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REVIEW ARTICLE

HIV Associated Neurodegenerative Disorders: A New Perspective on the Role of Lipid Rafts in Gp120-Mediated Neurotoxicity

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Abstract: The implementation of combination antiretroviral therapy (cART) as the primary means of treatment for HIV infection has achieved a dramatic decline in deaths attributed to AIDS and the reduced incidence of severe forms of HIV-associated neurocognitive disorders (HAND) in infected individuals. Despite these advances, milder forms of HAND persist and prevalence of these forms of neurocognitive impairment are rising with the aging population of HIV infected individuals. HIV enters the CNS early in the pathophysiology establishing persistent infection in resident macrophages and glial cells. These infected cells, in turn, secrete neurotoxic viral proteins, inflammatory cytokines, and small metabolites thought to contribute to neurodegenerative processes. The viral envelope protein gp120 has been identified as a potent neurotoxin affecting neurodegeneration via indirect and direct mechanisms involving interactions with chemokine co-receptors CCR5 and CXCR4. This short review focuses on gp120 neurotropism and associated mechanisms of neurotoxicity linked to chemokine receptors CCR5 and CXCR4 with a new perspective on plasma membrane lipid rafts as an active participant in gp120-mediated neurodegeneration underlying HIV induced CNS pathology.

Keywords: gp120, neurodegeneration, lipid raft, actin-cofilin rod, cholesterol, neuron, CNS.

1. INTRODUCTION

Human immunodeficiency virus (HIV), a member of the lentivirus genus of the Retroviridae family of RNA viruses, is the etiologic agent responsible for acquired immune deficiency syndrome (AIDS) and its associated complications, which has been responsible for 35 million deaths and more than 70 million infections worldwide since first emerging in the early 1980s [1]. As with other lentiviruses, HIV causes chronic disease in infected individuals with a characteristic period of clinical latency and persistent viral replication. Advances in understanding the virus and improvements in diagnosis, treatment, and outcomes of the disease have led to a substantial reduction in HIV-related mortality. However, as the epidemiology of HIV/AIDS shifts from an acute infection to chronic disease, new challenges emerge in terms of management of long-term infection and associated comorbidities that can have profound impacts on quality of life for infected individuals. HIV isolates are grouped into two types of which HIV-type 1 (HIV-1) is the strain responsible for the present worldwide epidemic of AIDS and is the principal subject of this review.

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1.1. HIV-associated Neurodegenerative Disorder

While the primary pathophysiology of HIV is typically associated with immune dysfunction and dysregulation, cognitive impairments have been a long recognized consequence of infection. The spectrum of progressive neurological complications of infection is characterized into three groups, ranging from asymptomatic neurocognitive impairments (ANI) to mild neurocognitive disorders (MND) and the more severe HIV-associated dementia (HAD) [2]. These cognitive dysfunctions referred to under the umbrella term HIV-associated neurological disorders (HAND) are diagnosed via neuropsychological testing and functional status assessments, and have been reported to affect 20-50% of HIV-infected individuals [3, 4]. Since the implementation of combined antiretroviral therapy (cART) as the primary treatment regimen for HIV/AIDS in the mid-1990's there has been a significant reduction in the incidence of the severest form of HAND, now rarely seen in developed countries in the post-cART era. However, the prevalence of milder forms of HAND remains stable and is expected to rise with the aging population of HIV-infected individuals [5]. In the United States, of the 1.2 million people living with HIV, an estimated 50% are older than 50 years of age, with an expected increase to 70% of the population of infected individuals by the year 2030 [6]. Complications from HIV infection may overlap with other age-associated neurodegenera-

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tive diseases such as Alzheimer's disease. Several groups have reported an increased accumulation of amyloid-β precursor protein (APP) in the brains of HIV infected patients [7-9]. It is hypothesized that neuroinflammation resulting from the activity of proinflammatory HIV proteins, including Tat and gp120, as well as prolonged cART and aging all, contribute to the overall increase in amyloid deposition. Thus, long-term infection with HIV might contribute to the worsening of age-related neuronal damage with evidence increasingly suggests that HIV infection exacerbates age-associated cognitive decline [10, 11]. With the shift of HIV infection to a chronic disease in the post-cART era, so too does the potential for significant effects on cognitive decline in the aging population of infected individuals.

HIV invades the central nervous system (CNS) during early infection and viral RNA has been detected in samples of cerebral spinal fluid (CSF) and in the brain within weeks of initial viral exposure with compartmentalized HIV replication detected in the CNS within four months of infection [12]. The early infiltration of independently replicating virus within the CNS may represent a mechanism by which the virus establishes a pharmacological and immunological sanctuary site, owing to the inability of most antiretroviral drugs to cross the blood-brain barrier and the restricted entry of lymphocyte populations into the brain [13-17]. Assessment of cognitive impairment in a population of long-standing aviremic patients yielded an estimated prevalence of HAND in individuals adherent to cART regimens at 69% [18]. Indeed, numerous studies support the occurrence of cognitive impairment in HIV-infected individuals despite cART-mediated suppression of HIV viral load in plasma [19-21]. HIV RNA viral load in the CSF has been reported to be significantly correlated with neurological dysfunction independent of plasma viral load [22]. Thus, evidence increasingly supports the CNS as a site of persistent low-level HIV infection.

HIV infects the CNS via a "Trojan Horse" model of blood-brain barrier (BBB) crossing that is characteristic of lentiviral spread in the bloodstream and CSF [23]. HIV infiltrates the brain via infected CD4+ macrophages and lymphocytes, allowing for transmigration of virus to perivascular spaces of the CNS while evading immune detection. Here, the infection is propagated in populations of perivascular macrophages and microglia [24]. Infected cells secrete neurotoxic viral proteins, inflammatory cytokines, and small metabolites that may contribute to further disruption of the BBB and promote continued influx of the virus [25, 26].

HIV infection of the CNS is associated with activation of microglia and astrocytes, as well as the induction of inflammatory and neurotoxic insults which contribute to the neurodegeneration and cognitive decline characteristic of HAND. Despite the prevalence of HIV-associated cognitive dysfunctions, the molecular and cellular mechanisms underlying HAND are poorly understood and are thought to consist of a combination of direct viral infection of cells of the CNS and indirect mechanisms involving host factors and neurotoxic effects of HIV-associated proteins.

Notably, HIV viral proteins Tat and gp120 have been implicated in affecting BBB integrity and promoting viral entry into the CNS. Tat (HIV *t*rans*a*ctivator of *t*ranscription), a viral regulatory protein responsible for activating viral transcription, is one of the first HIV proteins to be expressed following infection [27]. Tat has been recognized for its neurotoxic role in HAND and similarly to gp120 is found in extracellular spaces in a soluble form [28]. In the CNS, Tat has demonstrated neurotoxicity, including induction of neuronal oxidative stress via secretion of cytokines and chemokines and neuronal apoptosis via a pathway of NMDA receptor-mediated glutamate excitotoxicity [29, 30]. Tat has been detected in postmortem brains and CSF of HIV infected subjects, and numerous studies support Tat-mediated permeabilization of the BBB [31-33]. A recent study utilizing a Tat-expressing transgenic murine model to examine the influence of Tat expression on BBB integrity found Tat exposure is sufficient to destabilize BBB integrity and increase the presence of activated perivascular macrophages and microglia in an *in vivo* model of HAND [34]. These results support previous evidence that Tat-dependent disruption to the BBB may also contribute to glial activation and inflammation that underlie indirect neuronal injury observed in HAND [35]. While a role for Tat in HAND is clearly supported, Tat's contribution is beyond the scope of this review and is discussed elsewhere in greater detail [36].

Viral surface protein gp120 has also been linked to functional impairment of the BBB. As well as documented gp120 activation of proinflammatory genes and increased migration of monocytes across the BBB, a significant increase in permeability of brain endothelial cells in the presence of circulating gp120 has been reported [37, 38]. In both reports, removal of gp120 restored integrity of the BBB. The remainder of this review will focus on gp120-mediated neurotoxicity.

2. HIV GP-120

The entry of HIV to host cells is mediated by envelope glycoproteins gp120 and gp41. The non-covalently linked gp120 and gp41 interact to form a trimer of gp120/gp41 heterodimers where gp120 serves as a viral surface protein recognizing host CD4 and chemokine co-receptors and the transmembrane gp41 functions as a fusion peptide to assist in viral-host membrane fusion [39]. Given the relatively weak association between the two subunits, gp120 shed from the trimer is well documented [40, 41]. Indeed, high levels of monomeric soluble gp120 have been detected in secondary lymphoid organs of chronically infected individuals, while high levels of anti-gp120 antibodies have been detected in cerebral spinal fluid (CSF) of patients with HAD [42-44].

2.1. Gp120 Interaction with Membrane Lipid Raft Domains

Binding of viral surface glycoprotein gp120 to receptors is restricted to host-cell lipid raft domains (Fig. **1**) [45]. Lipid rafts are small (10-200nm), dynamic cholesterol and sphingolipid-enriched microdomains in the plasma membrane that serve to compartmentalize cellular processes, facilitating protein-lipid and protein-protein interactions and signal transduction events [46-48]. Membrane rafts have been implicated as critical signaling nodes of several neurological pathologies including Alzheimer's and Parkinson's diseases where they appear to play a role in mediating pathologic signal transduction characteristic of these neurodegenerative diseases

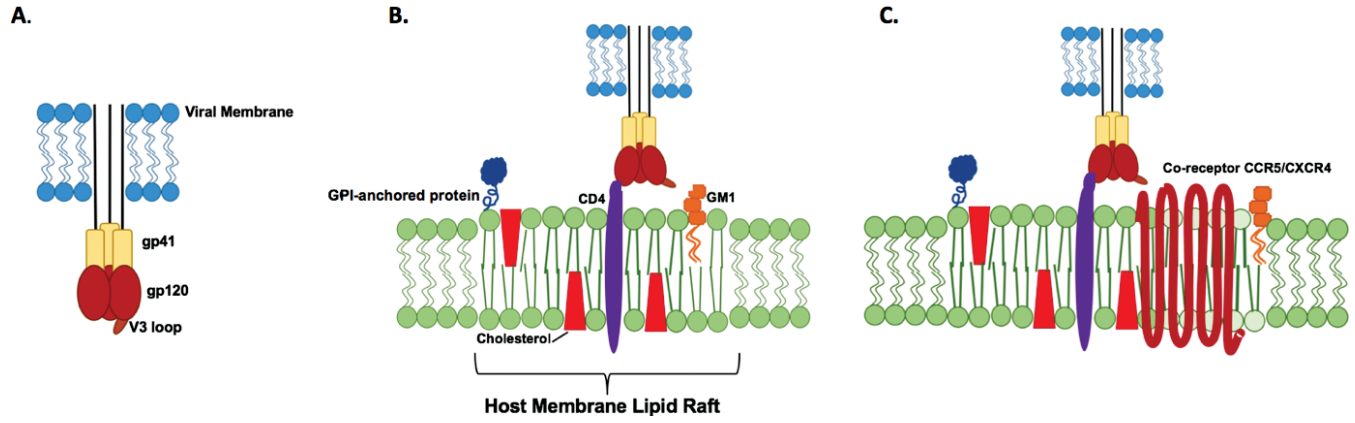

Fig. (1). Schematic representation of HIV-1 surface protein gp120 binding to host cell receptors in lipid raft domains. A. Viral membrane bound gp41/gp120 trimer. **B.** Binding to cellular CD4 receptor facilitates a conformational change in gp120 variable loop 3, allowing for further interaction with chemokine co-receptors CCR5 or CXCR4 (**C**).

[49, 50]. It has been demonstrated that many pathogens in addition to HIV exploit the lipid raft environment, including influenza and Ebola, at various stages of the viral lifecycle (viral entry, assembly, and budding) [51]. The life cycle of HIV is intricately tied to raft domains and requires interaction with raft-localized receptors for initial binding, viral-host membrane fusion, virion assembly, and subsequent release of viral progeny [52-55].

Several studies support the crucial role of rafts for HIV target receptor function. Fluorescence resonance energy transfer imaging has revealed a requirement for lipid rafts in the interaction between CD4 and co-receptors, thus suggesting receptor localization to lipid raft domains [56]. CD4 is enriched in lipid raft domains where it serves to facilitate coalescence of these domains and mediates the attachment and entry of HIV into host cells [54, 56, 57]. Site-directed mutagenesis has been used to identify a short sequence of positively charged amino acid residues in the cytoplasmic domain of CD4 controlling its targeted partitioning into raft domains [58]. In addition to CD4, HIV co-receptors CCR5 and CXCR4 are also found in detergent-resistant membrane fractions following exposure to HIV gp120. Host membrane cholesterol is but one particular lipid that is required for HIV infection, as evidenced by the blocking of viral entry following treatment with the cholesterol chelator methyl-β-cyclodextrin. Interestingly, the specific lipid environment of raft domains appears to be necessary for the conformational stability and function of these receptors and putative cholesterol binding sites in both chemokine receptors that appear to support their localization to raft domains have been identified [59]. These results support previous studies identifying a requirement for cholesterol in conformational integrity and function of both CCR5 and CXCR4 receptors [53, 60].

2.2. Gp120 Co-receptor Tropism

In most cases, gp120 binding to CD4 alone is not enough to induce membrane fusion, and interaction with a secondary co-receptor is required [61]. Gp120/CD4 interaction initiates a series of successive conformational changes in gp120 structure, ultimately exposing the third variable region (V3) loop which greatly enhances the affinity of gp120 to chemokine co-receptors CCR5 or CXCR4. Viral tropism is determined by V3-loop recognition of these co-receptors [62, 63]. Notably, viral transmission independent of co-receptor use has been demonstrated on rare occasions, and the establishment of an acute *in vivo* infection by a virus with a mutation in the gp120 V3 loop that prevented its binding with CCR5 and/or CXCR4 has been described [64]. Evidence of cell-to-cell HIV transmission activated by contact of infected and uninfected primary $CD4^+$ T cells in the absence of an appropriate co-receptor also has been reported [65]. Conversely, in the CNS, gp120 binding to both CCR5 and CXCR4 co-receptors independent of CD4 binding has been documented [66, 67]. While these represent instances of CD4/co-receptor-independent mechanisms of HIV transmission, it remains that most productive HIV infections are mediated by gp120 binding to *both* CD4 and co-receptors. Indeed, a "low-CD4" entry phenotype, characterized by gp120 capable of infecting cells expressing low densities of CD4, have been preferentially detected in the CSF of people with HIV-associated dementia. This may be suggestive of viral adaptation to the local cellular environment of the CNS allowing the virus to infect a population of cells expressing lower densities of CD4 [68].

Distinct strains of HIV can be categorized on the basis of cellular tropism tied to co-receptor preferences. Macrophage-tropic (R5) strains bind CCR5 receptors and preferentially infect peripheral blood mononuclear cells (PBMC), monocytes, macrophages, and T-lymphocytes, but not T-cell lines. T-cell tropic (X4) strains bind CXCR4 receptors of T-lymphocytes and T-cell lines, and dual-tropic (R5X4) strains bind both CCR5 and CXCR4 receptors [69]. Whereas R5 strains are detected throughout all stages of infection and disease, the population of viral strains in an individual often evolves during the course of infection, and an initial predominance of R5 strains gives way to the emergence of R5X4 and X4 strains in an estimated 50% of individuals as infection progresses [70].

The selective pressures driving the switch from CCR5 to CXCR4 receptor usage by HIV are not well understood, though there is evidence to support different hypotheses which may explain the underlying mechanisms. One hypothesis posits that the emergence of CXCR4-binding virus results from a depletion of susceptible CCR5-positive target

cells as infection proceeds. This hypothesis is supported by data indicating infected individuals heterozygous for a 32 base-pair deletion in the CCR5 gene, which results in lower expression of CCR5, have a higher incidence of X4 viruses when compared to infected individuals with normal CCR5 expression levels [71, 72]. Alternatively, a 2016 study provides evidence for host humoral immune pressure selecting against CCR5 variants facilitating the emergence of CXCR4 utilizing virus [73]. Although the mechanisms of tropism switch may not be clear, the switch in receptor usage has demonstrable clinical implications and the emergence of X4 strains have been linked to more severe illness and a more rapid progression to AIDS [74].

Feline immunodeficiency virus (FIV) represents an animal model of immunodeficiency with similarities in pathogenesis to HIV infection in humans, including subsequent FIV infection of the CNS in domestic cats that results in neurological symptoms comparable to those observed in HAND [76]. A recently published study investigating the role of FIV envelope glycoprotein gp95-mediated synaptic dysfunction found gp95 signaling through neuronal CXCR4 facilitates an elevation in intracellular Ca^{2+} and subsequent increase in synaptic activity [75]. Notably, the use of HIV antiretroviral drugs on FIV infected cats has been demonstrated to significantly reduce viral load, which may be suggestive of a similar mechanism for neuropathogenesis between the two lentiviruses, lending further support to a role for CXCR4 signaling in HIV neurotoxicity [77, 78].

2.3. Gp120 Neurotropism

Most cell types in the CNS express HIV target receptors CD4, CXCR4, and CCR5, suggesting that virus invading the CNS has the potential to infect many different cell types. CD4 and CXCR4 receptors have been detected on astrocytes, microglial cells, and neurons. CCR5 expression has been predominantly found on astrocytes and microglial cells, with less consistent evidence to support CCR5 expression on neurons.

Immunohistochemistry and flow cytometry have been used to demonstrate expression of CCR5 receptors on neurons of macaques and humans and in particular, on hippocampal neurons of patients with AIDS [72, 73]. Notably, neuronal expression of the receptor was decreased in the brains of AIDS patients with HIV encephalitis as compared to AIDS patients without HAND [79, 80]. In contrast, immunohistochemical analysis of the expression of CCR5, CXCR4, and several other chemokine receptors in brains of AIDS-positive and AIDS-negative patients found only CXCR4 expression on neurons whereas CCR5 expression was restricted to glial cells [81]. These results are in accordance with those of several other studies reporting CCR5 expression is restricted to glial cells of the CNS [82-84]. Whether the purported differences in neuronal CCR5 expression are the consequence of differences in experimental approaches or representative of true physiological differences in receptor expression has yet to be determined. There is some indication that the expression of chemokine receptors in cells of the CNS is dependent on cell culture conditions and the cytokine environment. Interestingly, a study of CCR5 expression in rhesus macaque brains found expression of receptors on cortical neurons increased with age and demonstrated differential expression in subpopulations of neuronal cells [85, 86]. Such dynamic receptor expression may be a factor in the inconsistencies reported above.

Though HIV co-receptors CCR5 and CXCR4 are widely expressed on cells of the CNS, productive infection appears to be restricted to microglia and macrophages, although limited latent infection of astrocytes has been demonstrated [87-90]. Productive infection of neurons has been reported but remains rarely observed [91, 92].

2.4. Mechanisms of CNS Dysfunction

Despite limited evidence supporting direct infection of neurons, it remains that synaptic dysfunction and neuronal cell death are prominent features of HAND and likely underlie the cognitive and motor dysfunctions exhibited by infected individuals [93-95]. Many studies investigating the mechanisms by which HIV impedes CNS function focus largely on neuronal cell death, which is attributed to both direct neurotoxic effects of soluble HIV proteins shed from infected cells, as well as bystander damage. The latter is a consequence of activated macrophages, microglia, and astrocytes releasing pro-inflammatory cytokines and chemokines leading to perturbed neuronal homeostasis [96-100]. While neuronal loss explains some degree of reported neurological complications of infection, neuronal death alone does not account for the cognitive impairments observed in HAND [101, 102]. HAND has been linked to impaired neuronal plasticity, characterized by synaptodendritic damage and decreased synaptic and dendritic density. Given the reduction of the severity of HAND, and in some instances the partial reversal of HAND symptoms, following initiation of cART, it is likely that a loss of synaptic plasticity and synaptodendritic injury account for much of the neuronal pathology observed in the HIV infected brain [103]. Indeed, synaptic dysfunction is emerging as an important neuropathologic mechanism underlying early CNS deficits and a role for several HIV proteins has been implicated in synaptic loss [75, 104, 105].

As previously described, FIV envelope protein gp95 has been implicated in CXCR4-mediated synaptic dysregulation. In this model, the intracellular Ca^{2+} increase is facilitated by the activation of both endoplasmic reticulum (ER) associated calcium channels and inositol triphosphate receptors (IP3Rs), and similarly to gp120, the activation of synaptic NMDARs. Notably, the neuronal nitric oxide synthase (nNOS)-cGMP pathway was found to be activated by *both* FIV gp95 and HIV gp120 stimulation of NMDARs. Gp95/gp120 interaction with CXCR4 promotes IP3 production and the nNOS induced activation of cGMP-dependent protein kinase II (cGKII). Subsequent phosphorylation of IP3Rs and serine 845 of AMPA receptor subunit GluA1 by cGKII leads to elevated intracellular Ca^{2+} levels and the promotion of AMPA receptor expression at the cell surface resulting in increased synaptic activity and Ca^{2+} hyperexcitation [75].

Gp120 is among the most potent HIV neurotoxins with deleterious effects reported *in vitro* for concentrations ranging from 1 pM to 1 uM [106, 107]. Such potency, in the picomolar and nanomolar range in an *in vitro* model is generally indicative of true *in vivo* biological relevance. There has been some controversy regarding the physiological relevance of gp120 concentrations used *in vitro* to extrapolate its *in*

vivo effects. Early attempts to quantify gp120 concentrations *in vivo* relied on capture enzyme-immunoassays which may be influenced by interfering plasma antibodies or limitations in the specificity of the detecting antibody [107-109]. While current understanding of *in vivo* concentrations of soluble gp120, particularly in the CNS, remains unclear, there is substantial evidence for a pool of soluble gp120 that has biological activity independent of productive infection [44]. Indeed, *in vitro* and *in vivo* studies have demonstrated gp120-mediated cell death in various neuronal populations and the resulting neuronal and synaptic loss, axonal retraction and dendritic simplification are similar to neuropathy observed in postmortem brains of HAD subjects [110, 111].

2.5. Gp120-mediated Neuronal Apoptosis

Several studies have identified a role for enhanced N-methyl-d-aspartate receptor (NMDAR) activation in gp120-mediated neurotoxicity. NMDA receptors are widely expressed on neurons and enhanced activation of NMDAR by high concentrations of endogenous glutamate results in the rapid influx of Ca^{2+} and free radical generation, ultimately activating pathways leading to neuronal apoptosis [111, 112]. Chronic NMDAR mediated excitotoxicity has been implicated in several neurodegenerative diseases including Alzheimer's disease and Huntington's disease and excitotoxicity in HAND has been linked to the gp120-induced release of excitatory molecules, including glutamate, from activated microglia and astrocytes in the CNS [90, 113-115]. Interestingly, gp120 has also been shown to act directly on NMDA receptors via interaction of gp120 V3 loop to the glycine site of the receptor and may be indicative of direct mechanisms of neurotoxicity induced by gp120 interaction with NMDA receptors [116].

In addition to NMDAR mediated neurotoxicity, gp120 has been associated with the generation of reactive oxygen species (ROS) and nitrosative stress which are potentially toxic for neurons. Astrocyte exposure to gp120 led to the production of ROS and tumor necrosis factor-alpha (TNF-alpha) in N9 murine microglial cells and stimulated upregulation of inducible nitric oxide synthase (iNOS), an important source of nitric oxide (NO) and nitrosative stress [117, 118]. In these indirect models of gp120 neurotoxicity, the release of excitotoxic mediators from surrounding macrophages and glial cells induces neuronal apoptosis via disruption of neuronal homeostasis. These results suggest that mitigating oxidative stress in HAND through the use of antioxidants to protect against neuronal apoptosis arising from indirect insults mediated by gp120 might be an effective therapy, and several studies have investigated this approach with varying degrees of success [118, 119]. Gp120 may also act directly on neurons to enhance NMDA-evoked calcium flux, possibly via modifications in spatial localization and focal density of NMDA receptors to lipid raft domains [120]. Gp120 has also been demonstrated to induce activation of nNOS directly in neurons, representing another potential source of NO and nitrosative stress, as well as a possible mediator of calcium hyperactivity and resulting synaptic dysregulation [75, 121].

Notably, neurotoxic effects of gp120 in the absence of NMDAR activation or production of inflammatory cytokines has been demonstrated. In the absence of productive viral infection of neurons, soluble gp120 in the CNS can induce neurotoxicity via direct interaction with chemokine receptors expressed on neuronal surfaces [122]. Gp120 induced cell death in human neuroblastoma cells through direct interaction with both neuronal CXCR4 and CCR5 chemokine receptors [123]. Further support for the role of chemokine receptors in mediating gp120 neurotoxicity comes from the discovery that endogenous chemokines are capable of blocking gp120-induced apoptosis of hippocampal neurons [124]. Interestingly, while endogenous CCR5 ligands, including macrophage inflammatory protein (MIP)1alpha, MIP-1beta, and 'regulated-and-normal-T cell-expressed and secreted' RANTES have been shown to slow progression to AIDS, the CXCR4 endogenous ligand SDF-1alpha has been demonstrated to induce neuronal apoptosis in some populations of cells following binding of the CXCR4 receptor [125, 126]. It should be noted that the majority of viruses isolated from the CNS are R5 strains, while R5X4 and X4 binding variants occur less frequently [127, 128]. Despite this, signaling through CXCR4 has been identified as an important pathway in gp120-induced neuronal apoptosis [3, 129].

2.6. Gp120-induced Synaptic and Dendritic Dysfunction

Synaptodendritic damage has emerged as a hallmark of HIV infection of the CNS, and several groups have reported a role for gp120 in synaptic dysfunction and dendritic simplification observed in HAND. An early neuro-histological assessment of synaptic and dendritic markers identified a strong correlation between presynaptic and neocortical dendritic damage and abundance of gp120 in neocortical gray and deep white matter [130]. The authors posit that in this model of neurotoxicity, glutamate signaling through NMDA receptors on dendritic membranes is a likely contributor to the observed pathology. Gp120 was similarly found to induce significant loss of synaptic connectivity in hippocampal neurons in the absence of neuronal apoptosis via an indirect mechanism involving the activation of NMDA and CXCR4 receptors, and requiring the release of cytokines from non-neuronal cells [131]. Gp120 was further found to induce gliosis and neuronal dendritic injury in a primary mixed human CNS culture analogous to dendritic injuries observed in postmortem brains of HIV infected individuals [132].

Subsequently, there is evidence suggesting that gp120 neurotoxicity is the result of spatially distinct mechanisms. In an investigation of HIV-associated sensory neuropathy, gp120-mediated axonal degeneration in rat spinal dorsal root ganglia (DRG) occurred as a result of two independent mechanisms [133]. Prominent axonal toxicity in the absence of apoptosis was observed only when gp120 was applied directly to sensory axons, with no effect when applied to cell bodies. Conversely, gp120 mediated neuronal apoptosis and subsequent axonal degeneration was found to be dependent on the activation of Schwann cells. Notably, gp120-induced direct axonal degeneration was found to be dependent on the local activation of the caspase pathway and mediated by activation of both CXCR4 and CCR5 receptors [133]. Blocking the CXCR4 receptor by monoclonal antibody prevented gp120-induced axonal toxicity and partial prevention of toxicity occurred by blocking the CCR5 receptor. These results highlight an intriguing mechanism by which axonal

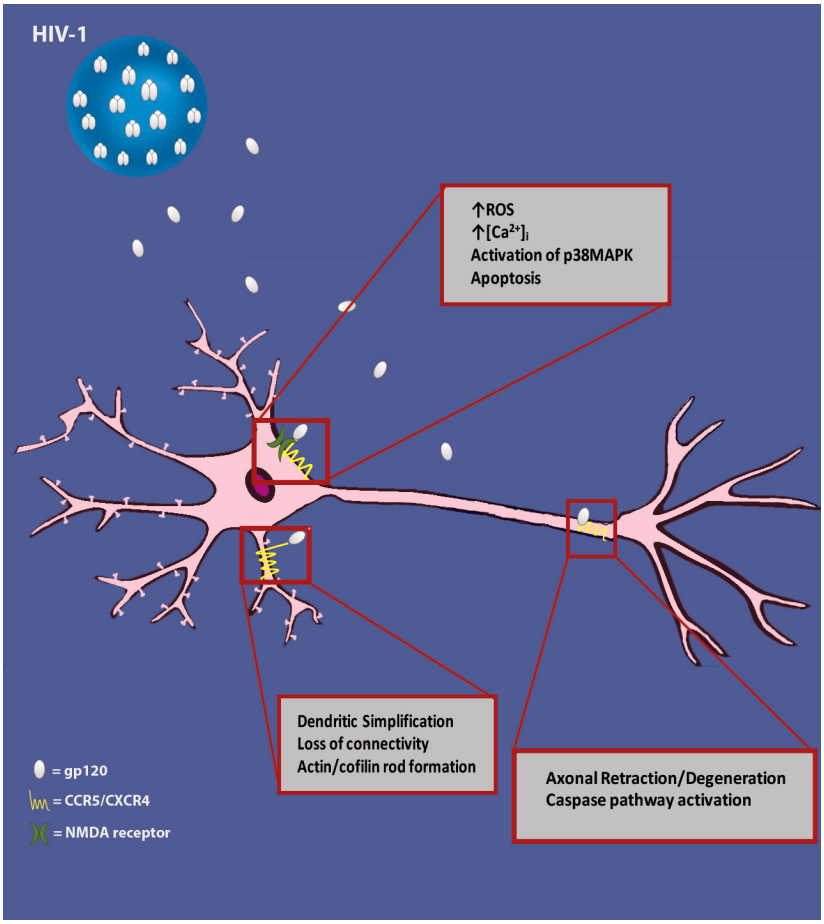

Fig. (2). Schematic diagram illustrating neurotoxic effects of soluble gp120 interactions with neuronal receptors. Direct interaction of gp120 with both chemokine co-receptors CCR5 and CXCR4, as well as N-methyl-D-aspartate (NMDA) receptors on neuronal surfaces leads to increased generation of reactive oxygen species and intracellular calcium influx and the activation of signaling pathways leading to cellular apoptosis. In the absence of apoptosis, gp120 has been demonstrated to induce dendritic and axonal degeneration leading to a loss of neuronal connectivity and formation of actin/cofilin rods.

degeneration following direct gp120 exposure occurs independently of toxicity in neuronal cell bodies.

3. RAFT-ASSOCIATED SIGNALING

Membrane lipid raft domains have been demonstrated to be critical sites of interaction between gp120 and chemokine receptors, mediating both internalization of the viral protein and activation of pathways contributing to apoptosis (Fig. **2**) [134, 135]. Lipid rafts also appear to be necessary for appropriate receptor conformation to support CXCR4-chemokine binding [136]. Gp120 coupled to CXCR4 in primary neurons induces neuronal apoptosis via activation of the sphingomyelin-catabolizing enzyme neutral sphingomyelinase (NSmase) [135]. In this pathway, activation of NSmase is regulated by the NADPH-oxidase (NOX2)-mediated production of superoxide radicals in neurons, leading to the increased production of ceramide, an apoptotic second messenger in a number of cell types including glia and neurons. SDF-1 was also found to induce neuronal apoptosis through the same NSmase-mediated pathway, confirming a role for CXCR4 in this pathway of pathologic signal transduction [135].

While overproduction of ceramide has previously been linked to neurotoxicity, ceramide is an important component of cellular lipid raft domains [137]. Experiments have demonstrated coalescence of lipid raft domains into larger platforms following ceramide generation by sphingomyelinases [138]. Gp120 binding to cellular receptors has been demonstrated to similarly induce lipid raft coalescence, leading to the increased size and stability of raft domains [120]. Raft coalescence has been proposed as a mechanism for clustering receptors and components of receptor-activated signaling cascades, and gp120-induced raft coalescence in hippocampal neurons has been found to promote the forward trafficking and surface clustering of NMDA receptors in rafts in a CXCR4 dependent manner [120, 139]. Prolonged surface clustering of NMDA receptors in lipid raft domains of neurons may represent an additional mechanism of gp120 mediated neurotoxicity via perturbation of intracellular Ca^{2+} flux or associated signaling events.

Notably, profound changes in lipid rafts are observed for a number of CNS dysfunctions, including Alzheimer's disease, mild cognitive impairments (MCI), CNS aging, and CNS trauma [50, 140-143]. Biophysical properties of lipid rafts are imperative for their signaling capacities and even subtle changes in lipid species rapidly affect overall membrane architecture influencing the structure and function of membrane proteins [144-148]. Presumably, entire signaling

cascades could be silenced due to imposed constraints on lipid-protein and/or protein-protein interactions. Emerging evidence attributes cholesterol a 'chaperone-like' allosteric function in stabilizing structural elements of many membrane proteins through a Cholesterol Recognition/Interaction Amino Acid Consensus sequence or CRAC motif (-L/V-$X_{(1-5)}$-Y/F-$X_{(1-5)}$-R/K-) or its reverse sequence (CARC motif) [149-154]. This sequence establishes interaction with the isooctyl tail (A, L, and V), the sterol ring structure (Y, F), and the 3β-OH group (R,K) of cholesterol. Spatiotemporal alterations of protein function by cholesterol interactions were demonstrated for neurotransmitter receptors (nAchR, 5-HT2B), CXCR4, the β-adrenergic receptor, Na^+K^+-ATPase, and the C99 domain of amyloid-precursor protein (APP) implicated in promoting the amyloidogenic pathway [59, 151, 153]. Aberrations in cholesterol homeostasis are commonly associated with degenerative CNS diseases including AD and MCI.

4. COFILIN-ACTIN RODS: A POTENTIAL MECHANISM FOR GP120-MEDIATED SYNAPTIC DYSFUNCTION

Protein association with lipid rafts as a mechanism for regulating signaling has been seen in a number of neurodegenerative diseases, most notably in Alzheimer's disease, where lipid raft-anchored cellular prion protein PrP^C serves as a receptor for amyloid-β (Aβ) oligomers [155, 156]. Previous research has identified a pathway mediated by ROS generation and NOX leading to the formation of cofilin-actin bundles (rods) initiated by the binding of diverse extracellular ligands, including proinflammatory cytokines and Aβ to raft localized receptors [155, 157]. Generation of rod-like inclusions is a cellular response to oxidative stress conditions and is characterized by the local dephosphorylation (activation) of cofilin and subsequent assembly of 1:1 cofilin:actin filaments that bundle into rod-like structures containing intermolecular disulfide cross-linked cofilin [158]. Rod formation may be a transient response to oxidative stress and rods can dissociate following relief from oxidative stress. Rod formation might also be responsible for the loss of synaptic function and decreased network connectivity observed in many neurodegenerative conditions including HAND [159, 160]. Although actin rod formation has not been identified in FIV *in vitro* or *in vivo*, considerable disruption to the neuronal cytoskeleton has been demonstrated both for filamentous actin structures as well as microtubule structures [161].

Indeed, cofilin sequestration into rods might have a neuroprotective effect due to its ability to promote mitochondrial-dependent apoptosis through its oxidation to form intramolecular disulfide bonds and its ability to target the tumor suppressor protein p53 to the nucleus and mitochondrion [162, 163]. However, persistent rods have been described in neurons during the progression of Alzheimer's and other neurodegenerative disease [164]. Cofilin-actin rods have also been observed in brains of AD model mice where their decreased abundance through inhibition of cofilin dephosphorylation or reduced cofilin expression results in normalizing cognitive function and long-term potentiation in brain slice cultures [165, 166]. In addition to synapse loss due to interrupted vesicular transport resulting from occlusion of neurites in which rods form, cofilin-actin rods have been linked to synaptic dysfunction via sequestration of cofilin from dendritic spines where it plays an important role in post-synaptic plasticity associated with learning and memory [167, 168]. Normalization of cofilin activity in dendritic spines through modulation of upstream kinases and phosphatases by cell-penetrating peptides has proven to be effective in many different neurological disorders, including AD [169]. Amyloid β induction of cofilin-actin rods requires the expression of cellular prion protein PrP^C in raft domains and is mediated by ROS generation by NOX [155].

A number of intriguing commonalities in the neuronal response to HIV gp120 stimulation and that of Alzheimer's associated protein Aβ have been identified, among them the enhanced lipid raft coalescence into stable macro domains, the activation of NMDA receptors localized to lipid rafts, and the NOX2 mediated generation of ROS [50, 120, 135]. Given these commonalities, it is quite likely that gp120 mediated stimulation of NOX2 also leads to the downstream formation of cofilin rods, thereby inducing synaptic loss and impaired synaptic function similar to what occurs in other neurodegenerative diseases such as Alzheimer's. Interestingly, gp120-mediated CXCR4 signaling has been demonstrated to activate cofilin in resting T-cells as a mechanism of facilitating nuclear localization and replication of the virus [170]. Rods have also been demonstrated to be induced by nitric oxide, a source of oxidative stress shown to be generated by both HIV gp120 and FIV gp95 via CXCR4 mediated signaling [75]. Further supporting the role of cofilin-actin rods in HIV-mediated synaptic dysfunction, data generated in our lab has shown gp120 is capable of inducing rod formation in rat E18 hippocampal neurons via CXCR4-associated signaling. Similarly to rod induction by Aβ, this pathway appears to be mediated by the presence of PrP^C in membrane raft domains (Smith L., Walsh K., Whittington L., Shaw A., Minamide L., Cartagena G., *et al.* in preparation).

Demonstrating the pivotal role of cholesterol as a "chaperone" allosteric modulator of NOX2-dependent superoxide generation would considerably advance our understanding of how membrane architecture regulates protein structure and function, potentially identifying putative interaction surfaces of NOX2 with cholesterol possibly through CRAC/CARC motifs and provide detailed insight into structure/function aspects of NOX2. Exploiting these findings could open new avenues for drug-discovery approaches to target NOX as well as novel therapeutic strategies to ameliorate gp120-induced oxidative stress in the CNS. Entire signaling networks could presumably be silenced by interference with annular lipids, boundary lipids, activating lipids, and/or "chaperone" lipids [147, 148, 171-173].

CONCLUDING REMARKS

Despite the reduction in incidence of severe forms of HIV associated neurocognitive deficits, prevalence of milder forms of HAND is expected to increase with the aging population of HIV-infected individuals independent of adherence to cART and represents a significant public health challenge. Gp120-associated receptor binding and activation of NOX2-mediated redox signaling have identified a potential mechanism linking gp120-CXCR4 induced ROS formation to

pathways of early synaptic dysfunction characteristic of other neurodegenerative diseases. Such pathways may contribute to the cognitive decline associated with HIV infection. The importance of membrane architecture as a regulator of protein function and the crucial role it plays in the HIV lifecycle highlights membrane rafts as a potential target for preventative/therapeutic approaches to HIV associated neurodegeneration.

LIST OF ABBREVIATIONS

AD = Alzheimer's diseases

APP = amyloid-β precursor protein

BBB = Blood brain barrier

cART = Combination antiretroviral therapy

CNS = Central nervous system

HAD = HIV-associated dementia

HAND = HIV-associated neurocognitive disorder

NOX2 = NADPH-oxidase 2

nSmase = Neutral sphingomyelinase

PrP^C = Cellular prion protein

ROS = Reactive oxygen species

TNF-α = Tumor necrosis factor α

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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