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Synaptodendritic injury with HIV-Tat protein: What is the therapeutic target?

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Neurodegenerative diseases are not considered reversible. Treatment is usually symptomatic. HIV infection also results in a neurodegenerative illness leading to a dementing process over a few months. Infection is present in glial cells and infiltrating macrophages while the degenerating neurons are not infected (reviewed in (McArthur et al., 2010)). This observation raised hope about the possibility of developing neuroprotective strategies for treating the disease. While this may still be a reasonable approach, unfortunately to date all such clinical trials have either failed or shown only minimal benefit (Kranick and Nath, 2012; Turchan et al., 2003). Interestingly, however, treatment of patients with HIV associated neurocognitive disorders with antiretroviral drugs has shown dramatic effects. A patient with profound HIV-dementia may show reversal of symptoms within a matter of weeks (reviewed in (Kranick and Nath, 2012)). This would only be possible if the neurons are dysfunctional and not dead since once a neuron dies it cannot be easily replaced. Thus upon suppression of viral replication the neurons should retain the ability to repair themselves. Neurons are unique compared to other cell types. They extend long processes which may range from a few millimeters to several meters. At the terminal ends of these processes, they form synapses with other neurons. Any dysfunction at the synaptic level can lead to the loss of transmission of impulses from one neuron to the next. Autopsy findings in HIV-infected patients clearly show evidence of synaptodendritic abnormalities (Masliah et al., 1992) and dysregulation of mRNA involved in synaptic function (Gelman et al., 2012). The loss of dendrites shows good correlation with the severity of neurocognitive decline and decreased gray matter volume on neuroimaging (Archibald et al., 2004).

It is clear that the synaptodendritic abnormalities are driven by viral replication in non-neuronal cells. There is also good correlation between the amount of virus in the brain and the degree of dendritic abnormalities in patients in the pre-antiretroviral era (Masliah et al., 1992). Using this rationale, if viral replication and production of viral proteins can be completely controlled, then HAND should be prevented. The use of antiretroviral drugs has led to a dramatic decrease in the incidence of dementia with HIV infection. However, the milder forms of neurocognitive impairment continue to persist even when the viral load is under excellent control in blood and cerebrospinal fluid (Heaton et al., 2011). What then

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could be driving the neuronal injury? While it is possible that viral replication might not be fully controlled in the brain, the evidence for that seems lacking. It is also possible that the antiretroviral drugs themselves may be neurotoxic; however, a closer look at the mechanism by which the available antiretroviral drugs control viral replication may provide important insights. Antiretroviral agents can prevent new cells from getting infected by blocking their entry into cells, inhibiting reverse transcription of the viral RNA or by preventing integration of the virus into the chromosomal DNA. If antiretroviral drugs are used after a cell has been infected and proviral DNA has integrated, protease inhibitors can prevent the cleavage of the HIV gag-pol polyprotein and thus prevent infectious virus from being formed. However, these protease inhibitors do not prevent the formation of early viral proteins which includes the Tat protein. Current guidelines for initiating antiretroviral infection call for treatment only when the CD4 cells fall below 500 cells/mm³, which usually occurs several years following infection. This provides ample time for the virus to be established with the brain. Thus Tat protein would be expected to be produced within infected cells. In vitro studies confirm the production of Tat despite the inhibition of viral replication by darunavir, a protease inhibitor in peripheral blood mononuclear cells. Further, Tat protein can also be found in the cerebrospinal fluid and in infiltrating macrophages in the brain of patients who have undetectable virus (Johnson et al., 2013).

Once Tat is released extracellularly from HIV-infected cells it causes neuronal injury to neurons in close vicinity and it can also travel along neuronal pathways to cause injury at distant sites (Bruce-Keller et al., 2003). While Tat can cause neuronal cell death in vitro, at lower concentrations it causes neurite retraction. Transgenic animals in which Tat is expressed in astrocytes show evidence of learning and memory deficits (Carey et al., 2012) and associated thinning of the cortex (Carey et al., 2013) and synaptodendritic injury (Fitting et al., 2013) which is also evident by direct injection of Tat into the brain (Lu et al., 2011). Importantly, Tat-induced synaptic injury is reversible (Shin and Thayer, 2013). Multiple mechanisms have been implicated in causing these effects. These include direct interactions with cell surface receptors on neurons such as the NMDA receptor (Li et al., 2008) the low density lipoprotein receptor-related protein (Eugenin et al., 2007; Liu et al., 2000) and several other receptors including integrins $\alpha 5 \beta 1$, $\alpha v \beta 3$ and $\alpha v \beta 5$; the vascular endothelial growth factor receptors and the chemokine receptors CXCR4, CCR2 and CCR3 and CD26 (Ghezzi et al., 2000; Noonan and Albin, 2000), and heparan sulfate proteoglycans (Tyagi et al., 2001). Tat can also activate glial cells which can result in the production of reactive oxygen species, cytokines, chemokines and block glutamate uptake. In fact Tat has been shown to bind to multiple proteins, and to RNA. A genome wide binding map of the Tat protein to the human genome shows that it binds to multiple host cellular factors that include transcription start sites of many genes and repeat elements or Alu sequences (Marban et al., 2011). In neurons, Tat activates the ryanodine receptor and glycogen synthase kinase 3 β (Peng et al., 2010, 2012) and inhibits vesicular monoamine transporter 2 (Theodore et al., 2012). By doing so, the protein serves as a master regulator of cell function and can control viral replication by multiple means.

In the current issue of *Experimental Neurology*, Dr. Rosemarie Booze's laboratory used a variety of deletion mutants of Tat to define the epitopes involved in synaptodendritic damage. They found that the terminal amino acids 73–101 which are formed by the second

exon were not critical for mediating these effects. They deduced that the cysteine rich region contained in residues 22–37 was critical for causing neuronal injury. This extends previous observations in which the neurotoxic domain was defined using overlapping synthetic peptides from Tat and found that the neurotoxic domain resided between amino acid residues 31–61. In this study, shorter 15 mer peptides failed to cause neurotoxicity (Nath et al., 1996). In a follow-up study, using a molecular approach, it was found that cysteine in position 31 was an important mediator of neurotoxicity (Li et al., 2008). Importantly, the Booze laboratory has confirmed this observation and has now identified the cysteine at position 22 as another critical residue for mediating these effects. One may ask however, if Tat has so many effects on cell function why then would defining the epitopes that cause neurotoxicity be important? HIV is unique compared to other viruses in that it mutates constantly even within a single individual which is called a strain; and signature forms of the virus can be identified in different geographical regions around the world which are called clades. Despite this, some parts of the virus remain relatively constant since any mutations in these regions would prevent viral replication. The cysteine rich region of the Tat protein is one such domain. This region has seven cysteines between residues 22 to 37. A mutation of cysteine in position 31 to serine has been identified in clade C and not in other clades, but mutations in any of the other cysteines prevent viral replication (Jeang et al., 1999). A Blast analysis of the cysteine rich region shows that mutations at specific sites may occur within this region (Table 1). These mutation sites are adjacent to the cysteines. Amino acid residues in positions 23 and 24 are commonly permutations of asparagine and threonine. At position 32 a conserved mutation of phenylalanine to tyrosine may occur and at position 35, it may be a proline, isoleucine, lysine or glutamine. All these changes are very conservative and would not be expected to have major changes in the charge or structure of the region. A Blast analysis of the clade C cysteine rich region also showed very similar mutations; hence, only the results from the clade B Tat are shown.

Thus from an evolutionary stand point one would wonder why the virus has conserved its ability to cause neurotoxicity. Does that provide a survival advantage to the virus? But from the point of therapeutic development, identifying such regions is important. Biological approaches such as antisense molecules or therapeutic vaccines could be targeted to this region. Pharmacological compounds that bind to this region could potentially be of therapeutic benefit.

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Table 1

Amino acid sequences of the cysteine rich domain of HIV Tat protein.

Clade B	Sequence ID
CTNCYCKKCCFHCQVC	HIV-BRU
CNTCYCKKCCYHCPVC	gb AAB68443.1
CTKCYCKYCCYHCPVC	gb AAW68101.1
CNNCYCKYCCYHCLVC	gb AFX98207.1
CNSCYCKKCCYHCPVC	gb ACB98575.1
CNNCYCKYCCYHCIVC	gb AAL06103.1
CNQCYCKYCCYHCLVC	emb CAA75935.1
CNKCYCKICCYHCPVC	gb AAD13362.1
CNACYCKKCCYHCPVC	gb AAO47176.1
CSTCYCKKCCYHCPVC	gb AAN73721.1
CNNCYCKRCCYHCPVC	gb AGF43770.1
CTTCYCKKCCYHCPVC	gb AGA93939.1