

Converging roles for sphingolipids and cell stress in the progression of neuro-AIDS

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1. ABSTRACT

Sphingolipids are a class of lipids enriched in the central nervous system that have important roles in signal transduction. Recent advances in our understanding of how sphingolipids are involved in the control of life and death signaling have uncovered roles for these lipids in the neuropathogenesis of HIV-associated neurocognitive disorders (HAND). In this review we briefly summarize the molecular mechanisms involved in the pathological production of the toxic sphingolipid, ceramide and address questions of how cytokine and cellular stress pathways that are perturbed in HAND converge to deregulate ceramide-associated signaling.

2. INTRODUCTION

Human immunodeficiency virus type -1 (HIV-1).infection is the most frequent cause of dementia in persons less than 40 years of age. Prior to the development of antiretroviral therapies (ART), neurological dysfunctions associated with HIV-infection (now collectively termed HIV-associated neurocognitive disorders; HAND (1)).occurred in nearly 20% of patients with acquired immunodeficiency syndrome (AIDS).and often progressed rapidly to death over a period of a few months (2, 3).Cognitive difficulties and behavioral abnormalities including psychosis, depression and psychomotor slowing were common findings. Neuropathological observations

revealed a subcortical dementia with a predominant involvement of the basal ganglia (4). The pathological hallmarks of HIV-1 infection in the brain are multinucleated giant cells that are the result of syncytia-inducing properties of HIV-1-infected cells. HIV-1 predominantly infects perivascular macrophages, brain resident microglia (de la Monte et al., 1987), and some forms of astrocyte (5). Although neurons themselves are rarely infected, dendritic pruning (6), loss of synapses (7), and cell death (6, 8), are common findings in the brains of HIV-1 patients with dementia. Neuronal damage and death are thought to be the result of indirect actions initiated by the virus and/or by viral proteins.

Since the availability of antiretroviral therapy, the incidence of HIV dementia has become less frequent and the clinical manifestations less severe. However, due to improved survival rates, the prevalence rates of HIV dementia continue to rise (9). Today, many HIV-1 patients live with a less severe form of dementia that is manifest as the chronic condition of minor cognitive motor impairment. Thus, despite advances in ART, neurological complications continue to compromise the quality of life for many individuals infected with the human immunodeficiency virus (HIV). The introduction of a successful neuroprotective therapy to combat neurological dysfunction in this population has been unsuccessful, likely due to the perturbation of multiple signaling pathways that result in cytokine imbalance, mitochondrial dysfunction, oxidative stress, channelopathies and abnormal lipid metabolism. In this review, we discuss our current understanding of the interconnectedness in these pathways and their convergence on molecular mechanisms that modulate sphingolipid metabolism.

3. DISORDERS OF SPHINGOLIPID METABOLISM AND NEURODEGENERATION

Sphingolipids are a class of lipids derived from the aliphatic amino alcohol sphingosine. The sphingosine backbone is O-linked to a charged head group such as ethanolamine, serine, or choline, and is also amide-linked to an acyl group, such as a fatty acid. This class of lipids make up approximately one third of the content in eukaryotic cell membranes, and are highly enriched in the central nervous system, especially in myelin where the proportion of sphingolipids are more than half the total lipid content. In addition to important structural roles, sphingolipid metabolites function as second messengers that modulate critical signaling functions for a variety of inter- and intra-cellular events. Sphingomyelins, ceramides, and gangliosides are the three main types of sphingolipids. Ceramide is the simplest sphingolipid, consisting of a fatty acid chain attached by an amide linkage to sphingosine. Sphingomyelin has a phosphorylcholine or phosphoethanolamine esterified to the 1-hydroxy group of ceramide. Glycosphingolipids are ceramides with one or more sugar residues joined with a α -glycosidic linkage at the 1-hydroxyl position.

In brain, the proper balance of sphingolipids are essential for normal neuronal function, as is evidenced by a number of severe neurological disorders that are the result of deficiencies in enzymes that control sphingolipid metabolism. For example, Niemann Pic disease (type I), involves a deficiency in sphingomyelinase (a phosphodiesterase enzyme that catalyzes the hydrolysis of sphingomyelin), and Tay-Sacks disease results from a deficiency in hexosaminidase (involved in the hydrolysis of certain sphingolipids). While these severe neurological disorders are the result of gross disruptions in sphingolipid metabolism, recent discoveries from a number of laboratories suggest that more subtle changes in sphingolipid balance may be intimately involved in a number of neurodegenerative diseases including Alzheimer's disease, Amyotrophic Lateral Sclerosis, Parkinson's disease, and HIV-associated neurocognitive disorders (HAND). (10-13). In particular, it is the accumulation of the toxic sphingolipid, ceramide that has been implicated in neuronal death.

4. MOLECULAR MECHANISMS OF CERAMIDE TOXICITY

The toxic properties of ceramide have been exploited as a means to arrest cell growth (for example, ceramide-coated balloon catheters are used to prevent stretch-induced neointimal hyperplasia that can cause secondary occlusion of coronary arteries after balloon angioplasty). (14), and to promote apoptosis (ceramide has been used to enhance the effectiveness of standard therapy in head and neck tumors and in neuroblastoma). (15, 16). In neuroscience, the roles for ceramide in normal and pathological events are just beginning to be understood. For example, although increases of ceramide have been associated with neuronal death in several neurodegenerative diseases including HIV associated neurocognitive dysfunction (HAND), Alzheimer's dementia and Amyotrophic Lateral Sclerosis (ALS). (see (10-13)), the exact molecular mechanisms that govern the propagation of ceramide-dependent cell death in neurons are currently poorly understood. Studies on mechanisms of immunological control suggest there may be several key points that control ceramide death signaling (Figure 1, 2). *The first* involves biophysical alterations in membrane structure that can result from a receptor-driven reorganization of cellular membranes. Ceramide has the spontaneous ability to form membrane microdomains and can act as a fusagen. The self-aggregating properties of ceramide can create signalosomes (17, 18), such as those involved in Fas-induced capping. The Fas/FasL interaction activates a caspase-8-dependent increase in sphingomyelinase activity and the resultant increase in ceramide generates large surface macrodomains (probably by the fusion of smaller raft, microdomains). where proteins of the death-inducing signaling complex (DISC) oligomerize (19, 20). *The second* level of control involves the interplay between ceramide-activated protein kinases (CAPK) and phosphatases (CAPP). Our understanding of the specific roles for CAPK in the initiation of apoptosis are just beginning to be understood and current evidence suggests that this death signaling

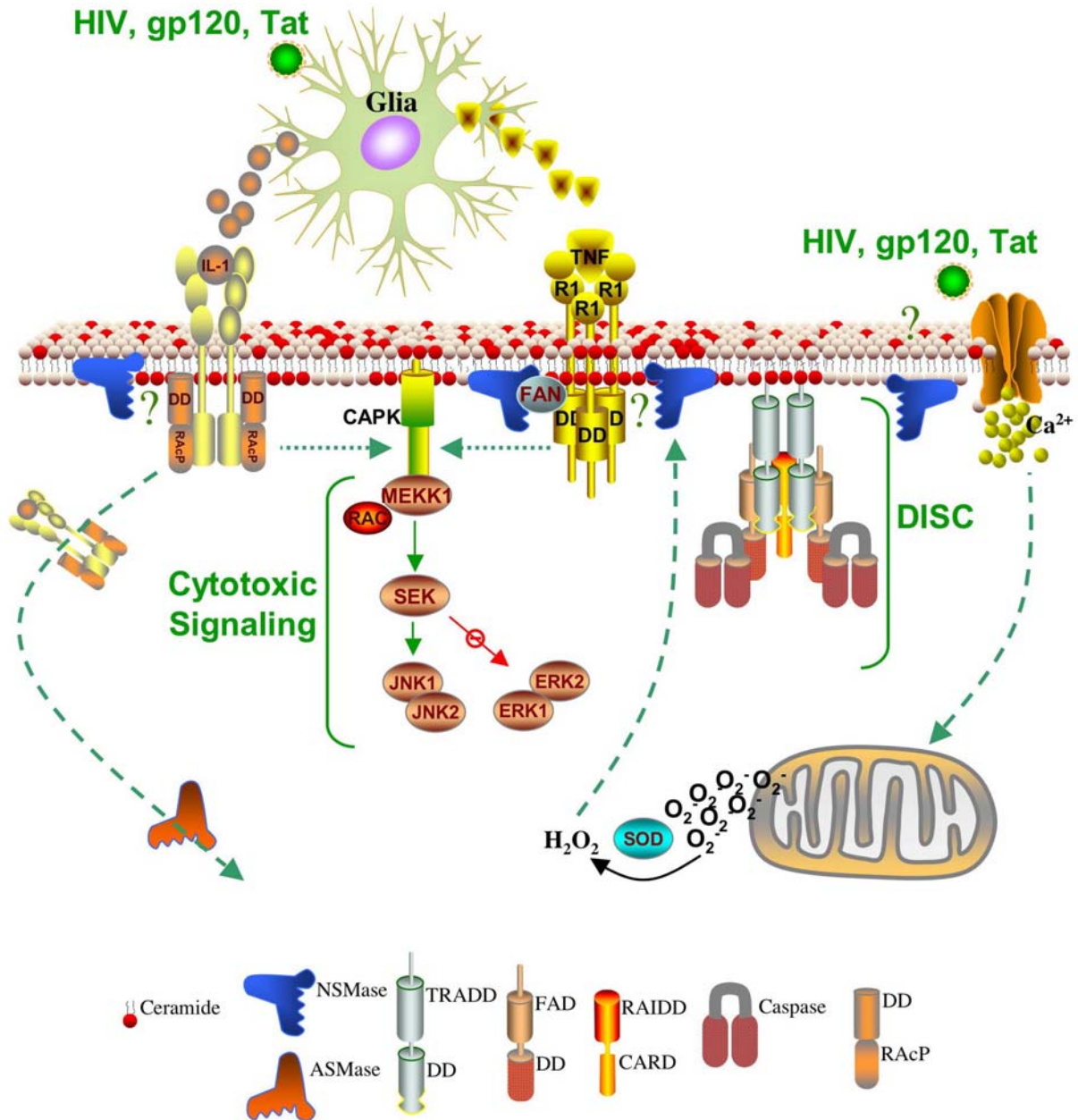


Figure 1. Molecular mechanisms of ceramide-associated death signaling. HIV and the HIV-proteins gp120 and Tat can perturb the function of glial cells in brain and deregulate cytokine balance. Two of the inflammatory cytokines that are increased in the brains of patients with HAND, IL-1 and TNF are potent inducers of ceramide generation by signaling that involves the hydrolysis of sphingomyelin by a neutral sphingomyelinase (NSMase). The TNF receptor R1 can signal to NSMase via the adaptor protein, factor associated with neutral sphingomyelinase (FAN), and also by unidentified mechanisms (indicated by ?). Likewise, the exact mechanisms that link IL-1 receptor activation to NSMase are unknown. IL-1 can activate acidic sphingomyelinase following ligand-induced receptor internalization by a mechanism that involves the adaptor protein RacP. Ceramide generated at the plasma membrane is critical for the assembly of proteins involved in the death inducing signaling complex (DISC). Shown are the assembly of TNF receptor associated death domain (TRADD), Fas-associated protein with death domain (FADD), caspase and RIP-adaptor with death domain (RAIDD), RIP-associated ICH-1/CED-3 homologous protein with a death domain (RAIDD), caspase recruitment domain (CARD), and associated caspases. Both IL-1 and TNF receptors can induce ceramide-dependent cytotoxic signaling via activation of ceramide-activated protein kinases (CAPK) that promote death signaling by mitogen-activated protein kinase kinase (MEKK), activation of Jun N-terminal kinase (JNK), and inhibition of extracellular signal-regulated kinase (ERK). HIV, gp120 and Tat-induced deregulation of calcium permeable ion channels and perturbed mitochondrial function can result in a leak of superoxide radicals (O₂⁻). Some free radical species, such as hydrogen peroxide (H₂O₂), promote the translocation of NSMase to the plasma membrane to generate ceramide.

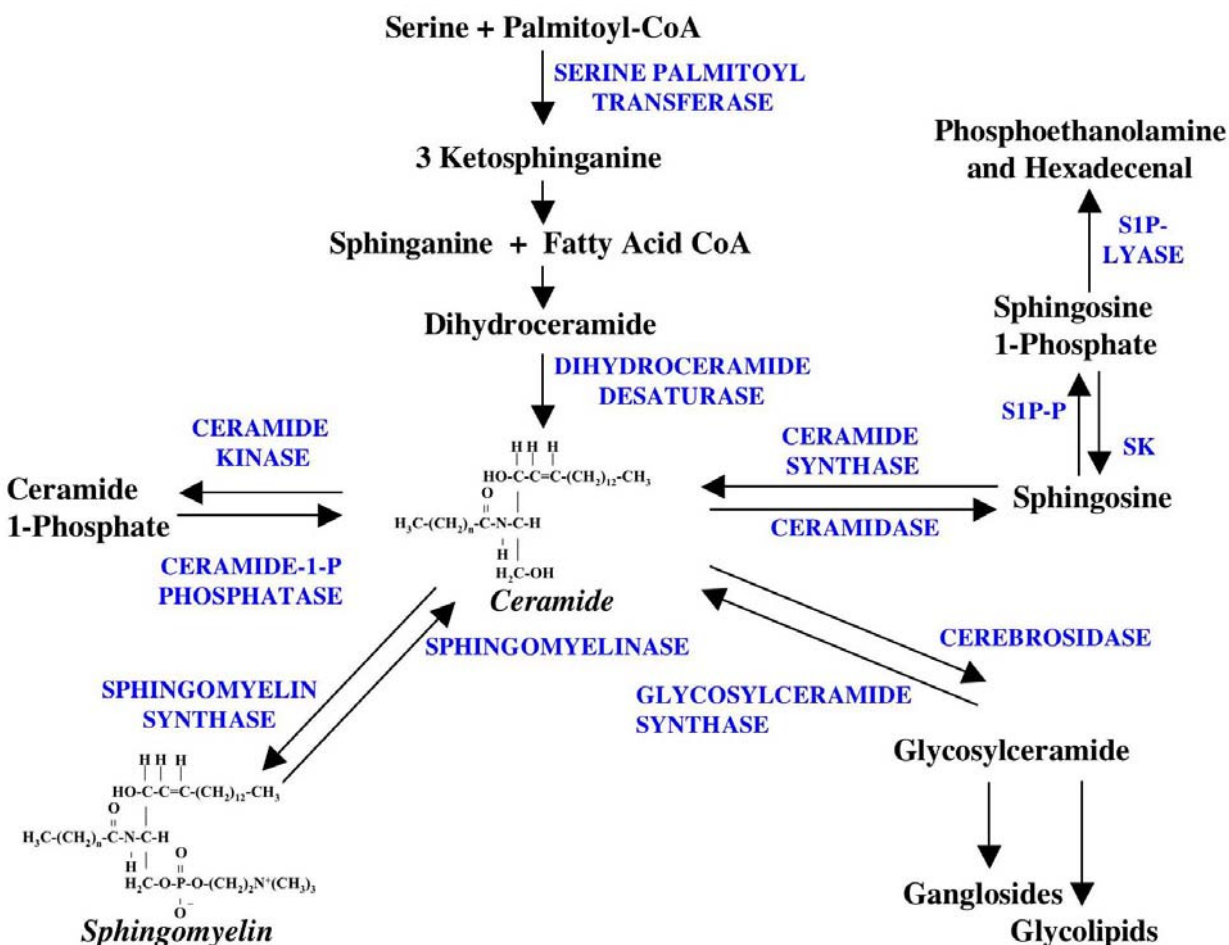


Figure 2. Biochemical pathways of sphingolipid metabolism. Catabolic and *de novo* pathways for ceramide production and related sphingolipids are shown. Enzymes involved in each of the pathways are indicated in bold blue letters.

involves recruitment of MAPK/ERK kinase kinase (MEKK1), activation of SAPK-kinase (SEK1), Jun N-terminal kinases (JNK 1 and JNK2), and inhibition of the survival factor extracellular signal-regulated kinase-1 and 2 (ERK1 and ERK2). (17, 21-25). In this CAPK-associated death pathway, it is thought that downstream signaling through the JNKs ultimately triggers apoptosis (24). CAPK-induced apoptosis may also involve a Raf-1 kinase, mitogen-activated kinase (MEK-1)/ ERK pathway that was demonstrated in astrocytes (26). However, the contribution of Raf-1 signaling to neuronal death is ambiguous, and ERK can have protective or apoptotic effects, depending on the mode and duration of activation. Potential roles for CAPPs in neuronal survival are uncertain, but may involve inactivation of the survival factor Akt1, p38 MAPK (27). A third level of control involves the mechanisms and site of ceramide generation. Ceramide can be generated *de novo*, through serine palmitoyltransferase, the rate limiting step in ceramide synthesis, or by sphingomyelinase (SMase), an enzyme that catalyzes hydrolysis of the phosphodiester bond of sphingomyelin to yield ceramide and phosphorylcholine. There are at least 5 different SMase

that differ in pH optimum, metal dependence and subcellular localization (see (28) for a review). Ceramide generated by the *de-novo* pathway, acidic SMase (ASMase) and a magnesium dependent neutral SMase (NSMase), have each been repeatedly implicated as effectors in pro-apoptotic pathways (reviewed in (29-31))

5. EVIDENCE FOR PERTURBED SPHINGOLIPID METABOLISM IN HAND

The first evidence to suggest that abnormal accumulations of sphingomyelin and ceramide may be involved in the pathogenesis of HAND was provided in a study of postmortem brain tissues from pre-ART patients (12). In this study it was reported that several forms of sphingomyelin and 4-HNE adducts accumulated in the medial frontal gyrus of HIV-infected patients with mild forms of cognitive impairment (MSK scores of 0.5 - 1.0), and there were additional accumulation of several forms of sphingomyelin and ceramide in multiple brain regions of patients with evidence of more severe cognitive dysfunctions (MSK > 1.0). Increased levels of

sphingomyelin and ceramide were also detected in cell-free CSF, with similar associations to cognitive status as was observed in brain tissues. In a subsequent study, a longitudinal design was used to determine if sphingolipid levels could be useful predictive biomarkers for HAND in subjects from the North Eastern AIDS Dementia Cohort (NEAD). The patients had cognitive testing and clinical data were collected at three time points (separated by 6 months) and a single CSF-draw (at the second visit). In this study, increased levels of vitamin E, a very long-chain triglyceride C52:0 (evidence of increased antioxidant defense) and decreased levels of an esterified form of cholesterol (evidence of neuroprotective changes in sterol content) predicted which patients would develop of deterioration of cognitive function 6-months later. A mild and stable neurocognitive dysfunction in this patient population was associated with accumulation of multiple long-chain sphingomyelins in a manner consistent with findings from an independent study that reported evidence of an expanded lysosomal apparatus in the subcortical white matter of patients with HAND (when overproduced, sphingomyelin becomes sequestered in lysosomes; as occurs in Niemann Pick disease). (32). More severe neurological dysfunctions were associated with the accumulation of ceramides and multiple lipid peroxidation products (33). Thus, these series of biomarkers reflect a neuropathological progression that starts with a neuropathology that is suppressed by an up regulation of endogenous defenses. When this defense system wears down, sphingomyelin accumulates in conjunction with mild cognitive impairment. Accumulations of ceramide species are associated with declines of cognitive function, suggesting that a conversion of this enlarged pool of sphingomyelin to ceramide triggered synaptic dysfunction and neuronal loss. Indeed, several *in vitro* studies have reported that an overabundance of ceramide can trigger apoptosis in neurons (34-36).

These sphingolipid-based biomarkers that were initially identified in brain tissues from The Johns Hopkins NeuroAIDS brain bank and CSF samples from patients enrolled in the NEAD cohort were subsequently confirmed in a series of cross-sectional studies that used CSF from a cohort of HIV-1 infected people in Baltimore (Ned Sacktor, oxidative stress cohort), HIV-1 infected women in Puerto Rico (Valerie Wojna, personal communication) and HIV-1 infected people in San Diego, California (Scott Letendra unpublished data). The combined findings from these cohorts suggest that sphingomyelins and/or ceramides with carbon chain lengths of 16 (C16:0) and 24 (C24:0, C24:1) are prominently dysregulated in the brains of patients with HAND. These two forms of sphingolipids are thought to be differentially regulated and preferentially reside in the plasmamembrane (C24:0; C24:1) and in the mitochondria (C16:0). (37), suggesting roles for perturbed plasmamembrane-associated signaling and mitochondrial function in HAND. These biochemical findings in patients with HAND were recently confirmed in brain imaging studies that reported elevated lipid metabolites in the subcortical white matter of HIV-infected patients with mild dementia, supporting the hypothesis that changes in brain sphingolipid metabolism are involved in the progression of

neurological dysfunction in HIV-infected patients (38). The similarity of biochemical and imaging findings from these diverse HIV-infected cohorts suggest that disordered lipid metabolism may be a ubiquitous event in the pathogenesis of HAND.

In tissue culture models of HAND, central roles for ceramide and NSMase in the HIV envelope protein, gp120-induced neuronal death have been recently suggested. In these experiments, pharmacological or molecular interference of NSMase-2 reduced gp120-induced generation of ceramide and protected neurons from gp120-induced death (12, 39). HIV Tat-protein induced neuronal death may also involve a NSMase, but additional roles for the *de novo* synthesis of ceramide have also been demonstrated (12). These experimental findings suggest that neurotoxic HIV-1 proteins may perturb sphingolipid metabolism and result in an overproduction of ceramide and increased lipid peroxidation, with consequent neuronal dysfunction and death.

6. THE RELATIONSHIP OF OXIDATIVE STRESS TO CERAMIDE PRODUCTION AND NEURONAL DYSFUNCTION IN HAND

Oxidative stress occurs when there is an imbalance between cellular oxidants and antioxidants that favors the overproduction of free radicals. Cellular targets including DNA, RNA, proteins and lipids can be modified by free radicals, with the typical result that function of the oxidized targets are compromised. Pathological studies in brain tissues from HIV-1 infected patients suggest that free radical production and oxidative damage play major roles in the pathogenesis of dementia. In brain tissues from HIV-1 infected patients, inducible nitric oxide synthase (produces the NO radical), and superoxide dismutase (catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide) mRNA were found to be elevated and nitrotyrosine staining of brain tissues (an indicator of peroxynitrite damage on proteins), was more intense and found in more brain regions of demented patients compared with non-demented patients infected with HIV-1. (40). In addition to evidence of oxidatively damaged proteins, there are also biochemical and immunohistochemical findings of increased lipid peroxidation in brain tissues and CSF from HIV-1 infected patients with dementia. (12, 41). Ultimately, lipid peroxidation also damages proteins, by covalently modifying proteins on cysteine, lysine and histidine residues, and by this mechanism, can impair the function of membrane ion-motive ATPases, glutamate transporters and synaptic proteins. (42, 43). As an example, HNE is known to mediate oxidative stress-induced apoptosis of cultured neurons (44) and can damage neurons and cause cognitive dysfunction *in vivo*. (45). These combined findings suggest that in HIV-infection, disruptions of redox balance that increase protein and lipid oxidation in brain may be pivotal events leading to the dysfunction and death of neurons. Consistent with this notion, several antioxidants, including trolox (vitamin E), L-deprenyl and diosgenin protected neurons in tissue culture and *in vivo* models of HAND. (41, 46, 47).

An abundance of evidence suggests that disruptions in cellular ion balance and mitochondrial function are decisive events that contribute to the formation of free radicals and neuronal dysfunction in HAND. When mixed human neuronal brain cultures were exposed to CSF from HIV-1 infected patients, mitochondrial function was perturbed to a degree that correlated with the severity of the persons' cognitive impairment at the time the CSF was acquired. (41). Several additional lines of evidence suggest that impairment of mitochondrial function by HIV-1 proteins is tightly linked to the dysregulation of calcium homeostasis. The HIV-1 coat protein gp120 can increase mitochondrial membrane permeability (MMP).by receptor-initiated actions at the plasma membrane that elevate cytosolic calcium (48-50), increase the transcriptional activation of p53 (51, 52).and enhance the expression of pro-apoptotic mitochondrial acting proteins such as Bax, while promoting Bad dephosphorylation. (53-55). Bad and Bax are pro-apoptotic members of the Bcl-2 family of mitochondrial regulating proteins. Although the viral regulatory protein Tat is not a structural component of the virion, it can be released from infected cells and perturbs ion homeostasis and mitochondrial function of neighboring cells by actions initiated at the plasma membrane. (56, 57). Tat can also disrupt calcium homeostasis by a mechanism distinct from the actions of gp120. Tat can induce phosphatidylinositol biphosphate (PIP2).hydrolysis to generate inositol triphosphate (IP3), a soluble messenger that binds and activates IP3-sensitive channels in the endoplasmic reticulum to release calcium. (58). This perturbation of calcium homeostasis can indirectly enhance calcium flux through NMDA receptors by recruiting calcium-dependent kinases to phosphorylate C-terminal regions on NMDA receptors that are critical for the modulation of channel activity. (59). There are in addition, direct and rapid actions of Tat that are mediated by the direct binding of Tat to NMDA receptors. (60, 61). These gp120 and Tat-induced perturbations of calcium homeostasis can increase calcium beyond the capability of energy dependent calcium pumps to remove calcium from the cytosol. When this occurs, calcium becomes sequestered into internal compartments including mitochondria. Sustained elevations of cytosolic calcium can overburden mitochondria and disrupt the electron transport chain with a resultant leak of reactive oxygen intermediates; primarily the superoxide radical (O_2^-). (62, 63).

The timeframe with which gp120 and Tat induce ceramide (within 3 h), perturb mitochondrial function (within 3 - 6 h).and increase oxidative stress (6 - 12 h).suggests that ceramide may be a triggering event in gp120 and Tat-induced oxidative stress. Indeed, studies on isolated mitochondria have shown that the synthetic cell-permeable ceramide analogs (C2-, C6- and C16-ceramides).rapidly reduce mitochondrial oxidative phosphorylation, inhibit the activity of respiratory chain complex III, and induce cytochrome C release by forming pores in the mitochondrial outer membrane that are permeable to molecules with a molecular mass less than 60,000. (64-66). There is in addition a strong link between cellular oxidant status and the activity of ceramide

generating sphingomyelinases (SMase). One mechanism that has been identified whereby cellular oxidants can modify sphingolipid metabolism is by controlling the cellular localization of SMase's. For example, the trafficking of a neutral sphingomyelinase (NSMase2).is sensitive to cellular oxidant status. Increased cellular levels of hydrogen peroxide (H_2O_2).can induce the trafficking of NSMase2 to the plasma membrane where it localizes into lipid raft domains, while the antioxidant glutathione, promotes the translocation and accumulation of NSMase2 in perinuclear regions (Figure 1).(67). These effects of reactive oxygen species on SMase activity are surprisingly specific to the form and concentration of the radical. While H_2O_2 can increase NSMase activity, peroxynitrate ($ONOO^-$).has little effect on NSMase activity; $ONOO^-$ can instead increase the activity of an acidic sphingomyelinase (ASMase). Evidence for a concentration-dependent effect of free radicals on SMase activity were provided in a series of experiments that demonstrated nitric oxide (NO), at low concentrations, can protect cells from apoptotic death by inhibiting the activity of ASMase (68-70), but at high concentrations, can induce death by activating both N- and ASMase's. (71). Although the exact mechanism (s).whereby cellular oxidation directs the trafficking and activity of SMase's are unknown, for NSMase2 it may involve the regulation of posttranslational modification. NSMase2 is palmitoylated on multiple cysteine residues that are critical for plasma membrane localization, and palmitoylation of cellular protein targets can be modulated by nitric oxide in a manner consistent with the effects of this redox molecule on the cellular localization of NSMase. (72, 73). Thus, one mechanism whereby gp120 and Tat may perturb mitochondrial function is by increasing the ceramide content of mitochondrial membranes. Perturbed mitochondrial function would further increase ceramide production by redox-regulated translocation of SMase's to the plasmamembrane. Although the convergence of these findings is intriguing, a direct link between the ceramide generating properties of these neurotoxic HIV-1 proteins and mitochondrial function needs to be determined.

7. THE CONTROL OF SPHINGOMYELIN METABOLISM BY INFLAMMATORY CYTOKINES AND THEIR RELATIONSHIP TO HIV-ASSOCIATED NEURODEGENERATION

Infiltration of lymphocytes and macrophages into the brain parenchyma with the subsequent activation of resident microglia and astrocytes is thought to be a seminal event in the pathogenesis of neuronal dysfunction in HAND. Changes in microvascular permeability with enhanced transmigration of monocytes and increased brain and CSF levels of a number of cytokines, including transforming growth factor- β (TGF- β), Fas-L, the interleukins IL-1 α , IL-1 β , IL-6, and tumor necrosis factor (TNF), have been reported in patients infected with HIV and are especially increased in those with HAND. (74-80).

Direct experimental evidence that cytokine imbalances can cause neuronal degeneration has been produced in rodent models of HAND. The inoculation of HIV-infected monocytes into the basal ganglia and cortex

of severe combined immunodeficiency disease (SCID).mice increased the expression of TNF α , IL-6 and vascular endothelial growth factor (VEGF). These cytokine changes were associated with neuronal damage, as was evidenced by decreased levels of the neuronal microtubule-associated protein-1 (81). Key roles for inflammatory processes in neurodegeneration associated with HIV-1 dementia were suggested in experiments where inhibition of TNF α and MMPs, or platelet-activating factor (PAF).reduced neuropathology in a rodent model of HIV-1 encephalitis. (82).Results similar to those obtained in models that use whole virus were observed when the neurotoxic HIV-1 protein gp120 or Tat were introduced directly into the lateral ventricle or hippocampus of rodents. Injections of Tat activated inflammatory mediators and adhesion molecules that increased that facilitated the transmigration of monocytes into brain. In this model, an initial infiltration of neutrophils is observed one day after Tat injection, followed by macrophage and lymphocyte infiltration and glial activation by 7 days. (Jones et al., 1998). Injections of Tat peptide fragments into rodent brain increased TNF α , IL-1 α /- β , and IL-6 and blockade of TNF α by pentoxifylline treatment decreased IL-1 levels and reduce the volume of the lesions, suggesting that the Tat-induced neuronal loss involves TNF α and IL-1. (83). Injections of the HIV-1 coat protein gp120 into neocortex of rats increased microglial activation, enhanced expression of IL-1 and induced neuronal apoptosis. (84-86). Neuronal damage following gp120 injection was preceded by microglial activation and by an early (within 24 h).enhancement of IL-1 expression, with a further enhancement of expression after 7 days of treatment. (86). It was concluded from these experiments that IL-1 plays a causative role in neuronal death, because an inhibitor of the interleukin converting enzyme (ICE; caspase-1), or an endogenous antagonist of IL-1 receptor type I (IL-1ra).minimized neuronal cell loss. (86, 87). The patterns of altered cytokine regulation in these rodent models of HAND are consistent with cytokine disruptions present in humans with HAND and implicate inflammatory cytokines as key regulators of neuronal dysfunction and death n patients infected with HIV.

Of the inflammatory cytokines known to be dysregulated in HAND, TNF α and IL-1 are among the best studied to have neuropathogenic effects (see (88).for a recent review).These two cytokines can initiate apoptotic signaling cascades by mechanisms that are dependent on the induction of ceramide (Figure 1). On the TNF α receptor there are two domains that can signal to modulate sphingolipid metabolism. A 75-amino acid motif termed the "death domain" links the TNF α receptor to ASMAse by a mechanism that may involve caspase 8; although there are reports that implicate (Brenner et al., 1998; Grulich et al., 2000).and those which exclude (Schwander et al., 1998).caspase 8 as an intermediate in TNF receptor activation of ASMAse and the generation of ceramide. A NSMAse activation domain on the TNF α receptor has been identified located adjacent to the death domain that is thought to interact with NSMAse by binding the linker protein FAN (factor associated with NSMAse activation).(Adam et al., 1996; Kreder et al.,1998). IL-1 can

likewise rapidly generate ceramide by promoting sphingomyelin hydrolysis (Mathias et al., 1993; Schutze et al., 1994). Although the exact domains on the IL-1 receptor that are necessary for signaling to sphingomyelinases are unknown, there is evidence that the IL-1 receptor type 1 signals to NSMAse, while internalization of the IL-1/IL-1 receptor complex is associated with activation of ASMAse and requires the IL-1R accessory protein, IL-1RAcP (Nalivaeva et al., 2000; Hofmeister et al., 1997). Ceramide generated through actions initiated at the IL-1 receptor have an array of biological effects including expression of prostaglandin, leukemia inhibitory factor, secretory phospholipase A2, matrix metalloproteinase-1, manganese superoxide dismutase and can modulate neuronal function by inhibiting L-type calcium channel activity and increasing the frequency of miniature inhibitory post-synaptic currents (Santana et al., 1996; Carlson et al. 1996; Reunanen et al., 1998; Pahan et al., 1999; Tong et al., 1999; Chik et al., 1999; Tabaraven et al., 2006). Ceramide-dependent death-signaling by IL-1 involves increased phosphorylation and activation of JNK by a CAPP and MEKK1 dependent pathway and/or inhibition of ERK1/2 through adenylyl cyclase (89)Huwiler et al., 2004; El Btaouri et al., 2006). These findings suggest that a dysregulation of brain cytokine levels in HAND may promote neuronal dysfunction and death by mechanisms that involve ceramide-dependent signaling pathways.

8. FUTURE DIRECTIONS

During the last few years, sphingolipids have emerged as bioactive molecules that modulate critical neuronal functions including survival, neurite outgrowth and synaptic integrity. Accumulating evidence suggests dysfunctions in brain sphingolipid metabolism that are critically involved in the neuronal dysfunction and death associated with a variety of neurodegenerative diseases. In HAND, there is an abundance of evidence that suggests key regulators of sphingolipid metabolism including cytokines, mitochondrial function and redox balance are deregulated in brain and there is evidence of perturbed sphingolipid metabolism. However, there are a number of fundamental question that need to be addressed. Many of the ceramide-dependent apoptotic pathways that are linked to cytokine signaling and oxidative stress have been determined in non-brain resident cells. The existence and potential differences of these signaling pathways in neurons and glia need to be determined. Likewise, it is not known if HIV or HIV-proteins perturb sphingolipid metabolism and ceramide-signaling in glial cells nd the contribution of these effects to glial and neuronal dysfunction. Although there is intriguing evidence that ceramide signaling may be critical to gp120 and Tat-induced neuronal death, the molecular mechanisms by which gp120 and Tat perturb sphingolipid metabolism need to be studied. While existing evidence points to roles for both NSMAse (hydrolytic).and de novo ceramide synthesis, determining the relative contributions of direct actions vs. the indirect actions (mediated by soluble factors released).of gp120 and Tat are critical to understanding the roles that sphingolipid signaling play in HAND. Molecular interference of specific targets involved

in sphingolipid biosynthesis and detailed lipid analysis of individual cell populations will begin to address these questions. The insights gained from these analyses will uncover roles for sphingolipid-signaling in both normal and abnormal brain function and may uncover new targets for neuroprotective therapy in HAND and other neurodegenerative diseases.

9. ACKNOWLEDGEMENT

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