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Emerging Role of Nef In the Development of HIV Associated Neurological Disorders

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Abstract

Despite adherence to treatment, individuals living with HIV have an increased risk for developing cognitive impairments, referred to as HIV-associated neurological disorders (HAND). Due to continued growth in the HIV population, particularly amongst the aging cohort, the neurobiological mechanisms of HAND are increasingly relevant. Similar to other viral proteins (e.g. Tat, Gp120, Vpr), the Negative Factor (Nef) is associated with numerous adverse effects in the CNS as well as cognitive impairments. In particular, emerging data indicate the consequences of Nef may be facilitated by the modulation of cellular autophagy as well as its inclusion into extracellular vesicles (EVs). The present review examines evidence for the molecular mechanisms by which Nef might contribute to neuronal dysfunction underlying HAND, with a specific focus on autophagy and EVs. Based on the these data, we propose an integrated model by which Nef may contribute to underlying neuronal dysfunction in HAND and highlight potentially novel therapeutic targets for HAND.

Keywords

HAND; HIV-1 Nef; Autophagy; Exosome

1. Introduction:

Human immunodeficiency virus (HIV) infection and its associated neurological consequences remain a consistent public health concern. Recent reports suggested that an additional 1.7 million people were infected with the virus in 2018, bringing the total number

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

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of people living with HIV to 37.9 million worldwide (UN AIDS 2019). Despite improved patient outcomes due to enhanced diagnostic testing and targeted therapies, there remains no effective vaccine for HIV, or a permanent cure for HIV-infected individuals (Davenport et al. 2019). Moreover, HIV continues to be a leading cause of morbidity across the globe (World Health Organization 2018). However, the implementation of combined anti-retroviral therapy (cART) has transformed this once fatal disease into a chronic, manageable condition (Deeks et al. 2013; Saylor et al. 2016). Following its introduction in the mid-1990s, cART has substantially increased the life expectancy of the HIV population, which is now approaching that of the general population (Fauci and Marston 2015; Trickey et al. 2017).

Although current treatments can control HIV infection, infected individuals maintain increased risks for numerous chronic comorbidities, including HIV-Associated Neurological Disorder (HAND) (Sengupta and Siliciano 2018). Amongst HIV infected individuals, HAND is estimated to afflict up to half of patients, even if cognitive deficits are not self-reported or viral loads are suppressed to undetectable levels (Heaton et al. 2010; Simioni et al. 2009). Broadly, these detriments can reflect impairments in several cognitive domains, including executive functioning (e.g. impulsivity, problem solving), memory (e.g. immediate retrieval, working memory) and attention (e.g. sustained attention, strategy shifting) (Clifford and Ances 2013). According to recently adopted criteria, HAND can further be delineated into asymptomatic neurocognitive impairment, mild neurocognitive disorder or dementia, depending on the severity of impairment and subsequent impact on daily functioning (Antinori et al. 2007). Thus, it is such neurocognitive impairments that remain a persistent challenge amongst HIV infected individuals, even those who adhere to therapeutic regimens and maintain long term viral suppression.

In the cART era, the prevelance and clinical presentaiton of HAND has shifted. Specifically, findings from the comprehensive CHARTER (the CNS HIV antiretroviral therapy effects research) cohort indicated an 80% reduction in the most severe forms of cognitive impairment since cART implementation, although milder cognitive deficits were found to persist at comparable rates. While only 2% of virally suppressed individuals are estimated to display severe deficits (i.e. compared to 10–15% pre-CART), more than half (52%) maintain detectable cognitive impairments upon neuropsychological assessment (McArthur et al. 2010). Subsequent reports disputed these findings and suggested the rates of HAND might be substantially lower, in part due to inconsistent testing criteria (Gisslén et al. 2011; McDonnell et al. 2014). Despite active debate surrounding the prevalence of HAND, it is evident that HIV infected individuals in the cART era can maintain significantly greater risk for the development and progression of neurocognitive deficits compared to the general population (Grant et al. 2014; Schouten et al. 2016). Furthermore, such likelihoods remain elevated even if alternative neuropsychological criteria used to asses HIV infected persons (group et al. 2016; Su et al. 2015)

Several key variables render HIV+ individuals at increased risk for HAND, including advancing age, drug use and co-infections (Table 1). Aging is particularly relevant in the cART era, where decreased mortality rates and increased life expectancies have resulted in approximately half of HIV+ individuals reaching at least 50 years of age (Goodkin et al. 2001). In turn, this cohort is consistently found to be at a significantly greater risk for

developing HAND (Chiesi et al. 1996; Janssen et al. 1992; L. Fazeli et al. 2014; Valcour et al. 2004). Notably, this age-associated risk for HAND could be due to long term adherence to antiretroviral treatment itself; for instance, cART induces metabolic changes (e.g. central obesity, dyslipidemia, and insulin resistance) which are themselves considered risk factors for dementia (Behrens et al. 1999; Falutz 2007; Narciso et al. 2001; Whitmer et al. 2008). In addition, illicit drug use, including methamphetamine and cocaine, is known to be significantly higher amongst HIV patients who develop HAND, compared to those who do not use illegal substances (Ayuso-Mateos et al. 2000; Byrd et al. 2011). However, it remains unclear if this association is mediated by pre-existing substance use disorders, which themselves can result in cART interruption and increase risk for HAND (Kamal et al. 2017; Meyer et al. 2013). Along with aging and drugs of abuse, several studies have also shown that HIV patients coinfected with other viruses or bacteria have increased risks for developing cognitive impairments (Cherner et al. 2005; Hestad et al. 2019; Marra et al. 2013; Ryan et al. 2004).

Although HAND's prevalence has shifted and risk factors have been identified, the continued growth of the HIV population and ensuing development of neurological impairments has increased focus on the neurobiological underpinnings of HAND. For instance, the implementation of a varieity of neurimaging techniques (e.g. functional magnetic resonance imaging (fMRI), positron emission tomography (PET)) has elucidated structural and functional alterations in specific brain regions (e.g. frontal cortex, hippocampus) that are associated with HAND (Ances and Hammoud 2014; Sanford et al. 2017). However, evidence has also begun to eluciate the molecular mechanisms that might contribute to neuronal dysfunction underlying HAND, including the aberrant consequences of HIV viral proteins.

2. HIV viral proteins in the development of HAND

Although HIV-1 was initially thought to target peripheral immune cells (T-lymphocytes, monocytes/macrophages, dendritic cells), subsequent findings illustrated that CNS immune cells are also susceptible to infection (e.g. macrophages, microglia and astrocytes) (Gartner et al. 1986; Koenig et al. 1986; Pope et al. 1994; Weissman et al. 1995). While the exact mechanisms of infiltration remain debated, the virus is thought to cross the blood-brain-barrier (BBB) within weeks of systemic infection through infiltrating monocytes or infected CD4+ T lymphocytes (Spudich and Gonzalez-Scarano 2012; Valcour et al. 2012). Once in the CNS, microglia and perivascular macrophages serve as persistent and latent viral reservoirs, provided that they are the only resident cells in brain parenchyma that have been shown to support productive HIV-1 infection (Chen et al. 2017; Crowe et al. 2003; Williams et al. 2001). Conversely, while limited evidence suggests neurons and other non-neuronal cells (e.g. astrocytes) may also be infected, it remains unclear if such cell types can support constitutive viral replication (Bissel and Wiley 2004).

Several mechanisms are suggested to contribute to HIV-associated neuronal dysfunction. A chonrically dysregualted neuroinflammatory profile may be a casual factor, provided that HIV elevates levels of various pro-inflammatory cytokines and chemokines in the CNS, including tumor necrosis factor alpha (TNFα), interleukin 1β, 6 and 8 (IL-1β, IL-6, IL-8)

and interferon α (IFNα) (Kaul et al. 2001; Tyor et al. 1992). Indeed, compared to uninfected controls and non-demented counterparts, HIV infected individuals suffering from cognitive dysfunction display significantly elevated levels of IFNα in cerebrospinal fluid (CSF) (Rho et al. 1995). Modified microglial functions may also contribute to HAND. Post-mortem tissue analyses indicate that microglia densities are positively correlated with the degree of cognitive dysfunction or impairment in HIV infected individuals. (Anthony et al. 2005; Ghorpade et al. 2005; Glass et al. 1995; Huang et al. 2011). Moreover, amongst virally suppressed individuals who remain cognitively sound, impaired executive performance is nonetheless associated with greater microglia activation across multiple cortical regions, whereas such an association is absent among uninfected controls (Garvey et al. 2014). In addition, abnormal astrocytic functioning may contribute to neurological dysfunction in HIV, given that the percentage of astrocytes harboring the virus is known to significantly correlate with increasing severity of cognitive deficits (Churchill et al. 2009). Consistent with the adverse effects of infected microglia, infected astrocytes can also compromise BBB integrity by altering gap junctions, and disrupt glutamate homeostasis via attenuated expression of amino acid transporters (Eugenin et al. 2011).

While some evidence indicates that HIV-associated neurocognitive impairments are caused by an altered neuroimmune profile or aberrant glial functioning, others suggest the adverse effects of specific viral proteins may ultimately contribute to HAND (Ellis et al. 2007; Nath 2002). The HIV genome is itself composed of nine genes that encode fifteen viral proteins (Frankel and Young 1998). *Gag, pol,* and *env* code for structural proteins (MA, CA, NC), enzymes (Pro, RT, IN, RNase H), and envelope proteins (gp120, gp41), while the remaining genes code for regulatory (Tat, Rev) and accessory proteins (Vif, Vpr, Vpu, and Nef). In addition to viral replication, these proteins maintain a plethora of roles, including the regulation of host-cell gene expression, metabolic modifications, and alterations in intracellular signaling cascades (Frankel and Young 1998; Swanson and Malim 2008; Wyatt and Sodroski 1998).

As suggested by initial investigations of Gp120, the neurobiological mechanisms underlying HAND may be due the adverse consequences of HIV viral proteins (Barnes 1987). For instance, Gp120 induces the production of proinflammatory cytokines, such as IL-1B, TNFA, and IL6, that subsequently elevates rates of apoptosis in both neuronal and non-neuronal cells (Cheung et al. 2008; Li et al. 2005; Yeung et al. 1995). Interestingly, such deteriorated neuronal variability could be inhibited by the application of anti-oxidant enzymes, suggesting Gp120's induction of ROS might also contribute to compromised neuronal functioning in HAND (Louboutin et al. 2012; Reddy et al. 2012). Gp120 has also been shown to disrupt ion regulation and glutamate homeostasis amongst neurons and glia, which can subsequently contribute to contextual memory impairments *in vivo* (Fernandes et al. 2007; Holden et al. 1999; Wang et al. 2004).

Along with Gp120, the HIV-1 viral protein R (Vpr) may also contribute to neuronal dysfunction underlying HAND. Indeed, Vpr is neurotoxic across a variety of primary cell models, including human hippocampal neurons as well as rat cortical and striatal neurons (Huang et al. 2000; Patel et al. 2000; Piller et al. 1998; Sabbah and Roques 2005). Such compromised viability could be due to direct consequences in neuron's themselves, given

that Vpr can impair the functional capacitates of neural mitochondria while also inhibiting axonal stability (Kitayama et al. 2008; Wang et al. 2017). Conversely, the consequences of this viral protein may be equally aversive across all CNS cell types; for instance, it can disrupt calcium regulation and induce pro-inflammatory cytokine expression in both neuronal and non-neuronal cells alike (Mamik et al. 2017; Na et al. 2011; Rom et al. 2009). Moreover, *in vivo* expression of Vpr in the rodent brain can result in significantly decreased performance across long-term, spatial memory tasks as well as short-term, working memory tasks (Torres and Noel 2014).

Along with Gp120 and Vpr, the Trans-activator of transcription (Tat) may play a role in facilitating HAND, especially given its persistent detection in the CNS despite viral suppression (Johnson et al. 2013). Indeed, Tat compromises a variety of homeostatic processes in the brain, including neuronal viability, synapse formation and neuroinflammatory profiles (Kim et al. 2008; Nath et al. 1999; Sabatier et al. 1991). In particular, recent *in vitro* evidence demonstrates Tat's capacity to not only induce and enhance aberrant protein aggregation, but also its ability to do so in concert with pathologically relevant polypeptides, such as Amyloid β (Aβ) (Fan and He 2016; Hategan et al. 2017; Rempel and Pulliam 2005). Moreover, a substantial body of evidence from preclinical models has illustrated both the exogenous application or endogenous expression of Tat in the CNS results numerous cognitive impairments across a variety of behavioral paradigms (Carey et al. 2012; Fitting et al. 2008; Fitting et al. 2018; Harricharan et al. 2015; Jacobs et al. 2019; Nookala et al. 2018; Zhao et al. 2020). Together, these data further suggest the abnormal persistence of HIV viral proteins in the CNS may contribute to neuronal dysfunction underlying HAND.

In addition to the previously characterized proteins, limited yet accumulating data also suggest a prominent role for the Nef protein in facilitating HIV-associated neurological deficits. Therefore, its association with the development of neurological impairments in HIV, as well as its potential pathogenic mechanisms in the CNS, warrant due consideration.

3. HIV Nef

The Negative Factor (Nef) protein is a multifunctional, 27– 34-kDa polypeptide that has historically been understudied in the context of HAND (Carroll and Brew 2017; Rao et al. 2014). Its gene is located at the 3'-end of HIV-1, HIV-2, and SIV, partially overlapping the 3' long terminal repeat (LTR) (Laprevotte et al. 2001). A combination of X-ray crystallography and nuclear magnetic resonance has characterized this viral protein's three-dimensional structure (Arold et al. 1997; Lee et al. 1996). Specifically, it is composed of a folded core (residues 55–65 and 84–203), with flexible N-terminal (residues 1–54) and C-terminal (residues 204–206) domains, as well as a central flexible loop (residues149–179) within the folded core (Geyer and Peterlin 2001). In addition, its myristoylated N-terminus allows for its association with the cytosolic face of cellular membranes and is required for Nef's cellular interactions (Fackler et al. 1997; Geyer et al. 1999). Such structural features may account for Nef's abundant interactions with host cell proteins (Figure 1).

As its name implies, Nef was initially considered an inhibitor of viral genome transcription, but studies have since shown that Nef is essential for the maintenance of high viral loads, and promotes disease progression to AIDS (Gorry et al. 2007; Hanna et al. 1998; Thompson et al. 2003). For example, HIV-1 particles produced in the presence of Nef are ten times more infectious than particles produced in its absence (Delassus et al. 1991; Kestler et al. 1991). Conversely, HIV strains that lack a functional Nef protein result in delayed disease progression; here, strains with deletions in the Nef gene, due to truncated 3'-LTRs, result in normal CD4 counts and lower viral loads up 14 years post-infection, even in the absence of cART treatment (Deacon et al. 1995; Learmont et al. 1999).

In addition to its roles in HIV pathogenesis, evidence also indicates a potential role for Nef in the HAND development. This postulation was spurred by initial post-mortem analyses of brain tissue from HIV infected individuals; while only half of all patients maintained detectable Nef-positive cells, this rate increased to ~85% amongst those individuals who met behavioral criteria for dementia (Ranki et al. 1995). Along with altered concentrations of Nef within the CNS, HIV individuals displaying cognitive deficits maintain specific structural subtypes of Nef, compared to patients who remain cognitively stable. Following structural bioinformatics, computational modeling and proteomic analyses, findings suggest the Nef protein in the brains of individuals suffering from HAND maintain distinct structural alignments that potentially alter its conformational transitions and subsequent binding potentials (Lamers et al. 2011). Moreover, the Nef structural signatures associated with HAND, including modifications to SRC homology 3 (SH3), apetela 2 (AP-2) and cytokine bindings domains, were found to be specific to the CNS, further suggesting a role for Nef in contributing to HIV associated neurological dysfunction (Lamers et al. 2018).

Nef may promote HAND through a number of complimentary mechanisms in the brain. Amongst neuronal and non-neuronal cell types, exposure to the Nef protein results in significantly decreased metabolic activity and increased rates of cell death (Trillo-Pazos et al. 2000). *In vivo* expression of Nef not only recapitulates such neuronal loss, but impairs locomotor activity and triggers a robust neuroinflammatory response, including an upregulation of interferon-gamma-inducible protein 10 (IP-10); furthermore, such increased expression of IP-10 has also been detected in the brains of HIV individuals suffering from severe cognitive deficits (van Marle et al. 2004). Along with neuronal loss, Nef may contribute to cognitive impairments by inducing the recruitment of peripheral immune cells into the CNS (Koedel et al. 1999). Specifically, transplantation of Nef-expressing glia into the rat brain results in increased recruitment of peripheral macrophages, as well as neuronal apoptosis and a neuroinflammatory profile; in turn, these animals maintain significantly decreased performance on a variety of cognitive measures, including spatial and non-spatial memory tasks (Chompre et al. 2013; Mordelet et al. 2004). Such Nef-induced recruitment of peripheral immune cells and ensuing behavioral impairments may in part be due to Nef's capacity to increase expression of CCL2 in the brain, a chemoattractant for circulating monocytes (Chompre et al. 2019; Lehmann et al. 2019) . Along with these mechanisms, emerging data suggest that Nef may facilitate neurological complications through two other pathogenic processes, cellular autophagy and extracellular vesicles.

4. Nef and Autophagy

Autophagy is a dynamic, self-digestive process that captures, isolates and degrades intracellular materials, such as damaged organelles (e.g. mitochondria), intracellular microbes and potentially toxic protein aggregates, in order to maintain a homeostatic cytoplasmic environment and ensure efficient protein quality control (PQC) (Kaur and Debnath 2015). This pathway is characterized by the formation of autophagosomes that sequester damaged cytoplasmic substrates, and ensuing fusion with lysosomes that leads to the degradation of targeted materials (Glick et al. 2010; Reggiori et al. 2012). Although it can serve as an innate self-defense mechanism to control viral spread, particularly follow initial infection, abnormal cellular autophagy is often correlates with HIV pathogenesis (Chiramel et al. 2013; Killian 2012; Lennemann and Coyne 2015).

Investigations have demonstrated such dysregulated autophagy may be caused by HIV-1 proteins themselves. For instance, following exposure to recombinant Tat, primary rodent hippocampal neurons display dose and time dependent decreases in levels of LC3, a protein which is otherwise responsible for initiating autophagosome formation; furthermore, such Tat-induced variation in autophagy is accompanied by elevated neurodegeneration *in vivo*, particularly in the CA3 region of the hippocampus (Fields et al. 2015; Hui et al. 2012). Similarly disrupted autophagic mechanisms are found in response to Gp120, while data from primary neuronal culture and transgenic models suggest this viral protein's effects on autophagy may be due to the modulation of mTOR-dependent signaling cascades (Fields et al. 2013; Liu et al. 2019b).

Similar to other HIV-1 viral proteins, Nef can significantly disrupt cellular autophagy (Kyei et al. 2009). Here, Nef's binding to beclin-1 (BECN1) and ensuing inhibition of autophagosome formation silence autophagy to the levels that are equivalent to uninfected cells (Campbell et al. 2015). The sequestration of BECN1 and inhibition of autophagy by Nef has been replicated in a variety of cell lines and primary cultures, while recent evidence also indicates this effect could be due to Nef's indirect yet further blockade of BECN1 by enhancing its binding to Bcl2, as well as Nef's capacity to prevent LC3 lipidation (Castro-Gonzalez et al. 2020). In turn, Nef-dependent inhibition of autophagy can significantly increase cellular susceptibility to apoptosis (Gupta et al. 2017).

The variation in autophagy within the CNS due to Nef may play a crucial role in HAND development, although further investigations employing neuronal cell models are necessary to validate this postulation. One study examined these claims directly utilizing primary human astrocytes expressing the Nef protein following adenoviral transduction. As indicated by the accumulation of ATG8 and p62, Nef emulated the autophagic blockade induced by bafilomycin treatment. Along with morphological alterations indicative of apoptosis, co-transduction with the tandem LC3 vector illustrated that Nef inhibited autophagosome fusion with lysosomes (Saribas et al. 2015).

5. Nef and Extracellular Vesicles

Extracellular vesicles (EVs), are membranous compartments that enable the direct exchange of materials between cells, including nucleic acids, lipids and proteins (Tetta et al. 2013). These vesicles comprise a heterogeneous population of membrane vesicles including microvesicles and exosomes with size variation between 50 nm and 500 nm (Raposo and Stoorvogel 2013b). Exosomes originate from the invagination of endosomal membranes that bud inwardly as intra-luminal vesicles (ILVs) during the biogenesis of multivesicular bodies (MVBs); in turn, MVBs can then fuse with lysosomes and be degraded, or fuse with the plasma membrane and release their ILVs as exosomes (Colombo et al. 2014). By endocytosis or fusion with the plasma membrane, exosomes subsequently release their content into recipient cells (Raposo and Stoorvogel 2013a).

Although they can transfer materials that enhance or maintain physiological homeostasis, a growing body of evidence suggests that exosomes may also facilitate pathogenic processes by transferring deleterious materials (e.g. viral proteins, aggregated proteins, miRNAs, cytokines etc.) (Arakelyan et al. 2017; Konadu et al. 2015; Levy 2017). Indeed, numerous viral proteins can be detected in excreted exosomes from HIV infected individuals, including Nef, Vpr, Gag, Pol and Tat (Anyanwu et al. 2018). As indicated by Gp-120 containing exosomes, such inclusion of viral proteins can double the rate of infection in naive tissue (Arakelyan et al. 2017). Likewise, exosomes from a variety of cell types (primary mouse astrocytes, CD4+ T cells, 293T, U373) can incorporate Tat; in turn, exposure to such Tat-containing exosomes impairs neural cell viability and blunts neurite elongation (Rahimian and He 2016a; Rahimian and He 2016b). Thus, Nef-containing exosomes may also induce similar dysregulations in the CNS and contribute to neuronal dysfunction underlying HAND.

Focus on Nef-containing exosomes was initiated due to reports of extracellular Nef and Nef-containing microvesicles in the circulation of HIV infected individuals, as well evidence that such soluble protein species reliably induce apoptosis in target cells (Fujii et al. 1996; James et al. 2004; Raymond et al. 2011). Subsequent investigations confirmed Nef's unique myristoylated N-terminus that enables its incorporation into exosomes to a degree that exceeds other viral proteins (i.e. Tat) (Lenassi et al. 2010). Moreover, Nef-containing exosomes are found across various cell models (Jurkat, SupT1, TZM-bl, HeLa, CIITA, Primary T-Cells) and remain functional following fusion with target cells, where they are capable of transactivating HIV LTRs and reducing viability by up to 70% (Campbell et al. 2008; Lenassi et al. 2010). Interestingly, recent *in vitro* and *in vivo* data suggest the Nef released by exosomes into recipient cells may impair functioning by augmenting pro-inflammatory signaling (i.e. TNFα, IL-6) as well as compromising a cell's lipidome (i.e. lipid raft rearrangements, altered cholesterol efflux) (Mukhamedova et al. 2019). Furthermore, inclusion of biologically functional Nef into exosomes appears to be a conserved process, as it has been demonstrated in both non-human primate models as well as primary human astrocytes (McNamara et al. 2018; Pužar Dominkuš et al. 2017).

Emerging evidence provided by the investigations employing neural systems indicate the adverse effects of Nef-containing EVs in the CNS (Table.2). Neural cell lines exposed to

plasma derived Nef-EVs from patients with HAD displayed several abnormalities, including enhanced APP expression as well as elevated production and section of toxic amyloid beta (e.g. $A\beta_{1-42}$); furthermore, compared to exosomes from cognitively sound individuals, exposure to exosomes from individuals diagnosed with HAND similarly induced increased levels of toxic Aβ (Khan et al. 2016). Provided their capacity to serve as latent viral reservoirs, it is notable that microglial derived Nef-exosomes may be particularly potent, as these vesicles can significantly increase BBB permeability and potentiate pro-inflammatory signaling in surrounding CNS macrophages (Raymond et al. 2016). In addition, Nef-containing EVs secreted by Nef transduced primary human astrocytes induce elevated oxidative stress following reuptake by proximal primary neurons (Saribas et al. 2018). Moreover, such Nef-exosomes can also impair functional efficiencies in neurons, as indicated by blunted action potential frequency and decreased spiking activity (Saribas et al. 2018).

In addition to the adverse effects of Nef on recipient cells, due to its strong association with EVs, Nef-EVs are also considered as a potential drug and antigen delivery tool. Recently, the exosomes carrying mutant Nef (Nef ^{mut}) has gained attention as a potential CTL vaccine candidate (Di Bonito et al. 2017; Ferrantelli et al. 2019; Ferrantelli et al. 2018). Nef ^{mut} lacks adverse effects of Nef associated with HIV pathogenesis, has high levels of incorporation into EVs, and acts an exosome anchoring domain that can fuse with heterologous protein and loaded with an antigen of interest. (Lattanzi and Federico 2012; Manfredi et al. 2016).

Conclusions

Human immunodeficiency virus (HIV) infection and its neurological comorbidities remain a major health concern worldwide, despite advances in treatment. HAND effect the majority of patients living with HIV, while advanced aged, illicit drug use, and co-infection increase the risk for such cognitive deficits. Within the CNS, a variety of cell types (i.e. microglia, perivascular macrophages, astrocytes) are susceptible to HIV-1 infection and subsequently release viral particles, such as viral proteins. In turn, HIV infiltration of the CNS is associated with several dysregulated processes that are thought to contribute to HAND, including an altered neuroinflammatory profile and aberrant functioning of non-neuronal cells. As exemplified by Gp120, Vpr and Tat, such dysregulated mechanisms may ultimately be a result of viral proteins themselves. In particular, recent evidence suggests Nef is a crucial modulator of neuronal functioning and may contribute to HAND, even in patients receiving cART, by altering cellular autophagy as well as its inclusion into EVs.

Although its functions within the CNS require further elucidation, we propose a mechanistic model by which Nef may contribute to underlying neuronal dysfunction in HAND (Figure 2). Specifically, we highlight Nef's capacity to inhibit autophagy across multiple cell types, which impairs the degradation of autophagosomes that contains damaged organelles and viral proteins, such as Nef itself. In addition, Nef is readily incorporated into, and translocated by EVs which can subsequently release functional Nef protein in adjacent cells including neurons. Moreover, given that autophagosomes can fuse with MVBs (i.e. amphisomes) and release their content as EVs, it is possible that Nef induced autophagy

dysregulation may potentiate the effects of Nef-containing EVs and vice versa (Sami Saribas et al. 2018). Along with its other aberrant consequences (i.e. metabolic alterations, neuroinflammatory modulation, recruitment of peripheral immune cells etc.), Nef's impairment of autophagy and inclusion into EVs may lead to its uptake by neurons and ultimately compromise neuronal functioning and contribute to cognitive impairments amongst HIV infected individuals.

Given the available evidence of Nef-specific mechanisms in HAND, several therapeutic approaches could prove beneficial and warrant further investigation. Pharmacological enhancement of neuronal autophagy or lysosomal degradation is one such approach, provided that impaired autophagy increases the secretion of Nef-containing exosomes that subsequently hinder neuronal functioning (Saribas et al. 2018). Indeed, the modulation of autophagy is increasingly recognized as a potent therapeutic strategy for a variety of neurodegenerative pathologies (Liu and Li 2019; Liu et al. 2019a).

In addition, the targeted inhibition of Nef on the surface of exosomes can inhibit exosomal reuptake at recipient cells, suggesting the inhibition of extracellular yet membrane-bound Nef (i.e. via monoclonal antibodies or nanoparticle antagonists) may help preserve efficient neuronal functioning (Khan et al. 2016). Such neurotropic immunotherapies warrant particular consideration, provided their increasing application in Alzheimer Disease. In light of rapid advancing gene editing techniques, it may also prove beneficial to target Nef transcripts directly, as functional Nef mRNA can be translocated between cells via exosomes, potentially exacerbating the pathogenic contributions of Nef in HAND (Abudayyeh et al. 2019; Khan et al. 2016).

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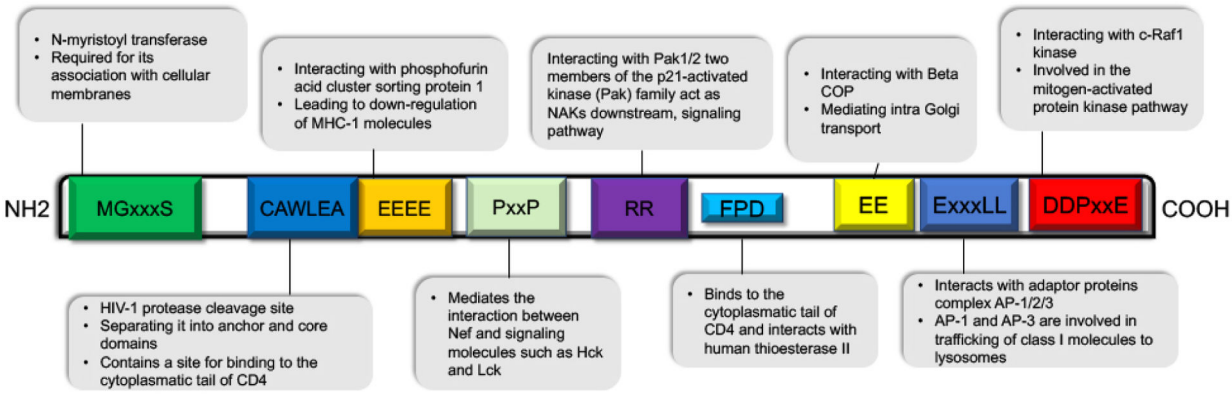

Figure 1: Structural motifs of HIV-1 Nef.

Conserved structural domains of HIV-1 Nef and their interacting proteins are depicted. Nef modification motifs: N-myristoylation site (MGxxx) and HIV-1 protease cleavage site (CAWLEA). Signaling motifs: Proline rich motif (PxxP), RR and DDPxxE. Internalization and trafficking motifs: EEEE, FPD, EE, ExxxLL, and DD.

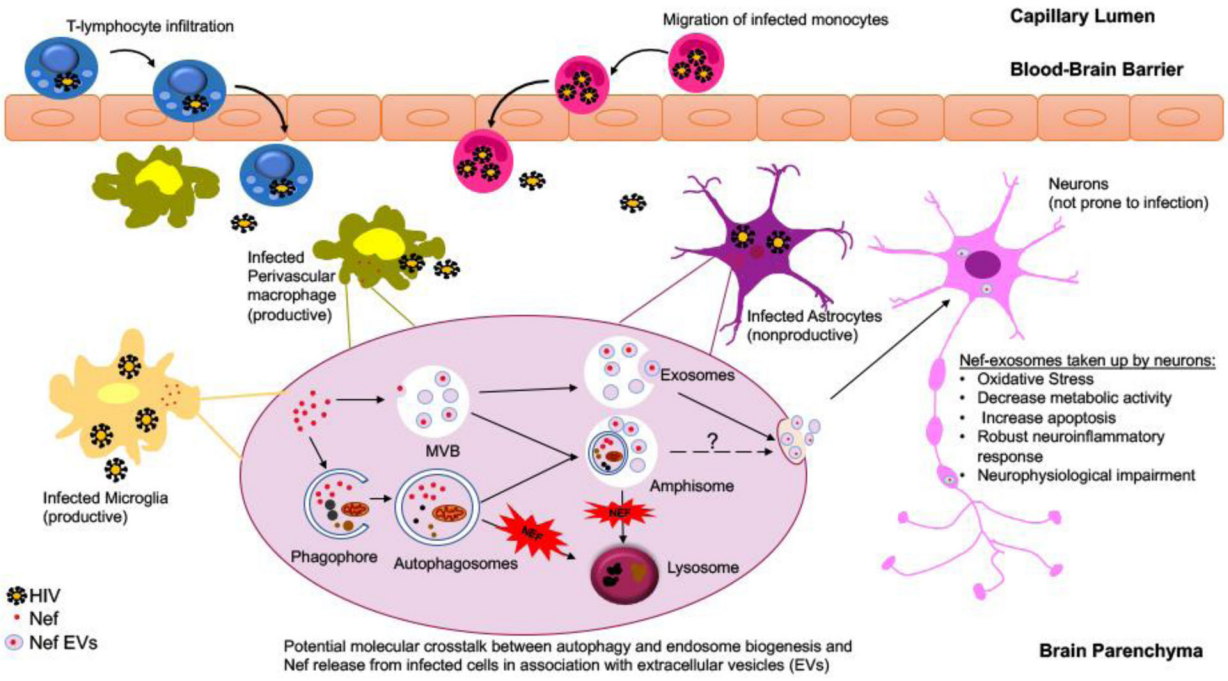

Figure 2: Potential molecular crosstalk between autophagy and endosome biogenesis leading to Nef release from HIV infected glial cells.

Several models were put forth to explain how cell-associated virus can be responsible for HIV infection of the brain. Infected T-lymphocytes and monocyte-derived macrophages can migrate into the brain from the peripheral circulation and seed the virus in the brain. While perivascular macrophages and microglia are considered as the viral reservoirs supporting the productive viral infection in the brain, HIV establishes infection in astrocytes by abortive infection leading to viral replication and release of viral proteins such as Nef. Limited yet accumulating data suggest a prominent role for Nef release from infected cells through two pathogenic processes, cellular autophagy and extracellular vesicle biogenesis and potentially by utilizing an establishing crosstalk between these two pathways. Within the HIV infected cells, Nef blocks autophagy by inhibiting the fusion of autophagosome with lysosomes. Autophagosomes may fuse with multivesicular bodies (MVBs) to produce organelles termed amphisomes, which can subsequently either fuse with lysosomes for content degradation or fuse the plasma membrane and secrete their content in the extracellular matrix. Released Nef exosomes can be picked up by the neurons leading to neuronal toxicity and impairment.

Table1.

Factors contributing the pathogenesis of HIV associated neurological disorders

Host Factors	Age	•	Decreased mortality rates and increased life expectancies
		•	Vulnerable to neuronal injury associated with HIV infection
	Neuroinflammation	•	Release of host chemokine and cytokine products and neurotoxin anti-viral products
	HAART	•	Long term adherence to antiretroviral treatment and increased life expectancies
	Substance Abuse	•	Narcotics, methamphetamine, and cocaine are associated with increase risk of HAND
	Co-morbid conditions	•	Increase risk of infection by other pathogens such as HCV
	Metabolism	•	Central obesity, dyslipidemia, and insulin resistance
Viral Factors	HIV-1 Genes and proteins		
	Tat	•	Compromises a variety of homeostatic processes in the brain, including neuronal viability, synapse formation and neuro-inflammatory profiles
	GP120	•	Induces the production of pro-inflammatory cytokines
		•	Increase in apoptosis in both neuronal cells, Induce ROS production, Disrupt ion regulation and glutamate homeostasis
	VPR	•	Impair the neural mitochondria, inhibiting axonal stability
		•	It can disrupt calcium regulation and induce pro-inflammatory cytokine expression in both neuronal cells
	NEF	•	Decrease metabolic activity and increase apoptosis
		•	Oxidative stress
		•	Up-regulation of IP-10
		•	Up-regulation of CCL2, chemoattractant for circulating monocytes

Table 2.

In vitro and ex vivo studies for Nef-EVs effects in the CNS

Model	Nef-EVs Donor Cells	Nef-EVs Recipient cells	Observed effects of Nef-EVs	Reference
***In vitro* cell culture**	Nef expressing primary human fetal astrocytes	Primary human fetal neurons	Oxidative stress and neuronal electrophysiological impairment	(Sami Saribas, Cicalese et al. 2018)
	Nef expressing microglia (CHME5)	In vitro Blood-Brain-Barrier model	Reduced the expression of tight junction protein ZO-1	(Raymond, Diaz et al. 2016)
	Nef expressing HEK 293T cells	In vitro Blood-Brain-Barrier model	Reduced transendothelial electrical resistance (TEER) and increased permeability of the BBB	(Raymond, Diaz et al. 2016)
	Nef expressing HEK 293T cells	Microglia (CHME5)	Induces microglial cytokine/ chemokine secretion	(Raymond, Diaz et al. 2016)
Ex vivo	Plasma derived Nef-EVs from patients with HAD	Neuroblastoma cell line (SH-SY5Y)	Enhanced APP expression and Increased expression and secretion of Aβ and Aβ peptides	(Khan, Lang et al. 2016)