EDITORIAL REVIEW

Comorbidities of HIV infection: role of Nef-induced impairment of cholesterol metabolism and lipid raft functionality

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Combination antiretroviral therapy has dramatically changed the outcome of HIV infection, turning it from a death sentence to a manageable chronic disease. However, comorbidities accompanying HIV infection, such as metabolic and cardio-vascular diseases, as well as cognitive impairment, persist despite successful virus control by combination antiretroviral therapy and pose considerable challenges to clinical management of people living with HIV. These comorbidities involve a number of pathological processes affecting a variety of different tissues and cells, making it challenging to identify a common cause(s) that would link these different diseases to HIV infection. In this article, we will present evidence that impairment of cellular cholesterol metabolism may be a common factor driving pathogenesis of HIV-associated comorbidities. Potential implications for therapeutic approaches are discussed.

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Keywords: cholesterol metabolism, comorbidities, extracellular vesicles, HIV, lipid rafts, Nef

Introduction

Despite effective control of HIV infection by combination antiretroviral therapy (cART), HIV-infected people remain at high risk for developing dyslipidemia, accelerated progress of atherosclerosis, metabolic syndrome, lipodystrophy, myocardial disorder, diabetes, abnormal hematopoiesis, cognitive impairment and many other metabolic comorbidities [1–4], which pose considerable challenges to clinical management of people living with HIV (PLWH) [5]. Effective prevention or treatment of these comorbidities requires identification of the key pathogenic factors that drive development of these diseases in patients with suppressed HIV load. So far, this issue remained contentious.

Antiretroviral therapy itself has long been considered the main reason for comorbidities in HIV-infected people. Indeed, some first-generation nucleoside reverse transcriptase inhibitors, in particular thymidine analogues (stavudine, zidovudine), have pronounced metabolic side effects and were strongly implicated in the pathogenesis of HIV-related lipodystrophy [6–8]. Similarly, the early generation of protease inhibitors, including such widely

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used drugs as lopinavir/ritonavir combination, has been associated with a high risk of dyslipidemia, metabolic syndrome and increased fat accumulation [9–12]. Progress in antiretroviral drug development during the last 20 years produced drugs from both classes with far fewer metabolic toxicities (e.g. tenofovir, emtricitabine, atazanavir, darunavir) [13,14]. Nevertheless, although discontinuation of anti-HIV drugs with metabolic side effects improves fat distribution [15,16], the negative consequences of previous exposures (legacy effect) persist and may contribute to increased risk of metabolic comorbidities [17]. Moreover, the newest addition to antiretroviral therapy (ART) regimens – integrase strand transfer inhibitors, and in particular dalutegravir – have been associated with excessive weight gain [18–20]. With regard to HIV-associated neurocognitive disorder (HAND), some in-vitro studies suggested potential neurotoxicity of various classes of cART [21,22], although relevance of this observation to HAND pathogenesis remains controversial [23,24]. Overall, antiretroviral drugs may contribute to metabolic abnormalities, but current ART regimens are unlikely to be the primary cause of HIV comorbidities. This leaves HIV itself as a primary suspect [25]. A study of 'elite controllers', a small group of HIV-infected people who control HIV replication to undetectable levels without anti-HIV treatments, demonstrated significantly increased coronary atherosclerosis and monocyte activation relative to uninfected individuals [25], indicating that the cause of this comorbidity is related to HIV infection.

Therefore, to find the cause of HIV-associated comorbidities, one has to look at indirect or direct effects of HIV itself. One indirect effect of HIV infection with a known influence on pathogenesis of various comorbidities is inflammation [26]. Inflammation due to early damage to gut mucosal tissue and subsequent leakage of microflora into the blood is a characteristic feature of HIV infection [27]. Given that recovery of gut mucosal tissue after ART initiation is slow, inflammation persists in treated patients for a long time (refer to [26] for an excellent review). Similarly, in elite controllers, despite little damage to lymphoid tissues [28,29], low-level HIV replication can be detected in the gut, associated with impairment of gut barrier function [30] and inflammatory responses [31]. However, while inflammation is strongly associated with HIV comorbidities, no evidence to support its role as a primary causative factor exists. In fact, inflammation may be a marker of the disease, rather than its etiological factor.

The other potential cause of HIV comorbidities is direct effects of HIV. Given that the number of HIV-infected cells in ART-controlled infection is extremely low, to instigate systemic diseases, such as metabolic or cardiovascular comorbidities, HIV must affect a large number of uninfected cells at various body sites. Such effects can be mediated by HIV proteins released into the blood within extracellular vesicles produced by HIV-infected cells. Since current anti-HIV drugs prevent infection of new cells, but do not block HIV transcription and translation in already infected cells, production of HIV proteins persists in ART-treated patients [32,33]. Extracellular vesicles containing HIV proteins, including Nef, are detected in a large proportion of ART-treated patients with undetectable HIV load, as well as in elite controllers [34–37]. In a recent study, Nef was detected in 83% of ART-naive individuals (median Nef level in the blood was 11.63 ng/ml and correlated with HIV load), in 47.4% of ART-treated patients with undetectable viral load (median Nef level was 8.25 ng/ml), and in 52.4% of elite controllers (median Nef level – 8.78 ng/ml) [37]. It should be noted here that due to Nef variability between the HIV isolates even within the same clade [38], immunological detection methods have inherently low sensitivity. The limit of detection of the assay used in the study by Ferdin *et al.* [37] was 5.46 ng/ml, so it is likely that lower levels of Nef were present in samples tested negative. In this article, we argue for the role of these Nef-containing vesicles as the pathogenic factor in HIV-associated metabolic comorbidities and propose a mechanism for this effect. We do not intend to provide a comprehensive review of existing literature on pathogenic factors in HIV comorbidities (the readers are referred to an excellent recent review [26]), but will focus on cholesterol metabolism and the role of Nef.

Nef is not the only HIV protein potentially implicated in metabolic disorders. HIV protein Vpr has been shown in mouse models to inhibit peroxisome proliferator-activated receptor gamma (PPAR-γ) [39], which is essential for adipocyte differentiation, leading to fatty acid accumulation and lipotoxicity [39]. Exposure of rat neurons to Tat led to upregulation of cholesterol biosynthesis genes and increased levels of free cholesterol and cholesteryl esters [40]. However, the role of metabolic mechanisms in the effects of other than Nef HIV proteins in HIV-associated comorbidities is not sufficiently characterized.

HIV infection, Nef, cholesterol metabolism and lipid rafts

Cholesterol is critical for HIV replication, as both HIV entry into and exit from the target cells occur through cholesterol enriched regions of the plasma membrane, lipid rafts [41–49]. Depletion of cellular cholesterol by chemical agents such as cyclodextrin [42,44,49–52] or as a consequence of genetic predisposition to high expression of ABCA1 [53,54], the cellular cholesterol transporter mediating cholesterol efflux, lead to suppression of HIV replication *in vitro* and control of HIV infection *in vivo.* Single nucleotide polymorphism in *PCSK9* gene controlling expression of LDL receptor was

associated with higher HIV load in HIV/hepatitis C virus coinfected women, presumably due to increased uptake of cholesterol by cells [55]. It is therefore not surprising that HIV evolved means to control cholesterol content of target cells, and Nef appears to be the main viral tool in this process.

HIV-1 protein Nef is a multifunctional protein responsible for many pathogenic effects of HIV infection. In virus producing cells, Nef inhibits an innate anti-HIV factor SERINC [56,57], thus promoting virus infectivity, and suppresses antiviral immune responses by down-modulating $CD4^+$, major histocompatibility complex (MHC)-I, CD28 and several other immune receptors on infected cells [58]. An important, but less appreciated pathogenic effect of Nef concerns cholesterol metabolism. Nef stimulates cholesterol biosynthesis and its delivery to lipid rafts [59,60] and inhibits cholesterol efflux by suppressing activity of cholesterol transporter ABCA1 [61]. The end result of these activities is increased abundance of lipid rafts in an infected cell, benefiting production of new virions [48,62]. Importantly, lipid rafts in HIV-infected or Nef-expressing cells are not only more abundant, but are also functionally defective [63].

However, cholesterol-related effects of Nef are not limited to HIV-infected cells. Nef is released from infected cells either as a free protein coming from dying cells, or being incorporated into extracellular vesicles [35,64]. Although most published reports identify these vesicles as exosomes [36,65,66], Nef incorporation into extracellular vesicles of other origin, such as microvesicles, cannot be ruled out [34,64]. These Nef-containing extracellular vesicles can interact with uninfected cells, impairing cholesterol metabolism on a systemic level [67,68]. Our results indicate that Nef-containing extracellular vesicles downregulate ABCA1, suppress cholesterol efflux and increase abundance of lipid rafts with corresponding stimulation of inflammatory responses, similar to the effects observed for endogenously produced Nef [69].

The accepted mechanism of Nef-mediated downregulation of cellular membrane proteins is Nef binding to the cytoplasmic domains and recruiting adaptor proteins to target these receptors to the endocytic machinery and degradation pathways (reviewed in [58]). Nef binds to ABCA1, however, it was found that downregulation of ABCA1 did not require a direct interaction of Nef with ABCA1 [70]. Instead, Nef-mediated transport of cholesterol to lipid rafts competed with ABCA1-dependent cholesterol efflux pathway altering functional properties of the rafts [63], displacing ABCA1 from the lipid rafts, and leading to its degradation in lysosomes and proteasomes [63,70]. In addition to this mechanism, Nef also affects de-novo production of ABCA1 by blocking the interaction between ABCA1 and calnexin, an endoplasmic reticulum chaperone necessary for proper folding and maturation of transmembrane glycosylated proteins destined for plasma membrane [71]. Nef binds to the cytoplasmic tail of calnexin causing structural changes, which affect interaction between the luminal domain of calnexin and ABCA1 [72–74]. As a result, maturation of ABCA1 and its functional activity are impaired, leading to accumulation of intracellular cholesterol and increased abundance of lipid rafts (Fig. 1). This finding presents an interesting conundrum. Given that many proteins, including HIV gp160 [75], mature through the endoplasmic reticulum, the effect of Nef on calnexin may potentially involve a large number of proteins and be detrimental both to the cell and the virus. However, there is certain selectivity in the effect of Nef: while it disrupts interaction between calnexin and ABCA1, the interaction between calnexin and gp160 was actually increased [74]. The mechanistic details of this selectivity, as well as identification of other proteins affected by the interaction of Nef with calnexin, await future studies.

Inhibition of ABCA1 may not be the only mechanism connecting Nef with cellular cholesterol metabolism and lipid rafts. In human aortic endothelial cells, factors secreted by HIV-infected cells inhibited cholesterol efflux to high density lipoprotein (HDL) without affecting ABCA1 or other known cholesterol transporters; contribution of Nef to this effect was relatively minor [77]. In the same model Nef was responsible for increased abundance of caveolae, a subset of rafts, while disrupting caveolin-dependent cholesterol trafficking, again without affecting ABCA1 [77]. Overexpression of caveolin-1 in macrophages restored Nef-induced impairment of cholesterol efflux without restoring ABCA1 abundance [78]. Clearly, an interplay between different and often redundant pathways involved in regulation of cellular cholesterol trafficking and lipid rafts determines the eventual outcome of Nef activity.

The mechanisms described above have been demonstrated for endogenously produced Nef. How exogenous Nef affects ABCA1 is still unknown. Given that Nef, added either as free recombinant protein or with extracellular vesicles, is delivered into the cells [34,69,79], a reasonable expectation is that the mechanisms described for endogenous Nef can be functional here as well. This assumption is consistent with our unpublished results showing that the compound blocking Nef-calnexin interaction (see below) inhibits ABCA1 downregulation by Nef-containing extracellular vesicles. Future studies will be needed to fully characterize the molecular mechanisms behind the effects of exogenous Nef on cellular cholesterol metabolism.

Cholesterol metabolism, Nef and comorbidities of HIV infection

Impairment of cholesterol metabolism and overabundance of lipid rafts are common elements in the

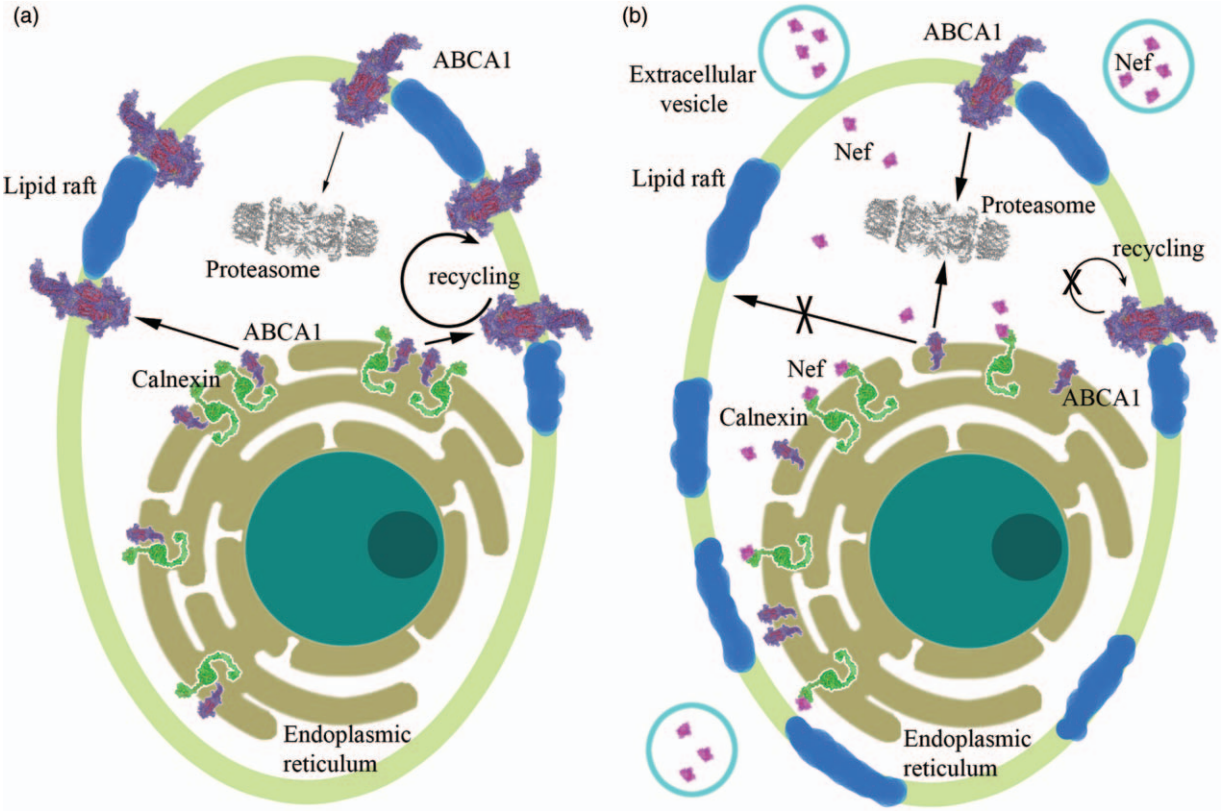

Fig. 1. Schematic representation of the effects of Nef extracellular vesicles on target cells. The cell plasma membrane is shown in light green, and membrane lipid rafts – in blue. Endoplasmic reticulum is shown in olive green around the cell nucleus. Nef, ABCA1, and calnexin are represented by their scaled down three-dimensional structures. (a) Cell not treated with Nef extracellular vesicles. In the endoplasmic reticulum, calnexin interacts with ABCA1 supporting ABCA1 maturation and transport to plasma membrane. ABCA1 is recycled from the cell membrane, and some is internalized to the proteasomes and degraded. (b) Cell treated with Nef extracellular vesicles. Extracellular vesicles carrying Nef molecules surround the cell and deliver Nef into the cell. Nef interacts with cytoplasmic domain of calnexin, which ostensibly causes changes in the calnexin structure, disrupting interaction of its intraendoplasmic reticulum domain with ABCA1. As a result, maturation of ABCA1 is impaired and it is targeted to proteasomes reducing cholesterol efflux. This increases cell's cholesterol content and changes abundance and properties of lipid rafts, leading to decreased recycling of the plasma membrane ABCA1 and its preferential targeting to proteasomes [63,69,73,76].

pathogenesis of almost any metabolic comorbidity associated with HIV infection and are the metabolic pathways targeted by HIV Nef, both produced intracellularly as the result of infection or secreted by the infected cells systemically. Most of Nef secreted by HIV-infected cells comes in extracellular vesicles [80,81], ensuring rapid systemic delivery of Nef to target cells and protecting it from being neutralized by anti-Nef antibodies [82]. It is therefore reasonable to suggest that disturbances of cholesterol metabolism and lipid rafts in bystander cells caused by Nef secreted by HIV-infected cells in extracellular vesicles contribute to pathogenesis of many comorbidities of HIV disease.

Inflammation

HIV infection is associated with a low-grade chronic inflammation. The reason for HIV-associated inflammation is thought to be a compromised gut mucosal epithelium leading to microbial translocation and 'leaking' of lipopolysaccharide (LPS) into blood [83]. Mechanistically, LPS and other microbial products induce inflammation through interaction with the inflammatory receptors. Lipid rafts host many receptors involved in inflammatory responses and play a key role in regulating their activity and, consequently, the severity of inflammatory response [84]. Many receptors are activated through re-localization to lipid rafts, including toll-like receptor 4, tumor necrosis factor receptor 1, CD11b, immune receptors B-cell receptor and T-cell receptor [85]. Augmentation of rafts results in potentiation of inflammatory responses and increased secretion of proinflammatory cytokines in response to LPS [86], while disruption of rafts is anti-inflammatory [87]. Formation of rafts stimulated by Nef may therefore potentiate inflammation in response to microbial translocation; whether or not Nef can also induce a sterile inflammation is less clear.

More generally, accumulation of cholesterol in cells involved in inflammation almost inevitably results, through the formation of rafts or other mechanisms, in a strong inflammatory response (for review see [88]). Inflammation, in turn, leads to accumulation of cholesterol in cells, forming a vicious cycle. Such vicious cycle would greatly amplify the effects of Nef on accumulation of intracellular cholesterol and inflammation, and both will feed into pathogenesis of a number of comorbidities of HIV disease, including atherosclerosis, lipodystrophy, dementia, diabetes and metabolic syndrome [83].

Dyslipidemia

HIV infection causes hypobetalipoproteinemia [low level of low density lipoprotein (LDL)], hypertiglyceridemia and hypoalphalipoproteinemia (low level of HDL) [89–91]. Hypobetalipoproteinemia is characteristic mainly for the untreated HIV infection, it is usually reversed upon commencement of modern ART regimen. Hypoalphalipoproteinemia is unaffected by ART treatment [91,92], and a leading cause of this condition is a deficiency or functional impairment of liver ABCA1, a key element in the pathway of HDL formation [93] and at the same time the very target of Nef. In addition to the reduced levels, the composition, size and functionality of HDL were affected in HIV infection [91,94,95], a finding also consistent with impairment of ABCA1. Generally, lipoprotein profile in PLWH is very similar to that in patients with Tangier disease, a monogenetic disorder where HDL fails to form due to familial ABCA1 deficiency [96], further pointing to the key role of ABCA1 deficiency in pathogenesis of HIV-associated dyslipidemia. However, no HDL turnover studies in PLWH directly supporting this notion were published and contribution of indirect mechanisms, such as inflammation and immune deficiency [94], to HIV-associated hypoalphalipoproteinemia cannot be ruled out.

Low HDL levels are commonly associated with high triglyceride levels due to lack of sufficient acceptor for triglycerides transferred from very low density lipoprotein (VLDL) to HDL through action of cholesteryl ester transfer protein. Deficiency of ABCA1 has been implicated in overproduction of VLDL by liver [97]. HIV-associated hypertriglyceridemia may, however, have additional contributing mechanisms due to insulin resistance prevalent in HIV infection [96,98], which, in turn, may be related to the impairment of cholesterol metabolism (see below). Remarkably, hypetriglyceridemia and hypoalphalipoproteinemia were mitigated in patients infected with Nef-deficient strain of HIV [99]. Mice injected with recombinant Nef displayed hypoalphalipoproteinemia, hypetriglyceridemia and reduced level of ABCA1 in liver homogenates [68], reproducing the observations in HIV-infected people and simian immunodeficiency virus-infected monkeys [67]. Mice injected with exosomes containing Nef also displayed hypoalphalipoproteinemia as well as reduced levels of ABCA1 in liver and peritoneal macrophages [69], providing in-vivo evidence for the effect of Nef on ABCA1.

Atherosclerosis

Hypoalphalipoproteinemia and hypertriglyceridemia caused by HIV, often combined with hyperbetacholesterolemia caused by some ART regimens, constitute a classical proatherogenic lipoprotein profile associated with elevated risk of atherosclerosis in HIV infection [100]. However, cardiovascular risk prediction based entirely on changes in lipoprotein profile underestimates the actual cardiovascular disease risk in HIV infection [101], pointing to the contribution of additional factors, both local and systemic. One of the systemic factors is elevated inflammation (see above), an important element in atherosclerosis [102]. A key local element in pathogenesis of atherosclerosis is accumulation of cholesteryl esters in the cells of vessel wall, macrophages and smooth muscle cells, with formation of foam cells. ABCA1 deficiency and impairment of cholesterol efflux are key causes of the formation of foam cells and development of atherosclerosis [103] and we have demonstrated that ABCA1 deficiency triggered by both intracellular and extracellular Nef causes formation of foam cells *in vitro* and *in vivo* and development of atherosclerosis *in vivo* [61,68,104]. Lipid rafts also play a direct role in accumulation of cholesterol in macrophages and formation of foam cells. Rafts are a location of TREM-1, an important contributor to the foam cell formation and atherogenesis [105]. Further, the current understanding of the mechanisms of ABCA1-mediated cholesterol efflux is that ABCA1 moves cholesterol from rafts to the 'activated lipid domains' where it becomes accessible to extracellular cholesterol acceptors [106]. Excessive rafts contribute to the impairment of cholesterol efflux by affecting this capacity of ABCA1. Lipid rafts also harbor CD36, a putative oxLDL receptor responsible for the uncontrolled cellular uptake of modified LDL [107]. Disruption of rafts with methyl-β-cyclodextrin and with apolipoprotein A-I (apoA-I) binding protein (AIBP) is antiatherogenic [108,109]. Thus, HIV Nef may contribute to the development of atherosclerosis in HIV infection through four interrelated mechanisms: causing dyslipidemia, potentiating systemic and local inflammation, and inducing accumulation of cholesterol and elevating the abundance of lipid rafts in macrophages and smooth muscle cells.

Diabetes

Type 2 diabetes is highly prevalent in HIV infection [110]. Defects in cholesterol homeostasis and impairment of lipid rafts are intimately linked to two key elements of pathogenesis of type 2 diabetes: impairment of insulin secretion from pancreatic β cells and insulin resistance. β Cells are very sensitive to excessive cholesterol, and impairment of cholesterol metabolism causes a sharp

decline in their ability to regulate insulin secretion in response to changes in blood glucose levels. Specifically, studies in mice have shown that increases in cholesterol levels in pancreatic β cells conditionally lacking *Abca1* led to markedly impaired insulin secretion [111]. Many effects of impaired cholesterol metabolism are thought to be mediated by overabundance of lipid rafts. In β cells, lipid rafts are critically involved in regulation of glucose sensing and insulin secretion through regulating the activity of glucose transporters [112], shifting neuronal nitric oxide synthase into a dimeric form [113], and regulating activity of SNAP receptor complexes [114]. Increased abundance of lipid rafts is associated with reduced glucose sensing and reduced insulin secretion [115]. Furthermore, accumulation of excessive cholesterol in insulin-containing β-cell secretory granules impairs their secretion [116]. Cholesterol enriched secretory granules and impaired glucose-stimulated insulin secretion were observed in cells lacking ABCA1 [117]. Lipid rafts play a critical role in proper compartmentalization of insulin signaling in adipocytes, important players in maintaining insulin sensitivity [118]; extracellular Nef inhibited glucose transporter type 4 (GLUT4) trafficking and glucose uptake in these cells [119]. In skeletal muscle cells, another tissue significantly contributing to insulin sensitivity, lipid rafts are involved in regulation of insulin-stimulated glucose uptake, influencing translocation of GLUT4 from perinuclear stores to plasma membrane [120]. Another contributor to pathogenesis of type 2 diabetes is dyslipidemia: both hypoalphalipoproteinemia and hypetriglyceridemia characteristic for the HIV infection (see above) are also important factors regulating insulin secretion and insulin sensitivity [121]. Taken together, these observations suggest that Nef-induced changes to cholesterol metabolism and lipid rafts are an important pathogenic factor in HIV-associated diabetes.

Hematopoiesis

Hematological abnormality is another important comorbidity of HIV infection [122]. It is characterized by reduced growth and differentiation of multiple hematopoietic lineages suggesting impaired functionality of early hematopoietic progenitors. Available data suggest an important role of HIV protein Nef in pathogenesis of hematopoietic abnormalities of HIV infection [123,124]. Prost *et al.* [123] described a mechanism where Nef acts as a PPAR-γ agonist reducing expression of signal transducer and activator of transcription 5. However, the molecular details of this mechanism remain unknown. Several lines of evidence support an important role of cholesterol metabolism in hematopoiesis and point to a possibility that impairment of cholesterol metabolism by circulating Nef may contribute to the abnormal hematopoiesis. Mice deficient in the two key transporters maintaining cellular cholesterol efflux, ABCA1 and ABCG1, displayed a myeloproliferative disorder manifested in profound leukocytosis and an expansion of the population of hematopoietic stem progenitor cells (HSPC) in bone marrow [125]. Significantly, this phenotype favored hematopoietic lineage decisions toward granulocytes rather than macrophages in the bone marrow, leading to impaired support for osteoblasts and decreased Cxcl12/SDF-1 production by mesenchymal progenitors [126]. Conversely, stimulation of cholesterol efflux by elevating expression of ABCA1 or levels of ABCA1 ligands, apoA-I or apolipoprotein E, had an opposite effect reducing HSPC proliferation and monocytosis [127]. Stimulation of cholesterol efflux and disruption of lipid rafts by apoA-I-binding protein (AIBP)-regulated HSPC emergence from hematogenic endothelium [128]. AIBP, ABCA1 and cholesterol efflux are important regulators of lipid rafts that control functionality of hematopoietic stem cells as many receptors involved in regulation of hematopoiesis are localized in rafts [129]. Quiescent HSPC contain very few rafts, and formation of rafts is a prerequisite for HSPC reentry into the cell cycle; inhibition of rafts induces hibernation of HSPC [130]. On the other hand, disruption of lipid rafts with phospholipase C-β2 promotes egress of cells from bone marrow niches, implying that rafts are required for retention of HSPC in bone marrow [131]. Taken together, these findings are consistent with a suggestion that impairment of reverse cholesterol transport, accumulation of cellular cholesterol and increased abundance of rafts, possibly due to action of Nef, produce a phenotype that mimics at least some elements of HIV-associated hematological disorder, such as anemia and thrombocytopenia. Furthermore, some other elements of hematological abnormality, such as leukocytosis, may have their origin in impaired hematopoiesis, contributing to, rather than originating from, HIV-associated inflammation.

Cognitive impairment

HAND is a frequent comorbidity of HIV infection. Effective treatment of HIV infection has reduced the rate of progression and severity of HAND symptoms, but the overall incidence of HAND (about 50% of HIV-infected subjects) remains unchanged [24,132,133]. HAND has all clinical hallmarks of a neurodegenerative disorder with progressive chronic loss of neurons, a spectrum of declining cognitive functions, together with behavioral changes and motor impairment. Neuroinflammation, demyelination, apoptosis and accelerated development of Alzheimer's disease were implicated as possible pathogenic mechanisms of HAND [134]. All these mechanisms have abnormality of cholesterol metabolism as a key element of their action.

Abnormal cellular cholesterol metabolism has been documented for classical neurodegenerative diseases: Alzheimer's disease, Parkinson disease, prion diseases and Niemann-Pick C disease [135–138]. A key element of neurodegeneration is neuronal dysfunction leading to neuronal death, and there is overwhelming evidence that

protein misfolding and subsequent oligomerization/aggregation is a primary cause of neuronal dysfunction and death in many neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease and prion diseases [139]. Many, if not most, neurodegenerative diseases have a 'prion-like' feature in their pathogenesis, where a misfolded protein causes cascading misfolding of other copies of itself [140,141] or of a different protein [142]. Two conditions are required for the misfolding process to spread throughout the brain, causing progressive neuronal dysfunction and death. The first is the initial presence of a misfolded copy of an amyloid-like protein, which is a result of a mutation, an infection, a trauma, or is a spontaneous event. The second is a high local concentration of normal protein to allow nucleation and propagation of misfolding cascade to occur [141]. Accumulation of various amyloidogenic proteins is a common feature of many neurodegenerative diseases, and most amyloid proteins involved in neurodegeneration are raft proteins [136,143–145]. Clustering of amyloidogenic proteins in rafts makes them susceptible to modification (e.g. phosphorylation), misfolding and aggregation when a misfolded copy of the protein becomes available. It is plausible that the abundance of rafts, and consequently the availability of sites where proteins are present at high local concentration, is a key 'permissive' element, or a risk factor, in the pathogenesis of neurodegenerative diseases, including HAND. The role of lipid rafts in inflammation has been described above, and these considerations apply to neuroinflammation characteristic to HAND. In addition, increased abundance and altered composition of lipid rafts may induce apoptosis of neurons and glia by triggering apoptotic signaling [146–148]. Finally, defects in myelination of axons is a common finding in postmortem analysis of brain samples from HAND patients [149]. Myelin is produced by oligodendrocytes from cholesterol, which in the brain is synthesized almost exclusively by glia [150,151]. Brain cholesterol is extensively recycled from broken down myelin [152]. This recycling, which depends on ABCA1 [153], is essential for both repair and production of new myelin sheaths [154–156]. Therefore, Nef-induced impairment of ABCA1 is expected to affect myelination.

These possibilities are not mutually exclusive, and Nef via its effects on cholesterol metabolism and lipid rafts may contribute to the development of HAND through all of these mechanisms.

Nef in the brain may originate from blood or may be secreted directly into CSF by HIV-infected cells in the brain [65,80,157]. HIV stays in the brain in microglial cells and astrocytes, as well as in perivascular macrophages migrating through blood–brain barrier [158]. Remarkably, HIV infection in the brain persists in treated patients without viremia [24]. The proposed lipid rafts-centered hypothesis unifies many of the known mechanisms of HAND, such as neuroinflammation, neuroapoptosis and connection with the Alzheimer's disease, demyelination, as well as common features of neurodegeneration in general, such as presence of misfolded proteins and impairment of lipid metabolism.

Potential therapeutic strategies

The prominent role played by cholesterol metabolism and lipid rafts in pathogenesis of HIV-associated comorbidities opens a possibility for new therapeutic approaches. Current approaches for treatment or prevention of HIV-associated atherosclerosis do not differ much from approaches used for general population (e.g. using statins). A more specific, and probably more effective, treatment would be to target the cause of HIV-associated cholesterol metabolism impairment, that is either the Nef-induced downregulation of ABCA1, or its downstream effect, modification of lipid rafts.

The first goal can be accomplished by stimulating the ABCA1 expression to counteract the effect of Nef, or by inhibiting the Nef activity. Among the most potent stimulators of ABCA1 expression are agonists of liver X receptor (LXR) [159]. LXR agonists are being developed for the treatment of atherosclerosis, as synthetic LXR agonists have been shown to inhibit the progression [160,161] and even promote the regression [162] of atherosclerosis in mouse models. They also were shown to attenuate inflammation and improve prognosis of neurodegenerative diseases in animal models [163]. However, introduction of LXR agonists into clinical practice was impeded by a significant limitation: LXR activation leads to increased fatty acid synthesis, accumulation of triglycerides and the development of fatty liver [164,165]. A new generation of LXR agonists that do not induce lipogenic effects but preserve ABCA1-inducing activity has been described [166–170], but so far, these drugs have not yet moved beyond Phase I clinical trials.

A search for inhibitors of Nef-mediated downregulation of ABCA1 has just started. Given that work on Nef inhibitors has produced a number of promising compounds [171–174], it appears that a large choice of potential candidates should be available. However, the N-terminal region of Nef responsible for ABCA1 downregulation [74] is distinct from regions of the protein responsible for downregulation of MHC-I, $CD4^+$ and SERINC5, which are targeted in most screens [171–174]. Modeling of Nef-calnexin interaction (Fig. 2) provided an opportunity for virtual screening of potential inhibitors and resulted in identification of the first compounds that specifically inhibit Nef interaction with calnexin and release the Nef-mediated blockage of calnexin-assisted ABCA1 maturation [73,74]. Development of these compounds for clinical use is ahead.

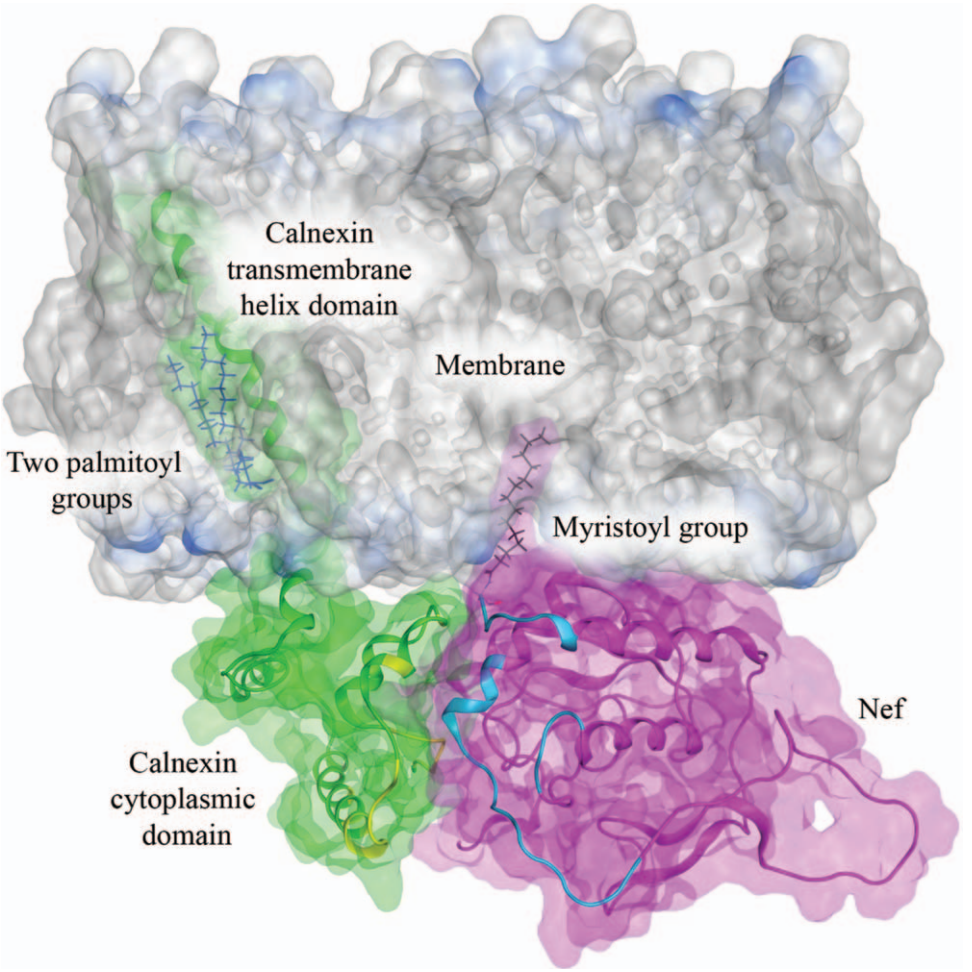

Fig. 2. Model of Nef-calnexin interaction. Interacting structures of Nef (green) and the calnexin cytoplasmic domain (magenta) are shown relative to the endoplasmic reticulum membrane. The interaction sites (shown in yellow for calnexin and cyan for Nef) were targeted in the virtual screening aimed at identifying potential small molecule inhibitors of this interaction [72,73]. The model has been adopted from our earlier results [73] and enhanced using molecular dynamics simulation (unpublished data).

Another potential therapeutic approach to alleviate Nef-mediated comorbidities is to reduce and normalize lipid rafts affected by Nef-induced changes to cholesterol metabolism. This appears to be a difficult proposition given essential functions that lipid rafts play in cell physiology, which requires therapeutic agents to discriminate between affected and unaffected cells and provide a measured effect that does not reduce rafts below physiological levels. Currently available lipid raft-targeting agents, such as cyclodextrins, do not fit these criteria and thus cannot be used for long-term treatment necessary to control comorbidities associated with chronic HIV infection. However, a recently identified innate factor, AIBP [175–177], ideally fits this requirement. This protein enhances apoA-I-mediated cholesterol efflux specifically from cells challenged by proinflammatory agents while sparing nonactivated cells [109,175,178–180]. Furthermore, AIBP appears to selectively target lipid rafts on activated cells, normalizing their abundance and function activated by inflammatory stimuli [178]. Our unpublished results demonstrated that AIBP reverses Nef-mediated effects on lipid rafts in monocyte-derived macrophages and normalizes their responses to inflammatory stimuli. Further work is warranted to evaluate the therapeutic potential of AIBP-derived agents for treatment of HIV-associated comorbidities.

Conclusion

Findings described in this review point to an important role that impairment of cholesterol metabolism and downstream changes to lipid rafts play in pathogenesis of HIV-associated comorbidities. While this pathogenic mechanism likely contributes to most metabolic diseases, there is a clear specificity in HIV infection related to the prominent role of Nef. Therefore, potential treatment strategies may combine HIV-specific, Nef-targeting

agents, with approaches stimulating cholesterol efflux and reducing lipid rafts in a nonspecific fashion.

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Conflicts of interest

There are no conflicts of interest.

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