HIV Associated Dementia:Dopaminergic Neuronal Apoptosis and Future Implications for Clinical Intervention

Working Draft Copy of Master's Thesis

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Special Thanks...

Well, I cannot believe how much I have learned in the past 8 months researching the causes and treatments for HIV Dementia. I was always interested in neuroscience and especially with how viral pathology can affect neurocognitive function.

I would like to thank my sponsor, Dr. Patric Stanton who taught me not only how to organize a thesis but how to ask the right questions of science and to not take anything at face value. As I enter my future career in medicine, I will not forget that advice. Dr. Stanton has been very patient with me he really inspired me to get into perhaps more research. I would also like to thank my two readers, Dr. Doris Bucher and Dr. Christopher Leonard. They are both very accomplished scientists in the field. Dr. Bucher has made some incredible contributions to virology especially with flu vaccines. Dr. Leonard has also made many contributions to the exciting area of neuroscience especially with his work on regulation of sleep and wakefullness.

I have to thank my wonderful family for supporting me so much these last couple of months, especially my mother Fauzia Ali for her moral support and to my Grandfather Mr. Ghulam Yasin for always believing in me. Also my father Dr. SM Yousuf Ali for being a role model, brother Tariq Ali and sister Shazia Ali for their unfaltering support. Mohammed Aamer, my cousin for his help the technical aspect of things. I also want to thank Dr. Sanjay Gupta of CNN for his support this year as I completed this work along with everything else.

Yet, the true inspiration for this research review will have to be my grandmother Mrs. Hamida Yasin, whose suffering from severe neurological impairment in ther last days of her time here gave me a new perspective on the importance of new research into causes of dementia. She also before she “crossed over” was my support as I began my masters degree.

And most of all, I would like to thank God for all of His blessings and in helping me complete this project.

Introduction

As HIV emerged on the scene in the 1980's, the world was overwhelmed by the threat of a new pandemic, yet little was known about the disease that led to AIDS. This is especially the case with regard to the devastating neurological damage the HIV virus can produce. Patients of Dr. Howard Gendelman at the University of Nebraska have provided much insight into the ravages of what is known as HIV Associated Dementia or HAD. Dr. Gendelman and his colleagues in 1986 pioneered the discoveries that led to a better understanding of HAD. In fact, Dr. Gendelman is still one of the most cited researchers studying the mechanisms of HAD. HAD is extremely troubling, even considering available new retroviral drugs, because most of those drugs do not adequately pass the blood brain barrier to reach the CNS. Many patients with advanced HIV infection may live an extended life even with a massive reduction in viral load. With HIV patients on cocktails of antiretroviral drugs along with protease inhibitors the expectancy of life has been shown to increase. Yet, these patients still exhibit severe neurological and psychological deficits that can severely degrade quality of life. HAD, is the area of research that has inspired great interest in the emerging field of neurovirology, and continues to be a top priority as HIV infected patients are often living much longer, lives in part due to HAART (Highly Active Antiretroviral Treatment). HAART has been the gold standard in retroviral therapy that includes the potent antiretroviral AZT (Zidovudine) with a combination of two nucleoside analogues and a HIV Protease (Gendelman et al, 1998). This combination

effectively inhibits HIV's ability to utilize reverse transcriptase and subsequently to replicate.

HIV Associated Dementia is a psycho-neurological complex that affects patients infected with HIV type 1, which is the most common form of the virus in the population. The complex has many stages, ranging from minor cognitive defects to a complete vegetative state. The course of the disease is often overlooked because patients presenting symptoms often have a decreased viral load in the blood. The HIV virus, however, finds a way of infiltrating the blood-brain barrier via infected monocytes (Gendelman et. al, 1998 p.37). Usually, the blood brain barrier is an efficient protective shield against pathogens, yet in this case the HIV virus has a way of undergoing mutations which make it able to interfere with specific adhesion molecules such as ICAM-1 and VCAM-1, that make up the specialized blood brain barrier. This will be described in detail as we touch on the mechanisms of how these coat proteins will affect the blood brain barrier. Furthermore, patients presenting with HAD often do not get tested for cerebrospinal levels of the virus, thus being misdiagnosed as having HIV Associated Dementia. HAD is a devastating disorder akin to Alzheimer's disease in which the patients can become mute, emotionally unable to express themselves and in some cases enter in a persistent vegetative state. Not only do patients of HAD have severe psychiatric problems and cognitive disorders, they also present with motor difficulties later as the disease progress. In this report, the hypothesis that specifically

dopaminergic neurons are being destroyed by HIV is suggested by the clinical similarities between HAD and Parkinson's disease. Parkinson's Disease is a motor deficit disease with the hallmark trait of loss of dopaminergic function and eventual dopaminergic neuron destruction. It is this parallel with the clinical manifestations which incited researchers to look into the possibility of similar molecular mechanism In addition to severe clinical symptoms, neurologists such as Dr. Gendelman (Gendelman et al, 1998), when first treating HAD patients, noticed a structural abnormality as well. In neuroimaging studies, the MRI of HAD patients showed a significant loss of white matter volume. Scans showed a rather large increase in the cerebral ventricles, another indicator of brain atrophy. On autopsy examination, brains of HAD patients confirmed this finding of white matter loss. It is found that the HIV virus itself secretes proteins that induce several apoptotic pathways that find an affinity to focus primarily on key dopaminergic nerve pathways found both in motor and memory regions of the brain. In addition, the HIV virus infects microglia, causing a disruption in critical glutamate balance towards an excess of glutamate leading to excitotoxicity. Current research into HAD is vital because the previous focus has been on reducing HIV mortality rates, rather than improving quality of life of those living with HAD. The World Health Organization, along with many private foundations, have reduced the cost of powerful anti-retroviral drugs and millions of HIV stricken patients around the world are now receiving them, yet most of them will have HAD that is misdiagnosed. This is evident as many HIV patients in rural areas are not even able to verify their own HIV positive status. Yet, with more awareness this is to surely change. As the population of

patients living with HAD grows, it will be increasingly important to find treatments that ameliorate or slow neurological sequelae of HAD.

Before we review what is known about the cellular mechanisms of HAD, and several hypothesizes that have been proposed to date, I want to ensure that we do not lose sight of the human aspect of this terrifying disorder. Here is some sample case studies described by Dr. Howard Gendelman, (Gendelman et al 1998). These cases are adapted from Dr, Gendelman's book The Neurology of AIDS. The patient is Garret Burton and he describes his life as was infected with HIV. He experienced severe motor difficulties and parathesias. He explains that his legs and feet were constantly in state that could be described being "asleep"-that is numb. The tingling sensation hampered his everyday activities especially in his profession as a piano player. The frustration is evident in his own accounts of how countless neurologists misdiagnosed his symptoms of HAD. He was told that he was experiencing constant dizziness due to hypoglycemia and that his mental status was due to a psychological problem. The most painful thing was that he was indeed disabled, but wasn't eligible for disability benefits because of his misdiagnosis. Eventually, a neurologist recognized the pattern of lesions in his CT scan, and then high HIV titers in his cerebro spinal fluid. His frustration with HAD is evident:

"The one thing I've avoided discussing in any detail is that part of the disease which for me carries with it the most pain, disappointment and sometimes anger and fear. The disease impairs all mental functions including memory and communica-

tion.... My family and I have to watch the disintegration of my personality, the gradual erosion of all those qualities of mind and spirit which we know is me" (Gendleman et al, 1998 p.597)

While Garrett found his HAD devastating, it pales in comparison to a mother's anguish while watching her young experience HAD. In this case, the HIV infection of the CNS made the patient completely unable to function both mentally and physically. In her mother's words:

"*All I knew for months was that something was affecting my daughter's brain. She couldn't think. She couldn't eat. She couldn't sleep. She couldn't get dressed. In short she couldn't function. I stopped planning a wedding with my son and his fiancé. This became a new role in my life: primary caretaker of my oldest daughter. Dressing her, bathing her, feeding her, putting her to bed*" (Gendelman et al, 1998, Foreword)

HAD is a devastating disease that arises from HIV infection of the CNS. Furthermore, many of the motor symptoms of HAD mimic those of Parkinson's disease suggesting a casual link between the two neurological sequale. In both syndromes the patient begins to exhibit motor difficulties ranging from diskinesias and often a abnormal gait. It even turns out that in some advanced stages of HAD, the patient will display a "mask like" face just as seen in Parkinson's disease. It turns out that certain dopaminergic pathways common to both diseases are found to be selectively ruined namely the nigro striatal pathway and meso-limbic pathways. This finding shows

common links that may help to not only further research on HAD but also subcortical dementias such as Parkinson's disease and Huntington's chorea as well. There is a vast literature just in the past 5 years exploring this link and suggesting new therapies focusing on dopaminergic pathway namely in helping to increase levels of dopamine. The issue may be a greater than normal breakdown of dopamine or an inhibition in the de novo synthesis thereof. However, the greatest significance of the research on HAD is to advance our understanding in the field of neurovirology. Infection of the CNS by a variety of pathogens demonstrated that the unity of the blood brain barrier is not what it was previously thought to be, especially in states of inflammation. Further concentration on the effects of specific cytokines which are released during the inflammation state on the blood-brain barrier may help us better understand how to prevent "breaches" in the blood-brain barrier, and in so doing to slow or even prevent the progression of HAD. HAD, as well as many other neurodegenerative diseases, occur at a slow and constant progressive rate and, although neuronal plasticity in geriatric patients with these diseases are mostly likely to be limited, there is hope by better understanding how to prevent CNS infection by HIV or how to restore normal neurophysiology to at least slow the progression of HAD. In addition, in this current day in age where bioterrorism agents may be deployed, it is hard to simply ignore the many implications of infection of the CNS. Therefore, evaluation of current research literature is central not only to understanding the etiology of HAD, but may be relevant to a wide array of other neurodegenerative diseases.

This report will look at the etiology of HAD from the molecular standpoint to help us better appreciate the detailed mechanisms that lead to clinical symptoms in HIV patients. First, the entry of the virus in to the CNS and the method by which it achieves widespread infection will be explored. Once the CNS is infected with the virus, then it is important to look at the virus's many mechanisms of actually triggering neuronal apoptosis. It has been shown that HIV's viral proteins themselves interact with neuronal homeostasis and can activate intrinsic apoptotic pathways. In addition, HIV has a way of affecting the neighboring support and microglial cells to deregulate the key balance of toxic excess neurotransmitters. Once, these support cells lose their ability to regulate excess transmitter s such as glutamate, release among other various substances, excitoxicity is thought to result in neuronal death. However, the most interesting aspect of the current literature is the finding that dopaminergic pathways seem to be specifically more vulnerable to apoptosis induced by HIV infection, explaining the dopaminergic clinical Parkinsonian symptoms of HAD. This finding may prove to be a very important factor in finding appropriate treatment for HAD as dopamine-replenishing drugs may be of benefit. Currently, patients and physicians resort to palliative therapies that focus on symptomatic relief, rather than looking at the root of the CNS infection itself. This report will examine key experimental data that clearly supports the current findings relevant to HAD etiology, to broaden our understanding of the mechanisms by which HIV may lead to HAD, and possible treatment modalities of the future.

Viral Infection of the CNS

In cases of HAD, the main issue at hand is the initial infection of the CNS, which then harbors the HIV virus while it destroys key neuronal circuits. Usually the Blood Brain Barrier (BBB) is a protective barrier against invading pathogens, yet current literature suggests that HIV viral proteins can mutate in ways that allow them to promote degradation of the integrity of the BBB enough so that the intact virus can enter the CNS as they disrupt junctional complexes. The BBB, though vital to protect the brain can be a cause for concern in limiting the penetration of antiretroviral drugs into the CNS. This makes the CNS a reservoir for HIV destroy neurons and reinfect the periphery. The main route of entry into the CNS for HIV that has been proposed is via the increase in the trafficking of infected monocytes. Usually, virally infected monocytes do not readily cross into the CNS, but in HAD patients the BBB is significantly compromised. Infected monocytes also are involved in the whole inflammation cycle that produces substances like TNF-Alpha among others that recruit cytokines. These cytokines are believed to contribute to a vicious positive feedback by further damaging the BBB and promoting further infiltration of infected monocytes. Determining how HIV gets into the CNS is vital for the development of treatments to prevent CNS infiltration before the signs of dementia appear when it may be too late to preserve neuronal circuits essential to normal cognition.

Research has focused on the sequences of events that lead to viral infection of the CNS. One theory being evaluated is that mutated forms of HIV induce special

proteins that recruit chemokines to promote degradation of the BBB by increasing inflammation, to facilitate further infection and sequestration Another way that HIV can compromise the BBB is by down regulating the production of key adhesion molecules that make up the tight junction complexes of the BBB (Persidsky et al, 1997) One important junctional complex protein is occludin. Occludin is a key protein that makes up the Zonula Occludens which in turn makes a seal between the adjoining endothelial cells in the cerebral vasculature (Junqueira et al, 2003) There is also evidence that the HIV virus can up regulate harmful adhesion molecules such as VCAM's which break down the tight endothelial layer of the BBB. Finally, current research looks at a very novel idea on how the HIV virus, after it initially infects the CNS, can continue to do so at a high rate by activating more infected monocytes to permeate across the BBB. It has been shown that, once HIV "activates" macrophages both in the brain and in the periphery through the release of chemokines. Shaker type Kv1.3 Ca+ activated potassium ion channels are opened that cause an osmotic shrinking of the infected macrophage. These vast populations of shrunken infected macrophages are small enough to "squeeze" through the already compromised BBB. Thus, the entry of HIV into the CNS occurs by many mechanisms, yet the interesting point is that not only does the HIV have a method of initially infecting the CNS, it also has a way to maintain infection by attracting more HIV particles to join in on the damaging invasion of the CNS.

It is known that HAD patients can be strikingly similar to those with viral encephalitis, because of initiation of the inflammatory cycle which increases production and secretion of cytokines during the course of infection. These cytokines are involved in degrading one the brains key defense mechanism, namely the blood brain barrier. Key cytokines such as Macrophage inflammatory protein (MIP-1 Alpha) and Macrophage chemotactic protein (MCP-1) are involved in allowing trafficking of the HIV infected monocytes across the BBB (Persidsky et al, 1999). These cytokines are released from infected astrocytes and other infected macrophages. These cytokines then are suggested to further add to the inflammatory response that necessitates the chemotactic attraction of more infected macrophages to the area in question. The infected macrophages are then found to attach themselves with high affinity to the CCR5 receptor in studies conducted by Von Marle and his colleagues (Von Marle et al, 2002). Such an attachment for the monocytes was necessary as a co receptor for entry into the CNS. This laboratory looked at how many divergent mutated forms of HIV may help to efficiently facilitate CNS infection. They devised a simple experiment in which patients with HAD produced by a certain form of HIV were examined. Using PCR analysis, Von Merle discovered that the C2V3 portion of the virus's protein coat was very actively found in regions with underlying HAD induced atrophy. This was a simple way to localize HIV virus to a specific region. A key point in this study is that, although both groups of patients had similar viral loads of HIV detected and both were on anti retroviral treatment, one group showed a difference. Although both groups were HIV positive, only one exhibited clinical manifestations of HAD. It was

these HAD patients who had a form of the HIV virus that actually was far more "diverse" in its genetic make up than that of a regular strain. It has been suggested that these mutated forms of HIV are the ones responsible for CNS entry of the virus. The V3 portion of that C2V3 envelope protein had a unique 7 amino acid sequence that exhibited high affinity to the CCR5 receptor, which is involved in allowing infected monocytes to cross the BBB. It may be that Von uncovered an important feature in these experiments, thought this was a study on 21 subjects, so the mutation discovered may or may not apply to the majority of HAD patients. This paper did not offer much more detailed support especially in the form of a molecular mechanism, of its claims beyond the fact that perhaps these mutations impart resistance to anti retroviral drugs. They claim that this finding might explain why patients with HAD do not see improvement of CNS symptoms when placed on anti retroviral therapy. Although Von Merle supplied only correlative, and not mechanistic, data, other studies bring up this idea again suggesting that the HIV virus may modify itself so that it can trigger the release of cytokines to help allow further entry of HIV infected monocytes across the BBB. Langford et. al (2002) confirmed this by examining the brains of HAD patients on autopsy and in in-vitro experiments using cultured neurons. PCR amplification of the HIV RNA showed the localization of the virus. She found that the micro endothelial lining was compromised and that more lymphocytes were crossing the BBB. Furthermore, the genetic make up of the HIV in areas of neuronal damage was found to be very different than that usually found in other HIV infected tissues. These authors used an elaborate HIV RNA quantification and PCR to examine the viral sequences of

regions of neuronal damage (Langford et al, 2002) and found that indeed the regions of primarily white matter(cingulate gyruses and basal ganglia) were where most of the HIV resided. PCR analysis also confirmed that HIV was working on its own as other viruses such as JC Viruses, Epstein-Barr virus and human herpes among other viruses were not detected. Strelow et. al (1998) showed that a specific variant of the Simian Immunodeficiency virus (SIV) was very effective in targeting the macro vascular endothelial cells leading to a defect in the BBB . Again the hypothesis was put forward that anti retroviral drug resistant forms of HIV may be responsible for the neurological damage seen in patients with HAD.

A most interesting and counter-intuitive observation is that it was found that the anti retro viral drugs normally given to HIV patients to help stop infection may actually be promoting compromise of the blood brain barrier themselves opening the door to more infection. It is known that DNA polymerase is inhibited especially at the level of the mitochondria and thus lipid metabolism is severely affected. (Langford et Al , 2002). A key problem is that these widely used protease inhibitors may mimic LRP (Low Density lipoprotein Receptor related protein), thus targeting the lipoid protective layer of the brain as seen in the BBB, as well as degrading important myelinated neuronal pathways. In fact on autopsy, most brains of patients affected with HAD exhibit numerous white matter lesions, indicative of demyelization. However, in these papers the primary question addressed was how a mutated form of the HIV virus might induce the production of cytokines, such as MCP-1 that elicit information

that allows the increased infiltration of infected monocytes across the blood brain barrier. This notion does makes sense, because HAD is characterized as an inflammatory state akin to encephalopathy as most HAD patients also present with structural features of HIVE (HIV Encephalopathy). In inflammatory states cytokines are actively recruited and the permeability of the vasculature is altered. Overall, PCR/ and DNA analyses have uncovered unique amino acid sequences of HIV's viral protein coat which are present in patients with HAD more so than in patients with HIV who are not neurologically affected..

The blood brain barrier is a very stable structure of endothelial cells involving several integral proteins that maintain tight junctions, yet in HAD, the integrity of the BBB is severely compromised. In patients with HAD the key integral proteins such as occludin and occludens, which vital to the maintenance of BBB tight junctions are often altered absent (Dallasta et al, 1999). Considering HAD a example of a type encephalitis , they observed an increase in inflamed and infected monocyte trafficking across the BBB, as others had. What was interesting here was that, consistent with HIV 's ability to cause demylellination, there were profound breeches in the BBB closely associated with the white matter. Upon further examination, Dallasta and colleagues found a decrease in the occludin. Occludin is the key component of strong tight junctions by anchoring endothelial cells together creating a seal. HAD patients exhibited a loss of occludin protein in areas of the BBB co-localized with the appearance of the inflammatory markers such as class II MHC.. It was further established

that these markers represented areas of inflamed astrocytes infected with HIV-1 as represented by microglial nodules. These findings are striking because they confirm that areas where the HIV virus infiltrates the BBB are precisely the areas where there is a loss of the integrity of the BBB.

In addition, HAD patients, experience many motor symptoms similar to those seen in Parkinson's Disease, Interestingly, and key portions of the striatum are the areas most affected by infected monocytes, and are the places where the BBB was devoid of occludin. To really confirm that these breeches in the BBB were the route by which infected monocytes entered the CNS Dallasta et. al traced infected monocytes by using CD8 markers and found a strong correlation with the presence of HIV and these areas of BBB damage. The areas where the CD8 labeled monocytes traveled to were precisely those where the BBB was compro-

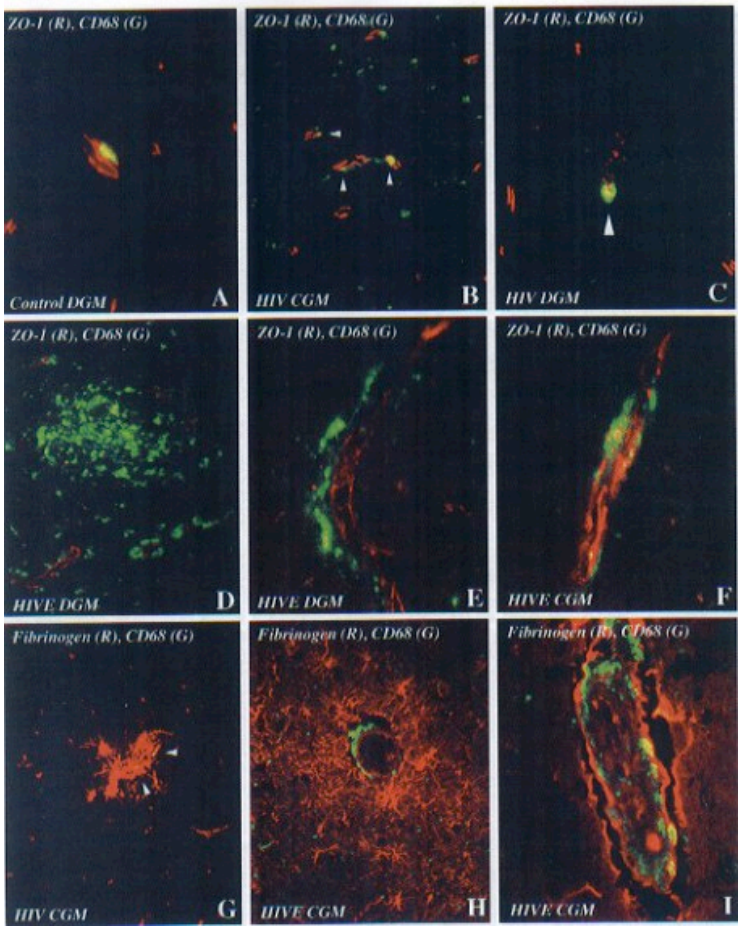

Figure 1: Tight Junction protein disruption is associated with marked serum protein extravastion and astrocytosis in HIVE. Notice in Panel I, the red labled fibrogen is extravasted signifying a markedly disrupted BBB. Adapted from Dallasta et al. (1999)

mised. Figure 1 illustrates immunoflorescence labeled confocal microscopy demonstrating that areas where tight junctions were altered and the BBB most compromised are also the areas where HIV infected monocytes infiltrated the most.

Another suggestion brought forth in this paper is that HIV may up regulate damaging adhesion molecules such as VCAM that contribute to further compromise of the BBB. Adhesion molecules work to compromise the BBB by adhering to and damaging the endothelium. Yet, one would think that, if HIV compromises the BBB, that penetration of antiretroviral drugs into the CNS would be promoted. Clinical data shows this, surprisingly is not the case. Though still quite unclear, the most plausible explanation is that ,while the HIV virus release inflammatory substances that breech the BBB, these breeches may be transient. An experiment that looks at the length of time a breech in the BBB created by HIV should be conducted, and might explain the nature of these breeches and the effects of occludin down regulation. Perhaps providing a way of protecting against BBB breeches could have a neuroprotective effect not only from HAD, but also other encephalitic insults. There are other observations that further also explain why antiretrovirals are not effective in HAD patients Langford et al. , (2002) found that anti retroviral drugs can produce mutated forms of HIV that are more prone to create breeches in the BBB. It may be that these drugs contribute to HAD sequale, rather than ameliorating it, explaining the resistance of HAD to antiretrovirals.

Crucial adhesion molecule found to be up regulated at the sites of HIV invasion is VCAM(Vascular adhesion Molecule). The BBB of HAD patients were not only made susceptible to breeches by the lack of integral proteins that make up tight junctions, but also by the activation of adhesion molecules such as VCAM because it along with E-Selectin helps to promote transendothelial migration of monocytes (Gendelman et al, 1998). In a study conducted by Persidsky and his colleagues in 1997 developed a model of the BBB, which showed that VCAM could actively rupture the BBB. The BBB model that was used here entailed an in vitro culture of endothelial cells assembled on a membrane associated with human foetal astrocytes. VCAN ruptured the BBB by creating a site where inflamed endothelium was being not only attached by VCAM, but other attracted monocytes. VCAM was found to be attracting other monocytes once the entire inflammatory cycle was initiated. On adding HIV infected monocytes to the BBB model as described, elevations in concentrations of cytokines and matrix metalloproteinase occurred, signaling the beginning of the inflammatory cycle. VCAM along with E-Selectin was also very highly found in BBB models where there was a correlated rise in inflammatory cytokines meaning that these adhesion molecules are contributing in some way to the inflammation seen in HAD.. These studies showed not only increased expression of activated VCAM on endothelial cells of the BBB, but also were strongly associated with an increase in HIV infected monocyte infiltration in to the CNS. To further confirm this association, Persidinsky examined electron micrographs, which revealed clearly that monocytes had infiltrated the BBB endothelium. In micrographs, the microvilli of the activated surface of the monocytes

can actually be seen "burrowing" into the tiny spaces between the endothelial cells (figure 2)

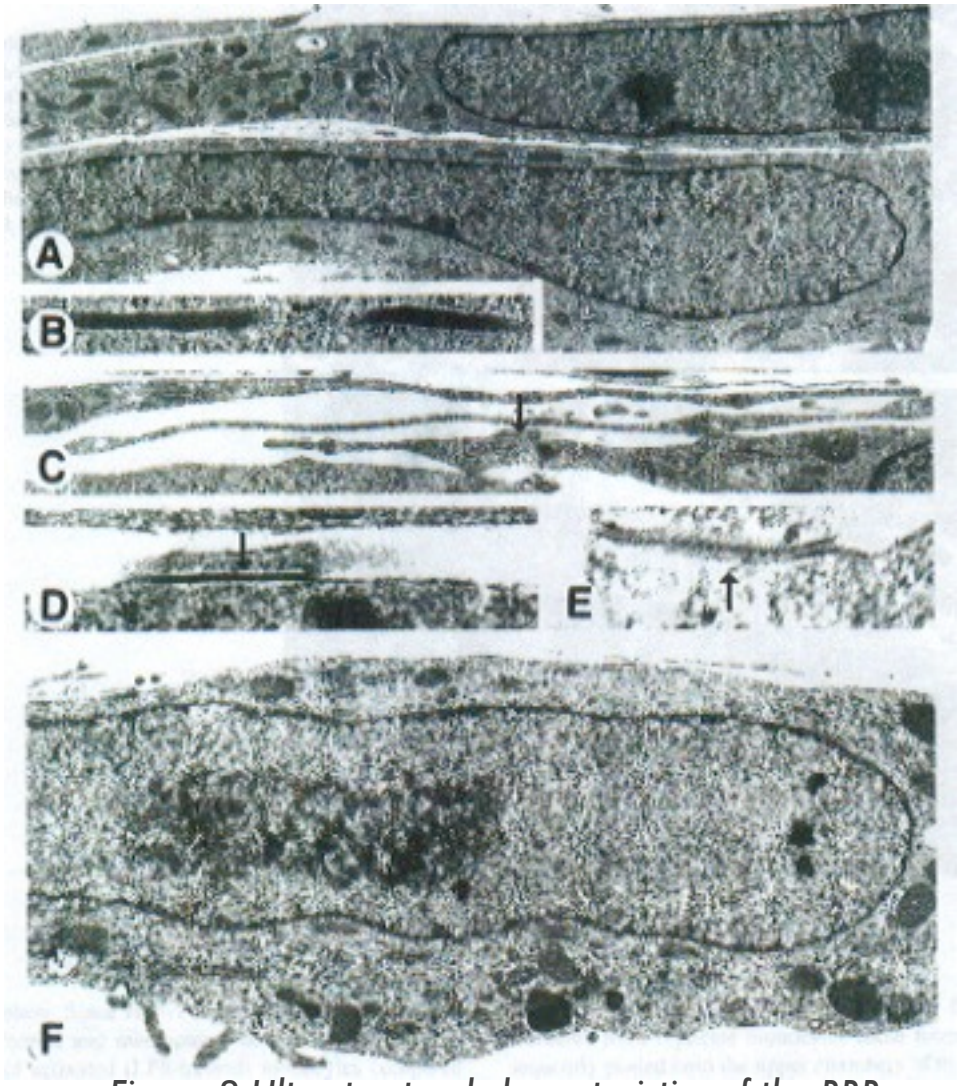

Figure 2:Ultrastructural characteristics of the BBB model constructs examined by Persidsky et al. This processes of endothelial cells form gap junctions (Arrows, C and D) and tight junctions (arrow E). In panel B, you can see the brain microvascular endothelial cells and the gap in between them. Adapted from Persidsky et al, (1997)

It was also shown that, although activated and infected monocytes behaved in this manner, inactivated monocytes did not. To ensure that it was indeed the increased expression of the VCAM and E-selectin that was creating tears in the BBB, the research team blocked VCAM and E-selectin using specific monoclonal antibodies. When both blocked activated monocyte-treated model BBB did not exhibit the same pattern of endothelial damage. These findings support the notion that an in-

crease in cytokines which then attract these adhesion molecules are a vital aspect of what allows the BBB to be susceptible to viral insult. Many studies have shown a consistent positive correlation between correlation between concentration of cytokine induced adhesion molecules and active CNS infection.

Although, a number of studies have examined activated monocytes further study of viral entry into the CNS is warranted. To directly test the importance of cytokines, I would develop an assay that would block the production of cytokines and examine the rate of monocyte infiltration. Such studies are needed to document a cause-effect relation between the rise of cytokines and monocyte infiltration. Yes, with cytokines there is an increase in the expression of adhesion molecules and the loss of key proteins such as occludin in tight junctions, but it remains an open question whether these are major causative events in compromising the BBB, or if there is a viral component independent of the cytokine cycle that promotes infection of the CNS? Further research to pin down this possible causal relation will be needed to identify new pharmacological targets that might slow or prevent CNS infection by HIV.

An alternative novel method of CNS infection has been proposed; that at macrophages may itself "shrink" or "swell" to breech the BBB and promote penetration of the HIV virus in to the CNS. Chung et al. (2002) examined a new angle of HIV 's ability to commandeer cells to infiltrate the CNS. They examined how mononuclear phagocytes, which are so dominant in certain cases of HAD infiltrated the CNS. It is infected mononuclear phagocytes (MP's) that not only allow the HIV virus to gain

easy entry into the CNS but also contribute to HAD 's pattern of apoptosis once they gain entry to the CNS. These MP s begin the secretion of cytokines such as TNF-Alpha from that activate intrinsic apoptotic pathways in neurons. These MP s are also significant in that they are also found in other neurodegenerative diseases similar to HAD. (Chung et al, 2002). Perhaps this can explain why so many of the motor symptoms of HAD are shared similar to diseases such as Multiple Sclerosis, where MP s are also highly active. Chung et al was recognized that MP s along with their counterpart , Monocyte Derived Macrophages (MDM) needed to first be activated by the HIV virus before being able to cross the BBB. However, the crux of their study is determining the mechanism by which phagocytes and infected MDMs become set in motion to cross the blood brain barrier. On examination of the surface of these activated MDMs, key potassium ion channels were found to be opened. Only blocking these surface ion channels via charybodtoxin prevented the subsequent activation of MDMs. These ion channels are actually employed by the HIV virus to shrink the volume macrophages via an osmotic mechanism. Macrophages then "shrink" just enough so that it can slip though the tight spaces between the endothelial layer of the BBB. In their study inflammation could actually be beneficial preventing macrophages from crossing the BBB, yet MCP and other inflammatory chemokines still are the substances that activate MDMs. However, it seems that when K channels are blocked via Charybdtoxin (ChTX) the effect of the chemokines specifically the MCP-1 type was diminished. Thus not only does this novel method of blocking ion channels affect the macrophages' ability to "shrink" and gain access to the CNS, it also prevents chemo-

tactic migration of additional monocytes. This study examined the functional importance of these ionic channels and it appears that the HIV virus uses these channels to restructure the monocyte carrier s so that it can infect the CNS. Also this study confirms that is not the HIV viral load that is the ultimate predictor of CNS infection, but rather the number of migrating MDMs found in the CNS. (Chung et al. 2001). Overall, this study encourages further examination of how infected macrophages' entry into the CNS is regulated and may provide vital insight on how to prevent CNS infection.

The key point in helping prevent the initiation or further progression of HAD is to prevent viral access to the CNS. Current work suggests that the key question is the integrity of the blood brain barrier. Cytokines released along with certain adhesion molecules such as VCAM readily creates breeches in the BBB therefore allowing infected monocytes to cross freely in to the CNS. In addition, the literature also suggests that although anti retroviral treatment may be beneficial overall, it still has the ability to prolong the course of HAD. Antiretroviral drugs appear to be able to cause viral mutations that cause the liberation of other inflammatory cytokines and down-regulates key tight junction proteins like occludin. It is HIV's ability to induce the down regulation of vital proteins involved in maintaining the BBB's characteristic protective tight junctions by which adhesion molecules prevent the passage of items into the cerebrovascular lumen. Although it has been suggested that anti-retrovirals could

cause mutated forms of HIV that create breeches in the BBB, it still doesn't explain why the powerful antiretroviral drugs can't by themselves cross the BBB to treat CNS infection. The question then arises, are these breeches in the BBB transient so that infected monocytes can freely enter and then suddenly close up to pharmacological therapy? What needs to be done is to examine why only monocytes cross freely and why not the ARV drug treatments. Perhaps this could help to design a better drug that could be lipophilic enough to cross the BBB in sufficient quantities. What, I suggest create a mutated/inactivated form of the HIV virus that could be associated with powerful antiretro viral drug therapy. However, this may have many obvious drawbacks. Other ways of ensuring that antiretrivirals could better cross the BBB in HAD patients could include cutting edge technologies such as intranasal delivery methods. Currently 5-HT receptor blockers, which are used for migraine headaches, are often administered as intranasal solutions. Research into intranasal delivery methods show that the unique connections of the nasal epithelium that connects to the trigeminal nerves and Olfactory nerves. The antiretrovirals could be some how aerosolized in a suspension that then could be

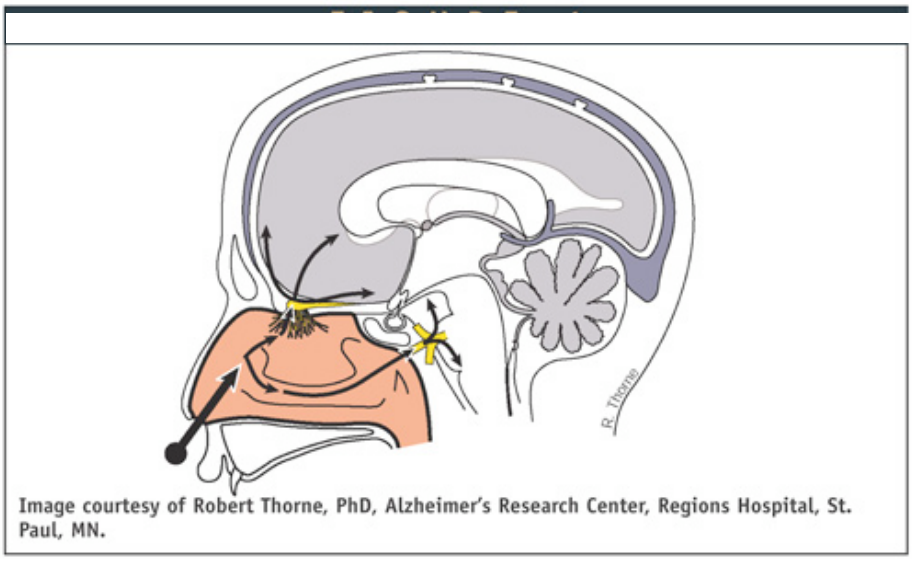

Figure 3: Proposed mechanism of intranasal drug delivery for CNS active drugs

taken intranasally to effectively cross the blood brain barrier(Frey et al, 2002). As seen in the figure 3, this methods could be an effective way to send antiretroviral drugs across the blood brain barrier. This could then ensure that CNS infection could be abated therefore slowing the progression or even eliminating apoptosis seen in HAD patients. The literature also points to evidence of down regulation of protein of the juntional complex; is there a certain gene that codes for expression occudin that could be resistant to HIV influence? If this was possible than surely, patients on first being diagnosed with HIV could be prophlactcally protected from the ravages of HAD by preventing HIV infection of the CNS. What impressed me were data suggesting that perhaps HIV is gaining access to the CNS by altering the morphology of macrophages so that it can use them as a carrier. Perhaps HIV is using the immunoprotected site of the CNS as a reservoir for viral particles as it is does in other tissue. The idea that HIV might open K channels to cause osmolarity induced "shrinkage" of macrophages to allow them to squeeze through the BBB is very ingenious. Perhaps future treatment modalities can focus on these K channels. As we seen in one of the papers (Chung et al,2002), the application of potassium channel blockers is indeed beneficial in preventing the HIV-induced increase in macrophage trafficking. Careful assays of CNS infection mechanisms could provide important new pharmacological targets for treating HAD patients. In addition, most experiments fail to examine HIV infection in vivo , focusing rather on models of the BBB. There is a need for an in vivo model for HAD in animals akin to models used to study other neurodegenerative diseases. Cytokines were also sited many times in the literature; perhaps there should be

more attention focused on looking at the cytokine receptors that initiate the deleterious effects leading to CNS infection. HIV is easily detected in the cerebrospinal fluid of patients, and in familiar cell populations of infected monocytes, which cross over into the CNS (Ho et al, 1985). As discussed, the endothelium of the BBB is the key area of interest because it is the site of insult in HAD and subsequently the "gateway" for further HIV infection. In fact there has been documented research about the endothelium and its associated basement membrane along with certain adhesion molecules such as VCAMs, which work in concert by a three-step method to allow the infiltration of infected lymphocytes. (Butcher, 1991;Lawrence and Springer, 1991; McEver et al, 1992; Shimizy et al 1992;Sloan at el, 1992.). This method entails loose interaction between the lymphocytes and then with the help of adhesion molecules firm binding to the endothelium and subsequent penetration through the basement membrane of the BBB Vasculature. To better understand the method

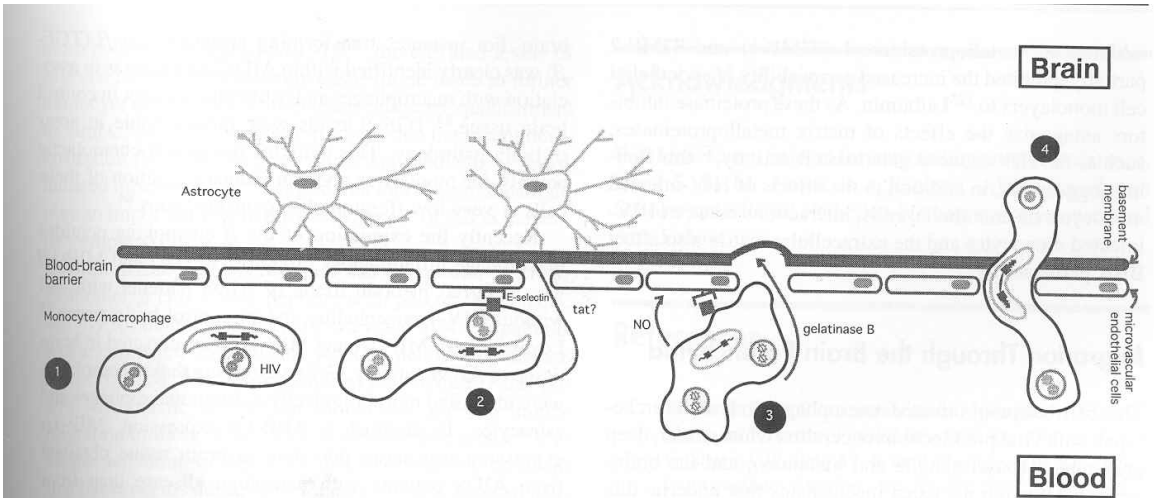

Figure 4: Mechanisms for early HIV-1 entry into the brain. HIV infects macrophages which then selectively induce E-selectin expression on brain microvascular endothelial cells that makes it possible for them to bind to the endothelium and secrete nitric oxide and gelatinase B which incresaes the permability of the BBB to more infected macrophages Adapted from Gendelman et al, 1998

in which HIV enters the CNS, Dr. Gendelman (Gendelman et al, 1998) depicts an accurate description in the figures shown.

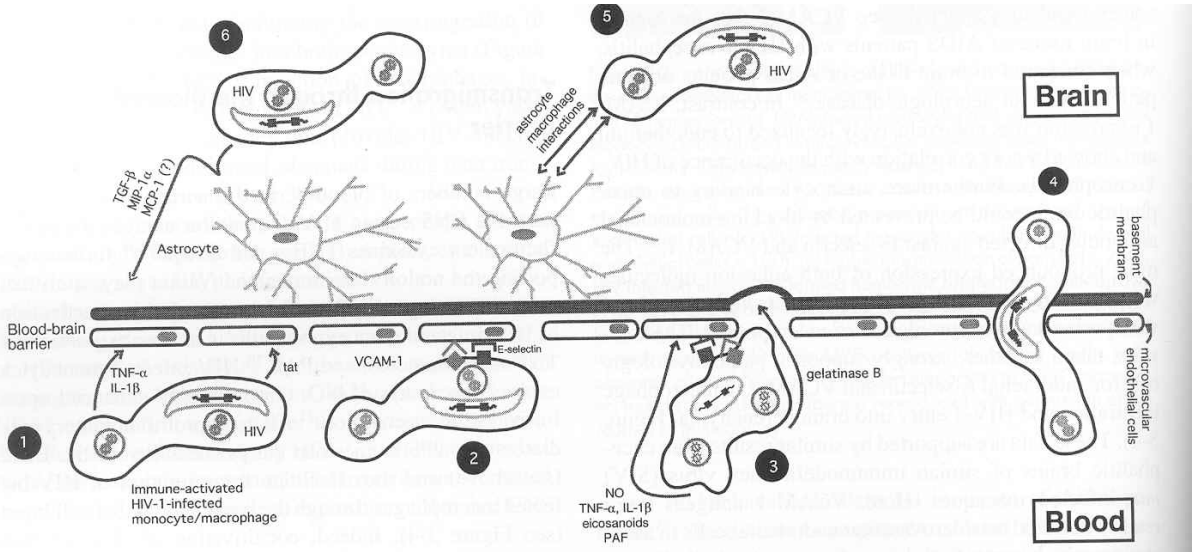

Figure 5: Mechanisms for HIV-1 entry into the brain during the onset of neurological disease. As patients enter advanced HAD, there are elevated levels of proinflammatory cytokines such as TNF-alpha. Also as seen here HIV-Tat induces vascular adhesion molecules such as VCAM-1 which allows more HIV infected moncytes and macrophages to cross the blood-brain barrier. Cytokines also shown include the monocyte chemoattractant protein (MCP-1). Adapted from Gendelman et al, 1998

Overall, The model of general encephalopathy correlated so nicely with HAD because simply, through the inflammation process, cytokines activate these lymphocytes which act as carriers of the virus into the CNS. There is a lot of research that is sited on the viral entry of HIV, yet to fully comprehend the mechanism and better yet to prevent HAD, much attention has to be given to how the HIV virus infiltrates the CNS.

HIV Viral Protein Direct Interaction

The HIV-1 Virus that is implicated in the progression of HAD, has been thought by some, to induce selective neuronal apoptosis through direct interaction between the neuronal membrane and viral proteins. That is, that two key viral proteins, gp120 and Tat which are part of the envelope glycoprotein and secreted respectively, are involved in direct neurotoxicity. This neurotoxicity would involve alteration of receptors on the membrane of neurons leading to aberrations in ion balance. These receptors that become altered in conformation may also inhibit proper communication between neighboring neurons. Maintaining functional synaptic transmission between neurons in the cerebral cortex is vital in tasks relating to memory and long-term potentiation especially with regard to Ca+ ion balance. HAD has the characteristic deregulation of neurons that eventually lead to individual neuronal apoptosis in key memory areas such as the CA1 area of the Hippocampus. Yet in some other cases, it is shown that the viral proteins themselves activate or inhibit specific transcription factors via extra cellular signalling mechanisms. These transcription factors could suppress synthesis of a range of proteins from proteins that help maintain the integrity of the plasma membrane to ones that trigger or suppress apoptosis. Transcription factors that become over active may also in turn affect receptor function leading back to another way neurons can die. The direct protein hypothesis looks at cultured cells with different amounts of Tat protein, gp120 protein to examine the method of apoptosis. Gp120 proteins are the key proteins associated with HIV's viral envelope. This

viral envelope is essential the one of the first set of proteins that neurons encounter and is thought to induce neurovirulence. Tat on the other hand is an element involved in the transactivation of transcription and it is needed to help regulate further HIV replication at the level of the mRNA. It is hypothesized that although gp120 can directly interact with the neuronal membrane, Tat must be released from surrounding supporting cells that have become infected. An example of Tat releasing cells would be the majority of the infected glial cells, as we will cover in a subsequent section of this report. In some instances research has shown that perhaps gp120 and Tat work synergistically to exert their deleterious effects. Furthermore, it has been observed that these proteins do not exert their outcomes on their own, but rather when they are in association with other HIV proteins in the viral package such as NEF and VPR. (Chen. et al, 2005) suggesting that perhaps these effects are dependant on HIV infection. Here we will look at three major accepted methods that HIV viral proteins are believed to damage neuronal toxicity as seen in the progressive illness of HAD.

The direct interaction of Tat and gp120 proteins are thought to be synergistic in eliciting neurotoxicity in HAD. These key viral proteins of HIV are implicated in working on the level of the neuronal membrane and thus altering ion balance through NMDA receptors. These NMDA receptors then regulate the flux of ions into the neuron. Interestingly, most in vitro studies of gp120ís actions show the most severe loss of neurons in the hippocampus. (Sabbatier et al., 1991). Haughley and his colleagues (2002) showed how gp120 might alter intra calcium release from IP3 sensitive Ca+

stores by first binding to a specific CXCR receptor. The CXCR receptor is a g protein coupled receptor that is the binding site for a specific chemo attractant, namely SDF (Stromal derived Factor –1(Gendelman et al, 1998 p.39). Now it is widely accepted that most ion imbalances that usually cause neurotoxicity are caused by glial cells not actively removing accumulating extra cellular ions such as Ca+ which then can hyper excite the neuron. With this in mind, it is interesting to see that the similar CXCR receptor that is involved in glial malfunction with associated ion imbalance is also found to be on the neuronal membrane. Haughley and his team demonstrated through a thorough examination of instances involving calcium deregulation induced HAD that the receptor once activated by Tat protein causes a rush of IP3 induced calcium release from intracellular stores. This internal release of Ca then subsequently triggered the activation of PKC. PKC is then thought to work downstream in helping to recruit harmful cytokines. The experiments that established this model were simple cell cultures. In addition NMDA receptors have been repeatedly implicated in toxicity and neuronal apoptosis in HAD (Gendelman et al, 1998 p.157) In Haughley's paper he examined membrane patches from human and cortical rat CA1 neurons with Tat. The results when extracellular calcium was removed showed a dramatic decrease in the membrane current suggesting that Tat along with gp120 may affect receptors that regulate the influx of Ca. It seems that gp120 also plays a role by acting as an allosteric modulator to the NMDA receptor that increases the affinity for its substrate. Now, the gp120 protein itself has not been found to bind to the NMDA receptors itself. At the level of the NMDA receptors is where we the synergistic effects of gp120

and the Tat protein. Is appears that while gp120 hypersensitization facilitates the easy binding of Tat protein to the NMDA receptor causing the observed toxic binding of excess glutamate into the neuron. Hypersensitive simply meaning, increasing the likely hood of binding to NMDA's substrate even in ow quantities. Furthermore, NMDA receptors also play a role in Calcium influx as well. However in Haughley's review no evidence was cited for a specific binding target that Tat might bind on its own. However, since it is known that Tat does indeed bind to CXCR receptors on red blood cells, it is reasonable to surmise that it may bind to the neuronal CXCR receptor as well. However, with the cited evidence in the literature, it seems that the theory that gp 120 and tat work together seem to make sense as many other original experiments prove that both proteins associated with the virus are required to produce neuronal apoptosis in cell culture.

Cell culture s are in vitro models, and they have provided useful correlates to the neuronal apoptosis seen in patients exhibiting HAD. Cell cultures allow manipulation that can examine molecular interaction rather than autopsy studies which only allow for structural examination. The studies of Nath and his colleagues (2000) further the idea that gp120 and Tat protein work in a synergistic way to induce apoptosis. They examine aborted fetus's neuronal tissues for reaction to gp120 and Tat protein. The results showed that, Tat and gp120 worked in concert to induce apoptosis; it was gp120 that actually seemed to cause most of the damage to neurons in vitro. They began by adding Tat and gp120 protein to healthy neuron cultures. The viral

proteins were immunoreactivley labeled and allowed to be incubated with foetal neurons and neurotoxicity was assessed. The dosages of the viral proteins were adjusted to account for which one was eliciting the neurotoxicity. Nath and his team saw that for gp120 levels as low as 500 pico molars was enough to create significant neuron death as measured by neuronal cell count. To establish if Tat and gp120 were working in concert with another, the investigators provided evidence of subthreshold levels of either protein together eliciting a response whereas each protein on its own would not contribute to neuronal death at subthreshold levels. This finding makes sense as it was earlier noted that gp120 sensitizes the NMDA receptor by increasing the affinity for its ligand. In addition this correlates with the assumption that gp120 may be inducing the extrinsic apoptotic pathway via the up regulation of apoptotic transcription factors. What was important in Nath's study is that it showed that the use of the selective NMDA receptor antagonist memantine reversed the damage caused by HIV 's viral proteins. Their data confirmed that a target of gp120 and Tat protein is NMDA receptor function, but did not demonstrate that. Tat protein necessarily bound directly to the NMDA receptor in its form that is not is altered by gp120. Thus, it is suggested that gp120 is needed to sensitize the receptor for Tat's binding to occur as noted in both Haughley's work. In all, there is not too much research that indeed Tat and gp120 work on the level of the neuronal membrane to be justify it as a only mechanism of apoptosis as there are many other venues of attack that Tat and gp120 take on neurons in HAD.

Another potential mechanism by which proteins from the HIV virus may induce apoptosis is through the up regulation of specific apoptotic transcription factors. Chen and his colleagues (2005) recognized that also gp120 must work on the CXCR receptor in same manner as Haughley noted in that it sensitizes the receptor for subsequent binding from the Tat protein. They demonstrated this quite simply by observing that a CXCR receptor antagonist would prevent gp120 from exerting its usual effects leading to neuronal apoptosis. Post mitotic NT2.N derived human neurons that they cultured with gp120 protein showed dramatic apoptosis as expected. Chen and his team also examined whether the addition of alcohol would potentiate the damaging effects of gp120. The micrographs depict the level of neuronal apoptosis that can be induced by ethanol. These neuron cultures also included the viral gp120 suggesting a

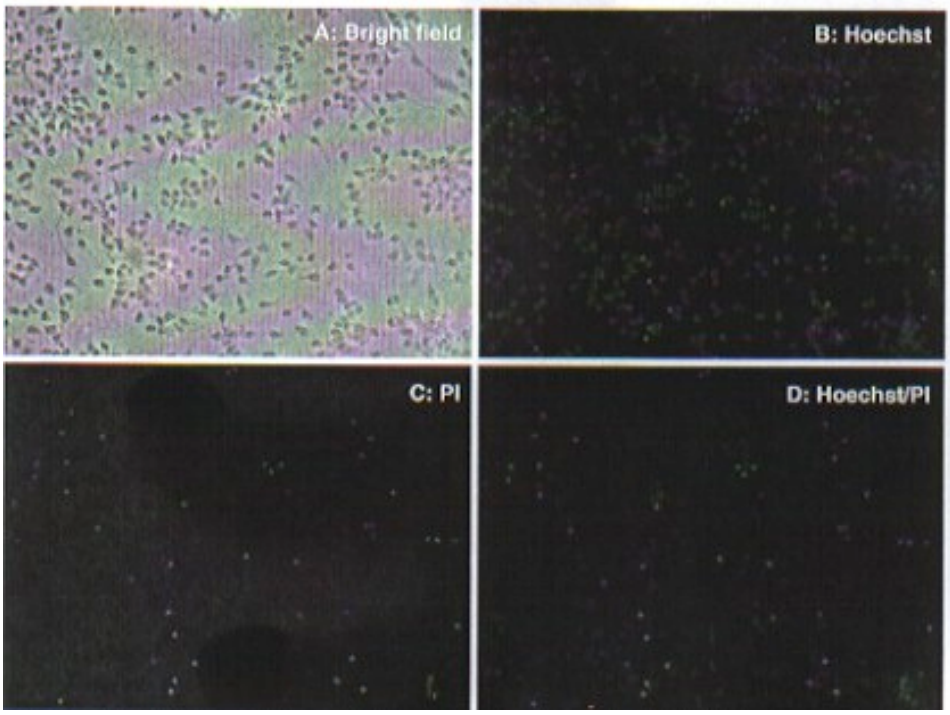

Figure 6:Neuronal apoptosis induced by ethanol can be demonstrated and quantified in human cultred neurons. Notice panel C which in the presence of ethanol show PI(Propidium iodide) positive cells which are indicative of apoptosis. Panel B uses Hoechst staining to show apoptosis. Panel A shows the morphology of these ethanol induced apoptotic neurons. Adapted from Chen et al, (2005)

remarkable link between the two. (See Figure 6). They had looked deeper into the mechanisms by which gp120 might exert its effects, learn that gp120, may have a high affinity for the glycine co agonist site on the NMDA receptor. Chen in his paper suggests that gp120 also binds to a "death receptor" leading to an up regulation in transcription of apoptotic factors. Chen refers to the CXCR4 class of receptors as the "death receptor" actively involved in the gp120 induced neuronal apoptosis. Gene chip analysis showed that gp120 is also involved in the up regulation of CASPASE 8 and 9 expression, enzymes that have been established to induce apoptosis in neurons. Interestingly enough, gp120 along with another HIV viral protein, VPR, also activates this same caspase initiating the same apoptotic pathway. Moreover, gp120 also up regulates TNFRSF5, which is a gene concerned with the extrinsic apoptotic pathway. The NMDA receptor mediated apoptosis is known as the intrinsic apoptotic pathway. Intrinsic pathways are concerned with how mitochondrial dysfunction and how the cell itself may contribute to apoptosis whereas extrinsic pathways are primarily concerned with gene expression and apoptosis associated with initiator caspase. Although this study primarily put the spotlight on gp120ís role in direct neuronal apoptosis, the Tat protein is known to also bind to the NMDA receptor (Song et al, 2003) as Chen notes, thus further confirming the idea that perhaps Tat and gp120 work synergistically to produce it effects. It appears that although both seem to bind to the NMDA receptor making it prone to dysfunction, only when both bind the results lead to apoptosis.

Singh and his team at the University of Kentucky look further into the role of Tat in the apoptotic pathway (Singh et al. 2004). Like Chen and his colleagues (Chen et al, 2005) in Singh's paper the importance of caspase in the apoptotic pathway is underlined as it relates to HIV proteins interacting with neuron cultures. Yet, as we know that gp120 activates the extrinsic apoptotic pathway via CASPASE 8 and 9, it appears that Tat works by stimulating endonuclease G. Yes, although on autopsy results of patients with HAD, there were a increases in caspase activation, Singh and his colleagues only discuss the importance of caspase 3 as being predominant The manner in which these caspase pathways are thought to work is through the proteolytic cleavage of apoptotic factors characterized as these caspase. Caspase 8 and 9 are the first to be activated from their pro-caspase form and then in turn cleave other caspase downstream. The activation of caspase 3 then induces the irreversible cleaving of the nuclear lamina, which eventually leads to the cells demise (Alberts et al, 2002). This is very interesting as noted in Chen's work caspase 8 and 9 were only of importance. Yet this paper demonstrates a CAPSASE 3 inhibitor reverses gp120 induced apoptosis. However, the highlight of this study is that it finally suggests a plausible role that Tat may also play in an apoptotic pathway. Endonuclease G is found to be target specific to Tat stimulation. Tat, although may play a slight role in activating caspase 3, it still did not exert its apoptotic results in that way. Observing that a caspase 3 inhibitor although prevented gp120 from inducing neuronal death, did not prevent the actions of Tat proved this. On the other hand gp120 failed to activate the Endonuclease G on its own. Although it may seem that Tat and gp120 are working

separately, the notion of synergistic action is also possible here. As Endonuclease G is activated, it waits for an appropriate signal from the apoptotic pathway for it to be released from the mitochondria into the cytoplasm. This is a mitochondria specific nuclease that translocate into the nucleus during apoptosis. While cytochrome c from the mitochondria activates an adaptor protein Apaf-1 that in turn activates caspase (Alberts et al, 2002). In spite of this observation, it could also be argued that Tat and gp120 can work separately as well. Singh and his team discovered that a massive load of Tat could still induce apoptosis in the presence of a caspase inhibitor. That is, even if the actions of gp120 were to be inhibited, Tat on its own can be sufficient to cause neuronal death. The big issue here with these studies is that, although they show some concerted effect of both Tat and gp120 through either the NMDA Receptor by way of activating the vital glycine site required for NMDA activation or apoptotic pathways, there is no clear consensus on whether these two proteins are indeed working together. It does correlate with the observed increases in Ca influx to suggest activation of the NMDA receptor via these viral proteins. Another fascinating transcription factor that interacts with HIV 's viral protein is NF-κB (Nuclear factor -κbeta). Knowing that the Tat viral protein causes an up regulation of NF-κB in T lymphocytes, Ramirez and his team (Ramirez et al. 2001) thought that Nf-κ-B might also be activated in neurons by Tat. Instead they found that Tat does not activate NF-κB in neurons but rather it was neurotrophins that activated it. What was surprising was that neurotrophins such as BNDF and NGF were actually using this transcription factor to

inhibit Tat from triggering apoptosis. It was shown in cell culture that when BNDF was applied to the infected neurons, the apoptosis was halted and on removal of these neurotrophins the protective effect disappeared. These results are shown as TUNEL –stained micrographs of cultured neurons with neurotrophins are shown in Figure 7. On further examination, it was found that neurotrophins act by activating the key anti apoptotic gene, BCL 2. This makes sense due to the fact that when Tat, which normally has been specifically implicated in activating apoptotic pathways, doesn't exert its effects when neurotrophins are present. . It seems that the Tat protein itself exerts its influence on apoptotic pathways via the caspase pathway as seen in the results of Singh and his team. Thus, when a counteracting anti apoptotic pathway is induced by these neurotrophins it may be enough to halt Tat provoked apoptosis.

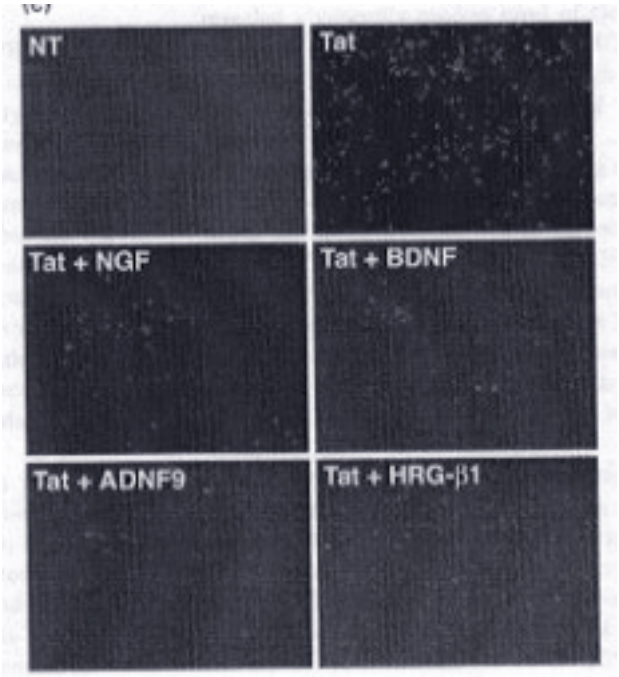

Figure 7:Neurotrophs protect neurons from apoptosis induced by HIV-Tat. As seen with Tat there is an increase in TUNEL signals meaning there is apoptosis. Yet, when NGF,BNDF and other neurotrophs, the TUNEL signal is diminshed

The main issue with this study as with all of the work that looks into Tat's interaction with neuronal apoptotic pathways is that these studies were examined in vitro with models "resembling" what would be found in vivo. HAD is a clinical finding and

more in vivo studies should be confirmed. Furthermore, in Ramirez's work it was shown that oxidative stress might activate the Tat protein to trigger apoptosis; this premise has not been frequently qualified in the literature. Oxidative stress is known to cause apoptosis and this study doesn't explain how Tat will, by itself, be activate the apoptotic pathway. They do, however note that, as Haughley and his colleagues observed, Tat contributes to apoptosis via the influence on IP3 sensitive Ca+stores via the activation of IP3 kinase that further activates an already gp120 sensitized NMDA receptors.

The ultimate question lies is how Tat itself directly interacts with the neuron causing cell death. In this paper, much was said about the neuroprotective effects of neurotrophins especially in their effectiveness to counteract the aforementioned Tat mechanisms on NMDA receptors. In this study, antibodies against Tat protein were shown to prevent apoptosis, but the mechanism of action of Tat was not elaborated and future research should look into Tat as an activator on its own of caspase 8 and 9 that trigger apoptosis. Perry et al. (2005) demonstrated that Tat on its own is the factor that activates the inflammation cascade seen in HAD by actually up regulating for PAF (Platelet Activating Factor) from mononuclear phagocytes. The Tat protein first increases TNF-alpha at the transcriptional level, and then in turn this leads to the release of PAF from the mononuclear phagocytes. It would make sense then to think of Tat as a factor that activates oxidative stress rather than oxidative stress activating Tat as Ramirez claims. This is because Tat by way of its function as a transactivation of

transcription directly up regulates factors that lead to increases in release of oxidative species. In Perry's et al, (2005) study, they go onto confirm that Tat does perform the function for which it named (TransActivator of Transcription) inducing the transcription of TNF-alpha, which. is known to induce apoptotic pathways. The consequences of the induction of PAF transcription are less clear. PAF is implicated in increasing the production of free radicals, especially in the mitochondria of affected neurons. The mitochondria are said to become "hyperpolarized" to the point where the ATP/ADP ratio is increased. This means there is an increased electron transport and thus an increased proton gradient produced within the mitochondria. So in essence mitochondria become very prone to malfunction by being too active. Perry and his colleagues showed that, by stabilizing the mitochondria of infected neurons with a simple K channel blocker (Tolbutamide), which is specific to mitochondria, the rate of apoptosis was reduced. So far these studies shed little light on how HIV 's proteins may actually cause apoptosis in themselves by directly activating specific transcription factors that up regulate apoptotic substances.

The viral proteins of HIV in HAD patients not only act at the levels of the plasma membrane and nuclear transcription factors, but also interfere with intracellular signalling mechanisms in between, such as tyrosine kinase, and with the recruitment of other cytokines. This action of interfering with signaling could be yet another way that transcription factors become modulated. The HIV-induced increase in cytokine release will promote inflammation, which might result in apoptotic neuronal loss.

A study examining how Tat and other HIV viral proteins can generate inflammatory products was performed by Johnston et. al (2001). They studied matrix metalloproteinases which are released by Tat infected neurons. Matrix metalloproteinases (MMPs) are known products of the inflammatory cascade and they are involved in degrading parts of the extracellular matrix. Increased MMPs were very prevalent in the CSF of patients who demonstrated clear dementia clinical symptoms. It has been suggested that Tat induces the transcription of MMPs. confirmed by Johnston et al (2001) rescued Tat infected neurons from apoptotic death with a MMP inhibitor. The most attractive point of this study is that these MMP inhibitors were active both in cell culture and in vivo. The animal models for HAD looked at the motor symptoms consistent with advanced stages of HAD. These motor behavioural studies focused on how Tat-infected mice tended to rotate ipsiversively. The study was carried out with the intra striatal injection of which these MMP inhibitors were delivered to Tat infected mice. Following that, they used immunocytochemical analysis to count neuronal loss. In addition these in vivo findings of the MMP inhibitor's ability to rescue neurons were further confirmed with behavioural tests, which showed improvement as the number of ipsiversive rotations decreased. Although, the neurons that were primarily infected with Tat were those of the hippocampus, a structure important for explicit memory consolidation, their findings focused on motor symptom logy similar to Parkinson's disease. This finding is striking as we later discuss the reason why HAD patients had selective DA neuronal loss and how essentially treatments for HAD be treatments already in use in Parkinson's disease. (See section on DA Selectivity). This study provides one of the

many possible mechanisms by way of MMP action by which HIV can cause apoptosis through actions of the Tat protein, yet it provides a an excellent example of HAD that can be attributed to a defective release of inflammatory substances. It is also interesting that Johnston et al, (2001) while uncovering evidence of apoptosis due to Tat's activation of inflammatory substances, they still mention Tat's other methods of apoptosis as seen in Nath's paper and Haughley's findings that I earlier discussed.

Singh and his colleagues (Singh et al. 2004) examined the involvement of caspase in the apoptosis by through Tat's activation of caspase -3 specifically. This paper also mentioned that most of the cellular signalling mechanisms such as the Tyrosine Kinases such as Flk-1/Kinase which is important for receptor for vascular endothelial growth factor (VEGF) were bound by Tat and gp120 leading to release of cytokines downstream. Shi et. al, (1998) also examined the role of TNF-Alpha in neuronal apoptosis demonstrating that oxidative stress was the cause of most cell death in response. TNF-Alpha is a cytokine that when released lead to lymphocyte apoptosis that also contributes to the general inflammatory process as seen so commonly in patients with HAD. Using DNA extract labeling through the TUNEL method in combination with immunofluorescence staining they found vast amounts of both Tat and TNF-alpha were found in the TUNEL-positive apoptotic neurons. Although Tat may disrupt normal neuron function by hyper activating NMDA receptors along with gp120 as discussed in detail earlier, it also works through TNF-alpha's ability to induce apoptosis via oxidative stress. As noted earlier, TNF-alpha is a cytokine that can induce oxi-

dative stress by their secretion from infected macrophages. Oxidative stress as being one of the ways TNF-alpha might work was confirmed by seeing a complete reversal of the oxidative apoptosis process simply by adding antioxidants to the cell culture. The most interesting finding in this paper is that it seems that Tat up regulates, as its name suggests (transactivator protein), the transcription of TNF-alpha. In turn TNF-alpha can up regulate the expression of HIV-1. The basic mechanism of TNF-alpha is to inhibit normally occurring antioxidants that are released from T-Lymphocytes, yet this mechanism is highly enhanced by Tat protein. Tat was found to inhibit manganese-dependant superoxide dismutase in T cells. (Westendrop at al, 1995) It seems that Tat activates important transcription factor in the release of not only TNF-alpha but also, Matrix Metalloproteinases, factors involved in inflammatory cascades. With a large amount of data showing that patients with HAD replicate the symptoms of encephalopathy, it makes sense that HIV viral proteins would be involved in triggering the release of inflammatory substances which often cause encephalopathy.

In searching for the mechanisms by which viral proteins of HIV directly interact with neurons to induce apoptosis, three major mechanisms have been highlighted; 1) NMDA receptor hyper activation, 2) induction of apoptotic transcription factors and 3) triggering of an inflammatory cascade. To date, both gp120 and Tat protein have been shown to be able to induce apoptosis, but, although these viral proteins on their own can cause damage to neurons, it is only when they work together that we see vast neuronal apoptosis. It appears that gp120 first sensitizes the NMDA receptor

which allows Tat to hyper activate it. Another important feature of the apoptotic pathways studied in these series of experiments is the whole suggestion that there is first an excitation of the neuron leading to activation of what is called the extrinsic apoptotic pathway. This theme of hyper excitation accurately describes what happens when a neuron's NMDA receptors are affected by the HIV Viral protein and equally when the mitochondria of infected neurons are found to be depolarized as seen in Perry's work (Perry et al 2005). In both of these instances we see a rise in intracellular [Ca]2+ before neuronal death from either the NMDA receptor malfunction or mitochondrial hyperpolarization.

HIV's viral proteins also seem to trigger an apoptosis through expressing inflammatory substances such as matrix metalloproteinases and TNF-Alpha which. This findings are consistent with, inflammation is seen in both HAD and HIV Encephalopathy where inflammation is also a hallmark feature. As we seen inflammation is a key mechanisms in promoting HIV infection of the CNS in the first place, by compromising the blood-brain barrier. In addition, the direct interaction of HIV proteins with intracellular proteins in vulnerable neurons that trigger internal apoptotic pathways and the induction of nuclear factors that up regulate apoptotic genes sequences via caspase activation. Studies done by Chen et al, (2005) along with others help to shed light on mechanisms that involve the interaction of HIV viral proteins and neuronal membranes. It remains for us to consider the possible implications for treatment options of these mechanisms for patients with HAD.

HIV Infection of Microglial Cells

One actively studied aspect of the neuropathogenesis of HAD is the involvement of astrocytes and other microglia. These supporting cells provide the CNS with many vital nutrients and defense mechanisms that maintain the homeostasis of the neighboring neurons. Certain crucial elements such as ion balance, excess neurotransmitter reuptake and neurotrophic activities are provided by astrocytes. In HAD, the HIV virus may produce neuronal apoptosis indirectly by affecting the integrity of protective astrocytes. Astrocytes play a vital role in maintaining the integrity of the Blood Brain Barrier. The end foot processes of astrocytes interact with key endothelial cells that comprise the BBB. In HAD, there is an overall increase in the trafficking of infected lymphocytes across the BBB, which could be due to compromised astrocytic function. Astrocytes secrete neurotrophic factors, as well as harmful inflammatory substances characteristic of HAD. It appears that astrocytes can be neuroprotective in HAD but can also aggravate CNS infections. Studies to date have suggested two roles for astrocytes in HAD, 1), Astrocytes where apoptosis pathways have been activated may harm neighboring neurons that depend on trophic support from astrocytes.2), astrocytes might through specific receptor expression and subsequent binding of inflammatory substances, namely chemokines, potentiate neurotoxicity on its on. The neurotoxicity comes as a result of the chemokines first binding to receptors on the astrocytes and consequently signaling apoptotic effects. Overall, the role of

astrocytes is one that cannot be ignored and it is the most common pathway of HAD to exert its neurological effects by indirectly inducing neuronal apoptosis.

The main functions of astrocytes and role in the CNS is that of both structural support in that it provides the framework for layers of membrane that insulate neurons. Also the glia provide important functional support by acting as managing extracellular concentrations of excess neurotransmitters and ions (Bear et al. p. 47). Astrocytes are an integral part of the microglial system that supports neurons and allows connection between certain elements in the cerebral vasculature and neurons. In another words the microglia provide the gateway between the cerebral blood flow and neuronal cell bodies. The astrocyte is classically known for its end foot processes that extend to surrounding blood vessels making up a vital component of the Blood Brain Barrier through formation of the glia limitans by their end foot processes. In addition, astrocytes play a major role in maintaining the extracellular ionic balance that neurons require. Excess Ca+ and K+ ions are buffered by these cells to maintain the required membrane voltage gradients neurons need to function. Not only ions are scavenged, but also excess excitatory neurotransmitters such as glutamate are also kept in check by astrocytic uptake. Most neuron loss, including that found in HAD, is due to excitotoxcity, as seen with HIV Tat induced Ca+ influx and subsequent gp120 NMDA hyper sensitization. This NMDA hyper sensitization caused by the gp120 viral protein creates the influx of Glutamate an excitatory neurotransmitter and accordingly excitoxic death. Needless, impairment in astrocytic function by HIV virus infec-

tion will have negative consequences for the neurons being supported by the many groups of astrocytes. These negative consequences range from the excitoxcity as described to a dangerous imbalance of K+ ions that ultimately lead to a loss of a solid resting membrane potential of the neuron. An alteration in the resting membrane potential can adversely affect the proper functioning of the neurons involved. Katherine Thompson and her colleagues at Johns Hopkins, (Thompson et. al, 2001) examined brains from post mortem HAD patients. , as well as 2 classes of living HIV patients, those who scored low on the Memorial Sloan Kettering scale that is the standard for diagnosing HAD, and patients who had HIV but no neurological deficit. The astrocytes were seen to express high amount of Glial Factor Associated Protein, GFAP as markers so that astrocytes can be qualitatively analyzed However, through their adept method of using dUTP nick and labeling techniques, they found an elevated number of astrocytes undergoing apoptosis. DUTP labeling examines fragments of DNA that are labeled. These DNA fragments are indicative of apoptotic events. There was a high correlation between the number of dying astrocytes and the rate of progression of HAD symptoms in the patients examined. They found that the majority of astrocytes that were succumbing to apoptosis were the once localized in the mid frontal gyrus and the basal ganglia, however only the patients with severe forms of HAD had more of the basal ganglia region showing the greatest increase of apoptosis. With all of the clinical data of showing HAD patients exhibiting motor disabilities and in addition the post mortem examinations of basal ganglia abnormalities, the basal ganglia seems to play a big role in the pathogenesis of HAD. The team at Johns Hop-

kins (Thompson et al., 2001) pinpointed where the actual HIV virus was being harbored in the brain of these individuals. Using in situ hybridization for HIV RNA, their study demonstrated that key regulatory proteins such as p40 were found in areas of great macrophage density near the blood brain barrier. In HAD patients the HIV virus was primarily localized to the regions with the highest density of astrocytes. This finding does make sense, given the astrocytic role in maintaining the BBB. The question arises, is that what makes some astrocytes so vulnerable to HIV infection? Are they just preferentially closer in proximity to the cerebral vasculature or is there some inherent attractor for the virus. In this section, we will examine possible receptor targets for cytokines and other inflammatory substances being expressed on astrocytes themselves. Astrocytes in the CNS are infected widely by the HIV virus, especially in advanced cases of HAD. While in some cases the HIV virus is thought to remain dormant in infected astrocytes waiting to be expressed and causing HAD. Thompson et al. (2001) have observed a high prevalence of HIV and related pathology of astrocytes in HAD as well as other neurodegenerative diseases, such as Multiple Sclerosis and Alzheimer Disease. These three diseases are often suggested in clinical case studies to exhibit similar neurological symptoms and radiological findings. A key shortcoming of this paper is the lack of astrocytic histological markers. Nevertheless, their results show a strong relation between HAD and astrocyte infection. The question arises- is this infection of astrocytes a nonproductive one that allows the virus to replicate in the CNS or simply a method to ensure CNS dysfunction directly. The virus could be utilizing the entry of the CNS as an immunoprotected site to replicate in-

stead of creating neuronal dysfunction. Future studies need to explore this topic since the HIV virus may stay dormant in the CNS by way of the microglial cells. Minagar et al, (2002) conducted a meta analysis that examined the possibility not only that HIV may be infecting astrocytes and causing a loss in their supportive function for neighboring neurons, but also that HIV may be using astrocytes to further their immunoprotected replication by damaging the BBB through way of the astrocytes. Thus allowing the free trafficking of HIV across the CNS. This review cited sources demonstrating high levels of HIV in the Cerebrospinal fluid of HAD patients, along with large microglial nodules in the brain tissue, an the ability of HIV regulatory proteins to induce expression of nitric oxide synthase. This finding seems very consistent with HIV' infection because it could be way of creating a localized vasodilation thus perhaps allowing for the further trafficking of infected Lymphocytes. As of yet, this is just a plausible conjecture, This review further suggested that astrocytes may not only be maintaining the integrity of the BBB, but also could, in fact, be an agent that HIV uses to recruit cytokines that can cause perforation of the endothelium by inflammatory substances. The studies cited in this review demonstrate the release of substances such as TNF-Alpha by infected astrocytes, possibly contributing to damage to the integrity of the BBB (Wilt et. al, 1995).

Again, the comparison of HAD to other common neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's disease is striking. In Multiple Sclerosis, reactive immune system players like activated T-lymphocytes can easily cross the BBB

in a manner similar to HIV infected subjects. The release of inflammatory products in MS is also similar to HAD. Overall, astrocytic studies noted here further validate the role they play in HAD and perhaps it is through the infection of astrocytes that allow for the rapid progression of HIV infection of the CNS. Köller et al's (2001) study focused on in vivo results which could make their work very important to understanding mechanisms of HAD. Their patient groups were divided into HIV and HIV positive patients, non-HAD patients and patients exhibiting some form of HAD. Spinal taps of HAD patients did indeed show a marked increase in HIV regulatory proteins such as gp 40. Consistent with a pro-inflammatory action, many inflammatory cytokines such as TNF-Alpha were detected. Koller et al (2001) sought to determine whether HIV affected the whole CNS, or if infection was localized to certain regions of the brain. CSF from HAD patients, when introduced in a culture of rat astrocytes, prevented astrocytes from taking up excess Ca+ ions, suggesting that soluble secreted factors are blocking Ca2+ pumps, channels, or glial buffering in some way. This does show support for the hypothesis that in HAD, HIV is actively killing neurons indirectly by disturbing Ca2+ homeostasis mechanisms. These authors suggest that their data might open up a new avenue for diagnosing HAD based on a simple spinal tap assay.

Yet, while soluble factors released by HIV-infected cells may affect astrocytes in important ways, the affects of these soluble factors to neurons cannot be dismissed. These soluble factors are recognized as Interferon gamma and prostaglandin E2 but their source of origin is unknown at the moment. Creating a simple assay for the ef-

fects of HIV-stimulated soluble factors on astrocytic function could be useful in better understanding the mechanisms of action of these factors. In addition, investigations are needed to determine whether astrocyte-astrocyte communication can allow single infected astrocytes to functionally alter neighboring astrocytes, or if the HIV virus itself must directly infect astrocyte. Understanding how astrocytes maintain homeostasis and how an infected astrocyte malfunctions will certainly shed light on how the HIV virus targets the cell. The question then arises of whether the HIV virus has a higher selectivity for astrocytes or not and why that might be.

Astrocytes appear to express membrane receptor that is exploited by either HIV proteins or inflammatory substances that are produced by infection to induce astrocytic proliferation. Many studies have described how inflammatory substances interact with receptors such as CXCR receptors. A study conducted by Okamoto and colleagues (2005) examined how the HIV virus may actually induce the up regulation of certain astrocytic membrane receptors The HIV virus first enters the CNS by way of infected macrophages that cross the BBB. Okamoto et al, (2005) suggest that the HIV virus needs macrophages and astrocytes to propagate infection of the CNS mainly through the induction of cytokines and through astrocytic proliferation. Astrocytes ae known if to be functional to be secrete vital neurotrophic substances. Microglia are of importance because of their "modulation of immune reactions as antigen-presenting cells"(Okamoto et al, 2005). The main crux of their work focused on how HIV may use astrocytes, perhaps not to replicate themselves, but instead to release inflamma-

tory substances and toxic HIV proteins such as Nef and Tat. Using a immunocytochemistry, they isolated a CXCR4 receptor that was specifically localized on the astrocytes of HAD brain samples. To confirm that the expression of the CXCR4 receptor was increased in response to being infected with HIV, the simple test of placing infected macrophages in healthy tissue culture was completed. As hypothesized, upon infection, healthy astrocytes clearly showed a marked increase in CXCR4 receptors expression. This result was very dramatic, since receptor up regulation in astrocytes occurred in as few as 5 days post-infection, which is considerably fast. It has been postulated, perhaps through the binding of these CXCR4 receptors to factors such as SDF-1 Alpha. that HIV can exert its toxic effects. CXCR4 receptors are basically G-protein coupled receptor that begins intracellular signalling cascades in response to inflammatory cytokines (Goldberg at al, 2001). For example as we saw in the section describing how the secreted HIV protein Tat can induce excitoxicity, Tat binds specifically to CXCR4 receptors, but those found on the membranes on neurons. Interestingly, the addition of toxic inflammatory substances such as SDF Alpha and gp120 do not have obvious toxic effects on astrocytes, even though they do show increased expression of CXCR4 receptors. It was only after an extraordinarily large dose of gp120 or SDF-alpha that they actually saw some abnormal proliferation of astrocytes. This finding that gp120 and/or SDF-Alpha can only in large doses cause proliferation suggests that perhaps the expression of CXCR receptors are necessary but not sufficient for the observed proliferation in HAD. It may be that additional substances, besides SDF-Alpha and gp120 found in the supernatant assists in binding to

the CXCR receptor or have separate effects that help to synergize binding to CXCR4. It could simply be that only when a large dose of the supernatant this 3rd player can reach a critical level. The study then goes on to demonstrate the ineffectiveness of antiretroviral drugs in stopping proliferation of astrocytes in HAD. This is consistent with the clinical observation that antiretroviral drugs are usually ineffective in treating HAD. Then again, it has been thought that anti retroviral are not effective because of difficulties in crossing the BBB, but even direct application to astrocytic cultures does not seem to prevent or reverse infection. These researchers concluded that even blocking CXCR receptors altogether still couldn't ameliorate the effects of SDF-alpha and gp120. So, indeed, there appear to be additional signaling molecules involved. Matrix metalloproteinases were suggested as a likely culprit, since their expression is also up regulated in HAD patients. Moreover, two specific matrix metalloproteinases (MMP-2 and MMP -9) are found exclusively in HIV patients who exhibit HAD, whereas HIV patients without neurological symptoms do not express these enzymes. HIV Tat can be an inducing factor for these types of MMPs in light of evidence that MMP's might "activate" the CXCR-4 receptor so that it can then bind to gp120 and SDF-Alpha, as results still show that CXCR-4 plays a role in the altered function of the astrocytes. In essence, HIV is disturbing astrocyte function by using the astrocytes themselves through the up regulation of CXCR-4 receptors, which in turn signal inflammatory substances.

Okamoto noted that these findings were consistent with other findings regarding Alzheimer's disease and multiple sclerosis. There are stages of HAD where patients may lose motor and memory function. Moreover, it has been discovered that MMP expression levels are also increased in arthritis, an inflammatory disease, leading them to suggest that HAD may also be associated with widespread inflammation. Since HAD patients are often classified as encephalopathies with pervasive astrogliosis. It is this astrogliosis that is believed to ultimately lead to neuronal death. Other studies highlight this notion of astrocytes expressing receptors that promote HAD's molecular mechanisms of damage. An interesting study by Dugas et al (2001) focused on how the HIV virus may infect astrocytes in a way that is classified as "non productive" (Dugas et al, 2001). That is, astrocytes are infected, yet neuronal infection is virtually undetectable. The distinction between non-productive infections versus a productive one is that the one productive one as Dugas puts it; the non-productive infection mainly uses "indirect" mechanisms to destroy neurons by way of the astrocytes. This is an important distinction because it basically localizes the bulk of HIV's mechanisms to the astrocyte. A very interesting and common theme throughout the literature is the similar symptoms of MS, Alzheimer's and HAD. Dugas et al (2001) discuss the role of IL-Beta as a pro inflammatory agent that has been implicated in the destruction of myelin, with possible importance in MS. Just as MS is a disease of demyelination, HAD patients also can show diffuse selective white matter damage. However it has also been shown that cytokines such as IL-Beta exert their effects mainly because HIV infected astrocytes overexpress cytokine receptors; such as CD23

which may make them more susceptible to cytokine-induced damage. It has been proposed that neuronal damage is not only caused by astrocyte's inability to scavenge excess free radicals, but that the infected astrocyte itself may be induced to release free radicals such as NO. In fact, when IL-Beta binds to the CD23 receptor, it can induce astrocytes to release NO. Blocking CD23 receptors with an antagonist (amino guanidine) prevented the release of NO from astrocytes. Furthermore, anti-CD23 antibodies have shown that HIV patients with severe neurological symptoms overexpress the CD23 cytokine receptor. It is striking, that HAD also shares some symptoms with Parkinson's disease in addition to MS. In patients with advanced HAD often show impaired motor abilities akin to that of Parkinson's. Lo and behold, this very same type of CD23 receptor was also over expressed in astrocytes of patients who died from Parkinson's disease.

Yet, this feature of HAD's molecular mechanism regarding astrocytes is further touched on by a study conducted by Goldberg and his colleagues who based their work on a CXCR3 receptor (Goldberg et al, 2001). Once again, like CD23 this receptor is involved in the binding the inflammatory cytokines which account for the bulk of the observed encephalitis seen in HAD Patients. Once more their work also confirms that this receptor can be found in patients with HAD symptoms and also patients who had other neurodegenerative diseases. They tended to find through immunohistological techniques that the CXCR3 receptor was over expressed in the astrocytes, yet interestingly enough; the CXCR3 receptor was also found on neurons in the

cerebellum. However, if CXCR3 receptors are involved in the inflammatory paradigm as suggested by this finding, one can ask why not we find profuse inflammation in the cerebellum. Furthermore, HAD patients experience ataxia (a sign of cerebellar defect) often only in advanced stages of the disease. Perhaps, the CXCR3 receptor on the neuronal cell body has a higher affinity for other substances than its astrocytic counterpart. In this study, clearly the astrocytes expressed a higher number of these receptors as seen in the astrocytoma model that was examined. The researchers also found the CXCR3 receptors in close proximity to the endothelial cells of the cerebral vasculature suggesting that perhaps the CXCR3 receptors maybe involved in the inflammatory cycle, which severely compromises the integrity of the BBB. Taken together, there is strong evidence in the literature suggesting that certain cytokine receptors may be especially expressed in HIV infection therefore helping to promote the alteration of astrocytes in way that it is deleterious to the dependent neurons in HAD pathology.

One more final common theme that is prevalent in the literature is the important role that astrocytes play in releasing specialized neurotrophic factors. It is considered that in HAD, patients whose astrocytes become infected lose ever so important functions that often leads the surrounding neurons to their demise. In a study conducted by Boven and his colleagues looked at how the brain might launch a counter offensive by over expressing nerve growth factors (Boven et al, 1999). This study looked at the proposed mechanism of how neurotrophins such as NGF may ac-

tually help in down regulating the enzymatic reactions that are responsible for the bulk of the free radicals and inflammatory responses seen in HAD. Although, I must question their sample size of 18 patients, they claim to have found a high corelation between the increase of neurotrophic factors released from the astrocytes in patients with HAD. Boven and his team suggest that perhaps the brain tries to counteract the effects of HAD by responding with neurotrophic factors. Even so, if there is a release in neurotrophic factors, they are of no use if there is not an adequate increase in specific receptors for the respective growth factors. This study fails to go into detail about the receptor targets of the observed increase in neurotrophic factors seen. To confirm whether these neurotrophic factors were localized in the astrocytes, the lab used glial factor marking protein and showed that the DNA of the expressed neurotrophs were restricted to the astrocytes. Yes, although their data does show this, it is hard to ignore the fact that it is these astrocytes that once infected lose their ability to exert any neuroprotective effect. Perhaps, as this study shows, astrocytes may still be able to release neurotrophic factors even though simple homeostatic roles have been hampered. Interestingly enough, according to the literature IL-Beta seems to regulate the release of neurotrophic factors (Boven at al , 1999). This seems to suggest that perhaps IL-Beta is working on those CXCR receptors earlier mentioned that are confirmed to be over expressed on the astrocytes in HAD. Furthermore, Boven and his lab confirmed the increase of these neurotrophic factors also in the endothelial cells of the BBB. They cite literature that shows that these neurotrophic factors are extremely important in angiogenesis. The basic conclusion from this and other papers cited in the

literature noting the release of neurotrophic factors is that conceivably science can help enhance this natural defense mechanism that the CNS launches against HAD that would aid in treatment options.

The roles of astrocytes in HAD cannot be underestimated. Figure 8 summarizes the roles that astrocytes play in HAD pathogenesis. In the literature cited there are vast implications for the role that astrocytes play with respect to the pathogenesis of HAD. The major functions of astrocytes are to supply homeostatic and structural support to the neurons and with an increase in CNS HIV infection, these main roles are altered. In fact, the astrocytes are themselves used as vectors for inflammation as cytokine receptors over express throughout the course of the disease. More research should be done however on how these astrocytes try to mount a counter defensive with the release on neuroprotective agents such as nerve growth factor. Also if there is an increase in these factors, much has to be done to look at the receptor targets for these factors. Are the receptors localized throughout the brain or are they limited to the site where HIV infection is the highest. With some evidence showing that there maybe large deposits of Nerve Growth Factor near the BBB in response to trafficking monocytes, this might open the avenue for research on treatment modalities that can harness this mechanism to help slow the progression of HAD. The endothelial cells may respond to the neurotrophins in way that is still not understood. Overall, the role of astrocytes in the progression of HAD is highly studied, yet there are many possibilities that have yet to be discovered.

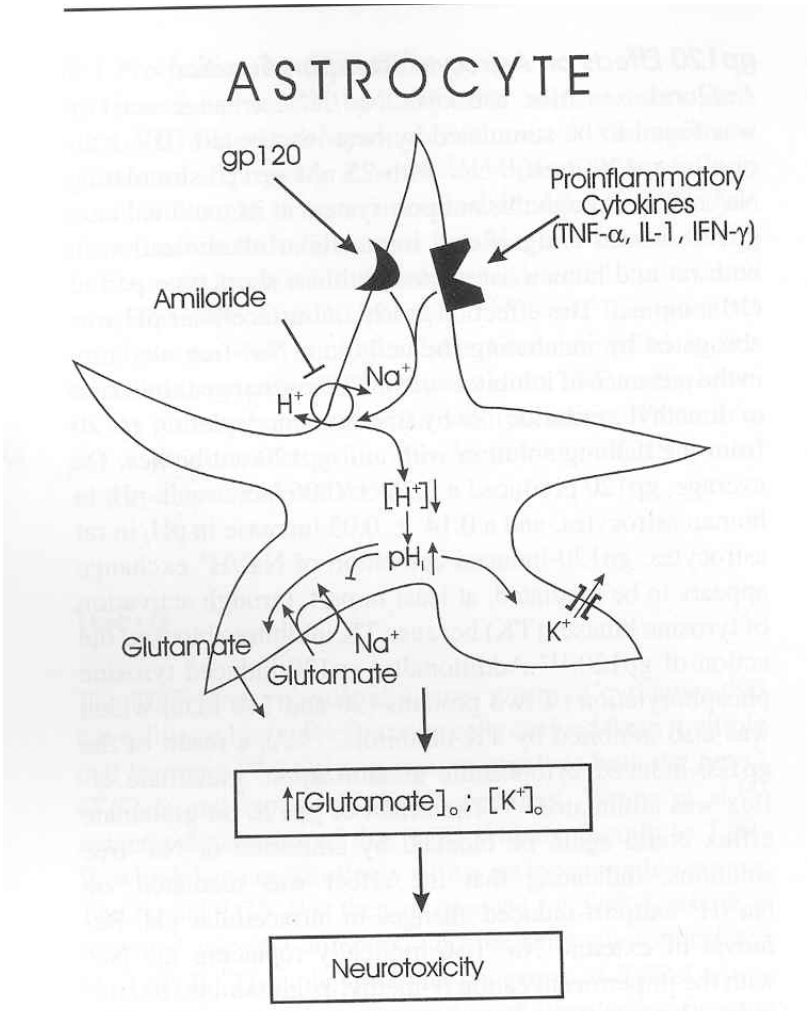

Figure 8: A schematic of how astrocytes play a major role in neurotoxicity related to HAD. Notice the actions of the proinflammatory cytokines. Adapted from Gendelman at. al (1998)

HIV Dementia and Dopaminergic Systems

Much of the literature on HAD emphasizes the importance of specific dopaminergic neural circuits to this type of dementia Through multiple mechanisms of pathogenesis, from astrocyte damage to direct activation of intrinsic neuronal apoptotic pathways, have been implicated in HAD, recent evidence has suggested unusual sensitivity to damage of dopaminergic systems. An Important dopaminergic system affected in HAD is in the striatum, particularly structures important in motor and memory function .An involvement of dopaminergic damage is suggested by the similarities between many symptoms of HAD, and those of other dopamine related illnesses. Most notably, Parkinson's disease shares many symptoms with patients in the advanced stages of HAD such as bradykinesia and altered gait to name some. Another finding that suggests that a link between dopamine receptors and HAD, is that HIV drug users exhibit a additive effect of HIV viral protein associated pathology along with commonly known effects of dopaminergic drugs of abuse. That is that HIV viral proteins were in effect acting like drugs of abuse in their mechanism as described in the following studies. As hypothesized by this observation, there is strong correlation between the mechanisms of oxidative damage to dopamine transporters as well as receptors in HAD common in both HAD and chronic drug abusers. In addition there is a decreased dopamine levels in the CSF of HAD diagnosed patients. This

observation of decreased dopamine levels in HAD patients lets us accept the possibility that HIV may be selectively targeting dopamine systems as this the most affected system through the course of the disease. This hypothesis if true suggests that drugs that enhance dopaminergic transmission might be a promising avenue for the management of at least some symptoms of patients diagnosed with HAD.

Fascinating studies have explored the parallel between patients who develop HAD after contracting HIV through the illicit use of drugs with chronic drug users. The illicit drugs in question are usually ones that interact strongly with dopamine receptors, such as methamphetamine and cocaine. The effects of drugs on dopaminergic targets in the brain are potentiated by HIV's viral proteins, suggesting that HIV may selectively target those neurons. Nath et al. (2002) focused on a population of patients with HIV who were also concurrently using drugs of abuse in their review. These drugs of abuse, such as methamphetamine, primarily exert their effect on dopaminergic transmission. Accordingly, HIV patients not only had an inflammatory encephalitis characteristic of HAD, but also exhibited significant dopaminergic damage. This led these authors to suggest a possible interaction between the viral proteins of HIV and drugs of abuse that could function synergistically to produce further damage to dopaminergic neurons. They specifically examined studies that produced data that showed that when neuronal cell lines were exposed to dopamine, cocaine along with supernatants from HIV-infected tissues that shown an increase in oxidative stress leading to cell death (Koutsilleri et al, 1997). The review noted that HAD patients are al-

ready found to be deficient in CSF dopamine concentration. Drugs of abuse exasperated reduction of dopamine to a greater extent. The evidence that suggests dopaminergic dysfunction in HAD patients is that small doses of neuroleptics that would be ineffective in normal individuals, elicit parkinsonian symptoms in these patients. Consistent with this hypothesis, HIV patients who are drug users are at a higher risk of developing HAD symptoms early in the course of their HIV infection. Nath et al. (2002) went on to test the interaction of HIV Tat protein with a dopaminergic drug of abuse in vitro, demonstrating an increase of oxidative damage to neurons in culture that was supra-additive compared to Tat or drug alone. The specific drug used in this assay was cocaine, a known drug that leads to oxidative damage. An interesting finding in their review pointed out the inflammatory mechanism of drugs of abuse. It was found that methamphetamine on its own produced an increase in release of inflammatory cytokines such as TNF-Alpha. TNF-alpha is also linked to neuronal damage caused by the viral Tat protein by way of Tat's bind into to certain cytokine receptors such as CXCR as noted earlier in this report. It has also been noted that heroin users exhibit up regulation of mu opioid receptors, but these receptors may be found on lymphocytes contributing to the idea that HIV may work with opiate drugs to destabilize neuroimmune function. (Chang et al, 1998). Consequentially, this indicates a possible synergistic mechanism in which HIV and opioids to allow further proliferation of viral particles within the CNS. This observation supports the possibility that drugs that up regulate mu receptors on lymphocytes might enhance infiltration of infected monocytes into the CNS in the first place. As Nath notes, mu opioid receptor agonists have

been shown to increase HIV expression and this could also explain why HIV drug users have an increased rate of developing HAD. Opioid antagonists may be a possible prophylactic approach to treatment in high-risk HIV Drug users. Furthermore, Opioid receptors are found to be very important in making the CNS vulnerable overall to many insults. An example of where opioids have been shown to make the CNS more vulnerable is the fact that opioids can worsen the damage to the basal ganglia after cerebral ischemia (Kofke et al, 1999) Furthermore the striatum, a key voluntary movement control area, contains high mu receptor density, and advanced HAD patients often show impaired motor abilities.

A number of other studies support the accelerated HAD symptom logy of HIV-positive drug users. Cass et al (2003) supplied evidence a link between Tat protein and Methamphetamine in decreased dopamine levels. This study, focused on nigrostriatal neurons, one target of methamphetamine actions. After establishing a baseline, the researchers injected a small amount of Tat protein directly into the striatal region of cannulated-mice and noticed a dramatic decrease in the dopamine levels. The dopamine levels were assayed by in vivo microdialysis. To measure the amphetamine – evoked overflow of dopamine. Tat and Methamphetamine both reduced levels of striatal dopamine release individually but produced a supra-additive effect when co-applied. The levels of dopamine were measured by measuring K evoked outflow of dopamine. They used microdialysis probes and lowered them into the striatum of rats and used two different dialysate perfusate solutions. One solution had the D-

amphetamine and one had 100 mM K ions. After a 2-hour equilibrium the dialysate samples were then analyzed for dopamine using high-performance liquid chromatography. Now the question may arise that perhaps any viral protein will create nonspecific effects that will damage the dopamine system, yet Cass and his lab accounted for this and accordingly used a heat inactive Tat protein and observed no effects. So they concluded that some activity of the Tat viral protein specifically promotes reduced dopamine transmission. On adding antioxidants to the cells, the effects of Tat were reversed, signifying that Tat must be involved in oxidative damage. This was also true when Tat showed a synergistic effects with cocaine; antioxidants reversed specific oxidative damage in this scenario as well. They vital point to Cass's work is that it showed this damage in the striatum which could account for all of the motor disturbances seen in HAD. Yet, dementia cannot be simply reversed with antioxidants so this result may not apply to the widespread dementia that is central to HAD. On further analysis, they observed that when Tat protein was applied to striatal neurons that there was an increase in dopamine metabolites such as Homovillic Acid which points to a metabolism shift toward the COMT degradation pathway. Taking this piece of evidence they did make a remarkable conclusion that may hold a pharmacological target for treatments. It is suggested here that Tat protein may induce COMT enzymes to hyper degrade dopamine and that perhaps COMT inhibitors might be of use. However, this conclusion is hard to single out especially with some of the evidence that points to Tat protein being involved in the inhibition of tyrosine hydroxylase which is the rate limiting step in de novo dopamine synthesis. The evidence put forth

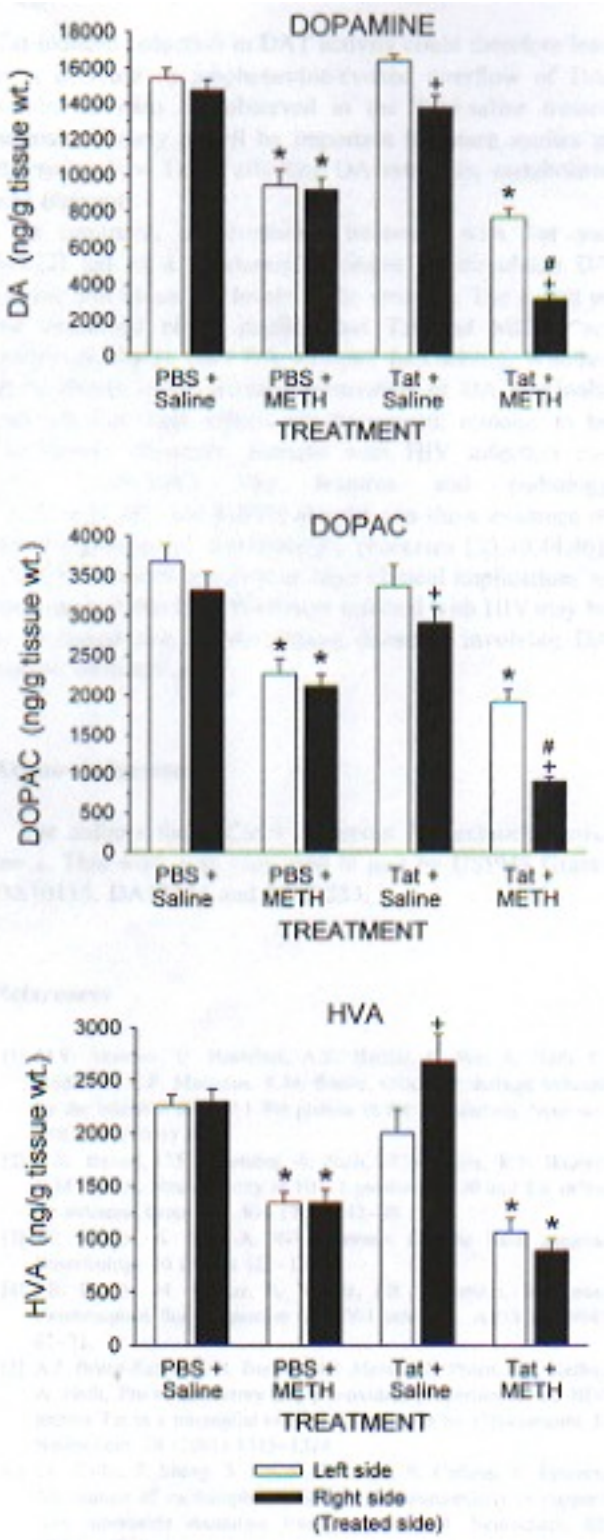

Figure 9:Post-mortem levels of DA and metabolites in striata of rats injected with PBS or Tat into the right striatum 1 day before treatment with saline or METH. Adapted from Cass et al, (2003)

for Tat's actions on the tyrosine hydroxylase enzyme is accounted for by observation that when Tat is applied to a catecholaminergic cell line the tyrosine hydroxylase enzyme does not become expressed (Zauli et al, 2000). This makes sems as Tat protein acts a tranasctivator of transcription. There are numerous reports that CSF levels of dopamine metabolites are low in HAD patients. This would not make sense if there were an induced form of COMT at work degrading dopamine, where one would expect increased metabolite concentrations such as DOPAC (Dihydroxyphenylacetic Acid). This is shown in Figure 9. Treatment might want to focus on either replenishing dopamine levels or finding some way to regulate the expression of the tyrosine hydroxylase enzyme. A pivotal study specifically linking Tat to dopaminergic transmission was found by Maragos et al (2002), which examined the effects of HAD on the basal ganglia. In this study, the authors compared the effects of Methamphetamine on tyrosine hydroxylase with

those of Tat, which both turn out to inhibit tyrosine hydroxylase. Evidence further suggesting a selectivity of HIV to dopaminergic terminals, was that specific dopamine transporters were examined to be involved as well. The dopamine transporters were assayed for their affinity for binding to available substrates. This was done by using a compound (3 beta- (4-iodophenyl) tropane-2 beta-carboxylic acid isopropyl ester). This compound binds to the cocaine specifically on the DA-1 transporter, and since it is iodinated, it can be followed. However, the most significant finding was that of the reduction of dopamine in the striatum of rats that were exposed to HIV as seen in the figure 10: This study showed that in as little as 7 days, CSF levels of dopamine dramatically, but without any change in dopamine metabolites in the CSF The lack of change in dopamine metabolites concentrations suggests a reduction in synthesis, rather than more rapid breakdown, of dopamine (Maragos et al, 2002). However, it is known that methamphetamine also affects tyrosine hydroxylase in its activity as well. It appears that methamphetamine and other agents, which affect dopamine, could work primarily on the enzymes that degrade dopamine as well. This is evident when both Tat and methamphetamine are combined in the experiment only a decrease in metabolites is seen whereas when methamphetamine is applied alone, the metabolites of dopamine are markedly in-

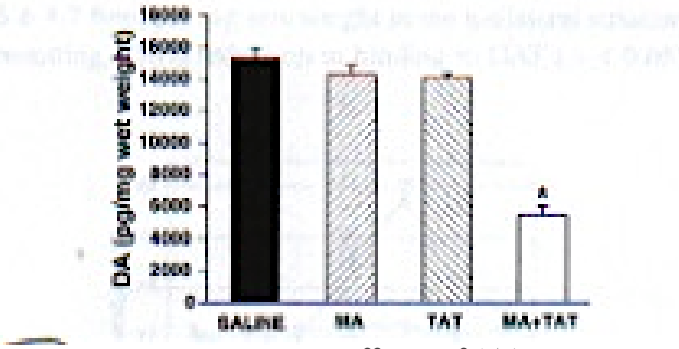

Figure 10:Synergistic effects of MA+Tat on striatal dopamine (DA) levels.Tat (20µg) was injected into the striatum and 24 hours later animals recieved IP injections of MA. Adapted from Marago et al, 2002

creased in the CSF. This suggests that methamphetamine might be involved in inducing the degradation of dopamine. On the other hand, when it comes to the activity of the dopamine transporter, it seems that methamphetamine and Tat are working synergistically to reduce the DAT 's affinity to bind to dopamine. Neither methamphetamine nor Tat, applied singly, produced an effect on binding ability of the DA-1 Dopamine transporter. Maragos et al, (2002) suggests that the Tat viral protein primarily affects the tyrosine hydroxylase enzyme but it may work in concert with drugs of abuse to affect the affinity of the dopamine transporter to bind dopamine. This is apparent with the damage to the dopamine transporters affecting their binding to dopamine by way of extensive oxidative damage.

As Amphetamine uses a mechanism that damages DA-1 transporters through reactive oxygen species, Tat is also known to induce the release of reactive oxygen species by way of increasing intracellular levels of oxidative species in mitochondria. This finding with Tat has been documented in hippocampal cells of rats (Aksenov et al, 2005). This may explain why HIV patients who abused drugs like methamphetamine have a greater risk of developing the symptoms of HAD. Moreover, these studies could suggest future pharmacological targets for treatment. A key area of future research could be on finding compounds that could regulate the expression of the tyrosine hydroxylase enzyme as well as to prevent oxidative damage done on the DA-1 transporters as described above.

Some salient evidence an HIV propensity to affect dopaminergic terminals in the course of HAD is found in clinical case studies HAD patients exhibit classic Parkinsonian symptoms similar to MPTP-exposed individuals (Koutsillieri et. al, 2002). MPTP is known to create damage via free radicals selectively in dopaminergic terminals of the striatum. HAD may be in due, at least in part, to similar oxidative stress produced by HIV inflammation (Itoth et al, 2001) went on to describe a possible mechanism by which HIV might affect dopamine systems selectively, by targeting tyrosine hydroxylase. Once again, this enzyme that can attributed to as the reason for such dramatic decrease in dopamine levels in the nirgastriatal area of the basal ganglia as there high Tat protein activity (Zauli et al, 2000). As Zaulli pointed out that Tat acts as a transactivator of transcription that affects the gene that expresses Tyrosine Hydroxylase. The main reason Koutsilleri and his colleagues focused on the link between dopamine and HIV is because it was observed that most patients who had advanced forms of HAD, were extremely sensitive to neuroleptics like metoclopopamide at such minute levels that should normally not elicit dyskinesia side effects. These observations are convincing because in part they confirm that there is a deficit in dopamine levels in HAD patients that correlates with the observed symptoms.

Simian Immunodeficiency Virus (SIV), a close analog of HIV, can also affect the dopaminergic systems in vivo. In test primates, investigators have reported reductions in CSF [dopamine] in SIV very similar to HIV. On histological examination of neural tissue, HIV infection associated with the appearance of a large number of viral

proteins such as gp120 and Tat, selectively in dopamine rich regions. Paradoxically, cell bodies of the basal ganglia remained rich with dopamine at a time when release is reduced. It was the axons and dopamine terminals at the axonal end were undergoing retrograde degeneration. These authors suggested that HIV viral proteins might first affect oxidative damage in the postsynaptic dopaminergic areas and then work their way to the cell bodies. If Koutsilleri and his team are right in this regard, this could explain why even when one neuron is affected other neighboring neurons connected to the axoaxonic network can be infected, more rapidly whereas if the cell body is first infected. Perhaps the HIV molecule finds a way to bind to microtubules in the axons that facilitate the retrograde movement to the cell body. Also by binding to microtubules it can be postulated that HIV's viral proteins can physically hinder the kinesin driven transport of dopamine secretory vesicles and/or important axonal transport of tyrosine hydroxylase. This should be further investigated, because of its potential of yet another pharmacological target for HAD therapy. Perhaps HIV can be labeled with a GFP protein and see if it is observed to bind with labeled microtubule elements. This would confirm a binding of HIV proteins with microtubule elements that could explain why axonal transport is possibly inhibited. With regard to therapeutic targets, this paper emphasized the potential of existing dopaminergic agents such as L-dopa to restore dopaminergic function in HAD. Yet, administration of L-dopa often causes dormant HIV to suddenly become activated accelerating CNS infection. However, one more successful treatment has been the administration of Monoamine oxidase B inhibitors. MAOB, the brain-specific enzyme that breaks down monoamines

such as DA, NE and 5-HT is widely used to raise [dopamine] and help prevent the production of free radicals such as MPTP, making them a second-line Parkinson's disease treatment. Yet, as this paper suggested, patients would have to remain on this therapy for an indefinite period of time, still it provides to reverse some Parkinsonian symptoms. However, as suggested earlier, MAOB Inhibitors may, at the same time, promote infection of the CNS by an unknown mechanism (Czub et al, 2001). Anyway these drugs could only help one of the many dynamic ways in which HIV leads to neuronal damage characteristic of HAD. Areas such as the frontal lobe and the hippocampus were involved. These areas are found to highly involved in memory and reasoning abilities-often the first to go in HIV demented patients. This review also cited the important neuroimaging studies that noticed marked hyperactivity in the subcortical regions of the brain involved in dopamine action In fact, as we will see later there are neuroimaging studies that show a decrease in dopamine transporter activity. Again in regard to treatment, the investigators noted the slight possibility of helping HAD patients with dopaminergic agents such as Selegiline. Yet the paradoxical downside of these treatments is that they seem to accelerate HIV in the CNS that further aggravates the problem. As seen in Czub et al's (2001) study, primates with SIV once given this very drug exhibited induced CNS vacuolization and enhanced viral replication. Wang et (2004) have recently reported that dopaminergic transporter protein expression is decreased in patients in the advanced stages of HAD. Wang thought this decrease in dopamine transporters signifies that there was extensive dopaminergic terminal injury as normally there would be high concentrations of dopa-

mine transporters in these terminals. This study used cocaine-binding affinity to the DA2 transporter sites as an indicator for DA transporter availability. The experiment was begun by dividing subjects into HIV patients that exhibited neurological symptoms and those that did not. The results were striking showing a dramatic decrease in DAT availability in key regions such as the caudate and putamen. Furthermore, as expected because the motor defects associated with advanced HAD, patients exhibited a decrease in dopaminergic activity and perhaps DAT availability, in the striatum. They went on to see through functional PET using the signaling from radiolabeled [11C} Cocaine to assay its binding affinity to dopamine transporter. This was carried out in HIV patients who did not exhibit neurological symptoms as hypothesized did not show a decrease in binding activity. It seems clear that HAD individuals exhibit evidence of attack on the dopamine systems by HIV viral molecules. however the question of dopamine selectivity was not addressed here.

Wang and his colleagues cite past studies conducted that show in vitro that the highest concentration of HIV is localized in the basal ganglia of the brain (Kure at al, 1990). MRI scans of HAD patients have shown increased gadolinium enhancement in the basal ganglia which, according to Wang et al (2004) could suggest that the BBB has been subject to insult that allows a free route for HIV entry into the CNS. Gadolinium only penetrates the CNS if the blood Brain Barrier is compromised. As noted earlier, regions of the brain that are mostly ravaged by HIV infection contain large

numbers of giant multinuclear cells; many of these areas are dopaminergically innervated.

While there is substantial evidence of selective dopaminergic impairments in HAD patients. HAD is a multifactor problem for which other mechanisms have also been proposed. These factors include a variety of ion channels whose function may be disrupted (Gelman et al, 2004). Yet, there many unexplained questions with regard to Gelman's study of acquired channelopathies in HAD patients as we will see. Gelman focused on over expressed ionic channel genes in the HAD patients. They found that calcium –driven K+ channel that prolongs after hyperpolarization and a metatropic glutamate receptor that regulates the opening of K+ ion channels. This paper claims that ion channel malfunctions may cause a hyper -excitation of particular neurons in the middle frontal gyrus that eventually succumb to excitotixic apoptosis. Yet, it can be argued that neurons are more susceptible to increased depolarization and ionic influx secondary to loss of astrocytic buffering of extracellular ions. It is difficult to assess whether direct actions of viral proteins on neuronal ion channels, or indirect astrocytic dysfunction, are primary causes of neuronal apoptosis. Gelman's paper does not properly address this question and it becomes a question which came first, "the chicken or the egg?" There is no clear causal relationship tested here for either dysfunction of ion channels or of astrocytic ionic buffering in neuronal apoptosis. This paper goes into great detail however of how ion channels may be abnormally over expressed at the level of RNA and this does indeed open the door to

many new avenues of research, yet the current evidence in not as convincing as the work done of ion deregulation via the astrocytes. Astrocytes have been shown to buffer extracellular ions more efficiently both in vitro and vivo studies than neuronal ion regulatory systems on their own. Gelman et al. (2004) claim that HAART or antiretroviral therapy can be beneficial for patients with HAD; yet the evidence in our opinion is not there in the literature. They claim that antiretroviral therapy leads to a resumption of normal electrophysiological readings. The electrophysiological readings were referring to the electroencephalographic recordings from relays of the thalamic relays and the basal ganglia. A usual finding in HAD patients ans well as patients suffering from other similar subcortical dementias is an abnormal brain wave oscillation. In fact with regard to their theory of "altered " RNA transcription they admit that it was difficult to fully determine if there was an altered mRNA concentration that could be directly correlated with changes in the neurons involved. Overall, this idea that ion channels might be the cause of excitoxicity may be one worth investigating further, but as of the current literature published, it does not affect the current accepted theories of HAD pathogenesis; in fact the authors themselves note that "More study is needed on ion channel expression and the posttranscriptional control of ion channel gating in demented people."(Gelman et al, 2004).

The current issues at hand is that there must be either a genetic mechanism and or some other mechanism that can be viewed as a potential pharmacological target for future therapy for patients suffering from HAD. Thomas et. al, (2005) tested the

possibility of genetic susceptibilities for microglia to be over expressed in some way. They believed that microglia are activated abnormally by HIV infection increasing production of toxic free radicals that selectively damage the dopaminergic systems. They observed was there was an increase in the expression of 116 specific genes associated with microglia involved. The microglia in question are the ones that are actively secreting cytokines and chemokines. They also however observed a down regulation in genes coding for microglia that were less anchored to the extracellular matrix and of those glia who tended to migrate more freely. More importantly, it was found that when the HIV protein Tat was included in the cell culture, it induced over expressed microglia that secrete chemokines characteristic to inflammation seen in HAD. To verify that it was indeed Tat that was activating these genes in the microglia, Thomas and his team compared the results to a known inducer of genetic over expression with an agent known as DAQ (Dopamine Quinone). DAQ, known toxin that affects dopaminergic neurons in a similar way as seen in HAD patients. DAQ also caused over expression of most of the same genes that induced the microglia to become hyper active and to release harmful cytokines and matrix metalloproteinases. Interestingly enough, it is these matrix metalloproteinases that have been implicated not only in damaging associated neurons, but also in creating breeches in the blood brain barrier. The paper focused on observations centered in microglia located in regions of dopamine activity. This finding could help focus treatment efforts, if further research is done to better understand how and where Tat induces the observed genes to become activated. Treatments might be centered on the specific binding mechanism

of HIV Tat as a preventive measure for patients who develop initial HIV infection. Many HAD patients still looking toward antiretroviral therapy and although some patients are responsive, the overwhelming evidence shows that, in most cases, they are ineffective.

By reviewing here much of what is known about the routes of CNS HIV infection and mechanisms of eventual neurotoxicity, it is clear that many questions remain to be answered. For example, (Shanbhag et al, 2005) have recently examined the effectiveness of antiretroviral therapy in children. Their findings show a marked decrease in children with neurocognitive effects as their viral load decreased, suggesting a surprising ability of antiretrovirals to reduce HIV-induced HAD ore age-related mechanistic differences in HAD in children versus adults. These children in this study were very young and it may well be that antiretroviral drugs were effective because the blood brain barrier is not fully developed in young children. This idea has to be further investigated because clearly there are some differences in the BBB of children and adults. Antiretrovirals have a difficult time entering the CSF in adults even tough evidence shows that the BBB is compromised. The only explanation for this could be that when the BBB allows the migration of infected monocytes, that there must be an additional factor beyond a simple breech in the BBB. Perhaps there is something intrinsic on the proteins that do make it across the BBB during the course of HIV CNSD infection. However, this study does underscore the importance of creating innovative vehicles for the delivery of antiretroviral drugs to the CNS in adults. Proper neutrali-

zation of HIV within the CNS will be the most logical way to prevent the progression of HAD.

Currently the main focus is to help HAD patients to better function by providing symptomatic relief. However as noted earlier Dopamine therapy is known to cause an acceleration of HIV infection in the CNS. New research has to focus on ways of targeting the neurons involved with perhaps a potent cytokine receptor blocker that could block the CXCR4 receptors involved in the recruitment of inflammatory substances that contribute to the overall expression of HAD. As we have seen the CXCR receptor is highly active in activating infected microglia and furthering the course of HAD. Currently therapeutic approaches have also focused on ways to transiently make the blood brain barrier permeable to powerful anti retroviral agents that could reduce CNS infection. This is best seen in pediatric populations whose blood brain barrier is not yet fully developed and in fact as we saw in the study conducted by Shanbahg et al, 2005, this is the case. In Our opinion, new avenues for treating HAD are to prevent it in the first place. This is best done with an early diagnosis and proper antiretroviral treatment. However, in adults as mentioned the use antiretroviral therapy is not effective mainly because poor CNS distribution of the drug. Drugs like Probenecid is currently being evaluated for its use to help remedy this problem. Probenecid inhibits the transport system involved in removing weak acids from the CNS and may aid in help keeping Zidovudine (AZT) drug levels high in the CSF (Gendelman et al, 1998 p.491) It is approaches like these that can be further investigated to

bring some new effective therapies. Research has been conducted on a new substance called Peptide T which is specifically targeted to help block the actions of gp120, yet unfortunately in phase 1 studies, adults did not show much benefit, Yet, it is important to note that this was conducted in pedaitric patients and may be someing to consider in the future (Gendelman et al, 1998, p492). Furthermore, with all of the excitoxic results being shown to be caused by the over activation of the NMDA Receptor, it would make sense to use it as a pharmacological target. The great research as we have noted is working on the usefulness of memantine on helping to reduce the neuronal apoptosis seen in HAD. With regard to therapy, the new and exciting focus is going to be on dopaminergic targets as it is getting more and more certain that dopaminergic neuronal circuits are involved in the pathogenesis of HAD. In light of this researchers at Johns Hopkins HIV Neurology Group(Turchan et al, 2003), have conducted a study of the aforementioned therapeutic modalities described. In the patients with HAD, they had tried different treatment approaches as outlined in the table 1.1. The highlight is how the most effective drug was Selegiline as a neuro protectant. Selegiline is a MAO B inhibitor, it is also used as a treatment therapy for Parkinson's patients whose dopamine levels are being enzymatically transformed to a powerful oxidant MPTP that is known to selectively destroy dopaminergic terminals. The study was a phase I study that showed a significant improvement in the motor skills and other neurocognitive values in the HAD patients.

As discussed earlier the use of NMDA receptor antagonists such as memantine were proposed to help block the onslaught of glutamate excitoxicity associated with HAD. Yet, the clinical results were rather disappointing as there really was no significant effect in the neurogognitive improvement of patients. In this study though a very novel idea was proposed in the possible neuroprotective features of phytoestrogen or estrogen like compounds. Phytoestrogens are commonly found in diets rich in soy and yams. It has been thought that these compounds can act as neuroprotective antioxidants. In this study Turchan and her colleagues looked also at the possibility of antioxidant actions of grape skins, peanuts and red wine as well. The mechanism that estradio might work as a powerful antioxidant through ensuring the mitochondrial ATP synthase is helping to maintain the proton gradient. These phytoestrogens have been shown to show some benefit in protecting dopaminergic neurons from MPTP neurotoxicity, which clearly demonstrates its importance. (See figure 11) The fact that most of HAD affected women who were not menopausal had amenorrhea

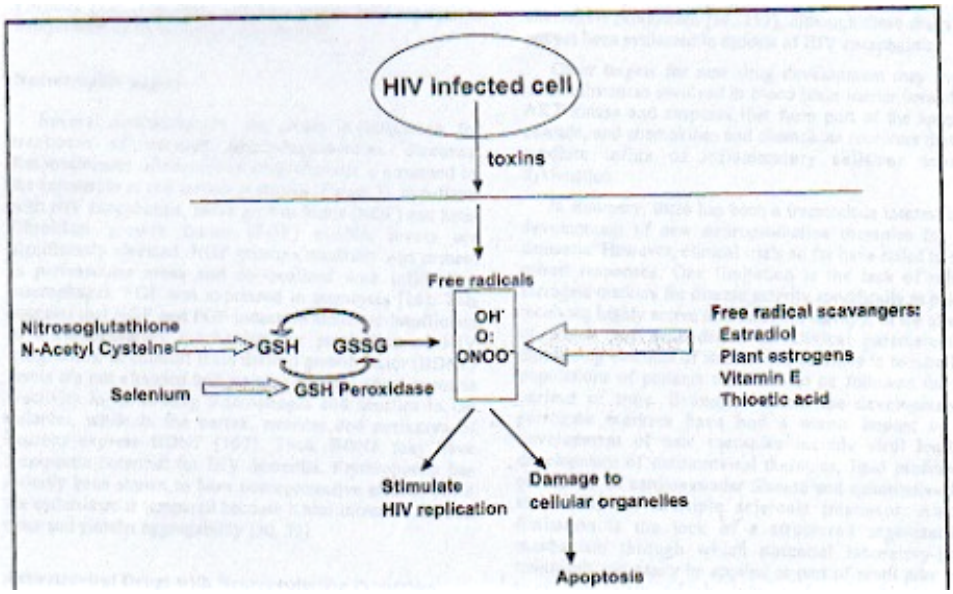

Figure 11: Role of growth factors and estradiol in treatment of HIV Dementia. Adapted from Turchan et al, 2003

alerted researchers to the loss of estradiol. They mention here that although pure estrogen can contribute to better neurocognitive functioning, it does carry the risks of estrogen related cancers and it cannot be used in males and children with out due side effects (Turchan et al, 2003). These results of therapeutic possibilities along with the evidence of dopaminergic neurons as being potential pharmacological targets have provided much avenues for research in the field of HAD. Yet, the clinical trials discussed in this report to date have only looked at a small patient sample and there needs to be more wide spread studies conducted to fully understand the methods of treatments possible.

Concluding Remarks

After a thorough review of the literature on HIV Associated Dementia (HAD), it is unfortunately easy to classify HAD an increasingly common disease, but one that does have a bright future for new therapies based on current research discoveries. As the population of HIV affected individuals who will survive for long periods grows due to effective antiretroviral treatments, quality of life will be more and more severely hampered by HAD. As the world works to curb the rates of viral infection the number of patients with HAD will continue to grow for quite some time. HAD creates a long-term care situation where many patients find so much neurological pain and cognitive impairment that quality of life becomes unacceptable. This makes the development of effective therapies crucial, especially with regard to preventing the onset of HIV Dementia by providing the CNS with adequate protective measures. As we noted there are many key targets and mechanisms that can be targeted by specific interventions. The direct protein mechanisms of HIV's Tat and gp120 can be blocked by preventing their binding to certain cytokine receptors and subsequent regulation of inflammatory substances. However, more importantly the issue of trafficking of infected monocytes into the CNS has to be addressed. However, the most exciting research has yet to begin with regard to the dopamine theory of HAD. With the reduction in levels in Dopamine levels in the CSF of HAD patients, the investigation has to now focus on whether the available dopamine is being highly degraded by COMT and

other enzymes or is it simply a reduction in the production of dopamine. In addition what is he mechanism in which dopaminergic agents that have been used accelerate HIV infection. This has been one of the major roadblocks in HAD treatment. Also there must be a way to prophylactically create a vaccine that could in theory prevent HIV individuals from succumbing to CNS infection. Perhaps a CSF injected vaccine may allow the neutralization of HIV before it has a chance to create HAD pathogenesis. Once this question is solved, new effective treatments can be made that are specific to HAD's mechanisms of action. As we have seen in this report, many other neurodegenerative diseases share similar features of HAD and by unlocking the mechanisms of HAD pathogenesis may shed new light on other diseases as well. As the world sees the rise in HIV infections, HAD will be an important problem to bring attention to. After all, since HIV patients are living longer, more has to be done to improve their quality of life as living with HAD can be devastating.

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