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NEUROPATHOGENESIS: ROGUE GLIA CAUSE MAYHEM IN THE BRAIN

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Abstract

Glia, including astrocytes, microglia and oligodendrocytes, are important components that maintain the architecture of the brain and in many ways contribute to the proper functioning of neurons. Glial cells vastly outnumber neurons in the brain and independently control several crucial brain functions. Impaired glial cells are the cause of several diseases, and pharmacological targeting to repair damaged glia will enable functional recovery in patients suffering from devastating neurological disorders. The interaction between glial cells and some patrolling immune cells in the brain comprise the brain-specific immune system that protects the brain from extraneous agents and repairs injured tissue. While this system can cope with minor insults and infections, when faced with significant challenges such as AIDS dementia, multiple sclerosis, Huntington's disease, Parkinson's disease, etc., an effective and balanced immune response that facilitates repair and protection is found wanting. Several debilitating neurological disorders are often associated with dysfunctional glial cells that have limited ability to repair the injured brain and even promote brain damage. In this discussion, specific signaling pathways in glia that are affected in AIDS dementia and periventricular white matter injury will be highlighted.

Keywords

• HIV Dementia • Microglia • Macrophage • Astrocytes • TLRs • Oligodendrocytes • Free radicals • Iron retention • Hypoxia

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TAT blocks Wnt in astrocytes to induce HAND

Can a 'serine', in place of a 'cysteine', induce changes powerful enough to cause human immunodeficiency virus (HIV)-associated neurocognitive disorder (HAND) or HIV-associated dementia (HAD)? Data obtained from a recent study [1], in corroboration with earlier results [2], seems to support this conclusion. Indeed, the result of this substitution causes sufficient molecular and physiological effects that lead to HAND/HAD. Over the years several pathways have been identified that HIV-1 sabotages to its advantage thereby rendering the host immune system compromised. The Wnt/β-catenin pathway is one of these pathways. In astrocytes, this protein complex, that regulates several different genes, seems to be involved in the host defense against HIV-1 replication [1]. HIV-1 has cleverly found a way to counter this protective mechanism by blocking Wnt/β-catenin activity through one of its viral protein, the transactivator of transcription or Tat protein. The HIV-1 *Tat* gene encodes for an 86-101 amino acid polypeptide that acts as the main transactivating factor of HIV-1. Tat protein can be actively released by HIV-1-infected cells and is detected in the serum of HIV-1- infected individuals. Tat also acts on different types of uninfected cells by interacting with several receptors belonging to the growth factor and chemokine families as well as heparan sulphate proteoglycans (HSPG) which, along with low density lipoprotein receptor-related protein (LRP), mediates the internalization of Tat inside the cells, which retains the capacity to transactivate viral and/or cellular genes [3]. From the perspective of the present study, HIV-1 Tat demonstrates strong monocyte chemotactic properties and an increased migration of monocytes to the brain, which is strongly correlated with HAND [2].

Interestingly, mutations in *Tat* seem to account for the difference in the effect of Tat protein in astrocytes, predominantly, the effect on dementia. Earlier work in the area of HAND/HAD revealed that the incidence of HAND/HAD in countries such as India was relatively low (about 1-2%) in comparison to the prevalence in North America and Europe that has been estimated at 15-30% [2]. Strikingly, the authors looked at the role of the Tat protein encoded by different HIV-1 clades since western nations harbored the HIV-1 clade B subtype, while in India the predominant subtype was clade C. Importantly, the authors revealed that the Tat protein, which has monocytic chemotactic function, showed important differences between subtypes. Cysteine (at position 31) was highly (>99%) conserved in non-subtype C viruses and more than 90% of subtype C viruses encoded a serine at this position. In fact, a variant strain, with a serine instead of a cysteine, possessed transactivation property of Tat, but was defective for chemotactic activity, which is key to the progression of HAND [2].

Progressing from this finding over a decade ago, the present study [1] demonstrates how the Wnt/β-catenin pathway suppresses HIV-1 replication in astrocytes, an important reservoir of HIV-1 in the central nervous system (CNS). Not surprisingly, the HIV-1 Tat protein through the dicysteine motifs, at positions 30 and 31, reduced Wnt/β-catenin levels, as measured indirectly using a TOPFLASH reporter

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construct. In an elegant model proposed, Tat associates with the transcription factor 4 (TCF4), but not β-catenin, which is modulated by the extent of interferon-γ (IFN-γ)-mediated inflammation. The Wnt/β-catenin pathway exerts its suppressive action by repressing HIV-1 long terminal repeat (LTR) activity in the absence of IFN-γ. However, with increased inflammation and higher levels of IFN-γ, the inhibition of β-catenin leads to increased LTR activity mediated by Tat through its partner, the positive transcription elongation factor (pTEFb) allowing for efficient viral replication. β-catenin is degraded through the association of Tat with TCF2 to allow for efficient HIV replication [1]. Very importantly, clade C Tat did not reduce β-catenin signaling, suggesting that the dicysteine motif is important for inhibition of β-catenin signaling. In the presence of high levels of β-catenin, HIV-1 replication in astrocytes is suppressed leading to dramatically lower incidence of HAND/HAD, common among HIV-1 patients infected with the clade C strain [2]. Thus, a single amino acid substitution might be partly or wholly responsible for HAND. An important read-out of glial toxicity in HAND/HAD is the release of neurotoxic factors [4]. In particular, HIV-1-infected astrocytes release bioactive substances into the cell culture supernatant that have a toxic effect on neuronal cultures. This could have been demonstrated in the present study to reveal how different HIV-1 clades damage neurons and whether the dicysteine motif is sufficient to exert neurotoxicity in HAND/HAD.

While the astrocyte is an important glial cell that has now gained substantial attention to its numerous roles in diseases and homeostasis of the CNS, another glial cell that also contributes to both pathology and normal brain development is the microglia [5]. Of hematopoietic origin (unlike the neuroectodermal origin of astrocytes), microglia release several neurotoxic factors upon HIV-1 infection and is an important HIV-1 reservoir in the CNS [4]. While it is known that Wnt-3a stimulation of microglia leads to enhanced expression of pro-inflammatory genes through the stabilization of β-catenin [6], there is no evidence for the effect of HIV-1 on the Wnt/β-catenin pathway in microglia and the findings of Henderson et al. [1] will serve to dissect the role of different glial cells in HAND. Needless to say, the role of the Wnt/β-catenin pathway in suppressing HIV-1 in microglia must be investigated and the role of Tat in encountering Wnt/β-catenin-mediated suppression must be demonstrated.

Based on the results of this study [1], one can speculate that the high levels of HIV-1 in microglia/macrophages could be due to the subversion of the Wnt/β-catenin pathway in these cells. A clearly understated phenomenon in this study is the role of microglia in this molecular and cellular symphony. Indeed, there is clear evidence for enhanced infection of astrocytes depending on the proximity of these cells to microglia/macrophages, demonstrating the importance of the cellular environment in regulating the permissiveness of astrocytes to HIV-1 infection. It would be interesting to identify whether there is differential activation and/or inhibition of the Wnt/β-catenin pathway in microglia and astrocytes. If there is a common β-catenin-related pathway that exists in astrocytes and microglia, the two important HIV-1 reservoirs in the CNS, efforts to target this pathway using drugs might be a reasonable strategy to flush out HIV-1. However, the complexity of the Wnt/β-catenin pathway could render a therapeutic strategy to combat HIV-1 difficult, if not impossible.

There are 19 Wnts and 10 Frizzled receptors and along with several co-receptors (for example, lipoprotein receptor-related protein, LRP) including molecules such as glypicans, a group of HSPG that assist in the binding of Wnts to their cognate receptors [7]. In the canonical pathway, where Wnts exert their effect through β-catenin, activation of the Wnt (Wnt1 or Wnt3a) pathway generally confers neuroprotection by inhibiting inflammatory events, including endothelial activation, monocyte migration and pro-inflammatory cytokine induction by macrophages. In contrast, Toll-like receptors (TLR) / nuclear factor κB (NF-κB) signaling promotes the production of pro-inflammatory cytokines and Wnt5a and through the non-canonical pathway, can enhance inflammation [8].

In a landmark study on HIV-1 associated neurovirulence, in human fetal astrocytes, HIV-1 Tat activated the NF-κB pathway, but HIV-1 Tat clones from individuals with HAD failed to transactivate HIV-1 LTR, while Tat clones from non-demented individuals without HAD were able to transactivate HIV-1 LTR in the U373 astrocytic cell line [9]. These results suggests that there is no definitive correlation between brain viral load and development of HAD and that Tat might contribute to HAD neuropathogenesis through various disease mechanisms with limited viral replication [9]. Putting the results of the present study in context, it might seem that activation of the Wnt/β-catenin pathway in the U373 cell line might be crucial to allow Tat to transactivate HIV-1 LTR, and this might be the missing link. Thus, these studies once again emphasize the marked effects of Tat molecular diversity on HIV-1 LTR transactivation. As a corollary, depending on the type of glypicans present on the cell-surface, cells can switch from a canonical to a non-canonical Wnt signaling pathway [10], which have vastly different outcomes on neuronal survival. Thus, targeting the type of Wnts and their receptors and co-receptors, with drugs to modulate the response of the cell during HIV-1 infection, must take into account pre-existing inflammatory responses as well as the cell types involved in the neuropathogenesis.

Germs and glia: drugs take a heavy Toll in HIV-1 neuropathogenesis

Currently, over 33 million individuals are infected with HIV-1 worldwide. In the absence of treatment, between 40% and 70% of infected individuals show neurological disorders. The most significant of these are primary HIV-associated brain disorders that are collectively termed HIV-associated Neurocognitive Disorders (HAND) [11]. A large number of patients who acquired HIV/AIDS early during the epidemic were intravenous drug abusers who suffered cerebral injury due to the drugs as well as secondary infections, associated with opportunistic bacteria and fungi. *Streptococcus pneumoniae* is the most common community acquired bacterial pneumonia in HIV-1 positive intravenous drug abusing patients [12]. In the nervous system, illicit drugs interact with their receptors that are often enriched in the brain centers controlling emotional responses, such

as the nucleus accumbens, causing dopamine release and changes in moods of drug abusers. However, with repeated use of narcotics, the responsiveness of the brain declines and higher doses are required to attain the same state of euphoria [12]. There is extensive loss and damage to neurons in particular areas of the brain due to drug abuse.

One major class of drugs used by abusers is opiates (e.g. heroin). Opiate abuse may disrupt the endogenous opioid system that is present throughout the brain, particularly in the basal ganglia but also on neurons and glia. Opiates can lead to respiratory depression, which contributes to the risk of bacterial bronchopneumonia in drug abusers. High levels of opiates in the circulation can also accelerate the progression to AIDS. Lymphocytes show increased expression of HIV-1 receptors in the presence of opiates. As well, increased influx of infected monocyte/macrophages is observed in the brain [13]. Although considerable evidence indicates that opiate abuse increases the progression of HIV-1 in the CNS, the mechanisms by which opiates in synergy with *S. pneumoniae* exacerbate CNS pathology and neurological complications are not well understood.

Macrophages from *S. pneumoniae*-infected HIV-1 positive patients, display a pronounced pro-inflammatory innate immune response [14], which is mediated by TLR. TLR are type 1 transmembrane proteins expressed in some innate immune cells, including microglia in the nervous system. Microglia are cells of myeloid origin and mediates phagocytosis, neuroprotection and neuroinflammation. Microglia also serve as the major target of infection by HIV-1 in the CNS and are the prime instigators of HAND. Microglia contribute to neuropathogenesis of HAND due to the presence of cell-surface receptors, such as TLR, that render these cells highly reactive to a variety of innate and adaptive immunological stimuli [15]. They recognize and bind conserved pathogen-associated molecular patterns (PAMP) shared by large groups of microorganisms. Following interactions with specific ligands, the cytoplasmic domain of TLR, through adaptor proteins, trigger activation of signaling pathways that ultimately induce an inflammatory response. For example, TLR-4 recognizes lipopolysaccharide (LPS) on bacteria and enhances this recognition with the assistance of LPS-binding protein (LBP). On the other hand, lipoteichoic acid (LTA) of *S. pneumoniae* binds to TLR-2 and signaling occurs with the assistance of cluster of differentiation 36 (CD36) molecule. LBP carries LPS to the CD14 molecule where it is then presented to the MD2-TLR-4 complex (Figure 1). The intracellular adapter protein MyD88 is recruited to the complex, resulting in the ubiquitination of IκB, which is targeted for degradation in the proteasome. This results in the translocation of the active form of NF-κB (p65/p50) into the nucleus to induce transcription of pro-inflammatory cytokines genes. While TLR-2 and TLR-4 recognize microbial components at the cell surface, TLR-3, TLR-7, TLR-8 and TLR-9 are expressed in endosomal compartments. TLR-9 recognizes unmethylated cytidine-phosphate-guanosine (CpG) DNA motifs in bacteria and viruses. Whether HIV-1 can form viral PAMP that activate TLR in microglia is unknown, but there is evidence that HIV-1 can activate TLR-7/8 and possibly TLR-9 in dendritic cells and macrophages [15].

In their paper, Dutta et al. [16] describe the impact of TLR in HIV-1 neuropathogenesis, opening up new avenues for therapeutic interventions. A principal finding of Dutta *et al.* was that activation of TLR (-2, -4 and -9) on microglia by morphine, HIV-Tat and *S. pneumonia,* led to an upregulation of pro-inflammatory cytokines, reactive oxygen species (ROS) and nitric oxide (NO). Microglia-derived free radicals and opioid-induced increases in free radical production are very important to immune signaling and the promulgation of brain inflammation through redox signaling in microglia [17]. This process is postulated to contribute to the increased prevalence of neuropathogenesis observed in HIV-1-infected opiate drug users [16]. While it has been previously established that morphine in combination with HIV-Tat induces a pro-inflammatory response in microglia, the synergistic impact of *S. pneumoniae* in HIV-1 neuropathogenesis has now been elegantly demonstrated in this paper [16]. A key question arising from this study and previous observations is how does morphine show differential activation of TLR in microglia and macrophages, both cell types of myeloid origin. Particularly, how does morphine induce upregulation of TLR-2, -4 and -9 in microglia [16], while downregulating TLR-4 in macrophages [18]?

Mononuclear phagocytes express classical μ, κ and δ (MOR, KOR, DOR) opioid receptors, functionally coupled to signal transduction mechanisms involving mainly G_i protein. Morphine is able to induce a significant decrease of TLR-4 (but not TLR-2) mRNA expression in macrophages, and acute morphine exposure can compromise the capacity of macrophages to respond to LPS [8]. This decrease in membrane levels of TLR, which binds to LPS and MD2, affects downstream signaling pathways. Pro-inflammatory cytokine production mediated by NF-κB is significantly reduced in macrophages, animals and patients under opioid treatment, causing increased susceptibility to opportunistic intracellular microbial and HIV-1 infections [18].

Microglia respond to morphine in different ways compared to macrophages. Morphine activates microglia as indicated by retraction of processes, membrane ruffling, activation of ERK1/2 and enhanced chemotaxis and phagocytosis. Morphine increases microglial production of NO, pro-inflammatory cytokines and neuroexcitatory molecules [19]. Intriguingly, it has been shown that morphine induces neuroinflammation by binding solely to the LPS-binding pocket of MD2 protein, an accessory of the TLR-4 receptor, resulting in TLR-4/MD2 oligomerization and subsequent TLR-4 signaling leading to activation of NF-κB in a similar fashion to the classic TLR-4 ligand, LPS [19] (Figure 1). More importantly, this process was not only observed in microglia, but also in endothelial cells lining the CNS, which are exposed to high blood-borne morphine concentrations as it transits to the CNS [19]. On the other hand, how does one explain the increase in TLR expression in microglia? Perhaps there are differential effects of morphine on microglia cell line (BV2 [19]) and primary mouse microglia [16] and as well, morphine may activate multiple pathways to induce neuroinflammation. In light of findings that opioid activation of TLR-

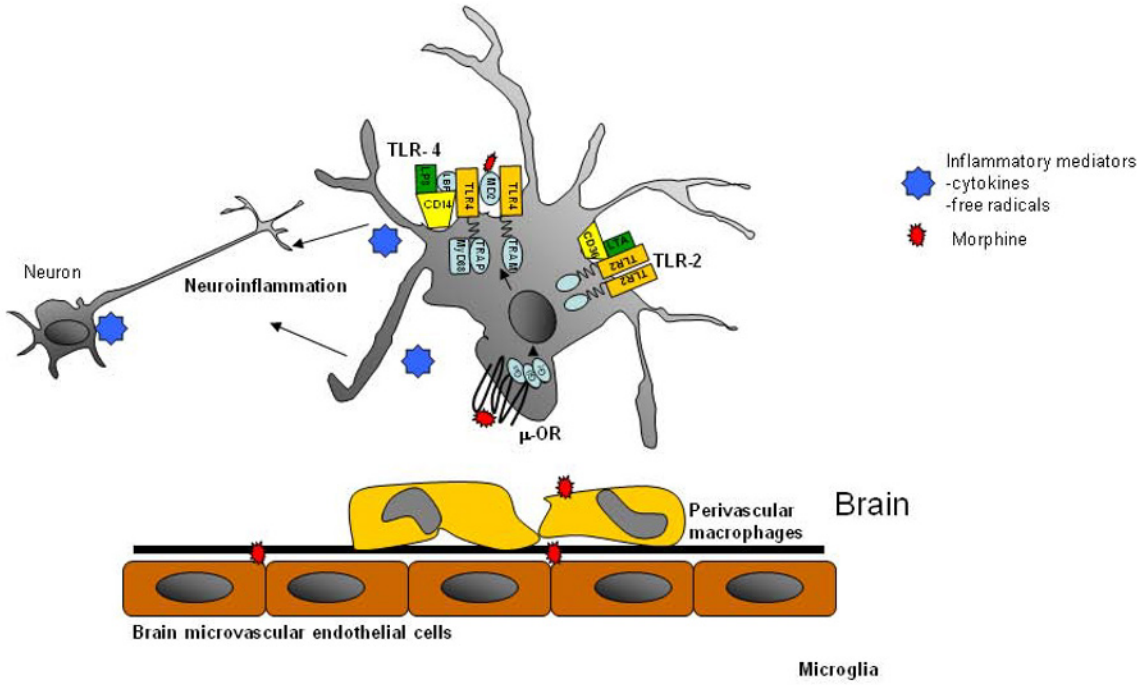

Figure 1. Microglia express both Toll-like receptors (TLR) and m opioid receptors (MOR). While lipopolysaccharide (LPS) binds to TLR-4-CD14 complex, the lipoteichoic acid (LTA) molecule of *Streptococcus pneumoniae* binds to the TLR-2-CD36 complex to initiate NF-κB signaling. Morphine binds to its cognate receptor, MOR and via unknown signaling mechanisms, increases expression of TLR. Morphine is also known to bind to the MD2 protein, an accessory protein in the TLR-4 complex. Binding of morphine to TLR and MOR increases the production of free radicals, pro-inflammatory cytokines and nitric oxide (NO) that results in neuroinflammation. The target cells are neurons that undergo damage and death leading to HIV-1 associated neuropathogenesis.

4 contributes to neuroinflammation [9], it could also be speculated that *S. pneumoniae* infection drives a pan-TLR response that is potentiated by morphine. Using an MOR-null mouse as well as MOR antagonists, Dutta *et al.* show that morphine increased TLR expression in microglia, suggesting that this is a MOR-dependent process [16]. Previously, they have shown that morphine impairs TLR-9-NF-κB signaling and diminishes bacterial clearance in resident alveolar macrophages [19]. These effects are independent from that of binding of opioids to the classical MOR. Dutta *et al.* show that in the presence of morphine, HIV-Tat and *S. pneumoniae* infections result in increased mortality, bacterial dissemination in the CNS, induction of pro-inflammatory cytokines in microglia and neuronal apoptosis, which are all TLR-dependent processes. But the increase in TLR expression is a MOR-dependent process. It could be hypothesized that both MOR and TLR are interacting with morphine and is necessary for neuroinflammation induced by morphine, HIV-Tat and *S. pneumoniae* (Figure 1), and a mouse model lacking MOR and TLR might be advantageous to test this hypothesis. Thus, morphine affects cytokine production in the periphery and in the CNS in different ways. Importantly, the contributions of perivascular macrophages (Figure 1) and infiltrating monocyte-derived macrophages in the CNS to morphine-dependent neuropathogenesis also remain to be identified. It would be interesting to determine whether the CNS microenvironment has an impact on how microglia and macrophages respond to morphine. Consequently, the response of TLR to morphine could be used as a means of differentiating between macrophages and microglia in neuropathogenesis. The synergistic effect of morphine in the induction of NF-κB by HIV-Tat and *S. pneumoniae* remains to be elucidated, and whether the cAMP-PKA-CREB signaling cascade is involved in morphine exposure and HIV-1 production in opioid receptor-positive HIV-1-infected cellular populations [13]. The present finding that morphine enhances TLR expression via MOR and NF-κB activation that is synergistically potentiated by HIV-Tat and *S. pneumoniae* in microglia suggest not only separate mechanisms of action, but might also be dependent on the cell type.

Opiate-induced exacerbation of the macrophage/microglial response to HIV-1 is likely to further contribute to neuronal dysfunction and death. As opiate abuse worsens disease outcomes and contributes to the development of HAND, identifying pathways involved in the synergy between germs and glia on one hand and opiates on the other will help in the development of novel therapeutic strategies.

Death by iron: how hypoxic microglia kill oligodendrocytes

Periventricular white matter injury (PWMI) refers to a spectrum of cerebral injuries that range from focal cystic necrotic lesions to diffuse

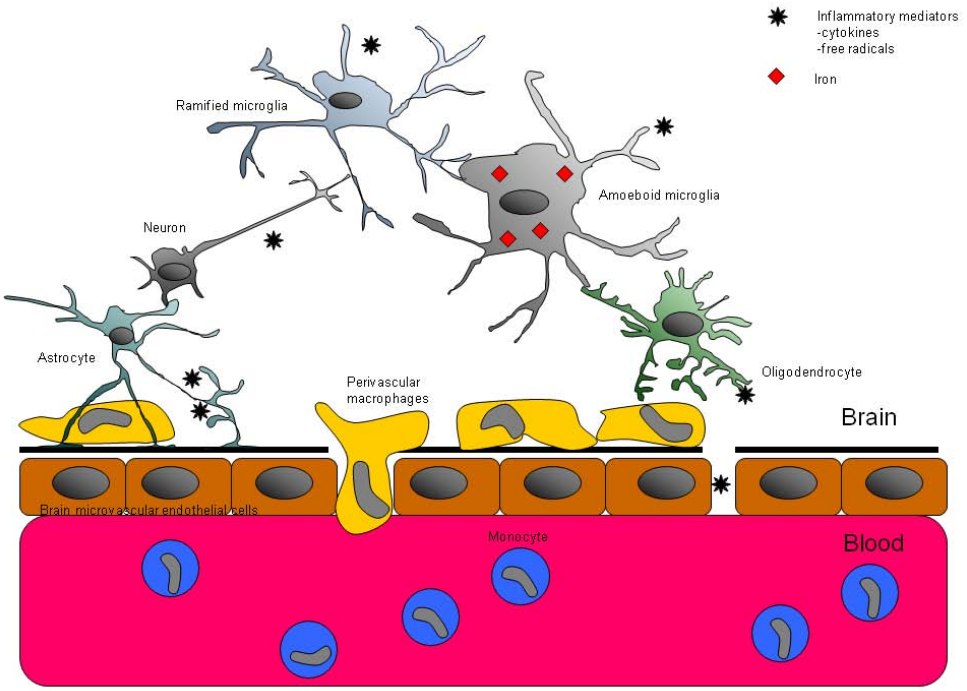

Figure 2. Amoeboid microglia accumulate iron during hypoxia and produce free radicals and inflammatory mediators including cytokines that cause death of oligodendrocytes. Normal ramified microglia on the other hand, contain low levels of iron, which increases significantly under hypoxic conditions.

myelination disturbances. Its major cause is hypoxia and is usually presented as symmetric lesions localized adjacent to both lateral ventricles [20]. It is the main cause of cerebral palsy in pre-term and neonatal infants. As well, globally, between 4 and 9 million newborns suffer birth asphyxia each year, leading to about 1.2 million deaths and an equal number of individuals with disabilities (National Center for Health Statistics, Hyattsville, MD, USA). Thus, there is a great need to understand the mechanism of PWMI and develop therapeutic interventions for this disease.

Pathological studies have shown astrogliosis and microgliosis in lesions of PWMI in human subjects [21]. Since microglia/macrophage activation is the first cellular event in and around the lesion following the insult, the role of microglia/macrophages in periventricular white matter neuropathogenesis needs to be elucidated. Microglia are the resident macrophages of the CNS and their numbers can range from 5% to 20% depending on the region of the brain [22]. Their functions include immune-surveillance and -response and phagocytosis in the nervous system. Microglia arise in the developing embryonic brain prior to vascularization. Fate mapping and lineage tracing studies showed that microglia are derived from primitive myeloid precursors in the yolk sac just before embryonic day 8 (E8) [23]. Precursors enter the developing brain and differentiate into microglia which guide invading vasculature to establish blood circulation in the developing CNS, in addition to its other functions [22].

Microglial progenitor cells that colonize the CNS during development might differentiate into amoeboid microglia [22]. Amoeboid microglia refers to microglial cells that penetrate the brain during early development, express surface antigens similar to, and share morphology and function with activated macrophages [22]. They can phagocytose cellular debris that arise during brain development and trim axonal projections and synapses; the latter process termed synaptic stripping [24], a part of a normal structural development of the CNS. Amoeboid microglia perhaps also transform into a ramified form with long processes as development proceeds, where the decrease in number of amoeboid microglia correlates to the increase in ramified microglia [22].

Ramified and amoeboid microglia could have distinct functions and responses to hypoxia. In this context, the paper by Rathnasamy *et al.* [25] describes the unique role played by amoeboid microglia during hypoxia in causing tissue damage mediated by iron. They demonstrated that hypoxia caused an increase in iron concentration and that the iron was specifically found to accumulate in lectin-positive microglia in the periventricular white matter of neonatal rat brain. Macrophages are able to regulate iron homeostasis by recovering iron from old red blood cells and returning it to circulation for binding to transferrin, a glycoprotein that binds to iron tightly but reversibly. Iron retention within activated macrophages is due to increased iron uptake and decreased iron export [26]. Iron is bound to transferrin and the hydrophilic nature of this complex would normally prevent its passage into the brain. However, brain capillary

endothelial cells express transferrin receptor 1 (TfR1), which facilitates iron movement into the brain. As shown in Rathnasamy *et al.* [25], under normal conditions, microglia contain low levels of iron, which dramatically increase under hypoxic conditions. On the other hand, subtypes of tissue macrophages regulate iron homeostasis differently, where alternatively activated macrophages are better at iron export than classically activated macrophages [26]. Alternatively activated macrophages (M2 polarization) function to dampen the inflammatory response, control growth and allow tissue repair. Classically activated macrophages (M2 polarization) are important drivers of the inflammatory response. M1 macrophages are activated by microbes and/or Th1 cytokines and are associated with the production of free radicals and pro-inflammatory cytokines. Thus, it has been shown that various tissue macrophages exert different functions depending on the stimuli.

Cellular iron homeostasis is regulated by two cytosolic proteins, the iron regulatory proteins (IRP 1 and 2) and the dysregulation of these proteins has been shown to be responsible for various neuropathologies. It has been shown that increased levels of IRP2 expression in M2 macrophages leads to upregulation of TfR1, which is associated with iron release [26]. However, Rathnasamy *et al.* demonstrated that amoeboid microglia (possibly inflammatory as evidenced by increased expression of TNFα and IL-1β) upregulate IRP and TfR as a consequence of hypoxia, and is associated with iron retention by the microglia [25], suggesting that the effect of upregulation of IRP and TfR is context-dependent. Furthermore, since differences in iron levels are associated with distinct phenotypical heterogeneity of M1/M2 macrophage populations, it could be speculated that similar heterogeneity might exist among microglia.

In the CNS, myelination and inflammatory processes involve iron. Traditionally, the view has been that oligodendrocytes, the myelin-producing cells of the CNS, possess high concentrations of iron, due to the enzymes involved in lipid production that utilize iron as a component of their catalytic center [27]. However, during hypoxia, amoeboid microglia have higher iron content than that found in oligodendrocytes. Iron is primarily present in microglia during the first two weeks of postnatal development but in the adult CNS these iron stores shift to oligodendrocytes to meet the requirements of myelination. Quite naturally, during CNS pathology, disruption of iron metabolism and homeostasis leads to oxidative damage in oligodendrocytes [28].

Rathnasamy *et al.* [25] show that hypoxia reduces the levels of the antioxidant enzyme, glutathione (GSH), in oligodendrocytes, making them vulnerable to hypoxia-induced death by free radical-mediated mechanisms. Along with death of oligodendrocytes, conditioned medium from hypoxic microglia also blocked oligodendrocyte proliferation, due to the pro-inflammatory cytokine, IL-1β [25]. Indeed, high levels of iron have been suggested to promote neuropathogenesis of multiple sclerosis (MS), which prompted clinical trials involving the therapeutic compound Desferal (Novartis International AG, Basel, Switzerland, generic name deferrioxamine) that was administered to MS patients. Results, however, were inconclusive and larger double-blind trials need to be performed to resolve the question of whether Desferal, indeed acts to suppress MS neuropathogenesis by limiting iron-catalyzed free-radical tissue damage, demonstrated in pre-clinical mouse models [27]. Unfortunately, contrasting results in mouse models also contributed to the discrepancies and currently there seem to be no active clinical trials with Desferal. The paper by Rathnasamy *et al.* [25] provides a mechanistic approach to the problem of iron overload in the CNS, which has also been suggested to be the instigator of MS neuropathogenesis. Recent controversial treatments for MS, such as balloon angioplasty is based on the hypothesis that MS is caused due to constriction of the neck veins leading to increase in iron load in the CNS [29].

In the light of these findings in MS patients, the study by Rathnasamy *et al.* [25] sheds light on iron-mediated neuropathogenesis since microglia seem to be the culprits, wherein iron from hypoxic microglia cause neonatal rat periventricular white matter damage through the production of pro-inflammatory cytokines and reactive oxygen/nitrogen species. In fact, in this study, it was found that the levels of iron in oligodendrocytes was low and did not contribute to the pathogenesis. Conditioned medium from hypoxic microglia contained a toxic cocktail of cytokines and free radicals that killed oligodendrocytes but was rescued by the iron chelator, deferoxamine. While this adverse effect can be prevented by deferoxamine treatment *in vitro*, it is not known if deferoxamine administered to neonatal rats can prevent hypoxia-induced PWMI. Another aspect that remains unknown is whether a similar mechanism contributes to the axonal damage seen due to hypoxia. The lack of myelin sheaths on axons, and loss of trophic support due to death of oligodendrocytes, causes axonal damage, but it is reasonable to speculate that free radicals and inflammatory cytokines produced by hypoxic microglia can damage axonal processes of neurons. Also, another interesting aspect of this study is the potential role of astrocytes in mediating oligodendrocyte death. Astrocytes are known to mediate oligodendrocyte death through a free radical-mediated pathway. Since hypoxic microglia are also known to induce release of TNFα and IL-1β by astrocytes, it is possible that in PWMI, microglia and astrocytes synergistically contribute to oligodendrocyte death (Figure 2).

Thus, the finding that amoeboid microglia accumulate iron during hypoxia and produce free radicals and pro-inflammatory cytokines, causing oligodendrocyte death, may underlie the mechanism of PWMI [25]. This study has important implications not only for the role of microglia in iron-mediated neuropathogenesis but also has far reaching implications in neurodegenerative diseases of the white matter.

Conclusion

Glial cells regulate several processes that are crucial for brain development, protection against insults and repair in the event of damage. There is ample evidence that glia, while making up 85% of the brain, are tasked with several goals during physiological and pathological processes. During HIV-1

infection of the brain, glial cells respond to the infection, while at the same time battling the neurotoxicity unleashed. The same is true when the nervous system is flushed with toxic components of recreational drugs and pathogens that opportunistically invade when the body is immune-compromised. The few studies that are highlighted here detail the intricacies of the processes involved. There might be other mechanisms that also play a role but remain undefined.

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