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INVITED REVIEW

Osteopontin/secreted phosphoprotein-1 harnesses glial-, immune-, and neuronal cell ligand-receptor interactions to sense and regulate acute and chronic neuroinflammation

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Abstract

Osteopontin (OPN) also known by its official gene designation secreted phosphoprotein-1 (SPP1) is a fascinating, multifunctional protein expressed in a number of cell types that functions not only in intercellular communication, but also in the extracellular matrix (ECM). OPN/SPP1 possesses cytokine, chemokine, and signal transduction functions by virtue of modular structural motifs that provide interaction surfaces for integrins and CD44-variant receptors. In humans, there are three experimentally verified splice variants of OPN/SPP1 and CD44's ten exons are also alternatively spiced in a cell/tissue-specific manner, although very little is known about how this is regulated in the central nervous system (CNS). Post-translational modifications of phosphorylation, glycosylation, and localized cleavage by specific proteases in the cells and tissues where OPN/SPP1 functions, provides additional layers of specificity. However, the former make elucidating the exact molecular mechanisms of OPN/SPP1 function more complex. Flexibility in OPN/SPP1 structure and its engagement with integrins having the ability to transmit signals in inside-out and outside-in direction, is likely why OPN/SPP1 can serve as an early detector of inflammation and ongoing tissue damage in response to cancer, stroke, traumatic brain injury, pathogenic infection, and neurodegeneration, processes that impair tissue homeostasis. This review will focus on what is currently known about OPN/SPP1 function in the brain.

KEYWORDS
CD44, extracellular matrix, integrins, microglia, splice variants, striatum

1 | INTRODUCTION

Our understanding that brain microglia display diversity in gene expression depending on developmental age, region, and in the context of health and disease has rapidly increased over the last several years. The advances emerge from the insights of genetic and molecular tools, which permit interrogation at the single-cell level. Microglia are the innate immune cell guardians of the central nervous system responding to infection, injury, or any chronic condition that disrupts normal homeostatic functions. While common to all the former insults is the initiation of an inflammatory response, the exact molecular mechanisms in each context differ and must be defined. Although not without obvious limitations, rodent models

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for the study of neuro-immune mechanisms are invaluable in allowing researchers to design targeted and carefully controlled studies not possible in humans. The barriers have been particularly acute for studying neurological complications and associated central nervous system (CNS) immune impacts of the human immunodeficiency virus type-1 (HIV-1). While antiretroviral therapy is highly successful in preventing the spread between people, it is not curative. Based on some estimates, approximately 30%-50% of those living and aging with HIV have an elevated risk for neurologic complications,[1] although other findings suggest a much lower prevalence.[2] The virus assumes latent infection in resting CD4 lymphocytes and brain microglia, which turnover very slowly by some estimates, with a half-life of four or more years.[3,4] Current therapies for HIV cannot eliminate these reservoirs and other viral tissue sanctuary sites.[5]

Several models of humanized mice have allowed recent advances in probing the molecular basis of neuro-immune interactions.[6-8] This review will focus on emerging insights on the role of osteopontin (protein name)/secreted phosphoprotein-1 (gene designation) at the intersection of the nervous and immune systems in the field known as neuroimmunology. Single-cell RNA sequencing from the last several years has implicated OPN/SPP1 as a highly expressed gene in microglia of the early postnatal brain[9] and in adults after injury.[10-12] Furthermore, OPN/SPP1 knockout mice are viable and show no gross physical or behavioral abnormalities but disorganized wound remodeling and defective macrophage infiltration after injury or infection is seen.[13] However, little is known about OPN/SPP1's fundamental basic molecular mechanism (s) of action in the injured central nervous system. We focus on how OPN/SPP1's innate immune function is activated in the context of viral infection and the ensuing impact on neurons and homeostatic regulation but believe the findings have broad applicability. Indeed, OPN/SPP1 is a potent sensor in the central nervous system responding to different types of acute or cumulative damage in cellular/tissue state or physiology that disrupts homeostasis, for example, in neurodegenerative disorders in which inflammation is a prominent component.[14] In multiple sclerosis and Alzheimer's disease (AD) models and humans, OPN/SPP1 is elevated in the plasma and CSF in those with advanced disease.[15-19] It is also a member of a highly expressed gene signature of so-called neurodegenerative microglia that includes ApoE.[10-12] Through ongoing research, we aim to gain a deeper mechanistic understanding of OPN/SPP1 function as an upstream regulator of neuroinflammatory signaling, identify the branch point(s) at which its downstream signals bifurcate to activate gene expression pathways that (1) are neuroprotective, (2) facilitate immune cell recruitment via tissue-level signals, and (3) modulate pro-inflammatory signaling via glial-neuronal crosstalk, all to restore homeostasis in the CNS.

2 | OPN/SPP1 STRUCTURE-ACTIVITY RELATIONSHIPS THAT INFLUENCE FUNCTION

Although first reported in osteoclasts and independently as a highly phosphorylated protein in bone matrix,[20,21] other cells including T, B, NK, and NKT lymphocytes, myeloid cells, osteoblasts, osteocytes, epithelial cells, and specific neurons express OPN/SPP1. Given this cellular distribution, OPN/SPP1 expression can be detected in joints, adipose tissue, liver, lung, and at the highest levels in brain tissue. Most OPN/SPP1 mRNAs encode a traditional signal peptide allowing cellular secretion and accumulation in blood, urine, bile, and milk.[22,23] No crystal structure is available for OPN/SPP1. However, biophysical modeling studies predict that the protein folds into two distinct alpha-helical and beta-pleated sheet conformations with a disordered tertiary structure.[24,25] Intrinsically disordered proteins have the property of forming more stable structures upon binding to their targets.[25] There are several excellent detailed reviews on the history of OPN/SPP1 discovery, its protein structure, and a general overview of its function in bone tissue, cancer, and angiogenesis.[14,26,27] An additional body of work has explored the role of a non-secreted form OPN/SPP1 involved in dendritic cell type-1 interferon immunity.[28-30] We will highlight herein what is currently known about key aspects of OPN/SPP1 function in the brain.

OPN/SPP1, a highly acidic protein of 45-75 kDa, participates in diverse cellular pathways through the use of (1) discrete functional domains, (2) by serving as a substrate for specific proteases, (3) post-translational modifications, and (4) the activity of splice variants. Indeed, variation in its apparent molecular weight seen upon native or denaturing SDS-PAGE reflects the type and extent of post-translational modifications. Specific amino acid motifs allow OPN/SPP1 to bind to integrins and CD44 and interact with calcium-binding factors and heparin.[31-35] Integrins form heterodimers and have the unique capacity to signal from the cell surface to the nucleus or in the reverse direction, sensing an intracellular signal transmitted outside the cell.[36,37] Integrins' additional layer of signaling specificity lies in their differential cell- and tissue-specific expression.

Cleavage sites in OPN/SPP1 for specific matrix metalloproteases and thrombin result in N-terminal and C-terminal peptides with biologic function controlling the polarization of Th_1 and Th_2 cell subsets.[38-43] Through the engagement of specific integrins, OPN/SPP1 can activate phosphoinositide-3 kinase and MAPK cascades leading to the inhibition of NF-kB from repressive complexes in the cytoplasm, allowing it to enter the nucleus and drive gene expression associated with immunity, vascular disease,[44] tissue remodeling, cell migration, or cell survival.[45] Using deletion analyses studies, we found that distinct OPN/SPP1 protein motifs such as the aspartate domain, C- and N-terminal peptides, and the calcium or CD44-binding domains possess fusion or adhesive properties that enhance HIV-1 cell-to-cell spread.[46]

OPN/SPP1 is phosphorylated on at least 25% of its acidic residues, depending on the specific cell type.[47,48] The highly acidic charge of OPN/SPP1 confers on the bone's ability to regulate mineralization in extracellular matrix (ECM).[47,49] N-linked and O-linked glycosylation[50-52] as well as transglutamination, which results in the increased polymerization of OPN/SPP1 in the ECM, can also play a role in either masking interaction domains or enhancing select functions of OPN/SPP1 in the particular cell and tissue relevant context.[53-56] Given the sheer number of acidic residues and, therefore, the limits of studying the direct contributions of phosphorylation to

OPN/SPP1 activity in vivo, most studies in this regard are biochemical, use modified peptides, mass spectrometry analyses, and cell-based assays to then infer the likely impact of phosphorylation on functions like integrin-binding ability.[23,57]

Therefore, a central key concept in understanding whether OPN/SPP1 function contributes to disease progression or resolution depends on whether the injury is (1) acute or chronic, (2) which cells express OPN/SPP1, (3) the type of tissue, and (4) what receptors are activated in the local microenvironment. For example, in multiple sclerosis, in which high OPN/SPP1 is associated with a worsening disease, studies from a mouse model of the disorder found that OPN/SPP1 through NF-κB and Fox3a activation promotes the survival of myelin-reactive T-cells.[58] In sporadic amyotrophic lateral sclerosis (ALS), a recent study using an expression-weighted cell-type transcriptome enrichment strategy on a human cohort and two different ALS mouse models implicated elevated OPN/SPP1 expression in perivascular fibroblasts (also known as vascular leptomeningeal cells)[59,60] and their altered function commencing before the manifestation of disease symptomology.[61] In contrast, in the eye, it was found that treatment of retinal ganglion cells having severed axons with OPN/SPP1 protein and insulin growth factor-1 (IGF-1) resulted in significantly enhanced regrowth of axons than with IGF-1 alone.[62]

Adding to the complexity of understanding OPN/SPP1 function in specific cellular contexts is the expression of distinct allelic variants resulting from alternative splicing.[63] The interaction surfaces of three verified allelic variants (OPN-a, OPN-b, and OPN-c), including integrin-, calcium-, and CD44/heparin-binding regions, have been identified.[31,47,50] The OPN-a transcript is selectively increased in HIV-infected human macrophages, and anti-sera that detect this variant was used in subsequent studies.[46,64,65] Future studies will ascertain whether specific OPN/SPP1 splice variants or peptides are expressed in brain glial and neuronal subpopulations and play differential roles in neuroinflammatory signaling. Given the need to substantiate form with function, identifying and studying the relevant tissue-specific OPN/SPP1 variant (s) would advance the field.

3 | A SENSOR OF CNS INJURY AND INDUCER OF NEUROPROTECTIVE SIGNALING

Several years ago, we and others reported that OPN/SPP1 in the cerebrospinal fluid was highest in HIV-infected individuals with moderate-to-severe cognitive impairment (CI).[64,66] In addition, both astrocytes and microglia in HIV-infected human brain tissue showed reactivity to monoclonal anti-sera against OPN/SPP1 protein. However, the levels in those with CI were significantly different in the latter.[65] Given this association and findings from an in vitro study of HIV-infected human macrophages where we found that OPN/SPP1 was a significantly upregulated gene,[46] we did not know whether the response was an effort of the host to protect cells or a stealth property of the virus from enhancing its replication potential. In addition, OPN/SPP1 can activate the transcription factor NF-κB, a potent activator of HIV gene expression in immune cells. Therefore, we hypothesized that increased OPN/SPP1 expression in the brain would enhance HIV replication and exacerbate viral-mediated damage to neuronal integrity. However, using primary cultures of rat cortical neurons, we found that, instead, OPN/SPP1 protected these cells from the damaging effects of HIV envelope protein on axonal and dendritic spine integrity.[67,68]

Interestingly, the mechanism involves a two-pronged activation of the mammalian target of rapamycin (mTORC1/2) pathway signaling.[67] G-protein coupled receptors, such as chemokine receptor CXCR4, but not CD4, are expressed on many neurons. However, the HIV envelope requires both of the latter receptors to successfully fuse to the target cell membrane and release its pre-integration complex into the cell. Interaction of the HIV envelope with neuronal CXCR4 or CCR5 chemokine receptors induces pathologic excitotoxicity and cell death.[69-71] HIV envelope engagement of CXCR4 alone on cortical neurons stimulates mTORC2 downstream signaling through stress glucocorticoid kinase 1 (SGK1) and phosphorylation of AKT at S473.[67] Concomitantly, OPN/SPP1 binding and signaling through β1 integrin receptors activates mTORC1 pathway signaling through p70 S6 kinase, pS6 ribosomal protein to increase axon length and the density of dendritic spines on cortical neurons.[67] Studies examining neurite regrowth after axotomy found that a combination of insulin growth factor-1 and OPN/SPP1 via mTORC signaling was superior at promoting regrowth of available corticospinal tracts than IGF-1 alone.[62] While mTORC signaling controls HIV latency,[72] autophagy, apoptosis,[73] and even homing of gut $CCR6^{+}CD4^{+}$ T-cells[74] before our study, little had been published about mTORC roles in HIV neuropathologic mechanisms.

Seminal studies showed that OPN/SPP1 plays a neuroprotective role in stroke.[75-77] More recent investigations suggest that the mechanism is partly related to the balance in inflammatory to anti-inflammatory signaling.[75] In a rat model of ischemia, intranasal delivery of an OPN/SPP1 seven-amino acid peptide confers protection against ischemia. The heptamer peptide downregulates the expression of IL-1β, IL-6, and TNF-α[78] through a putative mechanism involving αvβ3 integrin.[79] A very recent elegant study delineated a mechanism between T-regulatory cells expressing OPN/SPP1 that infiltrate the brain after chronic stroke injury and engage integrin-β1 expressed specifically on microglia to stimulate the proliferation and differentiation of new oligodendrocytes having the capacity to remyelinate the injured brain.[80] Details of the exact mechanisms remain to be further explored. Another recent study showed that dying neurons in the acute phase express OPN/SPP1 and induce similar expression in infiltrating monocytes that ultimately cooperate with other glial cells to phagocytize the damaged neurons.[81]

Hippocampal neurons play essential roles in the consolidation of memory learning and the regulation of emotions. The neurochemical messenger dopamine regulates reward responses and is impaired in untreated HIV infection and those experiencing long-term neurologic complications.[71,82] We found that OPN/SPP1 protected against HIV Env induced decreases in dendritic spine density that

depended on β1 integrin expression on rat hippocampal neuronal cultures devoid of glial cells.[68] Moreover, through the engagement of β3-integrin signaling, OPN/SPP1 alone increased spine density.[68] At least ten different integrin heterodimers are expressed in the central nervous system, and their roles in regulating the strength and propagation of electrochemical signals have long been known.[83] Multiple significant ligand-receptor interactions occur at the synaptic cleft.[84-86] The strength and timescale of these interactions are influenced by perineuronal nets, which support synaptic plasticity and protect cells from oxidative stress.[87-90] These cellular/molecular strategies to protect neuronal function are collectively known as homeostatic plasticity.[91,92] The disruption of homeostatic plasticity likely underlies several neurological and neuropsychiatric disorders, given the direct evidence for alterations in integrin expression and positive associations with specific allelic variants.[36] We found that changes in PNN density were regulated in a differential fashion by signaling through β1- or β3-integrins in the presence of HIV Env and OPN/SPP1.[68] Based on what is currently known about the contributions of PNN to signal propagation, we suggest that OPN/SPP1, in its role as an extracellular matrix protein, facilitates proper signal transmission. In the brain, glial cells can also express PNNs and a variety of other ECM proteins supporting the health and integrity of the basement membrane. As neuroinflammatory processes can disrupt basement membrane physiology, a fuller understanding of the mechanisms requires multicellular systems and in vivo models that would allow such contextual insights.[93]

The second important receptor for OPN/SPP1 is CD44, a widely expressed multifunctional transmembrane glycoprotein involved in activating cells as diverse as immune and epithelial cells and keratinocytes.[94-96] The complexity in CD44 expression lies partly in the alternative splicing of its ten exons and differential expression during development, adulthood, and post-translational regulatory mechanisms.[40,97,98] The most common form found on immune cells is called CD44 standard (CD44s), with variants designated as CD44v followed by the number of the exon (s) included in the mature transcript. There is evidence of a linkage between variant expression associated with pro-inflammatory cytokines like IFN-γ and function in health versus cancer.[33,99] In specific contexts, the function of CD44 may be regulated by the stability of transcript expression. In the brain, neurons, glia, and astrocytes, in particular, display marked region-specific heterogeneity in allelic variant expression.[100-102] Differentially expressed CD44 allelic variants have been found in the basal ganglia, hippocampus, cerebellum, and spinal cord.[100-102] CD44 activation has been reported for both pro-and anti-inflammatory signaling.[103,104] In a non-human primate model of viral-induced neuroinflammation, the trafficking of CD44v6 expressing monocytes to the brain predicted which animals developed encephalitis.[105,106] Importantly, with the CD44 interaction domain in OPN/SPP1 located at its C-terminus, cleavage of OPN/SPP1 by thrombin or MMPs leads to the exposure of cryptic epitopes that facilitate ligand-receptor engagement in cancer cells.[33] Whether similar such interactions occur among cells in the CNS remains to be defined.

The extracellular domains of CD44 variants are substrates for extensive glycosylation and other interactions within the ECM with proteoglycans, hyaluronan, growth factors, and cytokines.[96] Many peptidases and proteases that target OPN/SPP1 in the ECM can also modify CD44. This suggests that both ligand and receptor activity can be modified to satisfy gene expression needs in particular contexts. OPN/SPP1 interaction with CD44 on immune cells has been reported. However, far less is known about the functional consequences of OPN/SPP1 engagement of CD44 on astrocytes. Given astrocytes' critical trophic and neuroprotective roles, such signaling is expected to contribute to molecular mechanisms that resolve neuroinflammation, for example, in the context of HIV breach of the brain in which the function of astrocytes is dysregulated.[107-110] A common principle and critically important consideration when investigating and interpreting results are that the same protein, if expressed, often has differential functions in the developing versus adult brain and in health versus injury or disease. This strategy is a powerful demonstration of the economy and agile nature of the biological design. Given the multilayer context-dependent levels of CD44 regulation, there is a clear need for additional research. Most recent studies have reinforced the existing strong associations of CD44 with cancer pathogenesis. The rapidly emerging use of sc-RNA sequencing will be invaluable in allowing a deeper investigation of CD44v expression under health and disease and inform experimental designs to gain deeper mechanistic insights about OPN/SPP1-CD44 function in nervous system cells.

4 | CROSSTALK WITH A MICROGLIAL-NIGROSTRIATAL NEUROINFLAMMATORY NETWORK

Convincing evidence for the strong association between pro-inflammatory gene expression and many neurodegenerative disorders and neuropsychiatric conditions reported over the last 5-10 years has focused attention on the roles of non-neuronal cells in the initiation, development, and chronic persistence of neuronal dysfunction.

A unique vantage point provided by the study of HIV's detrimental impact on the CNS is that virus replication in brain microglia and macrophages and T-cells infiltrating across the blood-brain barrier (BBB) activates innate immune signaling.[111] Before much was known about HIV-1 pathogenesis, 40 years ago, individuals presenting with dementia were the defining hallmark of infection and a profound state of immunodeficiency. Today, one remaining but formidable barrier despite current life-sustaining therapies is the problem of long-lived peripheral and CNS tissue reservoirs from which replicating HIV can re-emerge.[112] Indeed, the former, combined with the fact that none of the therapies block HIV transcription, means that the body continues to, in a chronic fashion, respond through innate and adaptive immune response signaling.[113] In this regard, people living and aging with HIV infection are impacted by comorbid

conditions such as cardiovascular disease, metabolic disorder, and certain cancers, exacerbated by chronic systemic- and neuroinflammatory signaling.[114]

Given the strong association of OPN/SPP1 expression with HIV infection and neurologic disorder,[64,66,105] we hypothesized that this protein synergizes with HIV in worsening microglial activation and neuronal injury. We turned to an established humanized mice model shown to recapitulate aspects of HIV CNS dysfunction to test this hypothesis. Mouse neonatal pups (1-2 days old) of the NOD.Cg-Prkdc^{scid}-IL2r$^{\gamma tm1Wjl/SzJ}$ strain were engrafted with human CD34^{+} hematopoietic stem cells reconstituting mice with human T-cells, B-cells, and monocytes and hence providing cellular targets for HIV-1 infection. Global OPN/SPP1 expression was inhibited 2 weeks after HIV infection and in controls using previously validated aptamers shown to block both RNA and protein production.[115] To our surprise, we found that knockdown of OPN/SPP1 expression in HIV-infected adult CD34^{+} humanized mice exacerbated neuroinflammation as measured by the activation of microglia quantified using translocator protein (TSPO) positron emission tomography (PET) neuroimaging.[115] DPA-713 is a high-affinity ligand for the peripheral benzodiazepine receptor (aka TSPO receptor) expressed in the outer mitochondrial membrane and elevated in neurodegenerative disorders.[116-118] When microglia and, to a lesser extent, astrocytes are activated, TSPO receptor expression increases.

Interestingly, suppression of OPN/SPP1 in uninfected control mice did not change basal neuroinflammation.[115] These results demonstrate that OPN/SPP1, by mechanisms that remain to be defined, (1) senses pathologic changes in brain homeostatic states and (2) regulates the neuroinflammatory response.[68] Interestingly, in HIV-infected mice, a specific region of the brain known as the striatum, a region necessary for cognitive function, decision-making, and reward, showed intense labeling for TSPO.[115] Moreover, a subgroup of these TSPO+ neurons stained for tyrosine hydroxylase positive (TH+), a well-established marker for dopaminergic neurons. The highly selective reactivity of TSPO+TH+ neurons in the striatum was quite notable and suggested that these cells respond to/sense the inflammatory state of the brain.[68] These findings indicate that OPN/SPP1 has a role in regulating a "neuroinflammatory" pathway through dopaminergic nigrostriatal signaling. Interestingly, seven different dopaminergic neuronal populations have been identified in both mice and humans.[119-121] Much is known about the activation/disruption of dopaminergic signals in people with HIV[71,122-125] and from mouse models of HIV Tat/substance use disorders.[71,126-130] The vulnerability of striatal dopaminergic neurons was demonstrated in a simian immunodeficiency virus model by Scheller et al, and in people with HIV.[125,131,132] A series of seminal and follow-up studies showed that injury by HIV Tat alone and with opioids leads to selective loss of MOR+ dopaminergic neurons in the striatum.[71,124,133] Degradation of striatal and cortical circuits while aging with HIV and ART treatment continues to be reported.[122,123,134-137] Moreover, dysregulation of dopaminergic signaling by chronic, even low-grade system-wide inflammation is strongly associated with psychiatric disorders.[138,139] A study in 2021 found that brain levels of HIV RNA in human tissue from the National NeuroAIDS Tissue Consortium (NNTC) correlate with increased inflammation in the basal ganglia and white matter.[140] Recognizing the limitations of the available human studies, variation in radioligands, and how radiotracer uptake was measured,[2,141] several TSPO neuroimaging studies in people with HIV found evidence of microglial activation.[142-145] A recent study demonstrated in mice that long-term treatment with diazepam

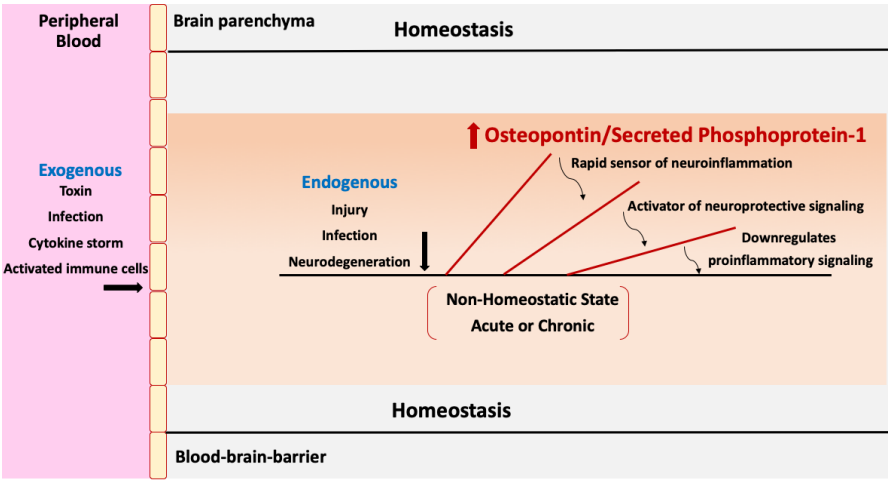

FIGURE 1 In the healthy brain, neuronal function or homeostatic plasticity is optimal with positive impact on cognition, mood, well-being, and behavior. Any disturbance to this homeostatic state due to exogenous factors circulating in peripheral blood that disrupt the function or integrity of the blood-brain barrier will induce an increase in OPN/SPP1 in perivascular cells, immune cells, glial, and specific neuronal cell populations. Likewise, infection and neurodegenerative processes occurring in the brain parenchyma will also elevate OPN/SPP1 expression. All of the aforementioned brain cell types express the receptors for OPN/SPP1, integrins, CD44, or a combination of both. The result is the activation of gene expression pathways that are neuroprotective and serve to downregulate the pro-inflammatory response as the damaging stimulus is cleared

accelerated microglia engulfment of synapses and cognitive impairment through TSPO, as the effects were mitigated in TSPO knockout animals.[146]

Importantly, we felt it necessary to first conduct our HIV rodent study without antiretroviral treatment, given some evidence suggesting that specific therapies may worsen neuroinflammation.[2] Seminal studies in the 1980s-1990s using postmortem brain and CSF from HIV-infected people reported on what has been rediscovered during the current COVID19 pandemic as a "cytokine storm".[147] Even now, many people with HIV on therapy continue to experience elevated levels of pro-inflammatory cytokine expression in plasma and the CNS in what is referred to as systemic inflammation.[113] To the best of our knowledge, our study represents the first report in a humanized mouse model for HIV-induced neuroinflammation as quantified by PET-neuroimaging.[68] The fact that this model could recapitulate this aspect of HIV CNS damage suggests that it is a suitable system to further investigate details of the molecular mechanisms.

Surprisingly, a comprehensive understanding of the molecular function (s) of TSPO in neuroinflammation is lacking. There was some evidence to suggest that TSPO played a role in steroid synthesis; however, embryonic knockouts of TSPO are viable and show no defects in this pathway, and microglia of both humans and mice lack the enzymes to convert cholesterol into steroids.[148] Other data suggest that TSPO may have an anti-inflammatory role,[141] decreasing NF-κB activation and pro-inflammatory cytokine gene expression.[149] Interestingly, immune cells express TSPO receptors through which their regulatory activity can be influenced by the binding and downstream signaling of benzodiazepines.[150,151] In addition, a recent study reported increased expression of the TSPO receptor on immune cells in treated HIV+ people suggesting a possible association with inflammatory signaling.[152]

5 | CONCLUDING REMARKS

Advances in molecular-based single-cell technologies and data-mining approaches[61,153] are helping us understand microglial phenotype and inferred functional heterogeneity in unhealthy, healthy, and aging brain.[154-158] Immune-altered microglial subtypes in the midbrain of healthy C57Bl mice displayed an inflammatory gene expression profile and appear primed for immunologic responses.[155,159] Alternatively, tolerogenic microglial subpopulations found in the cortex, hippocampus, and cerebellum are elicited after a second exposure to lipopolysaccharide.[160,161] With the continued refinement of experimental models, we are gaining a clearer picture of the intricate dance orchestrated between interactions at the blood-brain barrier,[162] in brain subcellular structures and individual players (glial, neurons, immune cells, and support cells) of the CNS and innate/adaptive immune systems.[163] An essential theme in strategies aimed at ameliorating the devastating impacts of neurodegenerative disorders, acute injury, and neuroinfectious sequelae such as HIV-1, prion disease,[164] or SARS-CoV-2, is shifting the neuroimmunological response from one that solely senses and responds to damage. To return to the healthy homeostatic state and minimize lasting damage to the CNS, there must be timely and efficient downshifting and switching from inflammatory to neuroprotective signaling. OPN/SPP1 has the ideal genetic architecture, molecular properties, and functional versatility to serve in such capacity (Figure 1). OPN/SPP1 engages the requisite integrin or CD44v receptors expressed in the affected local microenvironment outside or inside the cell and propagates the necessary downstream signals. However, while we have a few clues, much more research is needed in each relevant disease context to fully understand the molecular details in order to develop effective targeted therapeutic strategies.

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