Viral Burden and AIDS-related Neurodegeneration: the Role of Blood and Cerebrospinal Fluid

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Introduction: Prior to the era of antiretroviral therapy (ART), 30% of HIV infected patients developed neurological symptoms ranging from minor cognitive impairment to severe dementia. With the advent of ART, the prevalence of neuroAIDS declined, yet, as patients life-expectancy increases, so does the possible return of HIV associated dementia (HAD) (*1*). How HIV emerges in the brain is not fully understood. There are two theories that seek to explain the presence of virus in the brain. The prevailing theory states that the virus enters the brain via infected/activated monocytes/macrophages through the blood-brain barrier (BBB) (*2*). Once in the brain, the virus can infect microglia, macrophages, and other resident immune cells, but have minimal infectivity to neurons (*1*). Alternatively, virus may enter the brain via a direct infection of the choroid plexus, into the CSF, and eventually infect the brain (*3, 4*). Symptoms of dementia can be observed even in the absence of detectable viral infection in the choroid plexus, the region of the brain associated with production of cerebrospinal fluid (*5*). The metabolite ratio of N-acetylaspartate to creatine (NAA/Cr) has been shown to be a sensitive marker of disease and correlates well with markers of neuronal integrity (*2, 6*). Using an accelerated model of neuroAIDS, we tested the hypothesis that changes in NAA/Cr are more closely related to the viral burden in the peripheral blood, and not to the burden in the cerebrospinal fluid (CSF). Our data supports the theory that trafficking occurs through the BBB, and that virus is present in the CSF does not significantly contribute to the development of dementia.

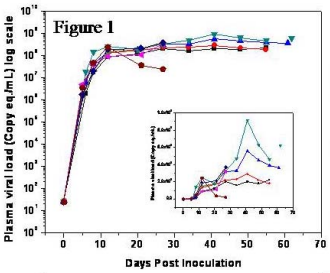
Methods: Eight rhesus macaques (macaca mulatta) were inoculated with 20 ng of SIVmac251 virus and their CD8+ T-lymphocytes were depleted with antibody targeted against CD8 (cM-T807) at 6, 8, and 12 days post inoculation (dpi) to facilitate rapid disease progression. Animals were scanned two times pre-inoculation, and biweekly until sacrifice at 4 weeks pi (wpi) (4 animals), or 8 wpi (4 animals), on a Siemens 3T Trio system. Single voxel ^{1}H MR spectroscopy was performed in the parietal cortex (PC), frontal cortex (FC), basal ganglia (BG) and white matter semiovale (WM) using a point resolved spectroscopy sequence (PRESS) with TE/TR = 30/2500 ms. Metabolite concentrations (NAA and Cr) were determined offline using the LCModel software package. Blood and CSF were drawn before every MR scan, blood was centrifuged, and plasma and CSF were stored at -80^{o}C until study endpoint. Viral burden was quantified using real-time PCR with a threshold sensitivity of 100 copy eq/mL, as previously described (*7*). Statistical analysis was performed using JMP 7.0 (SAS, Cary, NC). A general linear model was used to explore associations between viral RNA levels and MRS measures. The ability to utilize the baseline measurements with raw NAA/Cr values allowed us to use animals that were sacrificed with only three spectroscopic measurements (i.e. the animals sacrificed at 4 wpi). This is used as a probe for the initial events in the neuropathogenesis of HIV. To examine the later events in pathogenesis, we have utilized the changes in NAA/Cr from baseline.

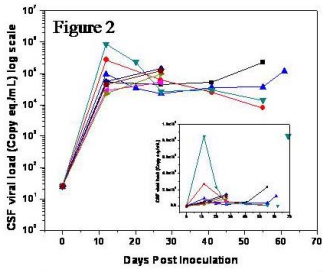
Initial events in SIV neuropathogenesis (n = 8)

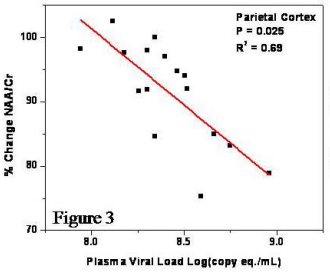
NAA/Cr vs.	Spectroscopy Brain Region	P-value	R^2-value
Plasma Viral Burden	PC	0.022	0.66
	FC	0.033	0.67
	BG	0.004	0.82
	WM	0.010	0.79
CSF Viral Burden	PC	NS (0.066)	0.60
	FC	NS (0.104)	-
	BG	0.031	0.77
	WM	0.030	0.77

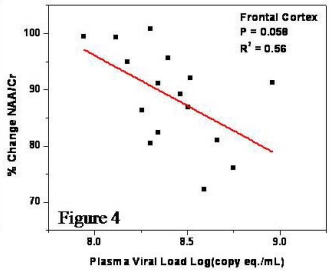
Late events in SIV neuropathogenesis (n = 4)

NAA/Cr vs.	Spectroscopy Brain Region	P-value	R^2-value
Plasma Viral Burden	PC	0.025	0.69
	FC	NS (0.058)	0.56
	BG	NS (0.152)	-
	WM	NS (0.083)	0.56
CSF Viral Burden	PC	NS (0.614)	-
	FC	NS (0.502)	-
	BG	NS (0.985)	-
	WM	NS (0.586)	-









Results: Analysis shows that NAA/Cr levels in all brain regions correlate well with the initial increase in viral load in both the peripheral blood and the CSF. The changes in NAA/Cr at later time points show trends/significant correlations with the peripheral blood viral load, except in the BG, but not with the viral load in the CSF. Interestingly, this pattern is mirrored by metabolic changes: the PC, FC, and WM all exhibit significant decreases in their NAA/Cr values ($P < 0.0001$ in the PC, FC, and WM), indicating neuronal injury and disease, while the BG is not shown to be changing as severely ($P = 0.004$). Statistical results are summarized in the table, figures 1 and 2 depict the viral loads in the plasma and CSF, respectively, and figures 3 and 4 show representative correlations between changes in NAA/Cr versus viral burden from the PC and FC, respectively.

Conclusions: Interestingly, in the initial SIV infection, we found that neuronal damage was associated with an increase in the peripheral blood and CSF viral burdens. At early time points, the viral load in both compartments shows a marked increase, which corresponds to the initial decrease in NAA/Cr. This observation has two consequences: (1) both compartments could lead to brain infection in parallel, or (2) the virus in the CSF may be derived from the peripheral blood. Further exploration of these relationships is required. At later time points of disease, our data suggests that virus present in the brain is as a direct consequence of the trafficking of infected/activated monocytes/macrophages through the BBB in corroboration with previous evidence (*1, 2*). Moreover, ours, and others, data suggests that the virus present in the CSF may not be greatly contributing to neuronal disease at later stages in infection (*5*). We also found that the severity of the disease only correlates when there is a highly significant level of damage. Furthermore, with the secondary increase of the plasma viral burden at 6 wpi, we observe dramatic decreases in NAA/Cr across all regions. The lack of correlation between damage and plasma viral burden in the BG may be due to the less severe drop in NAA. Finally, there are very few reports in the literature stating a relationship between neuronal disease severity and viral load. We suspect that our isolation of this relationship is due to a greater level of experimental control; we have a very homogeneous cohort to study (all are antiretroviral-naïve) and we know exactly when their inoculation with virus occurred.

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References:

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