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HIV-1 Nef in Macrophage-Mediated Disease Pathogenesis

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Abstract

Combined anti-retroviral therapy (cART) has significantly reduced the number of AIDS-associated illnesses and changed the course of HIV-1 disease in developed countries. Despite the ability of cART to maintain high CD4+ T-cell counts, a number of macrophage-mediated diseases can still occur in HIV-infected subjects. These diseases include lymphoma, metabolic diseases, and HIV-associated neurological disorders. Within macrophages, the HIV-1 regulatory protein "Nef" can modulate surface receptors, interact with signaling pathways, and promote specific environments that contribute to each of these pathologies. Moreover, genetic variation in Nef may also guide the macrophage response. Herein, we review findings relating to the Nef–macrophage interaction and how this relationship contributes to disease pathogenesis.

Keywords

cardiovascular disease; dementia; HIV-1 lymphoma; macrophages; Nef

INTRODUCTION

Combined anti-retroviral therapy (cART) has increased the life expectancy of HIV-1-infected individuals in industrialized countries. cART functions by targeting HIV-1 replication in CD4+ T cells, the primary cell-type infected by the virus, using a combination of drugs that affect the functions of several viral proteins necessary for viral entry, reverse

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transcription, integration, maturation, and assembly. Because HIV-1 infection is characterized by a large degree of genetic variability driven by high replication, mutation and recombination rates, the virus can develop resistance to cART [1]. Monocyte-derived macrophages (MDMs), which are not efficiently targeted by cART [2–4], are another primary reservoir for HIV-1 infection. Studies show that HIV-1 can be found in MDMs from patients on extended cART therapy [5, 6]. During late-stage disease when CD4+ T-cell counts drop to undetectable levels, HIV-1-infected MDMs continue to perpetuate and are found in different tissue types [7]. Moreover, it is well known that specific HIV-1 subpopulations can be detected in macrophages infiltrating brain tissues [8, 9]. In particular, HIV-1 productive infection has been shown to occur in perivascular macrophages [10]. Circulating monocytes also harbor HIV-1 and are permissive to viral replication after they differentiate into MDMs [11, 12].

Phylogenetic analyses of HIV-1 sequences outside the brain have provided evidence of similar genetic variants of HIV-1 within macrophage-rich tissues in different regions of the body, suggesting that viral populations in tissue-associated macrophages can migrate in either monocytes or macrophages [13]. Before the advent of cART, the role of HIV-1-infected MDMs in the development of AIDS was unclear; however, it is now evident that even when CD4+ T-cell counts remain high, macrophage-mediated diseases are a significant threat to HIV-1-infected individuals [14–16]. HIV-1-infected T cells rapidly undergo apoptosis due to the cytopathic effects of viral replication [17]. Occasionally, an infected T cell can revert to a resting memory state, which results in an integrated but transcriptionally silent provirus [18, 19]. MDMs can also be infected by HIV-1 for a considerable period of time before cell death occurs [17, 20]. In addition, it has been reported that MDMs are frequently infected with multiple HIV quasispecies, resulting in high rates of viral recombination [7, 21, 22]. Some studies have concluded that HIV-1 infection in MDMs may make them resistant to apoptosis thereby increasing the cell's life span [23, 24]. As a consequence, once they are HIV-1-infected, MDMs can affect the immunologic function and metabolism through altering cytokine immune-system signaling responses [25], increasing cholesterol efflux [26, 27], blocking normal immune responses [28] and inducing tumor-promoting factors [29]. As abnormal macrophage function is associated with many diseases that occur in non-HIV-1-infected individuals, it is not surprising that some of these same diseases are found more frequently in the HIV-1-infected community.

In the last decade, considerable research has focused on how specific HIV-1 proteins may alter macrophage function. One HIV-1 regulatory protein, Nef, has been directly implicated in "dysregulating" the HIV-1-infected macrophage's function. Three major HIV-1-associated diseases, AIDS-related lymphoma (ARL), metabolic syndrome, and HIV-associated dementia (HAD) are often considered separately in the literature; however, they are all macrophage-mediated disorders in which a Nef-association has also been considered. The aim of this review is to present in a coherent framework the recent findings concerning HIV-1 Nef and its association with macrophage-mediated disease processes.

I. Macrophages and the Immune Response

Monocyte-derived macrophages (MDMs) are the primary differentiated cells in the mononuclear phagocyte system. These immune cells are derived from bone marrow, distributed throughout the body, and display great structural and functional heterogeneity [17, 30]. MDMs are among the first immune cells to recognize pathogens and can both ingest them and signal other immune cells, primarily T-cells, to mount an immune response. In the CNS, the primary immune system cells are MDM-derived microglial cells and in general T-cells are not involved except in pathological processes [31]. MDMs are involved in many disease-related processes and generally increase in number locally during wound

healing, inflammation and malignancy. While MDM modulation of the immune response is usually beneficial, certain conditions can cause MDMs to mediate tissue destruction.

Virus infection is known to activate MDMs [30]. Herbein and Varin described three different macrophage activation states: (1) M1 is the IFN-γ classically activated macrophage that displays a pro-inflammatory response, (2) M2 is the macrophage activated by IL-4 and IL-13 that displays an anti-inflammatory response and, (3) dM represents a macrophage deactivated by IL-10, which leads to immune suppression [25]. Polarization of MDMs into classically activated M1 and alternatively activated M2 macrophages is critical in mediating an effective immune response against invading pathogens; however, this defense system is reversed during HIV-1 infection as the virus uses these pathways to facilitate viral dissemination and pathogenesis [32]. Inflammatory M1 macrophages are recruited to sites of infection, typically have a short half-life and may cause tissue damage. Noninflammatory M2 macrophages produce immunosuppressive cytokines that counteract M1 signaling and, in the context of cancer, may promote tumor growth and progression [32–34]. A more flexible description of macrophages has also been provided wherein there is less delineation between M1 and M2 macrophages, suggesting these phenotypes overlap. In this classification system, macrophages have “many different shades of activation that have yet to be identified, resulting in a ‘spectrum’ of macrophage populations based on their function [35].”

II. HIV-1 Nef

HIV-1 Nef is required to maintain high virus production within infected cells [36–38] and is unique to simian and human immunodeficiency viruses. The loss of Nef function results in delayed or absent AIDS progression [39–41]. HIV Nef RNA is the first transcript to accumulate in abundance after infection of macrophages and T-cells and is found at higher amounts than any other HIV regulatory protein [42, 43]. Additionally, the Nef protein promotes survival of infected cells [44] and increases the migration of monocytes [45]. Within the cell, Nef modulates a variety of surface receptors [46]. CD4 downregulation by Nef is required for viral spread in macrophages [47]. Downregulation of MHC class I receptors provides for protection against cell lysis [40, 48]. Nef reduces MHC class II antigen presentation as a strategy to evade MHC class II-dependent immunity [49, 50]. Nef has also been implicated in activating phosphatidylinositol 3-kinases (P13K), a process that can impair host cell response to infection. [51]. An interaction between HIV-1 Nef and calmodulin has been noted, suggesting that Nef might interfere with intracellular Ca^{2+} signaling and calmodulin-mediated processes such as inflammation, metabolism, apoptosis, nerve growth and the immune response [52]. Tetherin is a human cellular protein that inhibits release of virus particles by binding them to cell membranes; Nef counteracts tetherin, sequestering tetherin within intracellular macrophage compartments and allowing for more efficient viral diffusion [53–55].

The Nef protein contains a myristoylation domain that allows it to bind to lipid membranes, a transmembrane domain that includes a conserved core and a C-terminal flexible loop that is cleaved from the protein after attachment [Figure 1). These domains have been crystallized independently (1EFN, 1QA4, 2NEF). In one of these structures (1EFN), Nef is shown binding the SH3 domain of a Fyn tyrosine kinase, thus demonstrating one of the important interactions of the protein [56]. Other important binding domains in Nef have also been identified, including a cytokine-binding motif in the first alpha-helix [57] and an actin cortical patch-binding site within the Nef loop domain [58]. Nef is characterized by a large degree of genetic heterogeneity during chronic infection; therefore, it may evolve to optimize viral persistence during varied selective pressures *in vivo* [59, 60]. Additionally, Nef forms dimers localized to the plasma membrane and the trans-Golgi network [61]. Bentham et al. (2006) found that the myristoylation of Nef alone is insufficient for lipid

binding and suggested that it is a complex interaction of basic residues at the N-terminus, including electrostatic interactions that allow Nef to migrate and bind to target membranes [62]. Giese et al. (2006) agreed and elaborated that the association of Nef to lipid membranes is governed by a complex pattern of amino acid signature motifs that differentially contribute to individual Nef activities [63–65]. The association of Nef with the cellular membrane is believed to be essential for virtually all of its effects in infected cells [63, 64]; however, there is also a significant amount of cytosolic Nef [62, 66–69]. In fact, two recent studies have found that Nef travels from cells via exosomes that form at the plasma membrane [70, 71]. Recently, we have observed varied patterns of Nef staining within brain tissues (meninges and frontal lobe): (1) positive staining within perivascular macrophages, (2) large clusters of diffuse staining and (3) small spots of positive staining throughout the tissue that appears extracellular [Figure 2]. In contrast, similar staining with a p24 antibody in the same subject's brain tissues revealed that p24 was localized primarily within cells. As the number of known cellular interactions associated with Nef increases, it is likely that both the myristolic and cytosolic forms of Nef contribute to the biological activity of the protein and alterations in Nef will be linked to specific HIV-1 disease pathologies.

III. AIDS-Related Lymphoma

AIDS-related lymphoma (ARL) is a disease process wherein HIV-1-infected activated macrophages are found within B-cell lymphomas. ARL is similar to lymphoma found in non-HIV-1-infected individuals but it is highly metastatic and clinically severe [13, 17, 29]. Although early studies pointed to EBV coinfection as the primary cause of ARL, it was reported recently that approximately half of all ARLs do not contain detectable known oncogenic viruses [29, 72]. Recently, we have suggested that HIV may have lymphomagenic potential for a subset of tumors [72]. Xu and colleagues [73] demonstrated that HIV-1-infected macrophages form small virological synapses, termed nanotubes, which extend into B-cells in the presence of Nef. These nanotubes then exclusively transport Nef from macrophages into B-cells, evading protective virus-specific immunoglobin responses (IgG2 and IgA) at the mucosal sites of entry and interfering with the production of certain classes of antibodies in B-cells. The transfer of Nef to B-cells via nanotubes decreases CD40-dependent activation; interferes with germinal center factors such as Bcl-6, AID, and IRF4; and increases differentiation factors and receptors Blimp-1 and CD138 [73–75]. Swingler and colleagues [76] have also described an intricate relationship between Nef, macrophages and B-cells, indicating that the HIV-1 Nef protein carries a pathogenic determinant that governs B-cell defects in HIV-1 infection. Antigenic stimulation of B-cells by activated macrophages plays a central role in the pathogenesis of ARL [17, 77, 78]. HIV-1 Nef has been directly implicated in B-cell activation via production of soluble factors such as IL-2 and IL-6 and elevation of immunoglobulin [76]. In fact, HIV-1 Nef is absorbed by B-cells [73, 75, 79]. Salemi et al. [13] used a phylodynamic approach and found that HIV-1 migrates to sites of metastasis within the lymphatic system where it becomes compartmentalized within tumors. In addition, the study noted that tumor sites contain 100-fold more HIV-1 than normal tissues and that tumor tissues significantly exchange more virus with lymph nodes than with normal tissues. These results suggest an important relationship between HIV-1 evolution and lymphoma pathogenesis. It has also been reported that specific Nef signatures and three-dimensional structures were associated with viruses isolated from ARL tumor tissues [80]. The study found that ARL amino acid signatures mapped to regions of the Nef structure involved with SH3 binding, the internal core domain necessary for structural maintenance, and the flexible Nef loop domain. Interestingly, this latter amino acid signature exists in a Nef domain that was analyzed in Xu study [73], which determined that the motif was important for effective macrophage nanotube formation and the subsequent transfer of Nef into B-cells.

IV. Cardiovascular Disease, Atherosclerosis and Other Lipid Disorders

Metabolic syndromes comprise another category of HIV-1-associated diseases, especially in individuals on long-term cART. Due to both infection and antiretroviral drugs, the HIV-1-infected population has up to twice the risk, when compared to the uninfected population, of developing cardiovascular disease (CVD), atherosclerosis, dyslipidemia or insulin resistance [81–83]. Although abnormal lipid profiles in HIV-1-infected individuals were noticed before the introduction of cART, dyslipidemia has since been associated with specific antiretroviral drugs [84, 85], increased life expectancy [86], or the impact of HIV-1 on the macrophage reservoir [87–89]. The HIV-1 Nef protein has been implicated in contributing to macrophage cholesterol efflux, an important mediator in HIV-1 metabolic disorders [26, 27, 46, 90].

During the development of atherosclerosis in the uninfected population, fatty lipids are deposited within arteries. Phagocytic macrophages are then recruited to arterial lipid deposits where they become activated and develop fat-containing vacuoles. The damaged endothelium begins to produce pro-inflammatory cytokines such as IL-6. Damaged tissues attract more macrophages to the site of lipid deposition and eventually cause vascular breakdown, bleeding and a self-propagating inflammatory response to injury [91]. This process is similar to that which occurs during the perivascular macrophage inflammatory response related to HIV-1-associated dementia [92–94]; however, the atherosclerotic process differs in that the perivascular HIV-1-infected macrophages in atherosclerosis are associated with arteries, rather than the central nervous system (CNS) veins involved in HAD. Elevated cholesterol content alters plasma membrane morphology and induces cytoskeletal changes in macrophages [95, 96]. When cholesterol uptake from macrophages exceeds a certain threshold, the cells become foam cells and the chance of necrotic tissue formation increases. Foam cells are less nomadic than monocytes and macrophages [97] and express adhesion molecules on their surface [27, 95].

Although much is known about the biological pathogenesis of lipid disorders such as atherosclerosis, for a long time it was unclear why the incidence was elevated in those infected with HIV-1. In 2005, van't Wout et al. reported the effects of HIV-1 infection on cholesterol biosynthesis and uptake using microarrays [98]. The group used HIV-1-infected T-cells in culture and found elevated expression of seven cytosolic cholesterol enzymes that are key intermediates in cholesterol and sterol biosynthesis (IDI1, FDPS, SQLE, LSS, CYP51, HSD17B7, and DHCR24). Similarly, the low-density lipoprotein receptor (LDLR) and one cholesterol regulator (INSIG1) were increased in HIV-1-infected cells with an intact *nef* gene. In 2006, Mujawar et al. [26] demonstrated that the *nef* gene impairs ATP-binding cassette transporter A1 (ABCA1)-dependent cholesterol efflux from human macrophages, leading to chronic immune activation and inflammation. Morrow et al. made a similar observation [99]. ABCA1 is a major regulator of cellular cholesterol and phospholipid homeostasis [100]. ABCA1 deactivation is also implicated in Tangier disease, a rare genetic disease found in non-HIV-1-infected patients that is characterized by a severe reduction in the amount of high-density lipoprotein (HDL) in the bloodstream and accumulation of cholesterol in body tissues [101]. Besides atherosclerosis and CVD, Tangier disease also causes other pathological defects commonly identified at autopsy in HIV-1-infected patients, including neuropathy, splenomegaly, and hepatomegaly. Recently, a study of simian immunodeficiency virus (SIV)-infected Rhesus macaques, the primary animal model for HIV-1 infection, showed that when the animals were fed an atherogenic diet, HDL profile changes occurred similarly to those in HIV-1-infected individuals with lipid disorders [102]. In addition, the study found an abundance of Nef protein within atheromas at the time of necropsy as well as an abundance of Nef protein within the liver and plasma of atherosclerotic animals. Moreover, serum samples from the SIV-infected macaques also had suppressed cholesterol efflux in a Nef-dependent fashion [102]. These results indicate that

SIV infection is also a significant contributor to primary dyslipidemia through the ability of Nef to suppress ABCA1-dependent reverse cholesterol transport.

Lipodystrophy is the loss or gain of localized fat tissue and is frequently found in HIV-1-infected patients on cART. Although phenotypically much different from atherosclerosis or CVD, this medical condition may share a common pathological origin because it is also the result of metabolic changes that occur during long-time HIV-1 infection, cART therapy, or altered lipid mobilization [88, 103]. Lipodystrophy may also cause B-cell dysfunction [104] and is associated with impaired feedback of insulin on B-cells [105]. This association with B-cell dysfunction again highlights the newly identified connection between macrophages, Nef and the B-cell nanotube. It is possible that future studies may show that the transfer of specific Nef variants to B-cells also impacts the pathogenesis of metabolic disorders during HIV-1 infection.

To our knowledge, only one study has examined the molecular evolution of HIV-1 Nef during severe CVD and atherosclerosis [106]. The *nef* sequences were derived from several body and brain tissues of a patient on cART. Viral Nef populations found within tissues at autopsy were highly related and noncompartmentalized, even between brain and nonbrain compartments. This finding may be related to massive arterial leakage into tissues throughout the body before death. In significant contrast, a phylogenetic analysis of the env sequences from the same individual showed a higher degree of compartmentalization within brain tissues [13, 106]. The discovery of a more conserved HIV-1 *nef* gene and a compartmentalized *env* gene suggests that the patient might have possessed a genetic variant of *nef* that was under different evolutionary pressures in different compartments. This case suggests the possible existence of metabolic-disease-specific *nef* variants, an observation that will require follow-up studies.

V. HIV-1-Associated Neurocognitive Disorders

Neurocognitive changes have been reported in HIV^+ individuals since early in the AIDS epidemic [107]. These CNS symptoms were initially identified in persons with advanced HIV-1 infection and were first termed “AIDS Dementia Complex” and later “HIV-Associated Dementia” [108, 109]. The pre-mortem manifestations of HAD are often associated with one or more histopathological abnormalities on autopsy that were considered unique to HIV-1-patients. These features were defined as HIV encephalitis (HIVE), HIV leukoencephalopathy, and diffuse poliodystrophy [110–112]. HIVE was defined as multiple disseminated foci of microglia, macrophages, and multinucleated giant cells. If multinucleated giant cells were absent, the presence of HIV-1 antigen or nucleic acids was required to make this diagnosis [112]. HIV leukoencephalopathy was defined as diffuse damage to white matter that included myelin loss, reactive astrogliosis, macrophages, and multinucleated giant cells with little or no inflammatory infiltrates. Diffuse poliodystrophy was defined as reactive astrogliosis and microglial activation involving the cerebral gray matter [112, 113].

Continued investigation revealed that milder forms of neurocognitive impairment could be detected in many HIV-1-infected persons before the onset of advanced systemic disease [114]. Furthermore, a significant proportion of HIV-1-infected persons with documented pre-mortem HAD did not manifest the neuropathological hallmarks associated with HIVE at autopsy. Subsequent studies indicated that the postmortem finding most strongly associated with HAD was the number of activated macrophages present within affected areas of the brain [115]. In addition, dendritic loss [116], neuronal loss [117], and brain HIV viral load [118] are now considered important findings.

After the introduction of cART, the incidence of HAD declined dramatically [119]; however, milder forms of HIV-associated neurological disorders became highly prevalent, found in up to 50% of HIV-infected individuals [120]. The increased prominence of mild impairment led to a new research nosology called “HIV-Associated Neurocognitive Disorders” (HAND) in 2007 that includes asymptomatic neurocognitive impairment, minor neurocognitive disorder, and HAD [109]. cART-treated patients with HAND differ from pre-cART patients in many ways. They are less likely to have detectable HIV-1 RNA or have high levels of certain inflammatory biomarkers in their CSF [121]. At autopsy, they are less likely to manifest florid neuropathological changes [122, 123]. These observations have led to a reevaluation of the pathogenic mechanisms of HAND, including interest in persistent viral reservoirs, persistent CNS inflammation, and neurotoxicity, all of which are associated with the roles for activated macrophages and Nef [124–129].

HIV-1 enters the brain during early infection, as documented by case study and animal experiments [130, 131]. Infected CNS cells include circulating monocytes, macrophages, microglia, and astrocytes. There is minimal evidence for active infection/replication in either neurons or oligodendrocytes, although viral proteins have occasionally been identified in these cells. However, neurons and oligodendrocytes are victims of neuroinflammation and neurotoxicity, both directly (from individual neurotoxic viral proteins shed by HIV-1-infected cells), and indirectly (from neurotoxic soluble products in the HIV-1-infected environment) resulting in cognitive, motor and behavioral changes [132]. Importantly, cART does not entirely clear the CNS of HIV-1 [133]. In part, this is due to poor drug penetration of some regimens [134]. However, the predilection of HIV-1 to reside in CNS macrophages, microglia and astrocytes, which harbor a large reservoir of provirus and unintegrated DNA unaffected by cART, is also a likely factor [133]. Thus, progressive neuronal loss/dysfunction and CNS inflammation can be measured even in well-suppressed patients on long-term cART [135]. Consequently, the CNS should be considered an important viral reservoir, a source of viral escape [136] and a barrier to full HIV-1 eradication.

Circulating monocytes can enter the CNS and differentiate into many types of macrophages, including perivascular macrophages [137, 138]. These cells inhabit the Virchow–Robin spaces surrounding cerebral vessels. They are phagocytic and antigen-presenting cells that, when activated, can produce a large number of soluble substances important in the pathogenesis of HAND. They can be continually replenished by new recruits and act as a sensitive interface between the systemic circulation and brain [139].

Most HIV-1 is transported across the blood–brain barrier (BBB) in $CD14^+/CD16^+$ monocytes [117]. Expanded populations of these cells and high levels of circulating soluble CD14+ have been associated with HAND [140–142]. CD14+/CD16+ monocytes are especially likely to be HIV-infected, tissue-invasive, express pro-inflammatory cytokines that increase BBB permeability [143] and express chemokines such as CCL2 (MCP-1) [144] and adhesion molecules, such as PECAM-1, [145] that increase the trafficking of cells across the BBB [144] and perpetuate reinfection. Once they have differentiated into long-lived perivascular macrophages, they can infect new cells by forming tight cell-to-cell contacts using a matrix metalloproteinase-9 mechanism [146]. Perivascular macrophages are also considered the primary source of virus within the CNS [10]. Zink et al. [147] utilized the SIV-infected macaque model to determine the effect of cART on brain macrophage reservoirs. Despite a rapid decline in plasma and CSF SIV RNA and undetectable brain SIV RNA at the time of sacrifice, cART-treated animals had similar levels of brain macrophages and similar brain SIV DNA levels to those of untreated animals. They hypothesized that acute viral seeding of the brain occurs in early infection and that ongoing seeding is unnecessary for viral DNA to persist in the brain.

Whether macrophages are HIV-1 infected or uninfected but activated they still produce soluble substances that contribute to an increasingly toxic, inflammatory, and dysregulated CNS milieu and are implicated in neuropathogenesis [148, 149]. Substantial amounts of unintegrated (circular) HIV-1 DNA can persist for long periods in macrophages and can support transcription of viral genes, such as *nef* and *tat*, and can induce neurotoxic cytokines such as CXCL9 and CXCL10 [150, 151]. Exposure to *nef* and *tat*, and/or platelet-activating factor stimulates macrophages to produce the neurotoxin quinolinic acid in physiologically relevant concentrations [152]. The soluble products of HIV-exposed macrophages inhibit long-term signal transmission between neurons in brain slices, indicating yet another possible mechanism for HAND [153].

Monocytes that enter the brain during early fetal gestation differentiate into microglia. Microglia comprise a large and very stable population of fixed tissue macrophages (resident macrophages of the brain) that can perform phagocytic functions and are characterized by very slow cell turnover. Once introduced into the CNS, HIV-1 can infect these long-lived parenchymal microglia [154]. They are among the earliest cells to show the effects of HIV-1 in the brain [155]. Both infected and activated microglia can produce neurotoxic proteins and immunomodulatory substances such as TNF, IL-1β, and platelet-activating factor, excitatory amino acids, and inducible nitric oxide synthase (iNOS) [154, 156, 157].

It has been debated whether a viral component to the development of HAND exists due to the presentation of HAND with undetectable plasma viral loads. Instead, the initial HIV-1 infection may trigger an inflammatory response that leads to HAND. This trigger could permanently alter monocyte trafficking into the brain or switch brain macrophages to the pro-inflammatory M1 stage, eventually resulting in the wide range of neurological symptoms seen during HAND pathogenesis. Recently, a viral infection was simulated by the injection of poly-IC (inducer of an antiviral response) early in an animal's life that accelerated the development of neurodegeneration [158]. Similarly, a single injection of lipopolysaccharide (LPS) into the hippocampus of the transgenic mice model for Alzheimer's Disease (AD) led to the development of accelerated AD later in life [159]. Clearly in either of these cases or in humans with AD, the presence of HIV-1 or any other virus is not required to drive ongoing neurodegeneration. Similarly, in the case of chronic traumatic encephalopathy (CTE), athletes develop cognitive malfunctioning years or even decades after an apparently resolved brain injury and there is evidence of a continuing, persistent neurodegenerative process after the original injury [160]. CTE is thought to develop in part due to disruptions of the cerebral microvasculature and the blood–brain barrier that occur at the time of the traumatic injury. A subset of HIV-infected individuals could suffer from an experience similar to CTE, where they have an initial "hit" during primary encephalitis and subsequent "hits" during less severe HIV-related illnesses. Repetitive activation of brain monocytes/macrophages over years of infection and treatment could result in post-cART dementias and may even be associated with clinically observed mild cognitive motor disorders that can occur intermittently over the course of HIV-1 infection. CTE shares microscopic similarities to AD and clinical similarities to HAD because of the long time between the initial trauma and nerve–cell breakdown and death. In cases of CTE, AD and HAND, increases in β-amyloids in the CSF have been noted [161–163]. If a "viral-trigger" mechanism for the development of HAND is possible, then procedures under development to deliver cART more readily to the brain may not be the best medical treatment for such patients [164]. Furthermore, during AD, macrophages release sphingolipids that alter the functional plasticity of neurons [165]. To our knowledge, excess sphingolipids have not yet been examined as a direct or associated cause for HAD pathogenesis, but the possibility deserves further investigation.

VI. Nef as a Significant Contributor to CNS Pathogenesis

Nef plays a key role in producing a toxic environment in the CNS that leads to HAND. Furthermore, studies show that Nef-deleted viruses are less neurotropic [166] and neurovirulent [167] than intact Nef proteins. Nef itself is a neurotoxin, with structural and functional similarities to scorpion peptides [168]; it is directly toxic to astrocytes *in vitro*, and is also toxic to neurons, possibly by indirect, IP-10-mediated cytotoxicity [129]. Ranki et al. [169] reported Nef in astrocytes of patients with HAD and HIVE. Nef can attract migration of monocytes and macrophages in animal models when injected into the brain [170]. Acheampong et al. reported that Nef was able to induce BBB dysfunction by inducing apoptosis in human brain microvascular endothelial cells [171]. Interestingly, in another paper on Nef neuropathogenesis, he reported that the effects of Nef were increased by hyperglycemia, a highly pertinent observation given the growing number of HIV-1-infected persons with diabetes [172]. Spoerer et al. [173] reported that Nef disrupted the BBB impairment by a matrix metalloproteinase-9 mechanism that disrupts the tight junctions of the BBB [45, 128, 143], whereas other investigators have reported that Nef increases monocyte migration across the BBB by upregulating the production of chemokines such as CCL2 by HIV-1-infected astrocytes [45, 128, 143].

Nef can enhance viral replication and infectivity, downregulate CD4+ (thus increasing HIV-1 replication) [174], and downregulate MHC class I surface molecules (protecting the infected cell from lysis by cytotoxic lymphocytes and contributing to immune evasion) [175] [82]. All of these activities contribute to forming a robust and long-lived reservoir of HIV-1 in the CNS. Nef stimulates production of inflammatory cytokines by macrophages (such as MIP-1α, MIP-1β, TNF-α, IL-1β, and IL-6) [57], as well as the neurotoxin quinolinic acid [152] and modulates cell-signaling pathways [57], thus contributing to the indirect effects of HIV-1 on uninfected cells such as neurons. Verollet et al. reported that Nef could trigger the formation of multinucleated giant cells from HIV-1-infected macrophages, a histopathological finding that is considered a hallmark of the AIDS brain [171].

Whether expressed in infected monocytes/macrophages or introduced in experimental settings, Nef increases the transcription of genes for inflammatory factors such as macrophage inflammatory proteins IL-1β, IL-6, and TNF-α, and also corresponds to activation of NF-κB [176, 177]. Mordelet et al. grafted Nef-transduced macrophages into the hippocampus of rats. These transplants resulted in the expression of Nef by the grafted macrophages, recruitment of monocytes/macrophages into the area, expression of TNF, astrogliosis, and cognitive changes (e.g. reaction to spatial change) whereas these changes were not observed in control rats [178].

The reason that some HIV-1-infected patients develop HAD, others are HIV-1 infected in the brain with no signs of HAD, and others may never develop a significant HIV-1 infection in the brain may be associated with HIV-1 Nef signatures that influence protein structure or function. This concept is supported by studies of the varied prevalence of HAD within similar populations of individuals infected with different subtypes of the virus [179]. An earlier study by Salemi et al. [180] examined HIV-1 env evolution in the brain of a single patient with HAD and indicated that migration of viruses to regions of the brain associated with cognitive function (frontal and temporal lobes) was significantly higher than migration of viruses to other brain tissues. A study by Zhao et al. [106] showed that some patients may have no PCR-amplifiable amount of HIV-1 within brain compartments at the time of death. Moreover, a recent study employing the CD8-depleted macaque model of neuroAIDS has identified the presence of infected and expressing macrophages in the brain at 21 days post infection [181]. The study also demonstrated the presence of a high-frequency macrophage-

tropic viral variant in the bronchial lavage fluids and in brain macrophages from different infected animals that developed SIV encephalitis.

The degree of HIV-1 Nef evolution between lymphoid and brain tissues may be due to selection of specific Nef variants at the blood–brain barrier that are more favorable for expansion within the brain. Olivieri et al. noted differences among brain and lymphoid Nef isolates involved in the T-cell epitope, PACS1 and Src 3 family kinase binding [182]. Lamers et al. compared Nef sequences from patients with and without HAD and found several differences within the protein that could modify the degree of SH3 binding, alter structural orientation of the internal core domain and make available an additional cysteine residue in the variable loop domain that would be available for binding [80]. A study by Gray et al. found that "CD4 and MHC-1 down-modulations are highly conserved functions among Nef alleles from CNS- and lymphoid-derived HIV-1 isolates that may contribute to viral replication and escape from immune surveillance in the CNS" [183]. In a study of the evolution of Nef in SIV-infected pigtailed macaques, it was discovered that macaques that developed neuroAIDS selected for a mutation that contributed to its phenotype [184]. Brumme and colleagues [185] performed a large-scale multigene analysis and showed that Nef contains a dramatically high level of complex HLA-associated polymorphisms in comparison to other HIV-1 proteins; therefore, they concluded that Nef may "exhibit higher levels of mutational plasticity in response to selective pressures."

VII. Summary

The classic definition for HIV-1 Nef function is that it downregulates CD4 and MHC surface proteins and increases the ability of the virus to replicate and infect other cells. However, Nef is unique among other HIV-1 proteins because of its several cytotoxic effects that are not specific to virus-associated functions. Within macrophages, Nef is particularly destructive because of its ability to switch an important immune cell into a cell capable of causing damage and disease. In Figure 3, we summarize some of the important Nef-mediated processes in macrophages that have been associated with the pathogenesis of three major HIV-1-related diseases: lymphoma, dementia and lipid disorders. With the increased ability to control HIV-1 infection in T cells, further knowledge about macrophage-associated diseases related to the ability of Nef to bind cellular membranes, leak from infected cells and alter biochemical pathways may become evident. To this end, new therapies targeting Nef proteins or HIV-1 infection of macrophages should be developed and added to ongoing HIV-1 treatments regimens.

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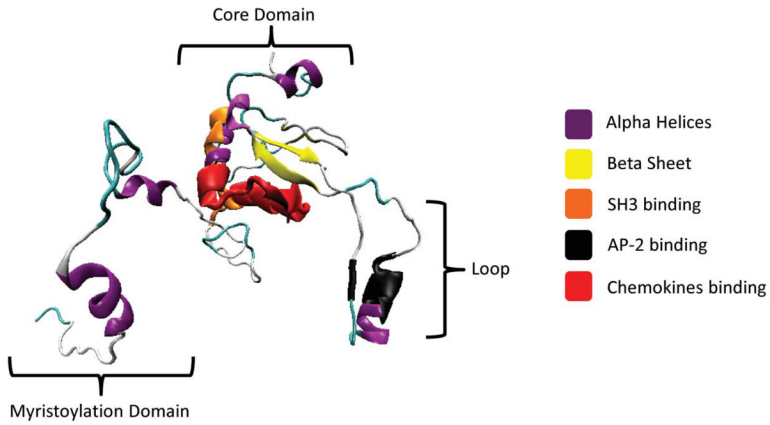

FIGURE 1.

Three-dimensional structure of HIV-1 Nef. The structure was generated as previously described [80]. Secondary structure and several important binding domains are highlighted as indicated.

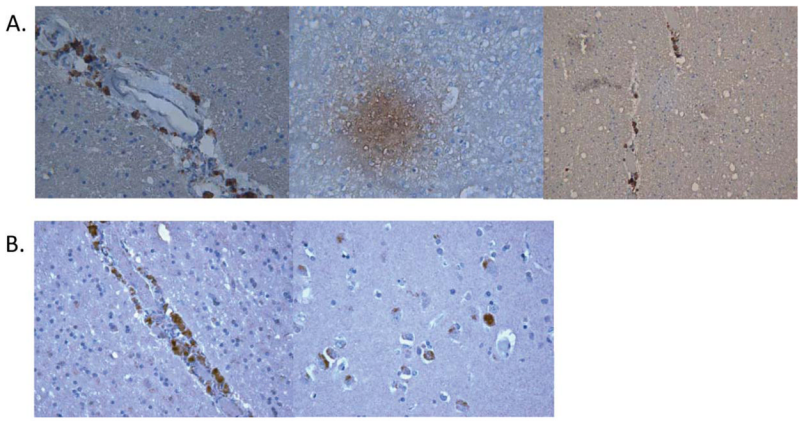

FIGURE 2.

Fixed brain tissues stained with Nef and p24 antibody. (A) Anti-Nef antibody positive staining in perivascular macrophages surrounding a meningeal blood vessel (left), large clusters of diffuse staining in brain parenchyma (center), and very small spots of positive staining within brain parenchyma (right). (B) Anti-p24 staining of macrophages lining a blood vessel in meninges (left) and localized to macrophages in brain parenchyma.

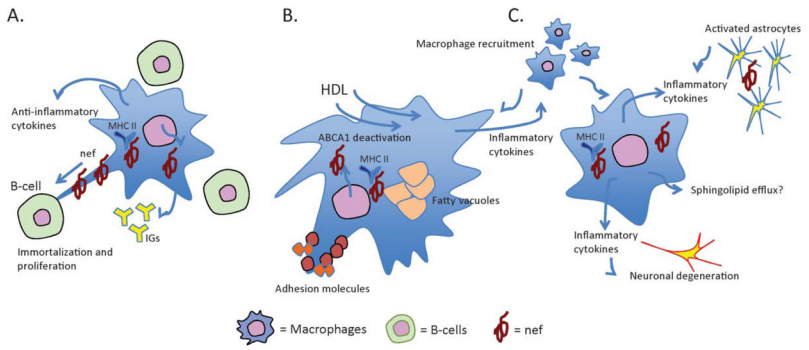

FIGURE 3.

Major HIV-1 Nef-associated macrophage responses linked to specific disease pathologies. HIV-1 infection causes morphology changes in both MDMs and B-cells. (A) During lymphoma pathogenesis, M2-activated MDMs produce anti-inflammatory cytokines. Nef stimulates MDMs to form conduits that selectively transfer Nef to B-cells, thus allowing the protein to bypass B-cell immunoglobulin receptors. Within B-cells, Nef interferes with germinal center factors and increases differentiation factors. MDMs present tumor-associated antigens to B-cells that result in B-cell immortalization and proliferation. Lymphoma tumors have increased rates of viral replication. HIV-1 migrates within MDMs to distant metastatic sites. (B) During lipid disorders, M1 MDMs produce inflammatory cytokines. Nef mediates ABCA1 deactivation that reduces cholesterol efflux and there is an increase in cholesterol enzyme intermediates. Fatty vacuoles form in the cell. Adhesion molecules appear on the surface of MDMs making them less motile. Additional macrophages are recruited to the site of inflammation. (C) During HAD pathogenesis, MDMs also produce inflammatory cytokines that trigger the recruitment of further MDMs into the brain. Cytosolic Nef stimulates astrocytes to produce cytokines as well with the overall result of neuronal degeneration. Nef may also stimulate shingolipid efflux, which could contribute to dementia pathogenesis. In all three HIV-1-associated pathologies, Nef can bind MHC class II receptors within and at the cell surface, SH3 molecules and the trans-Golgi network.