injury occurs followed by a secondary injury (7). The primary injury occurs at the moment of the concussion, and consists of tissue damage accompanied by features such as the rupture of blood vessels, neuronal damage, and haematoma. The secondary injury can last for days and involves several mechanisms, including cerebral edema and increased intracranial pressure, excitotoxicity, hypoxia, oxidative stress, and neuroinflammation (7).

Neuroinflammation secondary to TBI or SCI is primarily due to glial activation. Closed head injuries induce the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, and of several other inflammatory mediators [reviewed in (8)], with different kinetics, peaking 1-3 days after the injury. Along with pro-inflammatory cytokines, immune-regulating and anti-inflammatory cytokines, such as IL-10 and TGF-β, become detectable in the cerebrospinal fluid with a delayed kinetic, suggesting a counterbalancing activity (8). Post-TBI neuroinflammation involves microglia and astrocytes. Microglia activate immediately after the trauma, but may last for a very long time, up to many years post TBI [reviewed in (9, 10)]. A pro-inflammatory, so-called M1 phenotype, appears first, while an anti-inflammatory, M2, phenotype appears in later stages and may last only for brief periods. Eventually after up to 5 weeks post-TBI the M1 phenotype may prevail and perpetuate damage and tissue degeneration (10). A dichotomic view has proposed also for astrocytes, by classifying them in pro-inflammatory (A1) and anti-inflammatory (A2). As for microglia, this view may be useful as a simplified model, but is largely overcome by data showing that these cells have several phenotypes according to the different stimuli received. Nevertheless, the activation of pro-inflammatory astrocytes has been reported after TBI, starting from few hours after the injury and persisting for decades (10, 11). Neuroinflammatory mechanisms contribute to clear the injured brain and spinal cord of damage and to foster tissue regenerative mechanisms, especially remyelination. The consequences of TBI and SCI, however, include cell senescence (12), that may contribute to secondary injury. While this may be the substrate for neurodegenerative mechanisms secondary to repeated head concussions, this may also explain why the severity of long-term consequences after TBI or SCI is increased in elderly (10, 12).

Astrocytes also play an important role in the regulation of blood flow and maintaining the blood brain barrier; in order to perform this function, they normally surround blood vessels. After TBI, in addition to generalized increased reactivity and scarring, astrocytes expand their reactivity surrounding blood vessels, and also show decreases in the polarization of their endfeet (11, 13, 14). It is likely that this change in endfeet morphology leads to impaired function in the glymphatic system, resulting in impaired clearance of toxic substances, such as tau (15) (**Figure 2**). Astrocytes also show dramatic increases that persist for many years after specific types of TBI. Perl and colleagues report that strong astrocytic scarring occurs in specific neocortical sites after blast injuries, but not other types of TBI (11).

Immune cells are implicated in the pathogenesis of TBI and SCI, but they also impact immune functions. A CNS-injury related immune deficiency has been reported, and infections are one of the leading causes of morbidity and mortality after TBI and SCI (16). Also the gut-brain axis is affected after CNS injury, and gut-dysbiosis secondary to TBI may contribute to neurodegeneration (17).

Therapies for Neuroinflammation in TBI and SCI

Anti-inflammatory therapies or correction of gut-dysbiosis have been proposed to interfere with neuroinflammation secondary to TBI and SCI, to treat long term consequences of CNS-injury, along with several clinical trials. TBI and SCI and the underlying involvement of neuroinflammation are, however, extremely heterogeneous conditions, calling for a stratification of patients that may, or not, benefit from therapeutic intervention. An anti-inflammatory therapy targeting neuroinflammation may worsen CNS-derived immune deficiency, antibiotics to prevent infections may worsen gut dysbiosis, while dysbiosis correction always needs personalization of the intervention. There have been, however, research efforts trying to stratify patients according to peripheral inflammatory biomarkers to guide anti-IL-1β therapies (18), while integrated prognostic models to classify TBI patients in the emergency room to provide best treatment have also been validated in Africa (19). This said, steroids are largely used to treat brain and spinal cord edema secondary to traumatic injury, with all the consequences on immune system functioning.

STROKE AND NEUROINFLAMMATION

Globally, stroke remains a leading cause of dwindling economic fortunes with rapidly worsening epidemiologic indices, especially in low- and middle-income countries (LMICs) (20, 21). In low-resource settings with limited strength for acute interventional care (22), management of stroke patients is largely conservative and often the only available care. Understanding the role of neuroinflammation is sine-qua-non to improving outcomes, as inflammatory/immune response (23) in acute stroke is a major factor in stroke pathobiology (24). Inflammatory cells are involved in all the stages of acute stroke, from being potential risk factors to initial artery occlusion, brain parenchymal

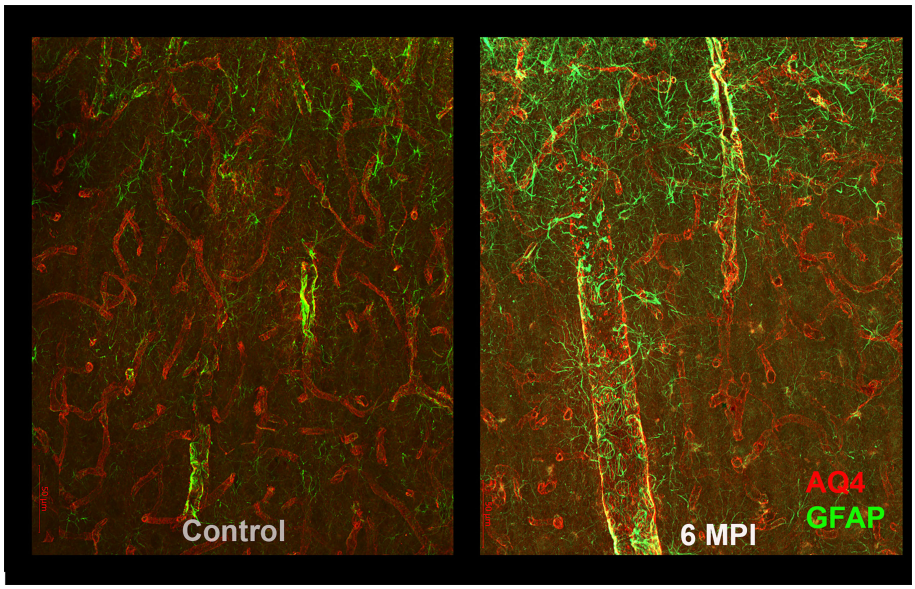

FIGURE 2 | As compared to controls (right panel), reactive astrogliosis can be appreciated as an increased GFAP reactivity and morphological changes in astrocytes 6 months after brain injury. These changes are likely to affect the blood-brain barrier and the glymphatic system and thus the clearance of potentially toxic substances such as tau.

damage, subsequent tissue repair (25), and development of various complications (26–28). Most of the complications and clinical deterioration in stroke are initiated and perpetuated by inflammatory cells and subsequent interaction with the immune system (29, 30). The ensuing "spillover-effect" leads to a systemic inflammatory response followed by immunosuppression aimed at dampening the potentially harmful proinflammatory milieu (31), thereby increasing susceptibility to post-stroke infections (16, 32, 33). This underlies the need to define the role of inflammation in the etiopathogenesis of stroke as it may offer a viable means of affordable intervention. Furthermore, stroke provides a template for the release of proinflammatory cytokines and recruitment of immune cells, which represent an important mechanism of secondary progression of brain lesion (27). Early neutrophil infiltration has been reported to be associated with larger infarct volume (34). Activated neutrophils cause the release of proteolysis enzymes such as acid phosphatase or reactive oxygen products, and exacerbate ischemic brain injury. In contrast to neutrophils, the role of different lymphocytes in acute ischemic stroke is mostly protective with release of anti-inflammatory cytokines to limit infarct size. Lymphocytes infiltrate the ischemic tissue and mediate the inflammatory response, where they increase the level of anti-inflammatory cytokines and suppress the production of proinflammatory cytokines (35). To this end, the neutrophil-lymphocyte ratio (NLR) as well as other risk markers (C-reactive protein (36, 37), Erythrocyte Sedimentation Rate, Ferritin), have been shown to be useful biomarkers of acute stroke severity and outcomes. In the longer term, particularly in patients with smaller stroke lesions, a repertoire of microglia and macrophages are recruited within the peri-infarct regions and even in subcortical grey and white matter to resolve necrotic or damaged tissue in a controlled manner (23). Current evidence suggests protective cellular mechanisms are established to repair or reseal the blood-brain barrier to prevent further damage.

Besides the role of inflammatory cells in stroke injury, it is important to recognise that the blood vessel wall undergoes inflammatory changes, which are constantly modified by age, diet, vascular and other lifestyle factors (38). Increasing age substantially reduces the inflammatory/immune response potential despite other acquired infections. In blood vessels, atherosclerosis involves significant inflammatory reactions (39) that occur during the entire process of onset, progression and rupture of atheromatous plaques (40). Among acute ischemic stroke subtypes, large artery atherosclerosis has a significantly higher inflammatory burden as determined by the NLR compared to other subtypes; low NLR positively correlated with lacunar stroke and transient ischemic attacks (TIAs) (41). A measure of inflammatory burden is now known as an emerging risk factor for incident stroke and may well predict outcomes. In the SIREN study, we found that 1 in 10 stroke cases reported antecedent history of febrile illness prior to occurrence of stroke suggesting that infectious exposures may be an important trigger of acute cerebral vascular event (42). Indeed, in previous candidate gene studies of the SIREN cohort, we demonstrated an association between the interleukin – 6 gene locus (rs1800796) and ischaemic stroke (43). Polymorphisms of the IL-6 gene regulate the circulating plasma level of interleukin – 6,

a pleiotropic cytokine, which plays critical roles in the acute inflammatory response and could trigger endothelial dysfunction and activation of the coagulation – fibrinolysis system.

CNS injury may also increase susceptibility to infection. This includes stroke, which may induce immunodepression leading to secondary immunodeficiency (CNS injury-induced immunodepression [CIDS] (16) and infection. Focal cerebral ischemia induces an extensive apoptotic loss of lymphocytes and a shift from T-helper cell (Th)1 to Th2 cytokine production. Secondary lymphatic organs like the spleen (44) and thymus may also atrophy after focal cerebral ischemia thus increasing the risk of infectious complications (45) Infections (particularly chest infection and urosepsis) remain a leading cause of death in patients with stroke (46, 47) Besides having a negative effect on outcome, infection plays an important role in extending hospital stays, worsening of neurological outcomes as well as development of more serious complications, and death (48). Emerging experimental and clinical evidence strongly suggests that brain–immune interactions play an important role for outcome after stroke. These interactions may have protective, destructive, or regenerative effects in the brain, and also impact the organism as a whole (48). Risk markers that define these interactions are relevant in predicting outcome and in risk stratification.

In conclusion, further characterization and knowledge of inflammatory and immune mechanisms of stroke and the consequences that lead to vascular cognitive impairment and vascular dementia (VaD) may pave the way for an Afro-centric as well as a tailored design of new treatment to manage varying stroke subtypes (49, 50) This knowledge may also lead to management of stroke and its complications *via* modulation of immune/inflammatory response.

ALZHEIMER'S DISEASE AND NEUROINFLAMMATION

Alzheimer's disease (AD) is a common dementing illness that manifests with progressive memory decline and cognitive dysfunction. A small fraction of familial cases, including Down syndrome patients (51), have been useful to understand pathological mechanisms and to identify heritable risk factors, although in the majority of patients, AD manifests in a sporadic form. In Africa, while there are several cases of FAD (Familial Alzheimer's Disease), most published reports indicate the majority of AD is late-onset (LOAD) in nature (52). A review of multiple articles that included population based studies from Burkina Faso, Cameroon, Ghana, Republic of Congo, Benin Republic, Kenya, Senegal, South Africa, Central African Republic, Tanzania, and Nigeria indicates that age-adjusted prevalence of dementia varied widely ranging from 2.29% (AD 1.41%) in Nigeria-Yoruba, to 21.6% (AD prevalence not reported) in the rural Hai district of Tanzania (53). The reported prevalence of dementia for the hospital-based studies ranged from 0.05% in southwestern Nigeria to 8.87% in Dakar, Senegal. Further, 6.9% of dementia cases were found in a hospital in Tanzania and 74% in a memory clinic in South Africa. Overall, the review highlights a wide variability in the prevalence of dementia in Sub-Saharan Africa (SSA); most studies suggest a lower prevalence of dementia compared with developed countries, which may be associated with the low life expectancy in the region. In general, the authors concluded that research on the epidemiology of dementia in older persons in SSA is limited, and recent studies suggest that prevalence rates in SSA may be similar to Western countries. More recently, Akinyemi et al. showed that the burden of dementia is rising in Africa at every age (52). Prevalence varies from 2.3% to 20.0% and incidence rates are 13.3 per 1000 person-years. The most common dementia subtypes are AD, vascular dementia and human immunodeficiency virus/acquired immunodeficiency syndrome-associated neurocognitive disorders. Culture-sensitive cognitive tools not influenced by language differences are needed for implementation of more detailed studies. As indicated previously, African populations are aging and thus correlates with increased prevalence of age associated disorders of the brain as AD (51, 52). AD is considered a multifactorial disease determined by interactions between environment, lifestyle, genetics and epigenetics (54). Several studies, including Genome Wide Association Studies (GWAS), established that neuroinflammation may contribute to AD pathogenesis (55–57). Many investigations on AD animal models, mostly exploiting lipopolysaccharide (LPS) to cause brain inflammation, suggest that neuroinflammation contributes to the disease directly by increasing amyloid β (Aβ) production, although this connection is still under debate (58, 59). In general, neuroinflammation is at the same time a reaction against and a contribution to the neurodegenerative pathology in AD. Among the many factors that directly give rise to neuroinflammation in AD, microglia, a population of resident innate immune cells in the central nervous system (CNS), have been recognized as a key player. Other important elements include complement proteins, CNS infiltrating mononuclear cells, cytokines, chemokines along with other factors that may drive neuroinflammation, such as systemic inflammatory events, obesity, ageing and traumatic brain injury. Due to the recognized role of neuroinflammation in AD, cells and soluble factors participating to the inflammatory reaction may become a target of therapy and/or biomarkers of the disease. Furthermore, considering the proposed impact of peripheral chronic inflammation on AD progression (reviewed in (60)), the effects of anti-inflammatory treatments on AD have been investigated and several studies indicate that the incidence of AD is reduced in nonsteroidal anti-inflammatory users, depending on the duration of the treatment and on the presence of other risk factors (61, 62). Not all investigations confirm the protective role of non-steroidal anti-inflammatory drugs, like ibuprofen, and some even worsen inflammatory progression (63). A recent study addressing the increased risk of AD in patients affected by rheumatoid arthritis (RA) indicates that RA patients treated with anti-TNFα therapy for RA showed a reduced risk to develop AD (64).

CNS Resident Myeloid Cell: the Multifaceted Role of Microglia in AD

AD is characterized by loss of neurons and synapses, amyloid plaques (extracellular deposition of Aβ aggregates), intraneural formation of neurofibrillary tangles, composed of hyper-phosphorylated tau (tau pathology) and neuroinflammation. Of note, proper microglia function protects brain tissue by limiting the toxic accumulation of Aβ, but in AD microglia become harmful, misbalancing normal clearance processes and promoting inflammation, mediating synapse loss and exacerbating tau pathology, that correlates with cognitive impairment (65). AD microglia, upon sensing these protein aggregates, starts a proinflammatory reaction that, due to the diffused presence of Aβ, may result in persistent inflammation. Under these conditions, microglia may shift from a protective to a harmful role and further contribute to the pathological process, accelerating the progression of neurodegeneration (66, 67). Age-related protein deposits, such as Aβ, cell debris or adenosine triphosphate, all constitute ligands that bind receptors expressed on microglia: danger –associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) including CD36, CD14, CD47, Toll like receptors (TLRs) and NOD-like receptor family, pyrin domain-containing 3 (NRLP3) inflammosome (68–70). This activation leads to the production of cytokines (CKs), chemokines and complement (C1q), sufficient to activate astrocytes to a neurotoxic state called A1, as described in animal models of neurodegeneration and in brain tissues derived from patients. A1 astrocytes can influence the interactions of microglia with neurons and be directly harmful to neurons and synapses (71). Indeed, the release of proinflammatory cytokines such as IL-1β, IL-6, and TNFα has been detected (72–74). In the brain, these cytokines activate protein kinases in neurons and inactivate phosphatases, resulting in a further increase of tau phosphorylation and toxic self-aggregation, further amplifying the immune reaction. Altogether, the proinflammatory environment in the AD brain may directly and/or indirectly contribute to neuronal damage by several mechanisms. For example, elevated TNFα in the CSF correlates with increased rates of cognitive impairment in AD patients. In addition, inducible nitric oxide synthase, toxic to neurons, is stimulated by CKs release and upregulated in the AD brain (67).

NRLP3 is a component of the innate immune system and activated in AD brains, forming the NLP3 inflammosome complex in association with other proteins, promoting the release of proinflammatory IL-1β and IL-18. Interestingly, recent evidence suggests the NRLP3 inflammosome is also activated in VaD as a result of chronic cerebral hypoperfusion (75). Mouse models of AD deficient for NLRP3 inflammosome are protected from amyloid pathology (76) and the inflammosome in microglia plays a role in AD progression and in the spread of amyloid pathology (77). In tau-mice, after uptake and degradation, microglia are involved in generating the seeding of tau (78, 79) Tau aggregation may be linked to NLRP3 activated microglia (80), although the role of tau peptides in NLRP3 activation is still a matter of current investigation (81). Furthermore, there may be interaction between components of the autophagic pathway, (autophagy in a healthy brain contributes to maintain a heathy environment) and NLRP3-mediated neuroinflammation (82). Protein quality control and autophagy are closely related to neurodegeneration and neuroinflammation (83). Increased autophagy limits the inflammosome activity, helping cells to return to a non-reactive state; impaired autophagy activates microglia towards a proinflammatory phenotype. This is further suggested by experimentally induced deficiency of microglia autophagy, with the consequent switch of microglia to a proinflammatory state and exacerbation of tau spreading and pathology (84). Among the identified risk genes for LOAD, innate immune response genes are a category well represented, including Clusterin, TREM2 and CD33 (85). TREM2 is a cell surface receptor highly expressed in myeloid cells, including brain microglia, that stimulates phagocytosis, suppresses TLR-induced proinflammatory CKs, and contributes to the normal function of microglia in clearing Aβ deposition in the brain (86, 87). Several mutated TREM2 alleles increase the risk of AD, and the missense variant R47H, depending on the population genetic background, is a major risk factor. The R47H variant of TREM2 reduces microglia capacity of phagocytosis and of clearance of debris and apoptotic neurons, contributing to the impaired protective action of microglia and to the shift towards a proinflammatory, harmful phenotype (88). In addition, a recent work further underlines the proinflammatory effect of TREM2 genetic deficiency in the AD brain, by detecting the increased gene expression of immune networks and pathways (89).

These observations led to the possibility of identifying promising biomarkers of AD progression. Microglia respond quickly to tissue damage, therefore *in vivo* imaging of microglia cell-surface and mitochondrial membrane ligands may track inflammatory events associated with neurodegeneration. The translocator protein TSPO, increasingly expressed during neuroinflammation, is one of these targets and radiolabeled ligands to TSPO are visualized by PET, enabling detection of increased microglia activation in AD animal models and in patients. Recently Furlan et al. characterized myeloid microvesicles (MMVs) produced by activated microglia (88). MMVs are neurotoxic after loading Aβ and freshly isolated MMVs from CSF of AD patients; they are also associated with white matter damage and mild cognitive impairment (90). Investigations on these biomarkers confirm the association between cortical activation of microglia and cognitive impairment and the relation between neuroinflammation and the severity of AD (91).

More recently, the role of microglia in AD has been further explored, taking into account tau seeding driven by amyloid, simultaneously investigating both Aβ and tau (92). This study used AD animal models and focused on the role of microglia in neuritic plaques associated with tau pathology. The authors reported that TREM2 depleted microglia increase tau pathology. Surprisingly, microglia repopulation also increases tau pathology in WT mice, whereas damage associated microglia (DAM) have a protective role. Therefore, the phenotypic switch of microglia, induced by several factors including TREM2, seems

crucial to the final role of these resident brain cells in limiting or inducing AD pathology during Aβ plaque-mediated tau deposition and spreading.

Innate Immunity: the Diverse Roles of Complement System to AD

The complement system, consisting of over 40 proteins in blood and other tissues, contributes to an innate and adaptive immune defense from pathogens and injury. Together with protective effects, the complement system has a plethora of roles in immune reactions, but hyper-activation of the system may participate in pathological reactions. Three different recognition pathways activate the complement system (classical, lectin and alternative pathways) and lead to activation of an enzymatic cascade in order to mediate effector functions. This cascade is independent from the activation pathway and includes opsonization, recruitment of immune cells, generation of the lytic membrane complex for targeted death of pathogens, increase of vascular permeability and cell polarization (reviewed in (93). In the brain, the main source of the C1q complement component is microglia (94). In response to complement activation, microglia mediate synapse loss in AD and trigger inflammation through the engagement of C3a and C5a receptors. Neurotoxic reactive "A1" astrocytes express complement proteins, potentially contributing, along with microglia, to complement-mediated neurotoxicity. Of note, during brain development, synapse pruning by microglia, namely the elimination of inactive synapses, involves the classic complement pathway, C1q and C3b, which are involved in this mechanism together with microglial complement receptor 3 (CR3) (95). Excessive complement dependent synaptic pruning associates with mouse models of neurologic disorders such as AD and experimental epilepsy (96, 97). CNS expression of complement proteins increases with age; it is interesting that AD-associated genes include CR1 (gene codifying complement receptor 1), which plays a role in phagocytosis, clearance of immune complexes and inhibition of complement (98). In the AD brain, C1 inhibitors are decreased together with increased level of activators, e.g. misfolded proteins, resulting in an imbalanced control of inflammation (reviewed in (99). The presence of activated complement in human brain tissues from AD patients suggests a role of complement in the inflammatory CNS environment (100); it is mainly associated with the Aβ plaques (C1q, C3 and C4) and to a lesser extent with neurofibrillary tangles and dystrophic neurites. Neuroprotection and reduction of synaptic loss has been observed in AD mice where C1q and C3 were inhibited or knocked-out. Some authors reported that inhibition of C3 results in increases of amyloid burden, indicating that this pathway may be involved in the clearance of plaques (101). The results of microglia depletion and of complement blockage in AD models are conflicting. Nevertheless, most studies suggest that blocking the complement activation pathway has a beneficial effect on AD pathology.

Several data sets suggest that complement-mediated functions may change during AD progression. Complement may initially be anti-inflammatory, since upregulation of C1q after initial tissue injury, in the absence of other complement proteins or other danger signals, enhances microglia phagocytosis while suppressing inflammation. Later on, the complement cascade is chronically activated by accumulation of Aβ and other activators in the absence of complement regulators. Finally, C5a is generated from plaques and engages microglia by inducing chemotaxis in the plaques and proinflammatory CKs production (102).

The Body Contribution: Proinflammatory T Cells Invade AD Brain

T lymphocytes are an important part of the adaptive immune response to infection and have specific receptors for antigens (T cell receptor, TCR), distinct from the innate immune system. T cells monitor the CNS for infection and injury, but patrolling of the CNS parenchyma is limited in non pathological conditions. In the brains of AD patients, the T cell number is instead increased, and $CD8^+$T observed in the AD hippocampus (103, 104). A recent work investigated T cell subsets in the peripheral blood of persons with AD and indentified a subpopulation, $CD8^+$ effector memory $CD45RA^+$ T cells (TEMRA), associated with mild cognitive impairment (MCI) and AD (105). These cells are $CD8^+$ memory T cells that have upregulated CD45RA, and are often senescent and terminally differentiated. These authors, by comparing a cohort of AD patients to healthy individuals, found a relation between $CD8^+$TEMRA cell concentration and cognitive decline. After *in vitro* stimulation with a mitogen, cells isolated from AD patients displayed an increased production of IFNγ, a proinflammatory cytokine. The analysis of brains from AD patients confirmed the presence of these cells in the hippocampus, in the proximity of neurons, Aβ agglomerates and meninges. T cells recirculate through the cerebrospinal fluid (CSF) and brain parenchyma; molecular analysis of this cell subset in the CSF of persons with MCI and AD identified a subpopulation that showed clonal expansion bearing a TCR that may recognize the same antigen. The same finding was described in patients with Parkinson's disease, indicating that the CSF during neurodegenerative diseases is a site of T cell expansion. In AD, clonally expanded $CD8^+$ T cells residing in the hippocampus express cytotoxic genes, e.g. increased granzyme levels (104). The obvious question is, which antigens recognize expanded T cells? Authors indicate that some of expanded clones were specific for Epstein-Barr virus (EBV). This finding does not indicate a direct cause-effect relation with AD, however it may indicate a link between infections with progressive neurodegeneration and rapid cognitive decline (106). Indeed, in animal models, T cell infiltration in the brain and amyloid accumulation are triggered by peripheral infections (107). Analysis of the TCR repertoire is an expanding field, and recent study of the TCR repertoire in the brain compared to the CSF and to peripheral blood has been performed in several neurological diseases (108–110) in the attempt to identify pathologically relevant clones. Therefore, these novel findings along with continuous increasing collections of TCR sequences from the brain, the CSF, and

peripheral blood of neurological patients, may allow us to compare TCR antigen specificity and identify T cell subsets that induce or restrain progression of dementia. This initial characterization of proinflammatory factors releasing T cells and infiltrating AD brains close to neurons, indicates a possible contribution of these cells to tissue damage and dysfunction in AD pathogenesis, determined by the activation of adaptive responses.

During aging, the immune system goes through a reorganization known as *immunosenescence*, where a state of chronic inflammation may damage the brain. In AD patients, and other dementias, this age-related change is exacerbated, accompanied by a skewed combination of innate versus adaptive immunity. Animal studies suggest that the adaptive immune system contributes to cognitive decline (104–106, 111). In AD, many genetic risk variants associate with innate immunity and may further drive the imbalance, due to immunosenescence or to the presence of more proinflammatory immune cells, that in turn determine the overall neurodegenerative processes. Further studies are needed to have a complete understanding of how the immune system is modified in people that develop neurodegenerative diseases, compared to people aging without cognitive decline, in order to develop therapeutically promising approaches and a new understanding of how the immune system may contribute to determining dementing diseases.

CONCLUSIONS

Non-communicable neurological disorders represent a major health care issue in Africa. In this review we summarized current knowledge for several of these diseases in the African context and highlighted the contribution of inflammatory processes to their pathogenesis. A common concern is the lack of epidemiological data for these diseases in most African countries. This knowledge gap does not allow clear evaluation of the extent of the problem on one hand, and clear planning of possible interventions on the other. We especially reviewed the neuroimmunological aspects of these diseases. Understanding the specific contribution of inflammation to degenerative processes, while characterizing both neurodegenerative disorders, post-acute phases of stroke, and TBI may elucidate possible therapeutic strategies, waiting for other, possibly more efficacious, neuroprotective approaches to be developed.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Neuroimmunology of CNS HIV Infection: A Narrative Review

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This short review provides an overview of the interactions of human immunodeficiency virus type 1 (HIV), immune and inflammatory reactions, and CNS injury over the course of infection. Systemic infection is the overall driver of disease and serves as the "platform" for eventual CNS injury, setting the level of immune dysfunction and providing both the HIV seeding and immune-inflammatory responses to the CNS. These systemic processes determine the timing of and vulnerability to HIV-related neuronal injury which occurs in a separate "compartment" with features that parallel their systemic counterparts but also evolve independently. Direct CNS HIV infection, along with opportunistic infections, can have profound neurological consequences for the infected individual. HIV-related CNS morbidities are of worldwide importance but are enhanced by the particular epidemiological, socioeconomic and environmental factors that heighten the impact of HIV infection in Africa.

Keywords: Africa, HIV, inflammation, central nervous system (CNS), cerebrospinal fluid (CSF), neuroimmunology, antiretroviral therapy (ART)

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INTRODUCTION

HIV is a retrovirus taxonomically grouped in the genus Lentivirus (1) that entered the human population through multiple zoonotic infections from simian immunodeficiency virus–infected nonhuman primates (2). Its double-stranded RNA genome is more complex than many other retroviruses, and in addition to structural genes it contains several regulatory and accessory genes that contribute to its detailed life cycle, protracted course and pathological consequences. While all viral proteins presumably play a role in the character of infection, some have been singled out as particularly important in determining the character of CNS infection and its consequences. These include, for example, the *env* (envelope) gene that determines T-cell or macrophage tropism (T- or M-tropism) that dominate in different phases of CNS infection (3); Likewise, the accessory genes, including *tat*, may contribute to neurotoxicity (4). HIV is also subdivided into four groups with several subtypes or clades (5). The importance of group and clade variations for neurological complications, particularly those related to direct CNS infection, remains incompletely defined (6). This review focuses on emerging concepts in the neurobiology of more "direct" CNS complications of HIV-1 infection, particularly HIV-associated dementia (HAD) and, by inference, also milder cognitive impairments.

CLINICAL BACKGROUND

HIV Epidemiology and Impact in Africa

Since its onset, the HIV pandemic has disproportionately impacted the African continent. While the first case definitions for AIDS were developed in 1982 (7), by the end of 2001 there were 40 million people living with HIV (PLWH), of whom 28.5 million (71%) were located in sub-Saharan Africa, at that time without access to antiretroviral therapy (ART) (8). While ART first became available to resource-rich countries in the 1990's, it took another decade of grass-roots political advocacy before ART first became more widely available in Africa through the United Nations Global Fund and the US President's Emergency Plan for AIDS Relief (9). Over the subsequent two decades, there has been tremendous progress in scaling up HIV care and treatment, and in 2021, 27.5 million PLWH globally were taking ART.

However, there remain important gaps. The prevalence of HIV in Africa varies widely among countries, from a low of <0.1% in Algeria and Egypt to more than 19% in South Africa, Botswana, Lesotho, and Eswatini (10). There remain 10.2 million PLWH who are not on HIV treatment, and in 2020 there were 1.5 million new HIV infections and 680,000 deaths (11). In sub-Saharan Africa, women and children are particularly vulnerable; in sub-Saharan Africa, women aged 15–49 make up 52% of new infections though they only represent 24% of the population. Older children (age 5–14 years) make up two-thirds of those not on treatment, and only 40% of children living with HIV had suppressed viral loads, as compared to 67% of adults (11).

CNS Disease in Africa

CNS complications of HIV are important causes of morbidity and mortality in Africa, and indeed globally (12). Descriptive epidemiology of HIV-associated CNS disease in Africa is limited by the availability of neurologists and advanced diagnostics such as computed tomography (CT), magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) analysis (13). Thus, many studies and clinical management decisions rely on syndromic clinical diagnoses with limited diagnostic precision, depending on the local resources. However, CNS opportunistic infections (OIs) are clearly common causes of hospitalization and may cause approximately 20% of deaths (14, 15). For disorders such as HIV-associated dementia (HAD) and, by inference, also milder cognitive impairments, diagnostic precision is even more limited.

Estimates of the prevalence of HIV-associated cognitive impairment have varied widely across the continent but are comparable to other world regions (16, 17) and have generally decreased as ART became more widely available (18–20). The prevalence of mild impairment was reported to be between 40 and 55% and moderate to severe impairment between 3 and 25% in two large multi-country cross-sectional and cohort studies using comprehensive neuropsychological test batteries in the African continent [the AIDS Clinical Trials Group 5199 (17, 21) and the African Cohort Study (22)], and in several larger studies from South Africa (23), Malawi (24), Tanzania (25) and Zimbabwe (26). Cognitive development is also impacted in pediatric HIV, where infants and young children with HIV do not perform as well as their HIV-exposed or HIV uninfected peers (27–30).

The variation in estimates of HIV-associated cognitive impairment in across Africa may be due in part to the use of tests with limited cultural validity, lack of well-matched norms and relying on screening tools with limited sensitivity and specificity when resources for neuropsychological testing are limited (31–33). In particular, the clinical relevance of mild impairment on neuropsychological tests in African populations is unclear (31) and test performance is impacted by literacy (22) and education level (23). HIV-uninfected individuals often perform poorly on tests (22), there is significant between country variation in normative data (21), and particularly among older individuals, there may be no group level differences observed between HIV-infected and -uninfected individuals (34, 35).

Pathophysiology: HIV Neuroimmune-Virus Interactions and Their Impact on the CNS

Among the viruses considered in this collection, HIV likely has the most complex and intimate interactions with the immune system and inflammatory responses, both outside (i.e., systemically) and within the CNS. In both systemic and CNS compartments these interactions change over the long course of chronic infection (36, 37). **Figure 1** diagrams these interactions, dividing the ***systemic*** (left) from ***CNS*** (right) processes. The elements in these two compartments interact, and more particularly, systemic HIV disease serves as the *foundation* for the CNS complications in several aspects. It establishes the conditions of immunosuppression and immune activation that underlie CNS vulnerability (37–40), and, more directly, supplies the key elements of neuropathogenesis, including HIV invasion and major blood-derived cells involved in CNS immune-inflammatory reactions. However, while CNS virus-immune interactions partially echo those occurring systemically, there are important differences, with the CNS interactions being highly compartmentalized despite these systemic origins (36).

In both systemic and CNS compartments the interactions of HIV and immune reactions evolve in important ways over the protracted course of chronic untreated infection. While the CNS infection echoes its systemic counterpart, it also diverges in important details, including in virus populations and particular inflammatory profiles (36, 41). If unchecked by ART this chronic course may be complicated by a range of disorders afflicting the brain, including major OIs and direct neuropathic HIV CNS infection (42, 43). Because of space constraints, this review omits detailed discussion of CNS OIs as well as disorders of the spinal cord and peripheral nervous system (PNS) that may be impacted by similar disease processes (44).

Progressive Systemic HIV Infection: Prerequisite and Facilitator of Major AIDS-Associated CNS Diseases

A number of the features of systemic HIV infection are important for the development of CNS HIV infection and disease. Ultimately, these stem from the fact that CD4+ T lymphocytes and, to a lesser extent, macrophages and related myeloid cells, are the main cellular targets of HIV infection (45–50). This targeted infection leads to progressive immunosuppression and also to

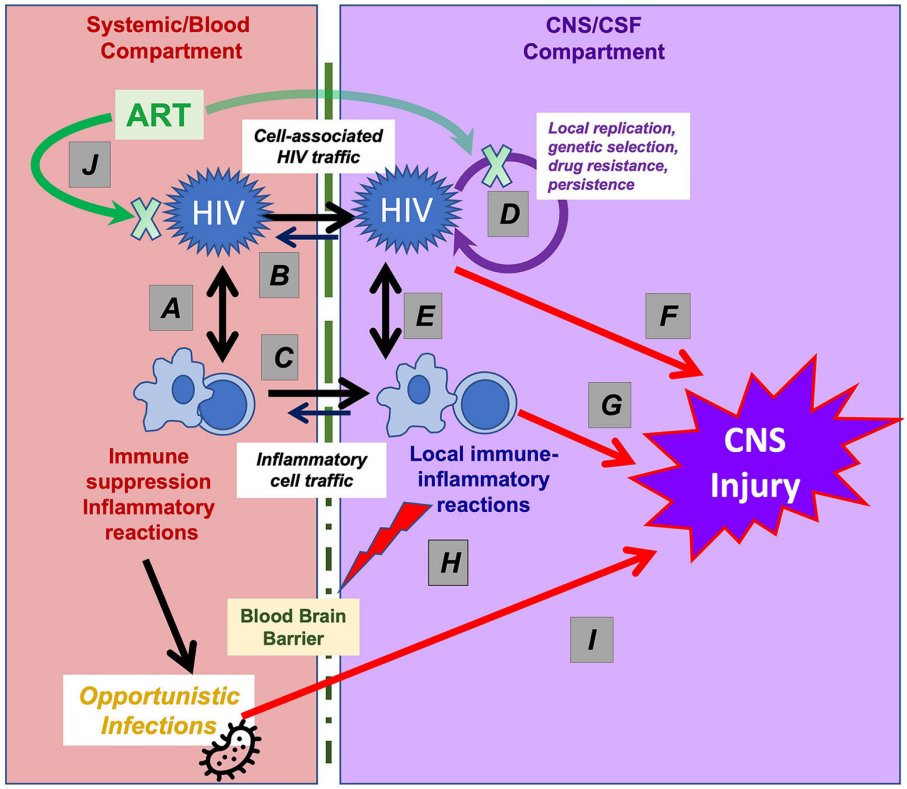

FIGURE 1 | Interactions of HIV and immune-inflammatory responses in systemic and CNS infection. This simplified schematic outlines the systemic and CNS viral-immune interactions that determine the immunopathogenesis of CNS injury. Systemic interactions (shown in the left part of the figure) establish the foundation for CNS vulnerability that are partly echoed by interactions within the CNS (right part of the figure), though with important differences. **(A)** HIV targets CD4+ T lymphocytes (and to a lesser degree myeloid cells) in which viral replication both sustains viremia and establishes long-term viral persistence, leading to gradual T-cell loss and immunosuppression and to lifelong infection. Virus-induced T-cell activation, in turn, enhances viral replication and dissemination. **(B)** Systemic viremia is the source of CNS HIV infection, beginning early in infection, likely mainly via infected T cells that migrate through the blood-brain barrier (BBB, depicted by vertical dotted line). **(C)** Cells important to the CNS inflammatory response also derive from blood sources; these include CD4+ and CD8+ T cells and macrophages that elaborate cytokines and other signaling and toxic molecules that contribute to the compartmentalized CNS inflammatory response within the CNS and are reflected in CSF. **(D)** HIV can replicate locally within these migrating CD4+ T cells and macrophages sustaining a genetically independent infection and perhaps establishing a longer-lived second viral reservoir within the CNS. **(E)** The interaction of the local HIV infection with "imported" inflammatory cells and native CNS cells (including astrocytes and microglia) establish an independent inflammatory milieu that evolves over the course of disease and is particularly heightened in HAD/HIVE. **(F)** Both HIV gene products and **(G)** host inflammatory reactions likely contribute to 'indirect' CNS injury. **(H)** inflammatory reactions can disrupt the blood-brain barrier, further exacerbating this injury. **(I)** CNS OIs may involve a similar pathway, first with loss of systemic immune surveillance allowing entry or activation of pathogens that then invade the CNS and cause neurological disease by direct injury or through a local inflammatory response. **(J)** ART reverses or mitigates all of these processes. By suppressing HIV replication, treatment fosters a variable degree of CD4+ T cells restoration and partial reversal of these pathological processes. Abrupt restoration of immunity may lead to robust local inflammation and the immune restoration inflammatory response (IRIS) with exacerbation of neurological symptoms and signs. The blood-brain barrier variably impedes CNS concentrations of certain drug components of ART, delaying or reducing local antiviral effects and, in rare cases, contributing to the development of neurosymptomatic CSF escape despite systemic viral suppression.

a state of enhanced immunoactivation, with both contributing to CNS disease consequences (51–54). HIV infection is chronic and persistent, but importantly mitigated by ART. It remains, however, a major challenge to therapeutic cure efforts (55, 56), and stopping ART almost inevitably leads to a return of viremia accompanied by CSF viral rebound (57, 58).

Complications of HIV vary with the stage of systemic disease progression, most easily assessed by the blood CD4+ T lymphocyte count (38, 59, 60). AIDS is defined by the development of major OIs (and, in parallel, including HIV-associated dementia, *HAD*) or by a CD4+ count falling below 200 per μl (61).

CNS OIs develop when there is loss of systemic immune surveillance that allows certain organisms to escape a latent or quiescent presence in the body (e.g., JC virus or *Toxoplassma gondii*) or to evade defenses that would otherwise prevent systemic dissemination (e.g., *Cryptococcus neoformans*); this is followed by subsequent failure of these same defenses to eliminate these pathogens within the CNS. The spectrum of common CNS OIs is relatively circumscribed and involves organisms of relatively low pathogenicity that are otherwise readily contained or prevented by T-cell/macrophage defenses in the normal host. In Africa where *M. tuberculosis* is common in the community, HIV infection also enhances susceptibility even

if this organism isn't readily classified as strictly "opportunistic" and is more common even at CD4 counts above those defining AIDS (15). However, the common CNS OIs generally occur at <200 CD4+ cells/μl (43). We emphasize this well-known susceptibility here because this also defines the susceptibility to HIV encephalitis (HIVE) and HAD which usually develops below this T-cell threshold (62), indicating that a similar level of immunosuppression is a prerequisite. In a sense HIVE might also be viewed as a CNS OI in which the same virus "creates the opportunity" through chronic systemic infection of CD4+ T cells before it can then "opportunistically" cause encephalitis. However, as discussed below, this does not apply to overall susceptibility to CNS HIV infection *per se*, but to "invasive" neuropathic encephalitic infection. In fact, low-grade HIV-1 meningeal infection is a common feature of systemic HIV infection that develops early in its course (37). The CNS is exposed to HIV very early in systemic infection, though it is often silent or accompanied by headache, fatigue or other unspecific symptoms. More rarely, acute encephalitis may develop during primary infection, likely involving an immunological pathogenesis (63). Over the course of chronic infection, milder neurocognitive impairment may develop and relate to low-grade forms of the viral and immunological processes that underlie HAD/HIVE, though these connections remain to be more precisely defined.

Systemic Origin of the Elements of CNS Infection

In addition to providing the background foundation and necessary level of immunosuppression for OIs and HIVE, systemic infection more directly underlies HIV CNS disease by providing both the invading virus and principal inflammatory cells that react to infection and contribute to immunopathology.

Most probably, HIV seeding of the CNS occurs *via* trafficking infected CD4+ T cells rather than by more direct virion penetration of the blood-brain barrier (64, 65). Infected cells entering the CNS can clonally expand and release (clonal) virus; this can then lead to further infection of susceptible cells, amplifying infection and establishing local replication (66). During later stage infection monocytes may also enter the CNS (65–70). This later CNS infection may be more *compartmentalized* with more notable evolution of virus populations independent of those examined in blood. Uninfected CD4+ T cells and monocytes may also enter the CNS contributing to amplified infection. This can also lead to local CNS HIV persistence after treatment, though, this has been less clearly defined, including the types of cells and anatomic locations, state of viral expression and mechanisms of replication control.

Dynamics of CNS Infection With Disease Progression: Transition From Meningitis to Encephalitis

A central feature of CNS HIV infection is its changing character with systemic disease progression. This includes shifts in the relation of CSF and blood viral populations (71–73), changes in the accompanying inflammatory profiles (36) and eventual shift in the main anatomic site of productive infection from the leptomeninges to the brain in some individuals. In the earlier phase of infection when blood CD4+ T cell levels are above 200 per μl, the leptomeninges are the most conspicuous location of chronic CNS HIV-1 infection so that a clinically silent aseptic meningitis is frequent. This infection is largely "equilibrated" with CSF HIV RNA concentrations maintained at levels near 10 percent of those in blood (37, 74, 75), and CSF and blood populations are genetically similar (76), presumably because of continuous and fresh virus traffic from blood to CSF. When CD4 cells fall below 50/μl, the ratio of CSF to blood virus deceases to near 1% blood HIV RNA levels as CSF pleocytosis also diminishes, consistent with a relation between CSF WBCs and viral load (77–80). The extent of penetration of infection into the brain parenchyma at this stage is uncertain, but if present it is largely clinically silent. Whether this early type of infection and inflammation is responsible for milder cognitive impairment is still not definitively established, though often presumed.

These relationships change in those who develop HIVE that presents clinically as subacute HAD (36). This condition usually develops after blood CD4+ cells fall below 200/μl and represents an extension of infection from meninges into the brain parenchyma. White matter abnormalities are usually prominent on MRI but gray matter also is frequently affected, particularly the basal ganglia (81–83). While inflammation in those without HIVE largely involves lymphocyte-related cytokines, as CD4+ T cell counts fall, macrophage-related inflammation increases. In those with overt HIVE there is augmentation of both lymphocytic and macrophage biomarkers (36). CNS viral populations in these individuals are more compartmentalized in relation to those in blood, and exhibit macrophage tropism (76, 84). While astrocytes can be infected by HIV, this is usually considered to be non-productive with limited gene expression; hence, their role in persistence and neuropathogenesis is still uncertain (85, 86). Importantly, neurons are not infected, and thus damage to neurons is largely or exclusively by "indirect" mechanisms, meaning that they are injured *from without* by signals and toxins released by neighboring cells rather than from direct effects of viral genes and their products expressed within these cells (87). Likely the external toxic signals are elaborated mainly from inflammatory cells, perhaps predominantly from macrophages and other myeloid cells. Late in infection HIVE also commonly disrupts the blood-brain barrier, further contributing to neuronal injury and dysfunction (36, 88, 89).

Impact of ART on CNS Infection and Disease

ART has had a profound effect on preserving CNS integrity, both in preventing HAD/HIVE development and in mitigating this CNS disease after it manifests (90, 91). This effect may be in part through preservation or restoration of immunity but mainly by more directly suppressing both systemic viremia and HIV replication within the CNS. As a result, HAD incidence is now markedly reduced and confined largely to those not receiving ART.

For individuals who present with HAD, having fallen through defects in the treatment network, ART can arrest and often reverse the severity of its impact, depending on the time frame of HAD development and treatment initiation. Diagnosis should be made quickly, and treatment begun rapidly. This is a setting

in which both the antiviral potency and CNS penetration of the components ART regimens are likely important (92, 93). In some of treated individuals the degree of short- and long-term recovery can be remarkable.

CSF Escape

This term refers to situations in which the impact of ART on CNS HIV infection is relatively reduced compared to that on systemic infection, leading to *CSF HIV RNA levels exceeding those of plasma* (94–98). Three distinct types of CSF escape have been defined: *asymptomatic*, *neurosymptomatic* and *secondary*. The most important of these is n*eurosymptomatic* CSF escape in which ART-treated individuals present with new or progressive neurological deficits (96–100). Most often, in addition to symptoms and signs of CNS injury and dysfunction, there is CSF pleocytosis, elevated CSF neurofilament light chain protein (NfL) concentration, and neuroimaging abnormalities consistent with active CNS HIV infection. Neurosymptomatic escape overlaps with pathologically-defined CD8 encephalitis (101–103). In most cases a background of reduced treatment adherence and drug resistance, at times in combination with insufficient CNS penetration of component antiviral drugs, can be identified (96). This provides further support for the need for targeted treatment of CNS, at least in some settings. Inflammation and immunopathology may be an important mechanistic component in this setting in which CD4+ T cell counts are higher than in HAD/HIVE because of the disproportionate systemic efficacy of ART that fosters CD4+ cell recovery and suppresses systemic viremia. CNS HIV isolates often exhibit drug resistance, though not always. The main avenue of treatment is changing the ART regimen to a potent antiviral drug combination that includes component drugs to which the CSF/CNS virus is susceptible and also achieve therapeutic brain concentrations.

The other two forms of CSF escape are of less clinical importance. Asymptomatic escape is an incidental finding mainly in CSF cohort studies. It is characterized by detectable CSF HIV RNA in the presence of plasma viral suppression; CSF HIV RNA levels are usually low with little or no pleocytosis. By definition these individuals lack new neurological symptoms or signs (104, 105). Secondary escape entails a disproportional increase in CSF HIV RNA in association with another CNS inflammatory process (usually another CNS infection) that provokes local HIV replication through recruitment of activated lymphocytes. Treatment of the provoking infection leads to reduction of the CSF HIV RNA elevation (79).

CNS Persistence and Cure

Despite the effectiveness of ART in suppressing systemic and CSF HIV infection, it does not cure HIV. When ART is stopped, viremia and CNS replication re-emerge (106). Because of this intractable persistence of HIV, efforts are now underway to effect a systemic cure using a variety of strategies (55). There is precedent with bone marrow transplant using an HIV-resistant donor. In one well-studied case, not only was there no evidence of viral persistence systemically but also no trace of virus in CSF (107). More broadly it remains an open issue as to whether the CNS serves as an independent viral reservoir that might require CNS-targeted cure strategies.

CONCLUSION

CNS HIV infection is a component of the "ecology" of HIV, an offshoot of systemic viremia that can lead to important morbidity and mortality. Fortunately, ART has a major impact on CNS infection and its effects, from Pre-Exposure Prophylaxis (PrEP) preventing initial infection, to early treatment of infection that likely reduces the CNS reservoir (91), to treatment of established HAD/HIVE. Thus, while additional interventions, including vaccines and cure efforts are welcome, widespread use of preventative and therapeutic ART continue to have a major impact on neurological disease in HIV and AIDS.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and contribution to the work and approved it for publication.

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Bacterial meningitis in Africa

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Bacterial meningitis differs globally, and the incidence and case fatality rates vary by region, country, pathogen, and age group; being a life-threatening disease with a high case fatality rate and long-term complications in low-income countries. Africa has the most significant prevalence of bacterial meningitis illness, and the outbreaks typically vary with the season and the geographic location, with a high incidence in the meningitis belt of the sub-Saharan area from Senegal to Ethiopia. *Streptococcus pneumoniae* (pneumococcus) and *Neisseria meningitidis* (meningococcus) are the main etiological agents of bacterial meningitis in adults and children above the age of one. *Streptococcus agalactiae* (group B Streptococcus), *Escherichia coli*, and *Staphylococcus aureus* are neonatal meningitis's most common causal agents. Despite efforts to vaccinate against the most common causes of bacterial neuro-infections, bacterial meningitis remains a significant cause of mortality and morbidity in Africa, with children below 5 years bearing the heaviest disease burden. The factors attributed to this continued high disease burden include poor infrastructure, continued war, instability, and difficulty in diagnosis of bacterial neuro-infections leading to delay in treatment and hence high morbidity. Despite having the highest disease burden, there is a paucity of African data on bacterial meningitis. In this article, we discuss the common etiologies of bacterial neuroinfectious diseases, diagnosis and the interplay between microorganisms and the immune system, and the value of neuroimmune changes in diagnostics and therapeutics.

KEYWORDS

bacterial, meningitis, Africa, pathophysiology, diagnosis, management

Introduction

Bacterial meningitis is characterized by an inflammatory process in the meninges of the brain and spinal cord due to a bacterial infection. It causes significant mortality and morbidity worldwide, with the major burden of disease in Sub-Saharan Africa (1). A global burden of disease study showed that meningitis caused 318,000 deaths worldwide (4.5 per 100.000),

resulting in 20,383 thousand years of life lost in 2016 (1). The incidence rates vary between 0.7–0.9 per 100.00 per year in the United States (US) and European countries, while in Africa, studies describe incidence rates between 0–40 per 100,000 per year (1, 2).

The epidemiology of bacterial meningitis varies widely by age (e.g., the higher incidence in neonates and elderly patients) (2). *Streptococcus agalactiae* (or Group B Streptococci) and *Escherichia coli* are the principal etiologies of neonatal meningitis (2). Recent epidemiological studies from Africa and the Netherlands show that between 2006 and 2014 of 1,412 episodes of community-acquired bacterial meningitis demonstrated that *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Listeria monocytogenes* accounted for 51, 37, and 4% of cases, respectively (3). *S. pneumoniae* and *N. meningitidis* cause up to 90% of cases in infants and children.

There are significant geographical differences in the epidemiology of bacterial meningitis worldwide. Sub-Saharan Africa, a region referred to as the "meningitis belt," has a large proportion of meningitis cases. Epidemic meningococcal group A disease outbreaks have recorded incidence rates up to 100 per 100,000 (4). The introduction of MenAfriVac (Serum Institute of India Ltd, Hadapsar, Pune, India), a conjugate vaccine against serogroup A *N. meningitidis*, in sub-Saharan Africa has virtually eliminated Group A meningococcal meningitis outbreaks. However, new epidemics in Burkina Faso, Chad, Mali, Niger, and Togo with other serogroups (W and C) are now occurring (4). A systematic review of bacterial meningitis in Africa found that the most common pathogens were *N. meningitidis* ($n = 2,433$; 56%), *S. pneumoniae* ($n = 1,758$; 40%), and *Haemophilus influenzae* ($n = 180$; 4%).

Clinical outcomes vary geographically, with mortality rates ranging from 6% in Germany to 54% in Malawi (5, 6). Similarly, neonatal meningitis mortality also differs between developing countries (40–58%) and developed countries (10%) (7). Low-income countries have a significant incidence of bacterial meningitis and higher rates of survivors with long-term disabling sequelae. In a meta-analysis of 18,183 survivors of acute bacterial meningitis, the risk for a major neurological sequela as, motor deficit, bilateral hearing loss, cognitive impairment, visual impairment, hydrocephalus and, seizures, was highest in Africa (25.1%) and Southeast Asia (21.6%) than in Europe (9.4%) (8). This discrepancy between outcomes is most because survival and neurological sequelae depend on a rapid diagnosis and early treatment, both of which are difficult to have in resource-limited settings where laboratory support and antibiotic therapy are scarce.

Bacterial meningitis is still a prevalent, often undiagnosed, fatal infection in many African neonates, with a high death and morbidity rate. In many African healthcare settings, lumbar punctures are performed infrequently, and bacterial meningitis goes undiagnosed (9). Primary and secondary prophylaxis are equally necessary for reducing newborn infections. Improved prenatal, intrapartum, and postpartum care, exclusive breastfeeding, and the avoidance of low birth-weight infants are all likely helpful. Socioeconomic and maternal education significantly impact mother and newborn health and must be addressed to prevent neonatal meningitis (7). Also, several African nations with the most significant risk of neonatal death have been affected by conflict, war, or other natural calamities (10).

This chapter describes in detail the different pathogens commonly causing bacterial meningitis in Africa, their prevalence, pathophysiology, factors associated with pathogen entry into the brain, the interplay between the pathogen and the immune system of the central nervous system (CNS), and the consequences of this interplay. We also outline strides and recommendations in the diagnosis, management and prevention of each pathogen-caused meningitis.

Neuroinflammation and role of microglia in bacterial meningitis

The pathophysiology of bacterial meningitis typically involves bacteria propagating into the brain through the bloodstream and then crossing the blood-brain barrier (BBB) however in a minor portion of cases, the bacteria enter directly through the cerebral tissue following skull fractures. Bacterial replication then occurs concurrently with the release of specific virulence factors, which trigger a cascade of signaling pathways that activate several transcription factors and initiate neuro-inflammatory processes that allow peripheral immune cells to enter the brain, causing BBB disruption. Thus, neuroinflammation, a process that should be a defensive mechanism, instead becomes dangerous for the host.

Bacterial infections of the brain are life-threatening because the brain is not easy to be reached by antibiotics because the BBB acts as a barrier between the brain and the systemic circulation (11). Nevertheless, death from bacterial meningitis does not occur because of the infection *per se*; the severe neuroinflammatory process that the host triggers in response to the infection results in the host's killer (12–15). Microglia are the resident immune sentinels of the brain with the primary function of eliminating invading pathogens by phagocytosis (12). Another function of microglial cells is to initiate a signaling process by releasing pro-inflammatory cytokines to recruit other immune cells, such as neutrophils, that reach the brain to help microglial cells in the process of pathogen elimination (12). Overall, neuroinflammation has a so-called "double-sword effect" even though the main scope of microglial pro-inflammatory response is for the host protection, the trafficking across the BBB of blood-borne immune cells usually causes severe disruption of the BBB with consequent intracerebral hemorrhage (12–19). Furthermore, activated microglia may secrete IL-1α, TNF-α, and C1q, generating reactive astrocytes known as A1; A1 astrocytes lose their capacity to support neuronal survival, outgrowth, synaptogenesis, and phagocytosis, and produces a neurotoxin that affects oligodendrocytes and can cause the neuronal death (20). In another study, newborn rats stimulated with LPS showed an increase in microglial cell activation in the hippocampus, cerebral cortex, and thalamus during their adulthood (21).

Microglia are very sensitive to external stimuli and can sense bacteria soon after they have invaded the brain (22, 23). Upon bacterial entry into the brain, microglia undergo a dramatic change in their morphology, and they are then classified as "activated" (23). Microglial activation occurs upon recognition by the microglial Toll-Like Receptors (TLRs) of specific pneumococcal components, such as peptidoglycan (PepG) and lipoproteins (LPPs) (24). PepG can cross the BBB, and it was recently reported that PepG originating from gut microbiota could modulate brain development (25).

When bacteria cross the BBB and invade the brain, microglia initiate their defensive action by eliminating the pathogens *via* phagocytosis (26). Endothelial cells line the internal walls

of the blood vessels, including the BBB, and can release pro-inflammatory cytokines in response to bacterial components that can infiltrate the brain (27, 28). During bacterial growth, peptidoglycan (PepG) is cleaved and detached from bacterial cells and several proteins, such as lipoproteins (LPPs). A question that the scientific community still has not addressed is whether microglia can be activated when bacteria are not yet in the brain.

In the case of *E. coli,* its replication occurs concurrently with the discharge of bacterial products, including lipopolysaccharide (LPS), DNA, and other cell wall fragments inside the subarachnoid space (29), identified as pathogen-associated molecular patterns (PAMPs) (30, 31). These PAMPs are detected by pattern-recognition receptors (PRRs) and non-PRRs, both of which are essential immune system components (31, 32). The PRRs are classified into several families, including toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and intracellular DNA-sensing molecules (30, 33), and the receptor for advanced glycation end products (RAGE), triggering receptors expressed on myeloid cells (TREM), and G-protein-coupled receptors (GPCRs) are examples of non-PRRs receptors (34). When immune receptors detect PAMPs, a cascade of signaling pathways is activated, promoting pro-inflammatory mediators. Cytokines, chemokines, and antimicrobial peptides are the mediators required to remove invading pathogens (35). During a meningitis infection, endogenous molecules released by stressed or damaged cells, known as damage-associated molecular patterns (DAMPs), activate the innate immune system by binding to PRRs and non-PRRs (30). The detection of PAMPs and DAMPs by immune receptors can result in adverse consequences, increasing the BBB permeability, allowing the peripheral immune cells to reach the cerebrospinal fluid (CSF), activating the glial cells triggering the neuroinflammation and long-term cognitive impairment in *E. coli* meningitis survivors (36, 37).

Pneumococcal meningitis

Bacterial invasion of the brain through receptor-mediated transcytosis

The Gram (+) bacterium *S. pneumoniae* (pneumococcus) is the major etiological cause of bacterial meningitis worldwide (38, 39). The main route for pneumococci to reach the brain is the bloodstream; bacteria travel in the blood and easily reach the blood-brain barrier (BBB), a specialized vasculature system that separates the brain from the rest of the systemic circulation (38, 39). The brain is defined as "immune privileged" because of the presence of the BBB. The primary function of the BBB is to protect the brain from harmful substances that can enter the brain and cause cerebral damage (40). Pneumococci exploit the so-called receptor-mediated transcytosis to interact with the BBB and enter the brain tissue (38), a mechanism in which surface-exposed proteins can bind to specific receptors that are exposed on the plasma membrane of the endothelial cells of the BBB; this binding is the first and fundamental step of the process of bacterial passage across the BBB (38). Below are the mechanisms or virulence factors that the bacterium uses to cross the BBB.

The pilus-1 and RrgA

The pilus-1 is a "hair-like" structure on the surface of the bacteria and is presented in approximately 20-30% of pneumococcal strains (22, 41, 42). The pilus-1, particularly the tip protein RrgA present on top of the filament structure, was previously reported to significantly enhance the capacity of *S. pneumoniae* to bind to the BBB endothelium (41). Pneumococci use RrgA to bind to the platelet endothelial adhesion molecule 1 (PECAM-1), and the polymeric immunoglobulin receptor (pIgR) expressed on the brain's surface endothelial cells lining the internal wall of the BBB vasculature. Through this binding, pneumococci can enter the brain (43). Before being a pathogenic bacterium, *S. pneumoniae* is a commensal colonizer of the human nasopharynx, and most of the time, this colonization is completely asymptomatic (22).

Choline-binding protein A, also known as Pneumococcal surface protein C

CbpA, also known as PspC, is a surface-exposed protein anchored to the choline of the pneumococcal cell wall (44). CbpA was previously described to bind to the laminin receptor (LR), an essential molecule in cell adhesion to the basement membrane of brain endothelial cells (45). More recently, CbpA was also described to mediate the adhesion of pneumococci to the pIgR expressed by the BBB endothelium. Interestingly, the exact interaction between pneumococcal CbpA and pIgR also mediates the adhesion to the respiratory epithelium and colonization of the pneumococcus in the nasopharynx (43).

Neuraminidase A

NanA, a sialidase that cleaves sialic acids on host cells, helps pneumococci to penetrate the BBB and invade the brain (46). NanA was described to promote pneumococcal adhesion to and invasion of brain endothelial cells; furthermore, this interaction between pneumococcal NanA and the brain endothelium is enhanced by the sialidase activity of NanA (46).

Pneumococcal phosphoryl-choline

The first study that investigated receptor-mediated adhesion by Cundell and collaborators showed that bacterial phosphoryl-choline (ChoP) played a vital role in interacting with *S. pneumoniae* and human endothelial cells (47). PAFr was proposed to facilitate the interaction of pneumococci with the BBB endothelium, and some studies hypothesize a direct binding between pneumococcal ChoP with PAFr (48). On the other hand, others seem to point toward a more indirect role of PAFr in pneumococcal meningitis pathogenesis in which, during the inflammatory events that the host triggers the bacterial infection, PAFr is activated by the release of pneumococcal components that lead to the infiltration of immune cells, like neutrophils, into the brain (48). Such immune cell infiltration leads to openings within the BBB endothelium that facilitate the passage of pneumococci into the brain (48).

Pneumococcal interaction with neurons

Neurons are the main cellular component of the CNS and are responsible for transmitting electrical and chemical signals critical for

all brain functions. Even though mortality due to bacterial meningitis is not dramatically high ranging from 10–30% globally (49–51), approximately 50% of survivors suffer from permanent neurological impairments, such as cognitive and motor disabilities and hearing loss, due to neuronal injury caused by the infection (52–54). The highest rates of bacterial meningitis worldwide belong to African children, and in almost one third of cases, *S. pneumoniae* is the etiological cause (55). Neuropsychological sequelae are frequently observed in African children that survive bacterial meningitis (55). Pneumolysin (Ply) is the pore-forming cytotoxin released by *S. pneumoniae* and can damage the host cells (56, 57). Generoso et al., have recently shown that the accumulation of pneumococci and toxic pneumococcal products, such as Ply, in the CSF compartments of the brain leads to neuronal damage with consequent dramatic impairment of neurological functions (58). Like it was previously described for other bacterial pathogens, pneumococci can exploit the interaction with the host cell cytoskeleton to invade neurons; neuronal cell death occurs due to cytoskeleton disruption (59, 60).

Diagnosis, clinical presentation, and treatment

Management of pneumococcal meningitis today

Cure and prevention of infectious diseases are usually resolved with antibiotics and vaccines. Like other streptococcal infections, pneumococcal meningitis is routinely treated with β-lactam antibiotics (61, 62). Two main problems related to antibiotic treatment in managing bacterial meningitis are (1) β-lactam antibiotics have poor penetration of the BBB (63), (2) due to the indiscriminate use of antibiotics in the last decades, the problem of antibiotic-resistance is a constant threat to face in clinics (64); bacteria are highly-versatile microorganisms and can change in response to antibiotics, and new antibiotics can be discovered, but bacteria can rapidly adapt and develop resistance (65). Preventing is better than curing, and to build immunity toward pneumococcal infections, the introduction of pneumococcal conjugated vaccines (PCV) in the early 2000s has decreased the incidence of invasive pneumococcal disease, clinically defined as any type of infection caused by *S. pneumoniae* (66, 67). A decrease in admission rates was observed in Ethiopia among children affected with pneumococcal meningitis, yet several thousands of cases have been registered, which means that vaccination has still not yet provided significant protection to prevent the disease (68).

PCV is based on polysaccharides that compose the capsule surrounding the pneumococcal cell, and they are poorly immunogenic. There are more than 100 serotypes of *S. pneumoniae*, and all these serotypes are defined based on the polysaccharide composition. The current PCV (PCV13) is protecting only against 13 serotypes, therefore, we can build up a strong immunity only toward infections caused by the serotypes included in the PCV. The negative downstream effect of the introduction of PCV has been that the incidence of invasive pneumococcal disease caused by non-vaccine-types has increased (69, 70). The sub-Saharan region has by far the highest burden of acute bacterial meningitis in the world (71). In Malawi, 7 years after the introduction of PCV13 in 2014, a recent study has shown that non-vaccine serotype invasive pneumococcal disease, including meningitis, has increased (72). Furthermore, PCV has not significantly boosted the local immunity of the brain against pneumococcal infections; despite vaccination programs, hundreds of thousands of meningitis cases worldwide still occur yearly (49, 73).

Current treatments and clinical diagnosis

Pneumococcal meningitis is routinely treated in clinics with ceftriaxone, a "broad spectrum" cephalosporin (50). High concentrations of ceftriaxone in systemic circulation lead to increased penetration of the antibiotic through an inflamed BBB (63). To suppress excessive neuroinflammation, which leads to BBB endothelium breakdown and consequent brain edema and life-threatening hemorrhages, antibiotic treatment can be combined with the use of corticosteroids (74). Typical symptoms of suspected pneumococcal meningitis are high fever, stiff neck, nausea and vomiting, mental changes, intense headache, and sensitivity to light (75). Besides the classic tests performed after sampling which include bacterial culturing and microscopy detection of bacteria (50), more rapid diagnosis can be performed through immunochromatographic test (ICT), which can detect around 30% more pneumococcal meningitis cases than what usually caught with CSF culturing alone (76).

New therapeutic and prophylactic approaches to cure and prevent pneumococcal meningitis

Blockade of host-pathogen interaction as an adjunct therapy to current antibiotics

It is important to have the availability of alternative therapies that can be used as adjunct treatments instead of antibiotics. Blood-borne pneumococci bind to PECAM-1 and pIgR expressed by brain vascular endothelial cells, and through this binding *S. pneumoniae* invades the brain (43). Iovino et al. have successfully shown that the administration of antibodies targeting PECAM-1 and pIgR *in vivo* significantly impairs pneumococcal invasion of the brain in mice, suggesting that the blockade of receptor-mediated adhesion and invasion can be a novel strategy to protect the brain from invading *S. pneumoniae* (43, 77). Bacterial adhesion to the BBB is only one step of a multi-event process during meningitis pathogenesis. Even though the blockade of bacterial interaction with the BBB can be achieved using meningitis animal models, the reality is much different because when patients are hospitalized with a diagnosis of bacterial meningitis, bacteria are unfortunately already in the CNS. In the brain, bacteria encounter neurons, and bacterial interaction with neurons causes severe and irreparable neuronal injury. Neuronal damage culminates into neuronal cell death, a pathological hallmark of all the impairments, so-called brain sequelae, which represent a dramatic issue in the burden of bacterial meningitis (52–54). Even if the bacterial infection is adequately cured, other eukaryotic cell neurons that have been damaged or killed by the bacteria cannot be replaced (78). For this reason, the World Health Organization (WHO) defines bacterial meningitis as a devastating disease. Recently, Tabusi et al. have shown a possible mechanism of neuronal cell death after pneumococcal

infection. Using human neurons *in vitro* and a bacteremia-derived meningitis mouse model *in vivo*, they found that pneumococci use the cytoskeleton protein β-actin through the pilus-1 adhesin RrgA and the cytotoxin pneumolysin (Ply) to adhere to neuronal β-actin filaments and invade neurons. Interestingly, blocking this pneumococcal-β-actin interaction using antibodies reduced neuronal cell death (59). Can this be the beginning of a new neuronal protective therapeutic strategy?

Boosting the brain's immune response to protect the brain from bacterial infections

A well-orchestrated host-inflammatory response is crucial in eradicating infections from the brain; however, excessive or prolonged neuroinflammation can cause severe damage to the host (79). Microglia are the first responders to fight microbes and the main potentiators of neuroinflammation in the brain (12). Microglia reactive states are sometimes divided into pro-inflammatory M1-like "classically activated" and phagocytic M2-like "alternatively activated"; however, this classification is an oversimplification since microglial cells can present a large variety of functional phenotypes (80). The M1-like skewed microglial response is the typical hallmark of neuroinflammation in the pathophysiology of bacterial meningitis (11, 72). Activated microglia release various cytokines and chemokines and acquire migratory, proliferative, and phagocytic properties (12). Even before the infiltration of other immune cells from systemic circulation, the pro-inflammatory cytokines released by microglial cells pass through the BBB, increasing its permeability, and blood-borne leukocytes then have an easier access into the brain (17, 39, 81, 82). Leukocytes, like other immune cells that enter the brain, are huge cells in terms of size, and this continuous cellular trafficking soon leads to the rupture of the BBB vascular endothelium, which is life-threatening (9). Modulating microglial responses boosting the phagocytic capacity, and suppressing neuroinflammation could bring important advantages in managing bacterial meningitis (12).

Meningococcal meningitis

N. meningitidis is an aerobic Gram (-) diplococcus species whose only host is human. It is found in the respiratory tract of healthy human beings but can cause devastating disease in those vulnerable. *N. meningitidis* is recognized as one of the three leading causes of meningitis in the world despite the presence of vaccines against almost five of its serotypes. Over 12 serotypes have been identified. However, only 5 of these have been identified to cause disease (10). *N. meningitidis* is grouped based on the surface polysaccharide capsule, and 13 meningococcal serotypes have been identified (A, B, C, D, 29E, H, I, K, L, Y, W-135, X, and Z). The majority of disease has been caused by A, B, C, Y, and W-135. Meningococcal disease in Europe and the Americas is mainly caused by serogroups B and C, whereas in Africa, the main causes are serogroups A and C. The capsule in serotype A is characterized by a non-sialic capsule with homopolymers of N-acetyl-D-mannosamine-1-P and (al-6) linked -N-acetyl-D-mannosamine-l-phosphate and has gene operon mynA-mynD. Sero-type C has a sialic acid capsule, homopolymers of sialic acid (a2-9)-linked- N-acetyl-neuraminic acid, and gene operon siaA and siaD (10).

Pathogen characteristics and virulence factors

Virulence of *N. meningitidis* is hinged on several factors, including but not limited to capsule polysaccharide expression, expression of surface adhesive proteins (outer membrane proteins including pili, porins PorA and B, adhesion molecules Opa and Opc), iron sequestration mechanisms, and endotoxin (lipooligosaccharide, LOS) (10). In addition to these specific virulence factors, *N. meningitidis* has evolved genetic mechanisms resulting in high-frequency phase, antigenic variation, and molecular mimicry. Capsule switching, due to the allelic exchange of capsule biosynthesis genes by transformation, is one example that can allow the meningococcus to evade immune detection (83).

While *N. meningitidis* can be both capsulated or not, most strains isolated in blood or have almost always been capsulated. The capsule protects the bacteria against antibody/complement killing and inhibits phagocytosis (84).

Pathogenesis and epidemiology

While *N. meningitidis* is an organism found in the nasal canal of healthy human beings, the rate of carriage and disease are variable and range from sporadic outbreaks, as seen in Europe, to epidemics in the African meningitis belt. To be able to survive, colonize and spread in a human being *via* the blood stream or CSF, the bacteria must harness specific properties, with its capsule being the main virulence factor, and its expression undergoes genetic regulation during pathogenesis. The capsule prevents cell adhesion and biofilm formation; thus, the expression needs to be downregulated or lost during carriage. However, the capsule is essential for survival in the blood and is thus upregulated during invasion into the bloodstream (85).

Adhesion to the mucosal membrane in the nasal canal is a key aspect of *N. meningitidis* pathogenesis. This is facilitated by the Type IV pili, which also play a key role in adhesion to endothelial cells, bacterial aggregation, twitching, motility, bacterial migration, and natural transformation (85, 86). The adhesion proteins then occur mediated by the opacity proteins, Opa, and Opc, with a typical integral membrane protein structure, which binds to carcinoembryonic anti-gen cell adhesion molecule (CEACAMs) receptor and extracellular matrix components (87). *N. meningitidis* has several adhesins enabling it to attach to several different receptors on the same target cell. It also means that it may respond differently or present differently during different stages of infection, thus mediating Neisserial adhesion to different cell types at different sites (86).

Following adhesion, the bacteria have to evade the complement system, which is the body's first line of defense. *N. meningitidis* achieves this through several mechanisms. The most studied is Factor H binding protein, which interacts with the Human Factor H, which is an inhibitor of the alternative complement pathway and therefore enhances the resistance of *N. meningitidis* while in serum to the complement system. There have been, however, other surface-exposed antigen components that have been found to inhibit the alternative complement pathway, thus suggesting that *N. meningitidis* has several mechanisms for evading the immune system, including NspA (*Neisseria* surface protein A), alkylated lipo oligosaccharide

(LOS), and Neisserial heparin binding antigen (NHBA), which all play a role in the evasion of the complement pathway (88).

Capsular polysaccharides modulate several pathways of the complement cascade, further improving survival of *N. meningitidis* e.g., serogroup Y and W135 enhance activation of the AP by enhancing C3 activation and deposition, serotype B, C, W, and Y capsular polysaccharide have been found to inhibit complement pathway by inducing less C4b deposition, thereby limiting the antibodies' ability to mediate bacterial extermination (89).

Given these and more factors, the bacteria can enter the bloodstream and CSF while evading the complement system. Once it arrives in the blood, it then multiplies rapidly to infectious levels causing sepsis or translocating, crossing the BBB, and causing meningitis. The ability to cause invasive disease is dependent on environmental factors, meningococcal virulence factors, and lack of protective immune response. Certain factors like tobacco smoking, exposure to low humidity, and other co-infections increase the incidence of invasive disease (10).

The global incidence of *N. meningitidis* disease varies greatly by geographical distribution. Globally, the incidence is 500,000 to 1,200,000 worldwide, with over 50,000–135,000 deaths annually (90). The incidence in Europe ranges between 0.3–3.0 cases per 100,000, while in Africa, the incidence is 10–1,000 cases per 100,000 during pandemics in the meningitis belt. The categories most at risk for developing the invasive disease include newborns, children under the age of five, adolescents, immunocompromised, and the elderly (90).

Diagnosis, clinical presentation, and treatment

Clinical presentation of *N. meningitidis* meningitis varies and may even appear benign but is characterized by sudden onset of high-grade fevers, headache, nausea, vomiting, unspecific rash, sore throat, and other upper respiratory tract infections. These symptoms can easily be confused with several other diseases, especially in areas of low incidence, and thus require that the clinician have a high index of suspicion as this type of meningitis will quickly progress to death. In the later course of the disease, it presents with neck stiffness, headache, photophobia, hemorrhagic or petechiae rash, altered mental state, and shock (91).

Early signs of sepsis, including tachycardia and hypotension, can be noted. A careful clinical examination should be performed with careful examination for a rash. The rash may initially appear as small papular, urticarial, or macular and later progress to petechia, purpura or ecchymoses, which are all early signs of thrombocytopenia, purpura fulminans, and DIC. The rashes can occur all over the body, but they are usually found on the palms and feet.

A positive Kerning's or Brudzinski sign (nuchal rigidity), fever, and altered mental status are the classical triad for a diagnosis of meningitis; however, these are rarely all present, and any two of fever, altered mental status, nuchal rigidity, and headache can be used to confirm the diagnosis of meningitis. Clinicians should consider *N. meningitidis* as the etiology if the patient presents with two of these plus a rash.

Purpura fulminans occurs when meningitis progresses further, due to vascular collapse initiated by LOS activating the release of inflammatory mediators characterized by cutaneous hemorrhage and skin necrosis due to vascular thrombosis. It can even lead to adrenal gland hemorrhaging and failure, termed Waterhouse-Freiderichsen syndrome and DIC; typically, the petechiae and erythema are seen on the skin but evolve into ecchymosis and later painful areas of necrosis with bullae and vesicles developing. Gangrenous necrosis may follow and lead to limb amputation and progression to DIC. Any evidence of bleeding from intravascular access, gingival bleeding, ecchymosis, or skin discoloration should be very concerning (92).

Diagnosis

The gold standard for diagnosis of *N. meningitidis* is by performing a lumbar puncture and collecting CSF for analysis. Based on patient history and clinical presentation, there needs to be a determination of whether there are higher chances of herniation prior to lumbar puncture. In a patient presenting with papilledema, seizures, and focal neurological symptoms, an LP may be delayed, and imaging, if available, done prior to the LP. CSF analysis should include Gram stain, protein, glucose, cell count, and protein count. Positive findings include increased opening pressure, pleocytosis of polymorphonuclear leukocytes, predominantly neutrophils, decreased glucose concentration, and increased protein levels. Gram stain may indicate Gram (-) diplococci; however, the gold standard for confirmation is CSF culture (93). Other tests could include PCR and latex agglutination to confirm *N. meningitidis*.

Treatment

Early recognition and initiation of treatment are vital in improving outcomes. Treating involves antibiotics, supportive care, coagulopathy management, contact tracing, and infection control.

Antibiotic treatment: Ceftriaxone and third-generation cephalosporins are generally preferable due to their high efficacy and easier dosing (94). Penicillin may also be used, and the dosing is 300,000 units/Kg/day IV or intramuscularly (IM), with a maximum dose of 24 million units per day. Penicillin is usually given as 4 million units every 4 h IV in adults and pediatric patients older than 1 month. High-dose penicillin is recommended for cultures with a sensitivity of penicillin minimum inhibitory concentration of 0.1 to 1.0 mcg/mL, although most clinicians will continue using third-generation cephalosporin instead (94). Dexamethasone dosing is 0.15 mg/Kg with a maximum dose of 10 mg every 6 h. This has no therapeutic benefit in meningococcal meningitis and, therefore, should be discontinued once this diagnosis is established. It is ideally administered 4 h prior to or concomitantly with antibiotics. It is not recommended if tuberculosis meningitis is suspected. Not recommended if meningococcemia with shock is suspected (94).

Vaccination

A lot of strides have been made in the prevention of meningococcal disease. Previously the main stay of the prevention of disease has been meningococcal polysaccharide vaccines; while these are inexpensive and more readily available, they are ineffective in infants and do not confer long-lasting immunity or provide herd immunity. Polysaccharide vaccines covering capsular groups A and C and A, C, W, and Y continue to play an essential role in emergency epidemic response in Africa. Also, recent reports indicate shortages of

polysaccharide vaccines during NmC epidemic in Nigeria and Niger, presumably because a number of vaccine manufacturers are phasing out the production of polysaccharide vaccines (95).

Currently, several conjugate vaccines are available on the market including Menveo, Menactra, Meningitec, Menjugate, NeisVac-C, MenAfriVac, and MenHibrix, each targeting various strains. These have been used to address the different strains with MenAfric vaccine leading to almost near elimination of strain A where it has been implemented.

Escherichia coli – Gram (-) meningitis

Pathogen characteristics and virulence factors

E. coli is a Gram (-) bacillus, facultatively anaerobic, and a commensal bacterium of vertebrates' gut, becoming an opportunistic pathogen in many intestinal and extra-intestinal infections. Pathogenic strains of *E. coli* are often categorized into pathovars, a group of bacterial strains that cause common illnesses and are designated using acronyms. The most common phenotypes identified are uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), neonatal meningitis *E. coli* (NMEC) is the cause of newborns infection and newborn meningitis, extra-intestinal pathogenic *E. coli* (ExPEC), intestinal pathogenic *E. coli* (InPEC), Shiga toxin-producing *E. coli* (STEC), typical and atypical enteropathogenic *E. coli* (tEPEC and aEPEC); adherent-invasive *E. coli* (AIEC). There are also hybrid pathotypes in the InPEC pathovars, including enterohemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC) (96–100). *E. coli* pathovars are clonal groupings distinguished by serogroup, a distinct variety based on lipopolysaccharide (LPS, O antigen), and serotype based on a combination of O antigen (lipopolysaccharide), flagellar (H antigen), and capsular characteristics (K antigen) (101). The MNEC pathovar is the most common cause of Gram (-) neonatal meningitis, and the serotype K1 capsular antigen is approximately present in 80% of the *E. coli* isolate from neonatal meningitis (101, 102).

Pathogenesis and epidemiology

Cases of *E. coli* meningitis may be classified into different scenarios: neonatal meningitis, trauma and neurosurgery, and community-acquired/spontaneous meningitis. *E. coli* neonatal meningitis is a significant source of mortality and morbidity, with case fatality rates ranging from 5 to 25% and neurologic sequelae affecting 25 to 50% of survivors (103, 104). Symptoms and signs of *E. coli* bacterial meningitis in adults are headache, neck stiffness, and altered mental status. However, the typical meningitis trio of fever, neck stiffness, and altered mental state were found in 25% of subjects in an observational cohort study of individuals over 16. The mortality rate of *E. coli* meningitis in patients older than 16 ranged from 36 to 53% (105, 106), and 64% presented unfavorable outcomes (107).

E. coli colonizes the gastrointestinal mucosa and translocates from the lumen of the small intestine or colon into the systemic circulation before entering the CNS across the BBB (107). Oral administration of *E. coli* K1 resulted in steady and sustained gastrointestinal colonization in newborn rats in an experimental meningitis model (108). Using the same experimental model, *E. coli* K1 colonized the gut and crossed the gastrointestinal barrier. The newborn rodent acquired a fatal systemic infection with the bacterium in the blood circulation and brain tissue (109). *E. coli* K1 crosses the BBB by interacting with CD48 on brain microvascular endothelial cells *via* a type 1 fimbrial adhesion (FimH), outer-membrane protein A (OmpA) *via* N-acetylglucosamine (GlcNAc) or glucose-regulated protein-96 (Gp96), and cytotoxic-necrotizing factor 1 (CNF1) *via* the laminin receptor (LR) (110). Cytotoxic necrotizing factor (CNF1) is a bacterial virulence factor predominantly associated with meningitis-causing *E. coli* strains (111). This toxin helps *E. coli* K1 invade brain endothelial cells *in vitro*, and the bacteria crossed the BBB in a newborn experimental meningitis model. An isogenic mutant missing CNF1 was less invasive in brain endothelial cells and less able to enter the brain in the meningitis animal model (112). In human brain microvascular endothelial cell cultures, a double-knockout mutant with deleted OmpA and CNF1 genes was less invasive (113); for additional details, refer to Figure 1.

Consequences of the interplay between the pathogen and the immune system

E. coli LPS binds to TLR-4, and the adaptor molecule myeloid differentiation factor 88 (MyD88) interacts with the interleukin-1 receptor-associated kinase-4 (IRAK)-4. The IRAK then interacts with the receptor-associated factor (TRAF) family and connects to the TAK1 [Transforming growth factor-β (TGF-β)-activated kinase 1]/TAB1 (TAK1-binding proteins)/TAB2/TAB3 complex. TAK1 phosphorylates the NEMO (NF-κB essential modulator)/IKK (inhibitor of nuclear factor-κB (IκB) kinase)/IKK complex, which phosphorylates IKB, allowing the transcription factor NF-κB to be released and translocated to the nucleus (30, 114), which in turn activates several genes involved in the production of pro-inflammatory cytokines such as interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha (TNF-α), and other inflammatory mediators (30, 105, 114). The flagella of *E. coli* and its protein flagellin binds to TLR-5, triggering the NF-κB and increasing the expression of the IL-8 chemokine. A preclinical study demonstrated that TLR-4 stimulation enhanced the phagocytosis of *E. coli* by microglial cells (115). Also, TLR-4 gene mutation was associated with an *E. coli* brain abscess in a twin pair of a newborn, according to a case report (116). Also, the MyD88-deficient animals could not prevent *E. coli* K1 neonatal meningitis, showing that MyD88 plays an essential role in early host defense (117). In mice, vulnerability to neurological morbidity changes intensely during the first few weeks of life. The neonatal brain is susceptible to infection leading to long-term neurological sequelae (118).

Diagnosis, clinical presentation, and treatment

Value of the neuroimmune changes in meningitis diagnostics and therapeutics

Significant rates of neurological morbidity and death continues to be associated with acute community-acquired bacterial meningitis. Differentiating between bacterial and viral meningitis remains a clinical challenge, particularly in individuals previously treated with antibiotics. Clinical studies and inflammatory biomarkers can help physicians with their diagnostic approach. The main characteristics of CSF bacterial meningitis, including *E. coli* meningitis, are the

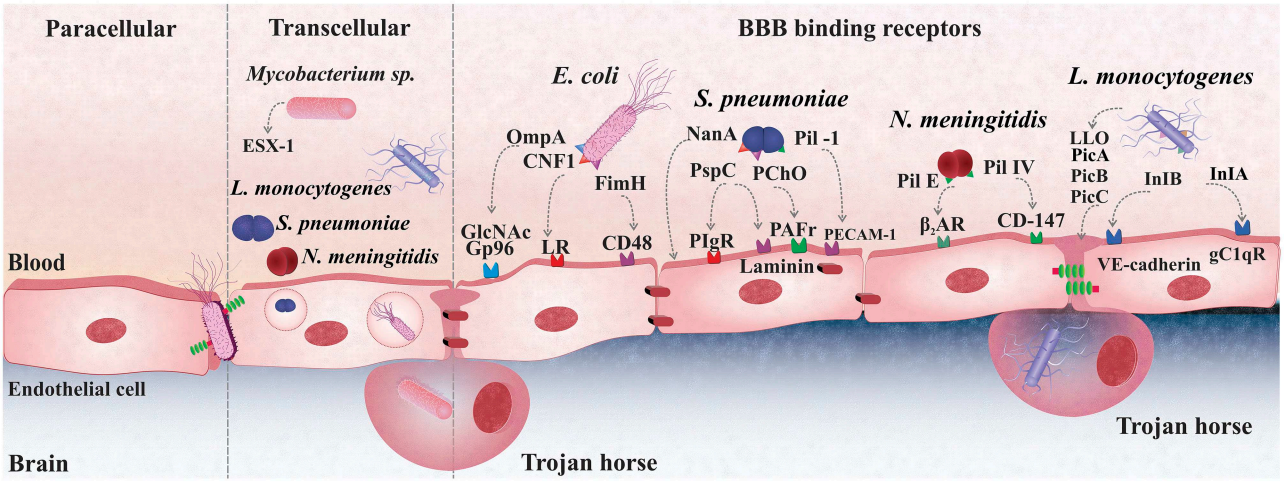

FIGURE 1
Bacterial mechanisms cross the blood-brain barrier (BBB). *S. pneumoniae* (pneumococcus) uses the pilus-1 and PspC (CbpA) to bind to PECAM-1 and pIgR expressed on the plasma membrane of brain endothelial cells; The PAF receptor plays also a role in pneumococcal adhesion to the BBB endothelium, although whether bacteria directly to it is still unclear; The laminin receptor (LR) mediates the passage of pneumococci from the basement membrane of the BBB endothelium promoting bacterial translocation into the brain. *E. coli* crosses the BBB *via* transcellular traversal or paracellular traversal. *E. coli* also binds with CD48 on brain microvascular endothelial cells *via* a type 1 fimbrial adhesion (FimH), outer-membrane protein A (OmpA) *via* N-acetylglucosamine (GlcNAc) or glucose-regulated protein-96 (Gp96), and cytotoxic-necrotizing factor 1 (CNF1) *via* the laminin receptor (LR). *L. monocytogenes* disrupts the phagosome membrane, releasing the phospholipases phosphatidylinositol-specific phospholipase C (Pic)-A and PlcB, as well as the toxin listeriolysin O. Internalin (Inl)-A and Inl-B bind to endothelial cells' receptors for the globular head of the complement component C1q (gC1qR) or VE-cadherin to cross the BBB. *Mycobacterium tuberculosis* infects brain tissue free or inside of macrophage, trojan-horse mechanism. The second mechanism is an active process that depends on an intact ESX-1, also known as type VII secretion systems, that induces phagosomal rupture in host phagocytes during transcellular migration. *N. meningitidis* uses Type IV pili and Opa, Opc binding proteins to adhere onto the mucocutaneous cells and uses Factor H to inhibit the alternative, complementary pathway.

presence of polymorphonuclear (PMN) cells (>1,000 cells/L, 80–90% PMN), hyperglycorrhachia (40 mg/dL of CSF glucose, glucose CSF/blood ratio ~0.4 in children, and ~0.6 in neonates), and high CSF protein levels (>150 mg/dL) (119). In addition, CSF Gram stain allows rapid and accurate identification of bacteria in around 60–90% of samples, with a specificity of 97% or greater (120). However, the percentage of a positive Gram stain is partly dependent on the specific bacterial infection causing meningitis. Gram (-) bacilli had a Gram stain positive in approximately 50% of cases (121).

Other biomarkers, including lactate, C-reactive protein (CRP), and procalcitonin (PCT), are used to differentiate between bacterial and non-bacterial meningitis. A total of 236 infants with meningitis were included in a retrospective analysis. The infants with bacterial meningitis had 22.88% of positive CSF culture results. The levels of lactate dehydrogenase (LDH) and high sensitivity CRP (hsCRP) increased in the CSF of bacterial meningitis patients compared with non-bacterial meningitis patients. The positive microorganism culture was associated with higher levels of LDH and hsCRP in the CSF of the patients (122).

The determination of the pathogen in bacterial meningitis is not simple and is often associated with secondary infections. In a case report of meningitis caused by *E. coli*, the pathogen was detected only in blood and urine cultures with negative CSF culture (123). In some cases, it is not possible to identify the primary focus of *E. coli* due to the early use of antibiotics, therefore the polymerase chain reaction (PCR) in the CSF can be a useful test in patients who received antibiotic treatment before the lumbar puncture (121, 124). In general, CSF cultures may be negative even when bacterial meningitis is diagnosed (125, 126). Therefore, in addition to investigating secondary infections, it may be necessary to investigate other rare causes such as strongyloidiasis and chronic organ insufficiency before considering the *E. coli* infection as having been community-acquired (124). In addition, *E. coli* is identified in the CSF through studies involving these cultured bacteria in the bloodstream, which has helped clinicians infer the source of *E. coli* (127); once it becomes a major pathogen that causes bloodstream infection (128).

Therapeutics strategies

Although *E. coli* meningitis can be effectively treated with antibiotics, bacterial meningitis is especially severe in newborns and premature infants. However, the increased death rate happens in low- and middle-income nations where invasive infections are common; several patients do not have access to antibiotics (129), and the incidence of antibiotic resistance makes effective therapy a challenge (130). In neonates, 21 days of antibiotic is recommended for Gram (-) bacilli meningitis. Gentamicin should be added for infants and toddlers with diagnostic *E. coli* meningitis until CSF is sterile. Ampicillin-susceptible: ampicillin 300.0 to 400.0 mg divided in 4 to 6 doses can substitute cephalosporin. Ampicillin-resistant: Ceftriaxone 100.0 mg divided into two doses OR cefotaxime 200.0 to 300.0 mg divided into four doses PLUS gentamicin 7.5 mg divided into three doses. Length therapy, 21 days (131).

In conclusion, *E. coli* meningitis is a leading cause of death and morbidity globally, particularly among newborns. A patient with a high bacteremia rate is more prone to meningitis. For example, bacteremia with more than 103 colony-forming units (CFU)/mL of blood is often more going to result in meningitis than bacteremia with lesser CFU/mL of blood (101). The *E. coli* then translocates

from the blood to the CNS, where it colonizes and causes meningitis. The inflammation causes further brain injury as well as long-term cognitive impairment. Pre-clinical models continue to further our understanding of the pathophysiology of *E. coli* meningitis and serve as a basis for developing new adjuvant and antibiotic treatments.

Tuberculous meningitis

Tuberculosis (TB) continues to be a massive global health problem. Africa retains the second-highest TB burden at 25% of the global incidence in 2019 (120). Long-standing challenges to the eradication of the disease include the lack of effective vaccination, poverty, lack of education, poor access to early and effective health care, HIV/acquired immunodeficiency syndrome (AIDS), and the emergence of multidrug resistance (132). Extra-pulmonary TB constitutes 16% of total global TB notifications, tuberculosis meningitis (TBM) is the most serious form of extra-pulmonary tuberculosis and the most common form of neuro-tuberculosis, leading to death or severe disability in half of the affected individuals (133, 134).

Pathogenesis and epidemiology

The systemic immune response to TB

TB is contracted through the inhalation of aerosolized mycobacteria tuberculosis (Mtb). The bacilli colonize pulmonary alveolar macrophages, which act as TB antigen-presenting cells to elicit an initial innate and consequent adaptive T-helper cell I (Th1) immune response (135). The inflammatory process encapsulates the infected cells in a granuloma and prevents the development of active disease in healthy individuals. However, in the very young, elderly, or immune compromised, the immune response may continue, resulting in active TB disease, destruction of the lung tissue, and potential dissemination of the TB bacillus to other organs (136, 137).

Dissemination to the CNS

TB dissemination commonly occurs hematogenously, and dissemination is often accompanied by miliary TB in children (138, 139). *In vitro* models have demonstrated that Mtb is able to invade the epithelial cells, replicate intracellularly, stimulate cell lysis and proliferate to neighboring cells (140). Further, Mtb is able to survive in infected macrophages and dendritic cells and may be transported out of the lungs to other organ systems (141), or it may invade and traverse vascular endothelial cells and be trafficked throughout the body in phagocytes (142). Host factors like polymorphisms in the genes encoding for antigen recognition and macrophage activation (143–145), perturbed pro-inflammatory cytokine release (146), and decreased vitamin D (133) may undermine the body's attempt to control the infection. Additionally, virulent TB strains may compromise the innate immune response, promote bacillary survival and replication, and cause more severe diseases like TBM (144, 147).

In the brain, Mtb can migrate across the protective BBB and blood-cerebrospinal fluid barrier (BCB) and enter the immune-limited domain of the CNS. *In vitro* and animal models have identified the Mtb gene Rv0931c (pknD) as a potential virulence factor that promotes CNS infection by enabling the bacilli to interact with extracellular factors on the brain endothelium leading to endothelial adhesion and rearrangement of the actin cytoskeleton of brain microvascular endothelial cells (142, 148). Another potential route of entry is the Trojan horse mechanism by which Mtb are trafficked across the BBB by infected macrophages (141).

Diagnosis, clinical presentation, and treatment

Diagnosing TBM remains challenging due to the non-specific nature of its presentation and the lack of clinical, laboratory, or radiological tools to enable a swift and definitive diagnosis. Early diagnosis and commencement of treatment are considered the most important determinants of outcome (149). Most institutions treat patients on the presumed diagnosis of TBM based on a combination of clinical, laboratory, and radiological criteria in conjunction with laboratory tests. However, there is considerable variability across these criteria and testing platforms (150). To aid in uniform case definitions for research, a consensus statement was developed, which allows comparison between studies (151).

In the early phases of the disease, patients may present with non-specific sub-acute symptoms that are challenging to differentiate from those of benign conditions like an upper respiratory tract infection, particularly in children (152, 153). Headache, vomiting, weight loss or failure to thrive, meningism, and a decreased level of consciousness are among the most commonly presented symptoms (152, 154–161). A recent TB contact may be reported in 20–66% of cases (152, 155, 160, 161) and a previous history of TB in 13–27% of cases (162, 163).

CSF microscopy and chemistry are essential in the presumptive diagnosis of TBM. Common findings include elevated white cell count with lymphocytic predominance, low glucose and high protein (150). However, atypical findings are reported (164). The culture of Mtb in CSF was considered the gold-standard diagnostic test; however, CSF culture positivity yields are notoriously poor and can take more than 40 days due to the paucibacillary nature of CSF. In a recent review Bahr et al. report that in 22 adult TBM patients with confirmed TBM using Xpert® MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA), more than 90% had a low to very low bacillary burden (165). Consequently, diagnostic tests lack sufficient sensitivity; smear microscopy is 10–15% sensitive, and sensitivity on culture ranges between 50–60% (33). Although Cepheid's latest installment Xpert® MTB/RIF Ultra (Ultra; Cepheid), is the currently recommended first-line diagnostic test for extrapulmonary TB, it's sensitivity for TBM ranges between 47.2–76.5% against the consensus criteria (34, 35) and in the region of 90% against culture (166). The performance improves with greater CSF volumes (which often are not accessible), and varies based on HIV co-infection (166, 167). It has therefore been suggested that diagnostic tests for TBM need to move beyond detecting the bacillus (165). Recently studies have examined the role of host inflammatory markers in both blood and CSF as possible diagnostic tools (162, 163). While these biosignatures demonstrate promise in small pediatric studies,

larger studies that include adults and HIV co-infected patients are required.

Radiology

Radiological findings suggestive of TBM include basal and leptomeningeal enhancement, hydrocephalus, tuberculomas, and infarcts, and are an integral part of the presumptive TBM diagnosis (151). The characteristic feature of TBM is the presence of an inflammatory exudate in the basal cisterns of the brain (134, 163, 168). It consists of chronic non-necrotizing and necrotizing granulomatous inflammation. The predominant location of exudate at the base of the brain has several important implications; firstly, all the major cerebral vessels originate from the base of the brain and are at risk of being encapsulated by the exudate. Secondly, the accumulation of exudate in the basal cisterns interferes with the circulation of CSF, causing hydrocephalus. Thirdly, it envelopes and compresses the local cranial nerves, including the optic and oculomotor nerves resulting in cranial nerve palsies (134).

Vasculitis

Exudate coats all the major vessels in the Circle of Willis, with a predilection for the middle cerebral arteries, as well as their small perforators (163). This results in inflammation of the vessel wall, vascular occlusion, and vasospasm, which put the brain at significant risk of ischemia and infarction, commonly seen in the basal ganglia (169, 170).

Hydrocephalus

The presence of exudate in the basal cisterns blocks the flow of CSF around the upper brain stem and may occlude the cerebral aqueduct. This precipitates hydrocephalus and raised intracranial pressure, which adds to the risk of ischemia. TBM-associated hydrocephalus may be communicating if the obstruction to CSF flow occurs in the subarachnoid space or non-communicating when the flow is obstructed at the cerebral aqueduct or the outlet foramina of the fourth ventricle (171). Although non-communicating hydrocephalus occurs in a minority of cases (172), it can be fatal to perform a lumbar puncture in these patients. Determining the communicating nature of the hydrocephalus is crucial to safe patient management (171).

Tuberculomas and abscesses

Tuberculomas and TB abscesses may accompany TBM or occur independently. Tuberculomas are granulomatous, with a necrotic center surrounded by lymphocytes and epithelioid cells, which may merge to form Langhans giant cells. They are bordered by astrocytes and associated with edema and vascular proliferation (134, 173). TB brain abscesses are less common (174, 175). They comprise necrotizing granulomatous inflammation in the form of an encapsulated collection of pus containing Mtb bacilli. Both these lesions may arise after the initiation of anti-tuberculous treatment, sometimes termed a "paradoxical reaction" or be associated with immune reconstitution inflammatory syndrome (IRIS) in anti-retroviral treatment (ART) naïve TBM patients started on ART and anti-TB antibiotics in close succession. Neurological TB-IRIS can cause dramatic deterioration in patients and is of particular concern in resource-limited settings (176).

Spinal TB

Spinal TB may develop from TBM or secondary to vertebral TB (134). Exudate may be present along the meninges, cord and nerve roots leading to spinal arachnoiditis, intradural (extramedullary) tuberculomas or intramedullary tuberculomas, and tuberculous radiculomyelitis (134, 173). Exudate in the caudal sac can lead to dry lumbar taps or a high CSF protein (134, 173).

Antimicrobials

Drug-susceptible TBM in children is treated according to the WHO regimen with isoniazide (H: 10 mg/Kg, max. 300 mg), rifampicin (R: 15 mg/Kg, max. 600 mg), pyrazinamide (Z: 35 mg/Kg) and ethambutol (E: 20 mg/Kg) once daily for 2 months. This intensive phase is followed by a continuation phase of H and R daily for 10 months (177). However, following compelling recent data, in 2021, the WHO published an alternative, shorter 6 months' intensive regimen of HRZE for children and adolescents with drug-susceptible TBM (178). Drug susceptible TBM in adults is treated with: H (5 mg/kg, max. 300 mg), R (10 mg/Kg, max. 600 mg), Z (25 mg/Kg), and E (15 mg/Kg) once daily for 2 months. This intensive phase is followed by a continuation phase of H and R daily for 10 months (135). The penetration of R and E into the CSF is low (10–20% and 20–30%, respectively) but high for H and Z (80–90% and 90–100%, respectively) (135). The most common side effects of first-line tuberculostatic drugs are hepatotoxicity (H, R, Z), orange urine (R), peripheral neuropathy (H), and arthralgia (Z) (135). Isoniazid-resistant TBM is treated with REZ-levofloxacin (Lfx).

Multidrug-resistant (MD) TBM is treated with second-line tuberculostatics from group A (Lfx or moxifloxacin, bedaquiline, linezolid), group B (clofazimine, cycloserine), and group C (E, Z, delamanid, imipenem-cilastin or meropenem, amikacin, ethioniamide or prothionamide, para-aminosalicyclic acid) if needed, depending on the drug susceptibility testing of the infecting strain. Initially, a minimum of four drugs for 6 months is followed by three drugs, for a total of at least 18 months (135, 179). The penetration of either Lfx or moxifloxacin into the CSF is 70–80%, for ethioniamide, prothionamide, or cycloserine 80–90%, for linezolid 30–70%, and for amikacin 10–20% 5. P-aminosalicylic acid and E do not penetrate the CNS well and should not be counted on as effective agents for MDR-TBM. Amikacin penetrates the CNS only in the presence of meningeal inflammation. There are little data on the CNS penetration of clofazimine, bedaquiline or delamanid (180). The most common side effects of the fluoroquinolones (levofloxacin, moxifloxacin) include nausea, tremors, headache, and confusion. Myelosuppresion and optic neuropathy can be caused by linezolid. Cycloserine causes CNS depression leading to depression, seizures, and neuropathy. Concomitant administration of pyridoxine (vitamin B6) is advised when the patient is treated either with linezolid or cycloserine. Nephrotoxicity, and ototoxicity are the most common side effects of the aminoglycoside amikacin (135, 181).

Host-directed therapies: Corticosteroids

In HIV-negative adults and children with TBM, a Cochrane meta-analysis (updated in 2016; 9 trials; 1,337 patients; 469 deaths) showed that steroids reduce mortality by 25% (95% confidence interval: 13–35%) at 3–18 months of follow up. In one of the nine trials of 545 patients with follow up at 12 months, the effect on mortality was no longer apparent. There was no significant effect on disabling neurological deficits (182). The number of HIV-positive patients was too small to draw conclusions, but trials are currently being conducted on that group of patients (183). The WHO guideline advocates initial adjuvant corticosteroid therapy with dexamethasone or prednisolone tapered over 6–8 weeks (184). Studies investigating the expression of leukotriene A4 hydrolase (LTA4H), suggest that mainly HIV-uninfected patients with a high pro-inflammatory response (TT genotype) benefit from immune suppression by steroids (185). A tailored approach to immunosuppression based on genotype might improve overall outcomes in the near future.

Aspirin

Acetylsalicylic acid or aspirin is a non-steroidal anti-inflammatory drug, originally derived from willow tree leaves (Salix). Aspirin inactivates cyclooxygenase, inhibiting the synthesis of prostaglandin and thromboxane A2 in platelets. A low dose of aspirin impedes platelet aggregation, while its anti-ischemic and anti-inflammatory properties are dose-related.

Arterial ischemic stroke, prior to or during treatment, plays a major role in irreversible brain damage and poor outcome. Schoeman et al. compared low- (anti-thrombotic) and high-dose aspirin (anti-ischemic) with a placebo in young children with severe tuberculous meningitis (186). The study did not find differences in motor and cognitive function after treatment. The authors debated whether thrombosis plays a major role in cerebral infarction and hypothesized that proliferative vasculopathy (vasculitis) and vasospasm are the driving forces in TBM related cerebrovascular disease. A study in 98 HIV uninfected adults by Mai et al. also compared high and low-dose aspirin (both 2 months from the start of tuberculostatics and dexamethasone) with placebo. In the per-protocol analysis the aspirin group showed less stroke or mortality compared to the placebo (high dose: 11%, low dose: 15%, and placebo: 34%) (187). A trial in HIV-infected patients has been registered and is currently underway (188).

Thalidomide

Adjunctive corticosteroids reduce cytokine production and dampen subsequent inflammation, improving TBM survival but do not prevent morbidity. Therefore, additional immunomodulatory drugs are needed to improve TBM outcome. Thalidomide is known to reduce the TNF-α levels in CSF of children with TBM. The first safety study in 15 children with stage 2 TBM indicated that thalidomide was well tolerated in doses up to 24 mg/Kg/day (189). Unfortunately, the subsequent randomized trial in 47 children with stage 2 and 3 TBM needed to be terminated because of adverse events and deaths (13%) in the treatment arm of the study. Also, motor outcome and IQ after 6 months of anti-tuberculosis therapy was similar in the two groups. The above mentioned 13% mortality, however, was lower when compared with other described cohorts of such young (mean age of 4 years) and ill TBM patients, and the observed anti-inflammatory effects of thalidomide confirmed its possible treatment role for intracranial tuberculous mass lesions (190). With a lower thalidomide dose of 3–5 mg/Kg/day compared to the first safety study, children and adults with TBM complications such as cranial nerve palsies, blindness due to optochiasmatic arachnoiditis (2 months of treatment), and mass lesions (4 months of treatment) had a satisfactory clinical and radiological response without severe adverse effects (191). In summary, thalidomide may be considered for patients with developing large necrotizing TB abscesses, forms of spinal TB, TBM IRIS, and blindness caused by raised intracranial pressure or vasculitis compromising the optic chiasm (191).

Hydrocephalus management

Hydrocephalus management includes medical treatment, neurosurgical intervention, or a combination. Medical management includes repeated lumbar punctures and the use of acetazolamide and Lasix followed by neurosurgery if intracranial pressure does not normalize (171). Surgical options include external ventricular drains (sometimes used as a temporizing measure in severe cases), ventriculoperitoneal shunts and endoscopic third ventriculostomy (171). To date, there have been no clinical trials evaluating the ideal course of treatment and management practices remain heterogeneous (150).

Patient outcome

A meta-analysis on treatment outcomes of children with TBM demonstrated a mortality rate of 19.3%, and neurological sequelae in 54% of children who survived (192). Cognitive impairment, learning disabilities, emotional and behavioral problems, or motor impairment are common sequelae in these children (193). The poor neurodevelopmental outcome is associated with various factors such as young age, HIV infection, ethnicity, clinical severity, and delayed presentation and treatment (152). Early recognition and management of sequelae in children with TBM and support, availability, and access to appropriate care for them and their families, emphasizes the needs which are present in this population of patients (177). A meta-analysis on treatment outcomes of adults with TBM demonstrated a mortality rate of 24.7%, and neurological sequelae in 50.9% of adults who survived (194).

Listeria monocytogenes

In the United States, *L. monocytogenes* accounts for 8% of all cases of bacterial meningitis, with the most common serotypes ½ b and 4 b (195). Preventive measures such as educational and awareness actions can be beneficial in the fight against bacterial meningitis. A study revealed that in the past 25 years there has been a decrease in the incidence of neonatal *Listeria sp.* meningitis possibly due to increased awareness of dietary restrictions for pregnant women (195).

Even though the incidence decreased, the rate of unfavorable outcomes among adults with *Listeria sp.* meningitis increased from 27 to 61%, with the emerging *L. monocytogenes* genotype sequence type 6 (ST6) identified as the main factor leading to poorer prognosis (196). A genomic sequencing study of

these *Listeria sp.* strains identified a plasmid containing the benzalkonium chloride tolerance gene that was associated with decreased susceptibility to disinfectants commonly used in the food-processing industry. Strains containing the plasmid also had increased minimal inhibitory concentrations (MICs) to amoxicillin and gentamicin, two commonly used antibiotics in the treatment of *L. monocytogenes* (197).

Risk factors for developing *L. monocytogenes* include age over 60 years, chronic steroid recipients, alcoholism, immunosuppression, and malignancy (196–198). In a review of 820 cases of neurolisteriosis, the mortality rate was 26%; patients with seizures and age >65 were at even higher risk (198). In another study of 1,959 cases of listeriosis in France, risk factors for mortality included age >65 years, underlying disease, and focal listeriosis (199). In a recent prospective study of 818 cases of listeriosis in France, of which 252 were neurolisteriosis, factors associated with 3-month mortality were cancer, multi-organ failure, bacteremia, pre-existing organ dysfunction, monocytopenia, and adjunctive steroids (200). This is the first study to date showing an increase in mortality with the use of adjunctive dexamethasone in *Listeria sp.* meningitis (200). Adjuvant steroids should be stopped if *Listeria sp.* is found to be the cause of bacterial meningitis (201).

Pathogenesis and epidemiology

L. monocytogenes is a food-borne pathogen that can cause gastroenteritis, bacteremia, meningitis, or maternal-neonatal infection (202). *Listeria sp.* has been isolated from water, sewage, dust, soil, and decaying vegetable matter (including silage and animal feed). Outbreaks of *Listeria sp.* infection have been associated with the consumption of contaminated coleslaw, raw vegetables, cheese, milk, contaminated turkey franks, cantaloupe, alfalfa tablets, diced celery, hog head cheese, and processed meats, thus pointing to the intestinal tract as the usual portal of entry (195). The largest outbreak was recently described in South Africa and accounted for 1,060 cases that were traced to processed meats (203). In addition, the infection can be transmitted from pregnant women to the neonate, since these women may harbor the organism in their genital tract and rectum and remain asymptomatic. Adults younger than 50 years who present with *Listeria sp.* meningitis should be screened for HIV infection (204).

Upon ingesting the bacteria, *L. monocytogenes* traverses the intestinal epithelium into the lamina propria and then disseminates to the liver, spleen, and brain (202). *L. monocytogenes* may cross the BBB by direct uptake from endothelial cells (202). Bacteria have been observed within endothelial cells, showing the ability of *L. monocytogenes* to invade cultured human brain microvascular endothelial cells, given that the listerial surface protein In1B is present. After entering cells, *Listeria sp.* utilizes listeriolysin O to escape from phagosomes by entering the cytoplasm (205). Once inside the cell, the resulting propulsion of *Listeria sp.* against the cell membrane facilitates that it can be phagocytosed by the adjacent cell, leading to further dissemination. *Listeria sp.* can also invade the CNS by transportation within leukocytes or *via* a neural route enabling cranial nerve invasion (202, 206).

Diagnosis, clinical presentation, and treatment

L. monocytogenes meningoencephalitis can present with seizures and focal neurological deficits such as ataxia, cranial nerve palsies, or nystagmus secondary to rhombencephalitis (e.g., brainstem and cerebellar involvement) (194, 197–199). In an extensive review of neurolisteriosis (199), the most frequent clinical findings were fever, headache, and altered sensorium, with <50% having meningeal signs. In the MONALISA study, a total of 818 cases of listeriosis from France were identified, of which 252 (31%) were neurolisteriosis. The most common presentation was encephalitis (87%), with brainstem involvement in 17%. Clinical findings included nuchal rigidity (65%), aphasia (19%), seizures (18%), and focal limb weakness (12%) (200).

The mortality in neurolisteriosis remains high. In a study of 375 patients, the mortality rate was 31%, with age and concomitant bacteremia as independent prognostic factors (207). In the MONALISA study, the 3-month mortality rate was also 30%, and the most important predictors were ongoing organ neoplasia, multi-organ failure, aggravation of any pre-existing organ dysfunction, mechanical ventilation, monocytopenia, bacteremia, and administration of adjunctive dexamethasone (200).

CSF

In the MONALISA study, the median CSF WBC was 457 cells per μl, the median CSF protein was 2.1 g/L, and the CSF to blood glucose ratio was 0.31 (200). The CSF Gram stain and culture in *Listeria sp.* meningitis is only positive in 24–32% and 80–90% of patients, respectively (120, 194, 199). In the MONALISA study, the diagnosis was established by CSF culture in 84%, with the other 16% being documented by either CSF PCR (positive PCR in 63% of all patients) or by positive blood culture (200).

Often typical CSF findings predictive for bacterial meningitis might be absent and about 11–30% of patients with bacterial meningitis show negative CSF culture results. In patients with Listeria meningitis this percentage may be higher (51, 208–210). Other pathogen detection tools such as next-generation sequencing (NGS) have been successfully used to properly detect *L. monocytogenes* in a case whose clinical manifestations were suspected as tuberculous meningoencephalitis (211). Furthermore, the detection of a combination of specific biomarkers activated in the immune response in Listeria meningitis may help in the differential diagnosis (212). Finally, the use of a real-time PCR assay to detect and quantify *L. monocytogenes* DNA through specific amplification of the *L. monocytogenes* hly gene in CSF helped to improve the sensitivity of microbiological diagnosis in 214 samples from patients with suspected listeriosis (213). Furthermore, the sensitivity of the CSF Gram stain and culture decreases significantly in patients with prior antimicrobial therapy prompting the UK guidelines to recommend routinely obtaining a PCR for the two most common meningeal pathogens (*S. pneumoniae* and *N. meningitidis*) in patients presenting with meningitis (194, 214). Novel multiplex PCR assay panels are now widely used, incorporating several viral, bacterial, and fungal targets that also include *L. monocytogenes* with high sensitivity and specificity (215).

Antibiotic therapy

Third-generation cephalosporins are inactive for *L. monocytogenes* meningitis (194). In patients with suspected *L. meningitis* (e.g., neonates older than 50 days or with cellular immunodeficiency), ampicillin or penicillin G should be added (120, 194). An aminoglycoside should be added in patients with proven neurolisteriosis because of *in vitro* synergy, enhanced killing *in vivo*, and efficacy in animal models (120, 194).

In the MONALISA study, the addition of aminoglycosides was associated with reduced 3-month mortality in 679 patients with *Listeria sp.* bacteremia and or meningitis but not in the subset of patients with neurolisteriosis (200). Nevertheless, it is important to emphasize that a controlled clinical trial comparing ampicillin alone with ampicillin plus gentamicin has never been done in humans with listeriosis (194). An alternative agent in a penicillin-allergic patient is trimethoprim-sulfamethoxazole, which is bactericidal against *Listeria sp. in vitro* (192).

Trimethoprim-sulfamethoxazole was used in 17% of 679 patients with listeria bacteremia or meningitis and was associated with a reduction in 3-month mortality (199). Both vancomycin and chloramphenicol have been associated with an unacceptably high failure rate in patients with *Listeria sp.* meningitis and should be avoided (194). Carbapenems are active *in vitro* and in experimental animal models of *L. monocytogenes* meningitis and have been used in up to 3.4% of patients with listeriosis (194, 199). The fluoroquinolones and linezolid have good *in vitro* activity against *L. monocytogenes*, but there is a limited clinical experience (194).

Adjunctive corticosteroids

The routine use of early adjunctive dexamethasone has decreased mortality in adults in pneumococcal meningitis in high-income countries and is advocated by the Infectious Diseases Society of America, United Kingdom, and European guidelines (194). This benefit, unfortunately, has not been seen in studies in low-income countries from Africa or Asia, as patients present too late with advanced disease when the disease process is already established (120, 194). In a randomized, double-blind, placebo-controlled study from Malawi, there were no significant differences in mortality at 40 days (56% in the dexamethasone group vs. 53% in the placebo group) or when the analysis was restricted to patients with proven pneumococcal meningitis (53% in the dexamethasone group vs. 50% in the placebo group) (216). However, in this trial, approximately 90% of the patients were co-infected with HIV and had the advanced disease; the delayed presentation was also associated with a poorer outcome, although adjusting for this factor in the analysis had no effect. In patients with bacterial meningitis that is subsequently found not to be caused by *S. pneumoniae*, dexamethasone should be discontinued, especially if caused by *L. monocytogenes* or *Cryptococcus neoformans,* as steroids increase adverse clinical outcomes (120, 194, 199).

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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