Commentary

YKL-40: A Candidate Biomarker for Simian Immunodeficiency Virus and Human Immunodeficiency Virus Encephalitis?

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Human immunodeficiency virus type 1 (HIV-1) affects more than 32 million people worldwide¹ and induces central nervous system-associated neurological dysfunction in at least 30% of infected individuals.² Cognitive disorders range from mild impairment of executive functions to frank dementia (HIV-associated dementia; HAD), and these are collectively termed HIV-associated neurocognitive disorders (HAND).³ Although the incidence of HAD has decreased with the use of highly active anti-retroviral therapy (HAART), it is only partially neuroprotective. The persistence and increasing prevalence of less severe HAND syndromes remains a serious concern, and the assessment of the risk of developing progressive disability in treated individuals is a clear priority. In underdeveloped countries where HAART availability is limited, increasing life expectancy remains the primary goal of HIV treatment, but our experience in the United States forewarns of the likelihood of increasing cognitive disability in these surviving populations.⁴ In this issue of *The American Journal of Pathology*, Bonneh-Barkay et al⁵ use one of several pigtailed macaque models to investigate a possible role for YKL-40, a secreted mammalian chitinase-like protein, as a predictive biomarker for simian immunodeficiency virus (SIV) encephalitis (SIVE), the primate homologue of HIV encephalitis (HIVE). Elevations in cerebrospinal fluid (CSF) YKL-40 preceded death due to SIVE by up to 8 weeks and correlated with CSF SIV viral RNA (viral load), indicating that YKL-40 could potentially serve as a biomarker for SIVE.

Primate Models of HIV Encephalitis

Defining the neuropathogenesis of HIV infection is critical for developing effective neuroprotective strategies, and identifying appropriate animal models and biomarkers of immunodeficiency virus neuropathogenesis is a significant challenge. The macaque models (*Macaca mulatta*,

rhesus; *Macaca nemestrina*, pigtail) of SIV infection currently provide the best opportunity not only for studying the virus-triggered pathways of neurodegeneration that lead to cognitive dysfunction, but also for testing biomarker validity and neuroprotective treatments. The SIVE model used by Bonneh-Barkay et al⁵ uses pigtailed macaques infected with a viral swarm (SIV/Delta B670) that efficiently produces immune suppression but varies in frequency and time to onset of SIVE. In this sense, it mimics the temporal variability of HAND progression.

Another closely related pigtail SIVE model (used by Clements, Zink and colleagues⁶) uses the B670 swarm with co-infection with a CNS-adapted molecularly cloned SIV strain, SIV/17E-Fr, and more consistently produces SIVE in a shorter length of time (~90% SIVE development in ~84 days⁶). This might be related to a more consistent loss of natural killer cell activity demonstrated in this effective model of SIV neuropathogenesis.⁶ Finally, rhesus macaques infected with other SIV strains (SIVmac239, SIVmac251, SIVmE660), with or without depletion of CD8+ T lymphocytes before SIV inoculation, are also used.^{7,8,9} Each of these models replicates some features of SIVE and several demonstrate associated neurobehavioral abnormalities, making them useful for SIV neuropathogenesis studies. Notably, by magnetic resonance spectroscopy (MRS), several macaque studies have also demonstrated characteristic brain metabolite alterations observed in individuals with HAND, further validating these macaque models.¹⁰

Identifying Predictive Biomarkers of HIV/SIV Encephalitis

In HIV neuropathogenesis studies, examination of autopsied brains of affected individuals suggest that significant and varying levels of irreversible structural damage often

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precede the presentation of cognitive disorders. Thus, defining the events that precede neurological symptoms and identifying "at risk" HIV-infected individuals are essential for developing rational neuroprotective approaches. A central strategy for defining such events lies in identifying expression of predictive biomarkers in the CSF compartment during different stages of SIV and HIV infection.¹¹ Investigation of the expression of CNS biomarkers and their relationship to SIV replication and development of SIVE in macaque models is thus clearly justified.

Markers of glial cell activation or virus replication are likely to be good candidates for predictive biomarkers of ensuing neuronal damage due to HIV replication. In addition, certain neuronal markers might predict ongoing injury as well as risk for progression of injury and cognitive decline. It is critical, however, to identify markers that *predict* neurodegeneration at stages of HIV infection that allow for therapeutic intervention. Macrophage-associated markers such as YKL-40 are likely to be altered during the course of SIV/HIV infection through both direct and indirect effects of viral replication. Because productive HIV infection is restricted to cells that express both CD4 and an appropriate chemokine receptor (CCR5 or CXCR4), the macrophage/microglia population is the primary target of HIV infection in the brain. The importance of HIV replication in brain macrophages for neuropathogenesis is supported by the observation that HIV swarms evolve toward enhanced macrophage tropism within the CNS compartment, and specific HIV envelope variants are associated with increased macrophage tropism and the presence of HAND.¹²

To use predictive biomarkers effectively, one must understand not only the pattern of HIV but also the course of brain HIV infection. Brain infection occurs early (weeks) after systemic infection and is associated with immune activation of both infected and noninfected glia (macrophages/microglia, astrocytes). Macrophage activation occurs both systemically and within the CNS, as evidenced by elevations of plasma sCD14 (lipopolysaccharide receptor) and the appearance of macrophage activation markers in the CNS.¹³ Macrophage infiltration into the CNS is enhanced through increased monocyte chemoattractant protein-1 (MCP-1/CCL-2) expression during infection, and this promotes a general enhancement of CNS immune activation.¹⁴ Neuronal damage likely begins later and progresses throughout the course of infection, resulting in both irreversible and reversible cognitive impairment. Although the predictive value of the CSF HIV viral load is uncertain, elevated CSF viral loads are associated with increased risk of HAND.^{15,16} Similar relationships between early virus replication, immune activation and ensuing neuronal damage are also found in the various macaque models of SIVE used by Bonneh-Barkay and colleagues⁵ and other groups, suggesting that multiple biomarkers of these processes might be useful and accessible in the CSF.

YKL-40 as a Possible Marker of SIVE/HIVE

In their study of YKL-40 (a glycosyl 1 hydrolase family member and a previously identified biomarker of tissue

inflammation and neoplasia¹⁷), Bonneh-Barkay and colleagues⁵ examined the protein's expression in the CSF and brain tissue of pigtailed macaques infected with SIV. Using the technique of surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy (SELDI-TOF-MS) to analyze CSF, the investigators found that four distinct spectral peaks were unique to SIVE, while four other peaks were found to be unique to animals without SIVE. Additionally, 14 peaks common between these groups demonstrated relative increases or decreases between the two groups. Among these, YKL-40 was identified and found to be elevated fivefold in CSF in SIVE cases versus non-encephalitis cases. These findings were confirmed by Western blot analysis of brain tissue. Although the origin and functions of YKL-40 in the CNS are unknown, YKL-40 is found in many non-neural cell types associated with inflammation and accompanying extracellular matrix (ECM) damage, including macrophages,¹⁷ it can bind to oligosaccharides in the hyaluronic synthetic pathway and to collagen (see references within Bonneh-Barkay et al¹⁷), it has mitogenic effects in synovial cells and chondrocytes, and it can suppress pro-inflammatory cytokine and matrix metalloproteinase production. Thus, the association between YKL-40, inflammation, and macrophages might have particular relevance for neuroinflammation associated with HAND.

In their study, the investigators present evidence that significant elevations of CSF YKL-40 consistently *precede* (2 to 8 weeks) the animal's death due to SIVE, and these elevations correlate with the CSF viral load in animals that develop SIVE but not in those who do not. Plasma levels of YKL-40 did not change from baseline in infected animals, suggesting that virus-induced YKL-40 expression and any resulting effects occur primarily within the CNS during viral replication, possibly qualifying YKL-40 as a compartmentalized predictive biomarker for SIVE. A potential link between elevated YKL-40 expression and the pathogenesis of SIVE neuronal damage was suggested by *in vitro* experiments demonstrating YKL-40 displacement of ECM-bound basic fibroblast growth factor (bFGF) and inhibition of bFGF-induced hippocampal neuronal axon sprouting. The displacement and inhibition of neurotrophic factors from the ECM by YKL-40 was proposed as a novel and indirect mechanism of SIV-induced neuronal damage.

A further link to HIV neuropathogenesis was provided by examination of CSF from HIV-infected individuals, which showed significantly elevated CSF YKL-40 levels in those with >10,000 CSF HIV RNA copies/ml. Although the frequency of HIV and HAND in the cases studied was not specified, high CSF viral loads are associated with increased risk of HIV and HAND (see¹⁶ and references therein). Because CSF YKL-40 is elevated in macaques before development of SIVE, YKL-40 could be a uniquely predictive biomarker for the development of HAND. If the predictive value of CSF YKL-40 expression is further validated in longitudinal studies of CNS HIV infection, this would represent a major step in progressing toward the eventual development of effective neuroprotective adjunctive therapies to HAART.

HIV Neurodegeneration Markers: Where Are We Really?

The search for biomarkers of HIV-induced neurodegeneration and/or glial activation in both primate models and human cohorts has been an arduous and only partially successful undertaking. Ideally, the most desirable marker would meet the criteria of high sensitivity and specificity along with high predictive value for identifying high-risk individuals before development of irreversible neurological damage. To date, a number of variably reliable CSF markers of either pathological damage or cognitive dysfunction have been identified.¹¹ Among these are the glial activation markers MCP-1/CCL-2 and other chemokines, neopterin, β -2 microglobulin, quinolinic acid, Fas, 4-hydroxynonenals and other associated reactive oxygen species,^{15,18–22} and the neuronal markers neurofilament light chain (NFL)^{23,24} and tau protein.²⁵ Each of these has been demonstrated to be associated with cognitive dysfunction in HIV-infected individuals, and MCP-1/CCL-2 (primarily derived from activated glia) and NFL have been found to be predictive of the development or worsening of HAND (CSF NFL: sensitivity 78%, specificity 67%).^{23,26,27} More recently, increases in CSF tocopherol and triglyceride C52 levels have also been shown to serve as predictive indicators of worsening HAND in individuals receiving HAART.²⁸ These latter unique markers are thought to represent an endogenous CNS antioxidant response, and it is intriguing to speculate that this response occurs during early glial cell activation induced by HIV replication. Such early postinfection glial cell activation has been demonstrated by detection of early elevations of brain myoinositol, another marker of glial activation, by MRS studies of HIV-infected individuals.²⁹ How YKL-40 compares with these glial and neuronal biomarkers in its predictive value for HAND remains to be determined.

Many questions about the ultimate validity of CSF YKL-40 as a predictive marker for HIV-induced cognitive impairment remain, including the CNS cellular origins of YKL-40, its kinetics of expression, its association with neurodegenerative pathways, and its association with cognitive impairment in individuals receiving HAART and adjunctive neuroprotective therapies. In extraneural tissues, YKL-40 expression has been detected in macrophages in various inflammatory conditions and in many tumor types (reviewed¹⁷). However, the cellular origin of YKL-40 within the CNS is undefined, and this clearly requires clarification. This is especially critical because macrophage/microglial activation might occur before astrocyte activation, and markers selectively released by these distinct cell lineages might therefore have different predictive values. The Bonneh-Barkay study also presents evidence that differentiated macrophages express YKL-40 *in vitro*, and the investigators further suggest (in data not shown) that HIV-1 infection of macrophages rapidly induces transient elevations in YKL-40 expression, although the kinetics of YKL-40 expression in HIV-infected macrophages and other CNS cell types are as yet unknown.

In these SIV-infected macaques, YKL-40 expression in the brain localized to perivascular macrophages (but not those in microglial nodules) and to areas surrounding activated astrocytes associated with microglial nodules, suggesting macrophage release and subsequent extracellular accumulation and association with astrocytes. This surprising accumulation is proposed to link YKL-40 directly to neurodegenerative pathways through interference with the neuronal binding and trophic activity of bFGF. The evidence for this is indirect, based on demonstration of bFGF displacement from ECM, perhaps through interactions with heparin sulfate in the ECM. However, the binding partners for YKL-40 are not completely defined, and YKL-40 apparently does not bind directly to the bFGF receptor. Despite these uncertainties, this suggests a role for YKL-40 in neuropathogenesis through interference with trophic functions of bFGF and perhaps other ECM-binding trophic factors. Because bFGF promotes the survival of neurons from multiple brain regions (neocortex, hippocampus, cerebellum, midbrain, others) and because expression of bFGF expression is altered in neurodegenerative diseases (Alzheimer's disease, AD; Parkinson's disease, PD), it may have a role in protecting the brain from pathological events.³⁰ Accordingly, because many growth factors are bound to components of the ECM,³¹ loss of activity of bFGF (and perhaps other ECM-bound neurotrophic factors) due to enhanced YKL-40 activity could indeed represent a novel mechanism of SIV/HIV-induced neurodegeneration. Determining the potential role for YKL-40 in such processes requires much additional investigation. Furthermore, although loss of several neurotrophic factors has been demonstrated in primary neurodegenerative diseases such as AD and PD, this has not been established in HAND. Alternatively, it is possible that YKL-40 accumulation could perturb supportive functions of astrocytes or induce production of neurotoxins by astrocytes. These questions are clearly worth further investigation in macaque SIVE models, in *in vitro* models of HIV-induced neurodegeneration, and in HAND.

Other major unanswered questions are whether predictive biomarkers such as YKL-40, MCP-1/CCL-2, NFL and others respond similarly and reliably to anti-retroviral therapy before the development of SIVE and neurobehavioral changes and whether macaque models will replicate responses of these markers in humans receiving HAART. As previously discussed, there are several macaque models used in studies of SIVE pathogenesis that could certainly be used to further test and validate a role for YKL-40 in neuropathogenesis and neurological outcomes in longitudinal studies. Along with a strong predictive value for HAND development, an ideal biomarker would also serve as an easily assayed predictor of neuroprotection in response to drug treatment regimens, not only in treatment-naïve patients but also in patients already receiving HAART. Several CSF biomarkers such as MCP-1/CCL-2 and NFL are expressed at lower levels during HAART than before HAART, and these lowered levels are associated with improvement in HAND symptoms.²⁷ Other potential biomarkers are under investigation in ongoing clinical trials. The relationship between

CSF YKL-40 expression, HAART, and the development and progression of HAND also requires investigation in longitudinal studies. Moreover, the effects of adjunctive (non-HAART) neuroprotective therapies (antioxidants, NMDA receptor antagonists, trophic factors) on predictive biomarkers such as YKL-40 need to be studied to determine the value of these biomarkers for defining the neuroprotective efficacy of combination therapy. If HAART is only partially neuroprotective, how do we assess these adjunctive therapies in real time in infected individuals? We need to urgently pursue the development of simple, rapid, and widely available assays of biomarkers suitable for both treatment-naïve and HAART patients.

Closing Remarks

HAND might be unique among neurodegenerative disorders in expressing a significant level of reversibility long after symptoms first develop, and effective “restorative” treatment might be possible in currently affected individuals. If a biomarker is linked solely to levels of SIV/HIV replication as indicated by CSF viral load, and it “normalizes” concomitantly with HAART suppression of CSF viral load, how do we then identify those individuals who nonetheless develop or progress to HAND? How do we apply neuroprotective adjunctive strategies in those patients? Perhaps predictive CSF biomarkers such as YKL-40 and/or others will eventually serve as adjuncts or even as surrogates for conventional specialized methods of neuropsychological testing and neuroimaging techniques used in individuals at high risk for HAND. Much additional study is required before YKL-40 can be validated as a predictive biomarker for the development of HAND. We should strive to fully define such biomarkers because of the potential benefit to millions of HIV-infected individuals worldwide.

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