# The Effects of Brain Infusion of Human Immunodeficiency Virus Glycoprotein (gp120) on Splenic Norepinephrine Turnover and Macrophage Activation.

by

Catherine A.Y. Vriend

A thesis submitted

to the University of Manitoba

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# THE EFFECTS OF BRAIN INFUSION OF HUMAN IMMUNODEFICIENCY VIRUS GLYCOPROTEIN (gp 120) ON SPLENIC NOREPINEPHRINE TURNOVER AND MACROPHAGE ACTIVATION

BY

CATHERINE A.Y. VRIEND

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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# ABSTRACT

It has been shown previously that immune cells produce cytokines such as IL-1ß (IL-1) which can signal the central nervous system and result in immune suppression mediated by the hypothalamic-pituitary-adrenal system and sympathetic innervation of lymphoid organs such as the spleen. IL-1 injected into the lateral ventricles resulted in suppression of splenic macrophage IL-1 production and in increased norepinephrine (NE) turnover in the spleen. In addition plasma corticosterone was elevated and T-cell proliferation and natural killer (NK) cell activity was suppressed. Human immunodeficiency virus (HIV) and coat protein, gp120, have been shown to induce IL-1 production in the brain and result in inhibition of T-cell proliferation and NK cell activity as well as increase plasma corticosterone. To determine if the IL-1 induced brain-spleen sympathetic signal could be a functional pathway for HIV induced immunosuppression, the present study initially compared the effects of central injection of gp120 with the effects of central injection of IL-1 on plasma corticosterone and splenic macrophage production of TNF-α. Adult male rats were injected in the lateral ventricle with 4 ug of gp120 or 5 ng of IL-1 or saline and plasma corticosterone and splenic macrophage production of TNF-α were assayed. Both gp120 and IL-1 significantly increased plasma corticosterone. It was hypothesized that gp120 and IL-1 would both suppress splenic macrophage production of TNF-α. However, gp120 significantly elevated TNF-α while the IL-1 response was not significantly different from saline. In the second experiment, rats were injected ICV with either 4ug of gp120 or saline and splenic NE turnover was determined using the rate of

decline of NE content in the spleen after synthesis inhibition. It was hypothesized that gp120 would increase splenic NE turnover in a manner similar to ICV injection of IL-1. In additional groups of animals, the IL-1 blocker, α-melanocyte-stimulating hormone, α-msh, was coinfused with gp120 or infused alone ICV in control animals to see if it would attenuate any expected increase in NE turnover induced by gp 120. gp 120 did not significantly increase splenic NE turnover while α-msh did. In those animals receiving both gp120 and α-msh, the gp120 attenuated the effect of the α-msh. These results suggest that gp120 in the brain modulates the splenic environment in a manner quite unlike ICV IL-1. Furthermore, endogenous α-msh may play a role in the increase in NE turnover in the spleen observed after ICV injection of IL-1.

# INTRODUCTION

A functional connection between the central nervous system (CNS) and the immune system has been proposed. Sympathetic innervation of lymphatic organs including the spleen is well documented (Felten, Felten, Bellinger, Carlson, Ackerman, Madden, Olschowka and Livnat, 1987; Nance and Burns, 1987) and lymphocytes express receptors for the sympathetic transmitter, norepinephrine (NE) (Roszman and Carlson, 1991). It has been shown that the brain possesses receptors for cytokines released by activated immune cells and that cytokines may act as neuromodulators (Farrar, Killian, Ruff, Hill and Pert, 1987). The suggestion has been made that the CNS, signalled by a neuromodulator produced by immune cells, provides a restraining influence on the immune response possibly through the hypothalamic-pituitary-adrenal axis (HPA) as well as the sympathetic nervous system (Besedovsky, del Rey, Sorkin, Da Prada and Keller, 1979; Sundar, Cierpial, Kitts, Ritchie and Weiss, 1990; Brown, Zuo, Vriend, Nirula, Janz, Falk, Nance, Dyck and Greenberg, 1991; Madden and Livnat, 1991). Interleukin-1ß (IL-1), a cytokine produced by macrophages, acts as such a neuromodulator and has been shown to have a number of CNS effects (Besedovsky, del Rey, Sorkin and Dinarello, 1986; Dinarello, 1988; Dinarello, 1991).

An immunomodulating pathway should be activated by naturally occuring infectious agents in the brain. The human immunodeficiency virus (HIV) is one such agent which has been demonstrated to impact on brain function (Ho, Poverantz and Kaplan, 1987; Price, Brew, Sidtis, Rosenblum, Scheck and Cleary,

1988). Involvement occurs early in the course of the disease, is often detectable prior to evidence of systemic infection (Navia and Price, 1987; Diederich, Ackerman, Jurgens, Ortseifen, Thun, Schneider and Vikadinovik, 1988) HIV may selectively infect brain macrophages and microglia which produce IL-1. There is no evidence of neuronal HIV infection and neuronal damage is thought to be brought about by macrophage or microglial secretion of cytokines or other toxic agents in response to HIV (Merrill and Chen, 1991. The known ability of HIV infection to induce cytokine production suggests that HIV could activate a sympathetic pathway to the spleen. Recent reports indicate that active infection of a cell is not necessary for IL-1 induction. The viral coat protein, glycosylated peptide 120 (gp120), can bind to macrophages and microglia and induce IL-1 secretion, as well as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and IL-6 (Wahl, Corcoran, Pyer, Arthur, Harel-Bellan and Farrar, 1989; Jordan, Walkins, Kufta and Dubois-Dalcq, 1991).

The following sections of this thesis will review the evidence for brain-immune modulation, a functional sympathetic brain-immune pathway and the role of IL-1 in induction of the immunosuppressive signal. This will be followed by an overview of HIV, its involvement in the brain and evidence for possible interaction with an IL-1 induced sympathetic pathway.

# BRAIN-IMMUNE PATHWAY

## Brain-immune Modulation

Research on brain-immune interaction has sought to demonstrate that a signal originating in the brain can have an effect on peripheral immunity. One approach has been to demonstrate a relationship between psychological state and immune responsiveness. This has ranged from clinical studies seeking a connection between depression and lymphocyte function (Wodaz, Rupprecht, Kornhuber, Schmitz, Wild, Braner and Riederer, 1991) and the effects of examination stress on Epstein-Barr antibody levels (Keicolt-Glazer, Glazer, Strain, Stout, Farr, Holliday and Speicher, 1986), and the effects of exposing rats or mice to various forms of emotion provoking stimuli on immune competence (Croiset, Heijner, van der Wal, de Boer and de Wied, 1990; Lyte, Nelson and Baissa, 1990). As well, stressors such as footshock, active avoidance and restraint, have been utilized to induce changes in immune function in animals (Keller, Schleifer and Demetrikopoulos, 1991; Berkenbosch, Wolvers and Derijk, 1991).

Another approach to demonstrating that a signal from the brain could affect peripheral immunity has been to condition immune responses. Conditioning implies an associative process occuring in the brain such that a cue paired with a known immune modulator becomes capable of inducing the immune effect when presented alone to the animal (Ader and Cohen, 1985; Spector, 1987). An audiovisual cue which had been paired with an antigen has been shown to elicit an allergic response when presented alone to rats (MacQueen, Marchall, Perdue, Siegel and Bienenstock, 1989). An odor cue previously paired with chemotherapy

has been shown to inhibit tumor growth (Ghanta, Hiramoto, Solvason, Soong and Hiramoto, 1990) and conditioned tolerance was observed when an odor was paired with an immunostimulatory drug (Dyck and Greenberg, 1991). Using a taste aversion paradigm in which saccharine was paired with an immunosuppressive drug, an immune suppression was observed upon reexposure to sacharine (Kusnicov, Husband and King, 1988). Recently, stress and emotion provoking stimuli have been paired with cues and have been shown to alter immune function following reexposure to the cue (Zalcman, Richter and Anisman, 1989; Lysle, Cunnick, Kucinski, Fowler and Rabin, 1990).

Brain-immune Neuro-pathways

In parallel with these psychologically based studies, a number of investigators have examined the physical mechanisms by which a signal originating in the brain could affect immunity. In this line of research, brain areas such as the septum and hippocampus have been lesioned (Nance, Rayson and Carr, 1987). Lesions of the hypothalamus, limbic system, brainstem autonomic nuclei and cortex were shown to result in altered immune function ( Reviewed in Felten, Cohen, Ader, Felten, Carlson and Roszman, 1991). Recently, septal lesions have been shown to suppress levels of splenic macrophage production of TNF-$\alpha$ (Wetmore, Green-Johnson, Gartner, Sanders and Nance,1993). However, the signalling pathway from brain to immune system has not been clearly defined. Stress research has shown that glucocorticoids are immunosuppressive and an HPA mediated pathway has been proposed (Bateman, Singh, Kral and Solomon, 1989). This model

presupposes that communication between the brain and immune system is bidirectional. That is, activated immune cells release factors which are capable of signalling the brain (Blalock, 1989). The brain has been shown to respond to immunization by changes in neuronal firing rates (Besedovsky, Sorkin, Felix and Haas, 1977; Saphier, Abramsky, Mor and Ovadia, 1987). In addition, changes in NE content and turnover were observed in key brain areas such as the hippocampus (Carlson, Felten, Livnat and Felten, 1987) and hypothalamus (Kabiersch, del Rey, Honegger and Besedovsky, 1988), the latter apparently induced by IL-1.

Considerable evidence has accumulated that the cytokine IL-1 is released by activated macrophages and results in activation of the HPA and an increase in circulating corticosterone followed by immune suppression ( Besedovsky et al., 1979; Besedovsky, del Rey, Sorkin and Dinarello, 1986; Besedovsky and del Rey, 1987). However, there is evidence of another pathway. Conclusive evidence of sympathetic innervation of the lymphoid organs has been demonstrated (Felten et al., 1987; Nance, Hopkins and Bieger, 1987; Nance and Burns, 1989). In addition, splenic denervation or chemical sympathectomy resulted in an enhanced immune response in the spleen as measured by the number of plaque-forming cells (Besedovsky, del Rey, Sorkin, Da Prada and Keller, 1979). As well, beta adrenergic antagonists attenuated shock-induced suppression of splenic lymphocye proliferation upon mitogen exposure whereas adrenalectomy did not (Cunnick et al, 1988). In addition, IP injections of IL-1 have been shown to selectively increase NE turnover in the spleen and lung (Akiyoshi, Shimuzu and Saito, 1990). Further studies have shown that the in vitro mitogenic response of splenic

lymphocytes can be inhibited by catecholamine agonists and immune cells have been demonstrated to express receptors for sympathetic transmitters and to respond to transmitter binding with altered function (Roszman and Caarlson, 1991, Heilig, Irwin, Grewal, and Sercarz, 1993). Recently, splenic nerve section was shown to abrogate the effects of footshock on splenic immune function (Wan, Vriend, Wetmore, Gartner, Greenberg and Nance, 1992).

In a series of studies bearing directly on this proposal, a small dose (5 ng) of IL-1 was infused into the lateral ventricles (ICV) resulting in suppression of the splenic T-cell proliferation response, natural killer cell (NK) activity (Sundar, Becker, Cierpial, Carpenter, Rankin, Fleener, Ritchie, Simson and Weiss, 1989) and splenic macrophage secretion of IL-1 in response to LPS (Brown, et al., 1991). Splenic sympathetic nerve cuts blocked the suppression of splenic macrophages induced by ICV IL-1 (Brown et al., 1991), and adminstration of a sympathetic ganglion blocker attenuated the suppression of NK activity and splenic T-cell mitogen responsivenes (Sundar et al., 1989). In both studies, adrenalectomy only partially lifted the immune suppression. Recently, we reported that ICV IL-1 significantly increased the turnover rate of NE in the spleen during the period of immune suppression. Collectively, these data provide strong evidence for an immunosuppressive sympathetic pathway between the CNS and the spleen which can be activated by the central administration of IL-1. IL-1 is of particular interest in these studies because of its key role in immune responses and extensive CNS effects which make it a likely neuroimmunomodulator.

IL-1

IL-1 is a 17 kd peptide produced by activated immune cells, which mediates the acute phase response to infection. This response includes hepatic acute phase protein synthesis, increases in circulating neutrophils and such central effects as fever induction, increased slow wave and non-REM sleep and release of the neuropeptides, CRF, ACTH, vasopressin, somatostatin and $\alpha$–msh. It is present in two forms, $\alpha$ and ß, which though they share little homology, appear to bind to the same receptors and have similar physiological effects. IL-1$\alpha$ is generally a membrane bound form while IL-1ß is freely secreted and appears to act in a paracrine fashion (Dinarello, 1984; Dinarello, 1991). It is the form utilized in this thesis.

In the rat, immuno-reactive IL-1ß (irIL-1ß) has been found in the hippocampus and the hypothalamus. The most prominant staining was in the hippocampus. Specifically, neuronal processes and terminals were found extending from the hilus of the dentate gyrus into the stratum lucidum and closely associated with the apical dentrites of the pyramidal cells in the CA3 and CA4 fields. Staining was also found in the basal dentrites of the pyramidal cells of the CA3. In the hypothalamus, irIL-1ß was present in the neurons of the paraventricular nucleus with beaded axons running through the lateral hypothalamus toward the medial basal hypothalamus. Positive fibers terminated in the suprachiasmatic nucleus, basal dorsomedial arcuate, internal and external zones of the median eminence and the posterior

pituitary. Fibers were also seen in the periventricular nucleus and the parvocellular area of the paraventricular nucleus bordering the third ventricle and the bed nucleus of the stria terminalis (Lechan, Toni, Clark, Cannon, Shaw, Dinarello and Reichlin, 1990). In addition to the hipppocampus and hypothalamus, irIL-1ß was found in the basal forebrain and the olfactory bulbs (Lechan, 1990). In the human, IL-1 has been found throughout the hypothalamus and the paraventricular nucleus of the thalamus. In addition, cell bodies have been reported near the anteroventral tip of the third ventricle (Breder, Dinarello and Sapier, 1988).

Following injection of IFNγ and LPS in the rat brain, IL-1ß mRNA was found at the point of injection and also within perivascular cells in the dorsal recess of the third ventricle ventral to the hippocampus and choroid plexus (Higgins and Olschowka, 1991). These were cells of CNS origin and not infiltrating macrophages. IL-1ß has been shown to bind extensively in the rat brain with very high densities of receptors found in the densely packed neuronal cell layers of the hippocampus and high densities in the cerebral cortex, the pyriform and cingulate cortex, the anterior dorsal thalamus and the ventromedial hypothalamus (Farrar, Kilian,Ruff, Hill and Pert, 1987).

As a lymphokine, the primary effect of IL-1 is the induction of IL-2, a T-cell growth factor which results in T-cell proliferation and clonal expansion. IL-1 induces T-cell maturation, enhances B-cell activation and proliferation and enhances NK activity. In an immune response, it is initially produced by macrophages which are those cells which are first to encounter an immune challenge and are responsible for antigen presentation to T-cells. IL-1 is secreted by peripheral blood monocytes

and by lung, peritoneal, splenic, liver, synovial and bone marrow macrophages. Of significance to the present proposal, IL-1 is expressed in the brain by astrocytes, ameboid microglia and by infiltrating maccrophages, and plays a role in recovery from brain injury (Dinarello, 1984, 1991; Guilian, Baker, Shih and Lachman, 1986). As a result of its CNS effects as well as its presence and binding in the brain, IL-1 may be considered a neurokine as well as a lymphokine and it has been proposed that IL-1 acts as a neuro-immuno-transmitter in the bidirectional communication between the brain and the immune system (Blalock, 1989).

α-MSH

The neuropeptide α-msh appears to function as a natural IL-1 antagonist. It can attenuate virtually all acute phase responses resulting from IL-1 induction including fever, hepatic acute phase protein synthesis, increases in circulating neutrophils and IL-1 induced non-REM sleep (Kluger, 1991). It has also been shown to block IL-1 induced hyperalgesia, induction of PGE synthesis, T-cell proliferation, ACTH release and increases in plasma corticosterone (Dinarello, 1991). While it does not inhibit $PGE_2$ once synthesized, it does block IL-1 induced fever at a much smaller dose than required of the $PGE_2$ blocker, indomethacin (Daynes, Robertson, Burnham and Newton, 1987; Dao, Bell, Jameson and Lipton, 1988). Administered ICV with IL-1, α-msh will block all known central IL-1 effects in a dose dependent manner (Weiss, Sundar, Cierpial and Ritchie, 1991). It enhances wakefulness and produces an EEG characteristic of arousal and attention. It has been proposed that endogenous IL-1 may be involved in sleep induction and that α-msh is part of a regulatory feedback loop restoring arousal (Opp, Obal and

Kruegor, 1988). The same may be true in fever, as IL-1 induces the parent molecule, pro-opiomelanocortin (POMC), circulating levels of α-msh are known to increase during fever (Weiss et al., 1991) and arcuate depletion of α-msh was noted after ICV IL-1 (Nance and Vriend, unpublished observation). α-msh is derived from POMC in the arcuate nucleus, intermediate lobe of the pituitary and the solitary nucleus. In the dorsal hypothalamus, it is synthesized de novo (Robertson, Gahring and Daymes, 1986; Lipton, 1990). The exact mode of IL-1 inhibition is not known. It does not block IL-1 binding and is thought to perhaps interfere with IL-1 signalling after binding (Lipton, 1989).

TNF-α

TNF-α is released along with IL-1 in the early stages of immune response by activated macrophages and was initially described as a cytotoxic molecule which in large doses causes hemorragic necrosis of tumors in vivo and has been shown to be identical with cachectin, a protein which causes the metabolic derangement leading to shock and the cachexia observed during severe disease (Ghiara, Boraschi, Nencioni, Ghezzi and Tagliabue, 1987). In smaller amounts it has been shown to interact synergistically with IL-1 in response to the same immune stimuli such as LPS. TNF-α is self inductive, as is IL-1, and both cytokines are mutually inductive. Like IL-1, TNF-α is responsible for the induction of acute phase proteins, stimulates the HPA, acts as an endogenous pyrogen, regulates cell metabolism and acts as a growth factor. In addition, TNF-α causes cytotoxic lymphocyte (CTL) differentiation, is a thymocyte comitogen and induces IL-8 during inflamatory responses. The TNF-α produced by activated macrophages in

turn induces IFN$\gamma$ production by NK cells. Subsequently, TNF-$\alpha$ and IFN$\gamma$ activate macrophage Ia expression and tumoricidal activity (Bonavida, 1992). The main difference between the effects of IL-1 and TNF-$\alpha$ is that the latter does not have a direct effect on activation of lymphocytes although it increases expression of HLA and IL-2 receptors on IL-2 dependent lymphocytes. Both cytokines are inhibited by prostaglandins, corticosteroids, IL-4, IL-6 and tumor growth factor ß, TGFß (Rosenblum and Donato, 1989). Despite its overlapping effects with IL-1, its synergistic activity with IL-1 indicates that TNF-$\alpha$ and IL-1 have different means of signalling cells. It may be that both cytokines are necessary for the full biological effect during an immune response and that the possible effects observed following exposure to one are attributable to the combined action of both (Neta, Sayers and Oppenheim, 1992).

# HUMAN IMMUNODEFICIENCY VIRUS

## The Virus

HIV is a viral particle approximately 100 nm in diameter with a lipid envelope composed of viral glycoprotein and infected cell membrane. It is a retrovirus and is charactized by a dense cylindrical nucleoid containing core proteins, genomic RNA and reverse transcriptase. Its most prominent immunosuppressive effect is the selective depletion of T4 or CD4 helper/inducer lymphocytes. It is the loss of the T4 cell population in the presence of an opportunistic infection which is diagnosed as acquired immune deficiency syndrome (AIDS).

The infection process begins with receptor mediated endocytosis, viral entry and uncoating. The genomic RNA is transcribed into DNA by reverse transcriptase and the DNA is integrated into the host genome during cell division by a viral encoded enzyme. At this point replication is restricted until the T-cell is activated by a mitogen, antigen or allogen and begins to proliferate. T-cell activation results in transcription followed by protein synthesis, post translational processing and glycosylation. Viral proteins and RNA are assembled at the cell surface and viral particles bud off. The T-cell dies in the process (Ho et al., 1987).

## Brain Involvement

Initially, it was thought that brain involvement occured as a result of opportunistic infection such as cytomegalovirus (Gray, Gherardi, Scaravilli, 1988). It is now known that meningoencephalitis occurs early in the disease process often before a patient is aware of other symptoms. The infection is easily overlooked,

characterized by headache and upon examination, aseptic meningitis is diagnosed. This initial brain infection appears to occur in nearly all cases and may be a necessary precurser to the development of AIDS (Diedirich, et al., 1988; Price et al., 1988). As the encephalitis becomes subacute, the patient may present with mild neurological symptoms and no or mild constitutional symptoms. These symptoms may be the primary indication of AIDS or the only aspect of the disease ever displayed and the mental health care worker is often the first practitioner the patient seeks (Navia and Price, 1987).

The neuropsychological symptoms first noticed are a slowing and loss of precision both in cognitive tasks and motor control. The patient must keep lists for normal activities and formerly routine complex activities take longer and have to be consciously broken down into component steps. The patient may become apathetic but not disphoric. Motor symptoms may develop later, not at all or be the only symptoms displayed. These include slowing of rapid alternating eye movement and extremity movements, abnormal release reflexes and unsteady gait. Neurological examination may show abnormalities on CAT scan, magnetic resonance imaging and EEG preceeding systemic development of AIDS. These are characterized as slow alpha waves, diffuse theta waves, atrophy and enlarged ventricles and parenchymal lesions or calcification (Perry, 1990).

Neuropsychological testing shows difficulty with complex sequencing, impaired fine and rapid motor movement and verbal fluency but not vocabulary. There is diminished performance under time pressure, with problem solving, visual scanning, visual motor integration and with alternation between two or more

performance rules or stimulus sets. Depression may coexist with, mimic or result from the complex but does not correlate with impaired performance. This characteristic testing pattern is diagnosed as AIDS dementia complex. Because of the relative sparing of cortical function in the early stages, it is termed a subcortical dementia and thought to caused by damage to the hippocampus and basal ganglia. A comprehensive study of HIV positive but asymptomatic patients indicated that they consistently scored significantly worse on these tests than a matched group of HIV negative subjects. Complaints about declining cognitive ability were more frequent in the HIV positive patients and correlated with poor test performance whereas the HIV negative did not (Stern, Marder, Bell, Chen, Dooneief, Soldstein, Mindry, Richards, Sano, Williams, Gorman, Ehrhardt and Mayeux, 1991). Further development leads to global loss of cognitive ability, mutism, weakness, incontinence and death in patients who have not already succumbed to opportunistic diseases (Price et al., 1988).

While 60% of all AIDS patients display symptoms, subacute encephalitis is apparent upon autopsy in 80 - 90% along with opportunistic infection and lymphoma. The usual physical manifestaions are gliosis of the cerebral cortex and subcortical nuclei, focal necrosis of white and gray matter, enlargement of oligodendrocyte nuclei, microglial nodules, multinucleated giant cells and demyelination. The nature of memory disturbance indicates that the hippocampus is an early target of HIV damage. The high concentration of IL-1 receptors would make this area particularly vulnerable to cytokine mediated damage. In vitro studies with hippocampal slices indicate that IL-1 inhibits long term potentiation (Katsuki,

Nakai, Hirai, Akaji, Kiso and Satoh, 1990), and HIV patients display disturbances of the theta rhythms which originate in the hippocampus (Perry, 1990).

Mechanism of Damage

HIV is now considered a neurotropic slow virus despite the lack of demonstrable infection in neurons. Macrophages and microglia also express CD4 although lower levels than T4 cells and infected macrophages and microglia are commonly found in brain tissue of HIV patients. Brain infection may occur according to the so called 'Trojan Horse' schema. In this model, the virus is transported across the blood brain barrier by a monocyte. Activation results in replication and cytokines or other chemotactic factors draw more macrophages to the area. Neurons and glia are damaged or functionally altered by cytokines or other toxic products and suffer 'innocent bystander' destruction. Neurons although damaged, remain uninfected whereas CD4-bearing glial cells eventually do become infected (Ho et al., 1987; Merrill and Chen, 1991). In the normal brain, monocytes are usually found only around the blood vessels. However, in the HIV brain they are found throughout (Peudenier, Hery, Montagnier and Tardieu, 1991). In addition, activated T-cells have been demonstrated to cross the blood brain barrier thereby providing another possible route of infection (Wekerle, Lenington, Lassman and Meyermann, 1986).

Early attempts to identify infected microglia and astrocytes were frustrated by the fact that infection was not detectable until the cells were activated (Tornatore, Nath, Amemiya and Major, 1991). Microglia are eventually killed due to cell fusion and the formation of giant multinucleated cells or syncitia. However, they can

reproduce virus without cell replication (Watkins, Dorn, Kelly, Armstrong, Potts, Michaels, Kufta and Dubois-Dalcq, 1990). Unlike T-cells, macrophages do not bud virus and die, but rather, HIV is treated like any other secretory glycoprotein. It is produced and assembled in the golgi and packaged in vacuoles. Exocitosis is suppressed and the infectious product is hidden from the host. At the same time cytokine production is increased causing damage to nearby neurons. It is thought that neuronal damage releases neuropeptides which stimulate the macrophage to produce more cytokines and virus. Latent provirus in the host genome DNA can be released by cytokines, mitogens, phorbol esters, infection by herpes or adenovirus and by sunlight exposure (Meltzer, Skillman, Hoover, Hanson, Turpin, Kalter and Gendelman, 1990). A low level of infection and nonproductive infection has been found in astrocytes in vivo and they are readily infected in vitro. Even with nonproductive infection, cytokine production increases (Leiberman, Pitha, Shin and Shin, 1989). Much of the neuronal damage may occur in the absence of productive infection. There is evidence of viral protein, gp120, binding to specific receptors, triggering a transmembrane signal and resulting in production of cytokines and other products which are toxic to neurons and potentiate viral replication in infected cells (Merrill and Chen, 1991).

gp120

gp120 is a viral coat protein which is incorporated into the infected cell membrane prior to viral budding. It is the portion of the viral surface which allows HIV to bind to the CD4 antigen on T-cells, macrophages and microglia (Ho et al., 1987) and gp120 binding can be inhibited in human peripheral monocytes by CD4

antibody (Jordan, Walkins, Kufta and Dubois-Dalcq, 1991; Wahl, Corcoran, Pyle, Arthur, Haril-Bellan and Farrar, 1989). Recently, brain derived cell lines, glioblastoma and neuroblastoma, have been demonstrated to have specific non CD4 binding sites for gp120 which are not blocked by CD4 antibody (Kozlowski, Sandler, Lin and Watson, 1991). A binding site 157 amino acids upstream from the CD4 site on gp120 is thought to be responsible for the neurotropism of the virus and binding of both regions is necessary for efficient viral entry (O'Brian, Koyanagi, Namazie, Zhao, Diagne, Idler, Zack and Chen, 1990). This site is the V3 loop and its presence on a viral strain indicates it is a neurotropic strain. Interaction of this area subsequent to CD4 binding appears necessary for viral fusion and entry. In T4 cells, gp40, transmembrane peptide of HIV, appears to be fusogenic but in macrophages and microglia the V3 seems to be more fusogenic resulting in the observed large multinucleated cells with vacuolar ballooning and death (Sharpless, O'Brian, Verdin Kufta, Chen, Dubois-Dalcq, 1992). One CNS derived virus isolate displays tropism for machophages and propagates well in those cells but not in T-cells and the V3 domain appears to be responsible (Liu, Wood, Levy, Cheng-mager, 1990). This same strain induced IL-1 and TNF-α in rat microglia and astrocytes in vitro. Productive infection did not occur and IL-1 and TNF-α were also induced by a mixture of gp120 and gp40. The gp120 epitopes responsible for IL-1 and TNF-α induction were found to be in the V3 loop and antibodies to these areas but not those to CD4 blocked IL-1 and TNF-α induction (Merrill, Koyamagi, Zack, Thomas, Martin and Chen, 1992).

In peripheral monocytes, gp120 has been shown to down regulate chemotactic ligand receptors, cause maturation and diminished ability to functionally migrate in response to stimulus. In addition, large amounts of gp120 can be shed by only a few early infected cells (Wahl, Allen, Gartner, Orinstein, Popovic, Chenoweth, Arthur, Farrar and Wahl, 1989). The neurotoxicity of gp120 is generally attributed to cytokine induction. However, a second factor has been demonstrated which apparently binds to the NMDA receptor and contributes to neuronal damage (Guilian, Vaca and Noonan, 1990; Dreyer, Kaiser, Offerman and Lipton, 1990). In any event, neuronal damage would also result in induction of IL-1.

## THE PRESENT STUDY

Based on the observation that gp120 infused into the rat brain induces IL-1 expression in the brain and elevates HPA activity while suppressing peripheral immunity (Sundar et al., 1991) and that IL-1 injected ICV increases NE turnover in the spleen (Vriend et al., 1992), I have measured splenic NE turnover following brain infusion of gp120. The hypothesis was that gp120 is a naturally occuring (HIV) neurotoxin which induces IL-1 and results in stimulation of a functional immunosuppressive sympathetic pathway. Support for such a mechanism would be increased NE turnover in the spleen, suppression of splenic macrophage activation, and elevated plasma corticosterone levels.

In the first experiment, animals were injected ICV with 4 ug of gp120, 5 ng of IL-1 or saline. Animals were sacrificed at 2 hours post injection and spleens were collected for TNF-$\alpha$ production as a measure of macrophage activation and blood samples collected for plasma corticosterone analysis. It was expected that both

gp120 and IL-1 would decrease splenic macrophage production of TNF-α and elevate plasma corticosterone. In the second experiment, animals were injected ICV with gp120 or saline. Immediately after injection, half of the animals in each group received intraperitoneal (IP) injections of AMPT, a NE synthesis inhibitor. The remainder received saline IP. These animals were sacrificed at 4 hours post injection. Four additional groups of animals received saline IP and gp120 or saline ICV. One gp120 and control ICV group were sacrificed immediately and formed the 0 hour groups.

Splenic NE turnover was determined using the rate of decline of NE content from 0 to 4 hours post injection in the manner of Brodie (Brodie, Costa, Dlabac, Neff and Smookler, 1966). The remaining two groups were sacrificed at 4 hours and served as controls for AMPT activity and any effect gp120 might have on splenic NE content. Elevations in plasma corticosterone were considered an indication of successful injection.

It was hypothesized that relative to controls, gp120 and IL-1 would both suppress splenic macrophage production of TNF-α while elevating plasma corticosterone in the same way that IL-1 ICV suppressed splenic macrophage production of IL-1 while elevating plasma corticosterone. Further, it was hypothesized that relative to control, the gp120 group would demmonstrate an increase in NE turnover in the spleen. Two additional groups of animals were used to examine the effect of α-msh on gp120 induced changes in splenic NE turnover. One group received gp120 and α-msh ICV and a control group received only α-

msh. Both groups were administered AMPT IP and sacrificed at 4 hours. It was hypothesized that $\alpha$-msh would block the effects of gp120 on NE turnover.

Table 1

Experimental Design: Effects of gp120 and gp120+α-msh on Splenic NE Turnover

| n | Group | Hour |
|---|---|---|
| 15 | Sal ICV/Sal IP | 0 |
| 10 | gp120 ICV/Sal IP | 0 |
| 10 | Sal ICV/AMPT IP | 4 |
| 10 | gp120 ICV/AMPT IP | 4 |
| 10 | gp120 ICV/Sal IP | 4 |
| 10 | gp120 ICV/AMPT IP | 4 |
| 10 | gp120+α-msh ICV/AMPT IP | 4 |
| 10 | α-msh ICV/AMPT IP | 4 |

# METHOD

## Subjects

The animals used for these experiments were 100 experimentally naive, male Sprague-Dawley rats obtained from Charles River, Quebec, and weighing 300-350 g. The rats were housed individually in polypropylene cages and maintained on a 12:12 lighting regime with food and water ad libitum. Animals were routinely handled so that baseline corticosterone values would remain low during each experiment.

## Surgery

Seven days prior to each experiment, animals were anesthetized with 50 mg/kg nembutal. A cannula was stereotaxically implanted in the lateral ventricle of each rat with coordinates 0.8 mm posterior to bregma, 1.3 mm lateral and 4.5 mm deep (Paxinos and Watson, 1981). Animals were food restricted 12 hours prior to surgery and prior to anesthesia were injected SC with 0.02 ml atropine (0.5 mg/ml ) to reduce respiratory problems during anesthesia. Animals were anesthetized with 50mg/kg nembutal. Immediately after surgery, they were injected IM with a broad spectrum antibiotic.

## Procedure

In the first experiment, rats were infused with 4 ug of gp120 (American Biotechnologies) in 20 ul saline, 5 ng of IL-1(Hazelton Labs) in 5 ul saline or saline (lactated Ringers solution) at either 20 ul (large volume control) or 5 ul (small volume control) over a 10 minute period while unrestrained. At 2 hours post injection, animals were decapitated and spleens and trunk blood collected. Spleens

were assayed immediately for TNF-α production and trunk blood was spun down for plasma which was frozen for later corticosterone determination. In the second experiment, animals were injected ICV with 4 ug gp120 in 20 ul or 20 ul of saline over a 10 minute period while unrestrained. Additional animals were injected with 2.5 ng α-msh (Sigma) with gp120 or in saline. Immediately after ICV injection, animals received 300 mg/kg AMPT (Sigma) in 0.4 ml saline or saline IP. Rats were sacrificed at 0 and 4 hours and spleens and trunk blood collected. Spleens were rapidly frozen on dry ice and stored at -80°C for later NE analysis and blood was spun down and plasma frozen for corticosterone determination.

TNF-α Assay

Immediately after removal, spleens were disaggregated through stainless steel wire mesh and washed in RPMI 1640. Red cells were removed by $NH_4Cl$ lysis and remaining cells were washed, resuspended in RPMI 1640 with 0.1% BSA. Cells were plated 1 ml/well at $10^6$/ml in 24 well plates and adhered for 2 hours at 37°C, 5% $CO_2$. They were then washed 3 times with RPMI and 1.0 ml of media or 1ug/ml LPS (Salmonella typhi) was added. Plates were incubated for 1 hour and supernatent collected and frozen for assay. Adherent cells were removed from the plates with 0.1M NaOH for protein analysis. Protein per well was determined using a Micro Protein Determination Kit (Sigma Diagnostics).

4 X $10^4$ TNF-α sensitive L929 cells were added to wells of 96 well flat bottom microtiter plates in 50ul of media consisting of RPMI containing 15% (v/v) FBS plus 4 ug/ml Actinomycin D (Sigma). After 2 hours of culture at 37°C in 5%

$CO_2$, eight doubling dilutions were made from each sample. 50 ul of each dilution were added in duplicate to the wells. The plates were further cultured for 20 hours, then pulsed for 2 hours with 50 ul of neutral red dye (0.05% in .85% NaCl) at 37$^{o}$ C. Plates were washed twice with PBS and cells were lysed for 20" with 100 ul/well of 0.05 M sodium phosphate in 50% ETOH. The optical desity of each well was read spectrophotometrically at 550 nm. Data are expressed as ng/pg protein.

Corticosterone Assay

Vials of plasma and standards (corticosterone diluted to 1 mM in redistilled ETOH and then further diluted with charcoal stripped serum to final concentrations of 0.0, 50, 100, 200, 400, 600, 1000 nmol) were thawed and 10 ul added to 0.5 ml assay buffer (0.82% anhydrous sodium acetate, 0.01% sodium azide, pH 5.2 with 0.01% BSA added). Duplicate 100 ul volumes of unknown and standard dilutions were gently vortexed with 100 ul of antiserum (diluted 1:4 with physiological saline containing 0.1% sodium azide then further diluted 1:100 with assay buffer). 100 ul of $^{3}H$ corticosterone (New England Nuclear, 250 uCi in 7.5 ml of re-distilled ETOH and further diluted with assay buffer to 25,000 cpm/100 ul) were added to each tube, vortexed and incubated at 4°C for 90 min. After incubation, 1 ml of charcoal/dextran (0.025% dextran T-70 and 0.25% Norit A charcoal in assay buffer) were added to each tube, vortexed and incubated at 4°C for 10 min. Tubes were then be centrifuged at 3000 rpm for 15 min at 4°C, and

supernatents decanted into vials for scintillation counting on a Beckman ß scintillation counter. Data are expressed as nmol per liter.

Norepinephrine Assay

Frozen spleen was weighed and homogenized in 0.1M perchloric acid containing 0.1 mM EDTA to yield a final tissue concentration of 20 mg per ml. Homogenates were centrifuged at 1500 rpm for 10 min and 1.0 ml of the supernatant was taken for alumina extraction using the ESA Plasma Catecholamine methodology with an internal standard. Samples were separated by HPLC using an ESA Model 5700 solvent delivery system with a CSC-S ODS2 5-$\mu$m column. Analysis and quantitation was accomplished with a Coulochem 5100 A electrochemical detector and Shimadzu CR601 Chromatopac Integrator. Data are expressed as nanogram NE per gram wet tissue weight.

Statistical Analysis

Corticosterone and TNF$\alpha$ data was analysed by analysis of variance with a post-hoc analysis by Scheffe. Regression coefficients for the decline of splenic NE were determined by a least-squares linear regression of the log NE content of the tissue vs time (0 and 4 hr). A planned comparison was made between the control and gp120 regression lines from the AMPT IP groups by means of a t-test for identical slopes (Kleinbaum, Kuper and Miller, 1988). Additional planned comparisons were made between the control regression line and the gp120 + $\alpha$-msh line as well as the $\alpha$-msh line. $\alpha$ was set at .05 for all analyses. The NE turnover rate was determined according to Brodie et al. (1966). This method allows for the

calculation of the rate constant of amine loss after synthesis inhibition or the fractional turnover rate expressed as $k(hr^{-1}) \pm SE$, the turnover time in hours and the turnover rate expressed as ng/gwet tissue weight/hr.

RESULTS

In the first experiment an initial ANOVA on the four groups (gp120, IL-1, small volume control and large volume control) revealed significant differences in corticosterone production ($F_{(3,10)} = 63.227$, $p < 0.0001$). Subsequent post-hoc analysis indicated that both gp120 and IL-1 were significantly elevated over control ($p < 0.0001$) and that the IL-1 and gp120 groups did not differ significantly from each other.

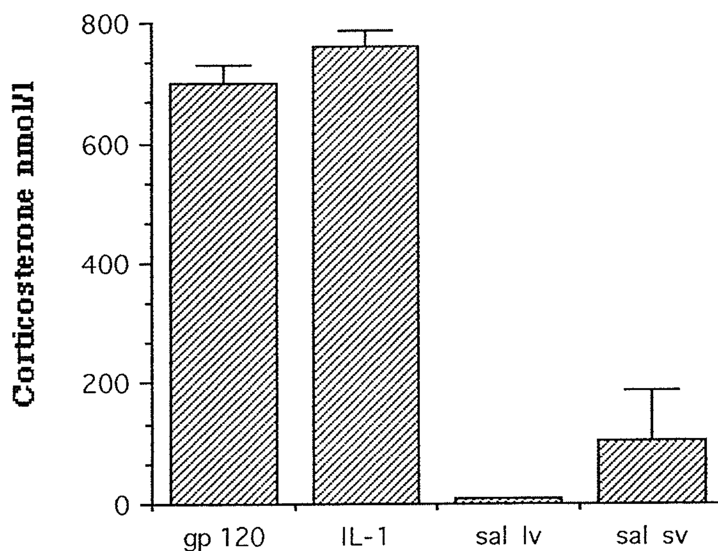


Figure 1. Effect of ICV injection of gp120, IL-1 or 5 ul or 20 ul of saline on plasma corticosterone levels. Rats receiving gp120 or IL-1 had significantly higher corticosterone levels than saline controls ($p<0.001$).

Since there was no difference between the small and the large volume controls in the corticosterone analysis they were collapsed for the TNF-α data analysis and an ANOVA was run on the 3 groups (gp120, IL-1 and saline with two levels of LPS stimulation, 0 and 1ug.) which was significant ($F_{(2,11)} = 7,184$, $p< 0.01$.) Post-hoc analysis revealed that the gp120 group was significantly different from the IL-1 group ($p< 0.02$) and the saline group ($p< 0.02$) and the saline and IL-1 groups did not differ from each other. TNF-α was significantly elevated in the gp120 group at 0 LPS stimulation and further elevated following stimulation with 1ug LPS. The saline group was slightly but insignificantly elevated of at 1ug LPS which was not apparent in the IL-1 group.

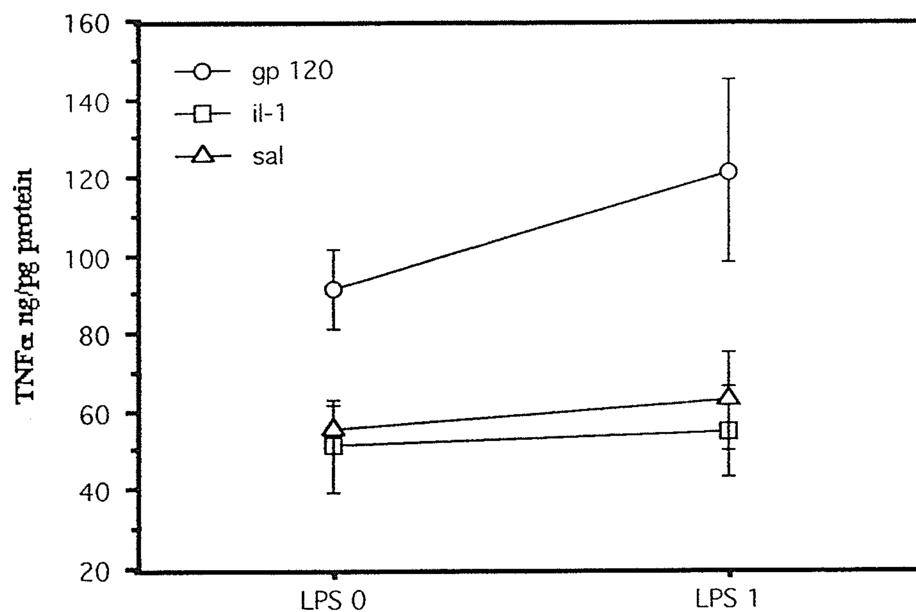


Figure 2. Effect of ICV injection of gp120, IL-1 or saline on splenic macrophage production of TNF-α. Rats receiving gp120 produced significantly higher levels of TNF-α with and without LPS stimulation ($p<0.02$).

In the second experiment, gp120 ICV did not increase splenic NE turnover as there was no significant difference between gp120 and saline with regard to NE turnover. On the other hand, α-msh injected alone ICV resulted in a significant increase ($T = 2.00$, $p< 0.02_{\text{one tail}}$ and $p< 0.05_{\text{two tailed}}$) in NE turnover which appeared to be attenuated by coinjection of gp120. Saline ICV resulted in a turnover rate of 83.87 ng/g/hr. gp120 ICV resulted in a rate of 89.38 ng/g/hr and α-msh resulted in a rate of 144.56 ng/g/hr. The coinjection of gp120 with α-msh brought the turnover rate back down to 101.52 ng/g/hr. (See Table 2)

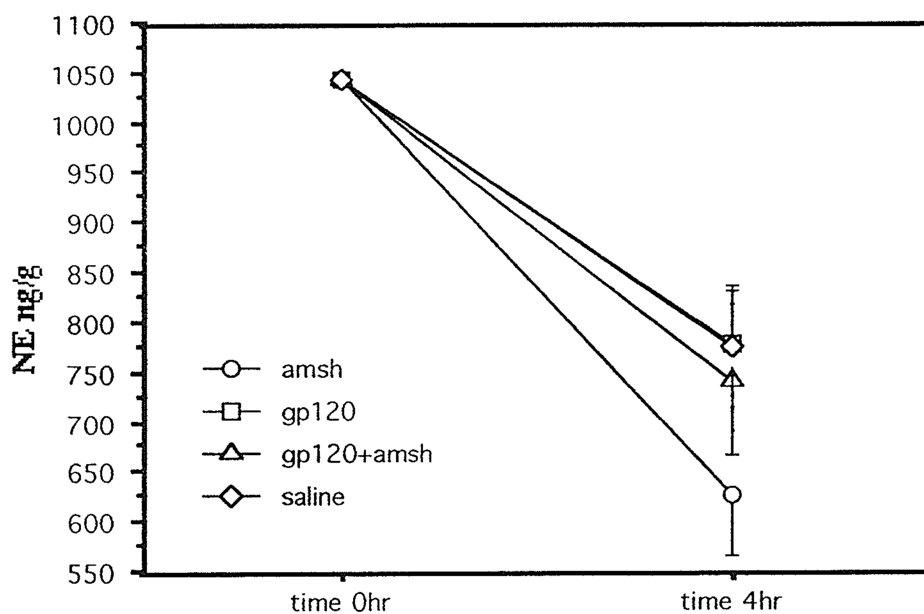


Figure 3. Effect of gp120, gp120 plus α-msh, α-msh or saline on the decline in splenic NE content 4 hrs after NE synthesis inhibition.

Table 2

Effects of gp 120 and α-msh on Norepinephrine Turnover in the Spleen

| group | rate constant of amine loss k ($hr^{-1}$)±SE | turnover time hr | turnover rate ng/g/hr |
|---|---|---|---|
| Saline | -.076 ± .001 | 13.16 | 83.87 |
| gp 120 | - .081± .021 | 12.35 | 89.38 |
| α-msh | -.131± .014 | 7.63 | 144.56* |
| α-msh+gp120 | -.092± .018 | 10.87 | 101.52 |

*The slope of the decline of NE after α-msh is significantly different from the slope of the decline of NE after saline ( $p<.02$ ).

gp120 and α-msh both elevated corticosterone in the AMPT animals but the elevations were not significantly different from the saline AMPT group. gp120 coinjected with α-msh in AMPT animals did result in a significantly ($p<0.02$) higher level of corticosterone relative to the saline AMPT group.

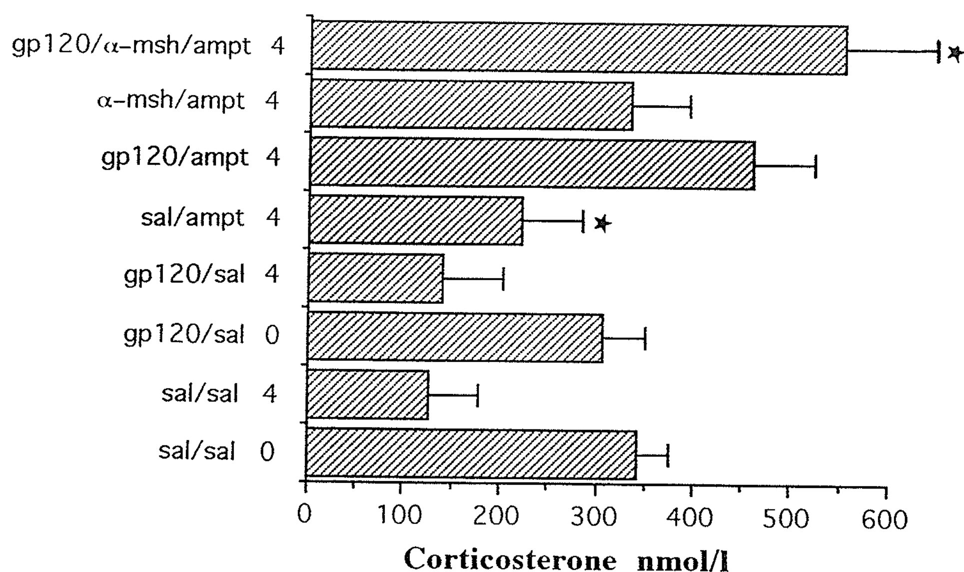


Figure 4. Effect of gp120, gp120 plus α-msh, α-msh or saline on plasma corticosterone levels at o hr and 4 hrs post injection, with or without AMPT. gp120 plus α-msh with AMPT resulted in significantly higher levels of corticosterone than saline with AMPT ($p<0.02$).*

# DISCUSSION

Previous studies in the rat with ICV injection of gp120 have resulted in IL-1 induction in the brain along with increased plasma corticosterone, decreased mitogenic response to Con A of blood and splenic lymphocytes, and decreased NK cell activity. In addition, these effects were blocked by coinjection of α–msh (Sundar et al., 1991) These results were consistent with previous studies in which IL-1 was injected ICV in the same lab (Sundar et al., 1989) as well as our own (Brown et al., 1990.) In a similar paradigm, we observed a suppression of splenic macrophage activation as measured by splenic macrophage production of IL-1 as well as significantly increased sympathetic neural activity as measured by splenic NE turnover (Vriend et al., 1992). These observations formed the basis for the hypothesis tested in the present study, that is: gp120 in the brain would suppress splenic macrophage activation as measured by splenic macrophage production of TNF-α and increase NE turnover in the spleen. It was expected that coinjection of α–msh would attenuate these effects as it did in the Sundar (1991) study. The results of the present study do not support this hypothesis. In our lab, gp120 injected ICV enhanced TNF-α production and did not result in increased NE turnover in the spleen. On the contrary, gp120 appeared to attenuate the increase in NE turnover which resulted from an injection of α–msh. gp120 did, however, result in increased plasma corticosterone levels at 2 hours post injection.

The experimental designs for the two studies were similar in some respects but the differences in designs may well account for the differing results. In the Sundar experiments, blood and spleens for corticosteroid determination and

immunological studies were collected from animals prepared for saline perfusion just prior to the perfusion. That is, animals were under halothane anethesia and the peritoneal cavity was opened. Under these conditions IL-1 induction showed no clear time or dose response and IL-1 induction was noted in only half of the gp120 treated animals at 100ng, 1ug and 4ug, at 2 hr, 6 hr and 24 hr post injection. In other experiments using hippocampal injection of lesser amounts of gp120 which induced IL-1 in the brain more consistently, animals underwent acute cannulations such that they were under anesthesia and mounted on the stereotaxic frame at the time of perfusion and a control group of only 2 animals was included. These conditions in our lab elevate serum corticosterone levels such that it is difficult to distinguish the effects on serum corticosterone from that resulting from either IL-1, LPS or saline (unpublished observation.) In addition, surgery and anesthesia are known to induce immune suppression (Katafuchi, Take and Hori,1993). Recently, it has been reported that surgery and anesthesia can induce release of IL-1 receptor antagonist at 2 hours after commencing surgery while having no effect on circulating IL-1 levels (ONuallain, Puri and Reen, 1993). If the same phenomenon is present in rats, it would further confuse interpretation of the data from acute preparations.

In those experiments which were not acute, injections of gp120 took place 4-6 days post cannulation, at a time when macrophages responding to surgical trauma are still present and gliosis is still underway (Giulian, Chen, Ingeman, George and Noponen, 1989). Glial cells as well as macrophages produce IL-1 when perturbed (Giulian, Vaca and Naponen, 1990). In the present study, animals were allowed a

7 day recovery period to allow for completion of gliosis and outmigration of scavenger macrophages. All animals were sacrificed while awake by decapitation within 90 seconds of removal from the cage before corticosterone levels had an opportunity to rise. In addition, all animals were housed one per cage and were well handled prior to the experiment. Thus, they were calm enough at sacrifice that handling gloves were not necessary and baseline corticosterone levels were very low. In the Sundar study, animals were housed 2 per cage in enclosed, microisolator cages within laminar-flow racks and routine handling was not reported. Such conditions could lead to differences in pre-experiment immune status as well as different neuroendocrine profiles.

An additional problem with the gp120 preparation was noted in our lab and confirmed in discussions with the supplier, American Biotechnologies. gp120 is a sticky peptide which has a tendency to precipitate out of solution upon one repetition of a freeze thaw cycle or when either substantially diluted without a carrier protein (prohibited by experimental design) or further concentrated after production. Therefore, individual aliquots of gp120 at the required concentration were prepared for our studies by ABT and thawed once on the day of injection. At the time of the Sundar study, the company was not aware such precautions were necessary.

The results of these experiments suggest that the effects of exogenous and endogenous α-msh on NE turnover in the spleen should be further explored. This observation has not been reported previously and is contrary to the hypothesis that α-msh acts primarily as a specific IL-1 blocker (Lipton, 1990). While there is

evidence that IL-1 administered centrally suppresses splenic immune responses and that the HPA and splenic sympathetic nerve both appear to carry the suppressive signal (Sundar et al., 1989; Brown et al., 1990; Vriend et al., 1992), little is known about the initiation of the signal from within the brain after exposure IL-1. Presumably, exogenous or induced IL-1 would bind to receptors present close to the site of injection, periventricular areas and in the ventromedial hypothalamus (Farrar et al., 1987). The consequences of binding appear to be changes in NE content and turnover in the hypothalamus as well as CRF and ACTH release which would initiate the HPA signal (Kabiersch, et al., 1988; Besedovsky and del Rey, 1987). It is also known that exogenous IL-1 results in the release of vasopressin and somatostatin as well as α–msh (Dinarello, 1984;1991). IL-1 positive fibers have been found to terminate in the basal dorsomedial arcuate and external and internal zones of the median eminence, areas containing α–msh neurons as well as those staining for ACTH. As mentioned previously, α–msh has been thought to be part of a regulatory feedback loop restoring arrousal after the sleep inducing effects of central IL-1 (Opp et al., 1988). The same appears to be true in fever induced by IL-1 and many other central and peripheral effects of IL-1. Rather than referring to α–msh as a specific IL-1 blocker, it may be more accurate to view it as a mechanism for down regulation of IL-1 following IL-1 induction by an immune response. Pituitary release may serve to attenuate the peripheral and inflamatory effects of IL-1 and central activation may attenuate the central effects of IL-1. How central attenuation occurs is unknown and generally described to be indirect. α–msh does not displace IL-1 from the CNS, is not a receptor antagonist and

appears to act for only 1 to 1.5 hours (Lipton,1990). It has recently been found to attenuate fever induced by TNF-α as well as IL-1 (Martin, Catania, Hiltz and Lipton, 1991). Interestingly, in rabbits neither endotoxin or CRF injected IV caused α-msh release but did stimulate ACTH and corticosterone release. Pretreatment with dexamethasone abolished the ACTH and corticosterone response to both stimuli and had no effect on circulating α-msh after CRF stimulation. It did, however, result in significant α-msh release after an endotoxin dose which previously had no effect (Catania, Martin and Lipton, 1991). This would imply an interaction between cytokines and perhaps glucocorticoid receptors in the release of α-msh. Glucocorticoid receptors and IL-1 staining cells and receptors are located in many of the same brain areas(Farrar et al., 1987; Breder et al., 1988; Lechan et al., 1990; Berkenbosch et al., 1991). In summary, little is known about the releasing signal for α-msh into the circulation nor how its central effects are mediated other than to conclude that it is by an indirect action. The results of this study suggest that the central effects of α-msh might be mediated by the sympathetic nervous system. α-msh is released by the pituitary into the circulation as a hormone, but it also functions as a peptidergic neurotransmitter in neuronal and fiber systems forming short loops to primarily the limbic areas and projecting to the brainstem. In cats, α-msh cell bodies are found in the medial basal hypothalalmus and the lateral hypothalamus. Fibers extend into the paraventricular mucleus of the thalamus, the rostral amygdala, the parabrachial nucleus and the nucleus of the solitary tract. α-msh axons are seen diffusely in various cortical areas but most

extensively in limbic cortical regions (Rao, Hu, Prasad and Jayaraman, 1987). More recently in the rat, retrograde labelled neurons from the parabrachial nucleus have been traced back to the lateral hypothalamus. 50% of these retrogradedly labelled cells were in the arcuate nucleus and stained for ACTH or α-msh. Similarly stained neurons were found in the retrochiasmatic area (Moga, Saper and Gray, 1990). α-msh receptors have been found in the septal area, hypothalamus, thalamus, epithalamus, olfactory striatal complex and midbrain. Peptide cross talk may exist as these receptors recognise both α-msh, NDP-msh and ACTH (Tatro, 1990). These areas are considered potential target sites for thermoregulation, pituitary regulation and effects on learning and behavior. Certainly, these studies indicate that the hardwiring exists for α-msh involvement in sympathetic nervous system function. α-msh activity as a neurotransmitter may be mediated by CRF, AVP or any of the other peptidergic transmitters in the hypothalamus or respond independently to the same neuronal signals as CRF.

Recent studies have demonstrated that ICV infusion of CRF suppressed LH realease and this suppression was prevented by coinfusion of α-msh. IL-1 infusion was found to reduce LH and stimulate prolactin. Both effects were prevented by coinfusion of α-msh. However, coinfusion of α-msh with CRF did not prevent increased corticosterone (Shalts, Feng, Feris and Wardlow, 1992). This is in agreement with the results of our study in which α-msh did not prevent gp120 induced corticosterone increases. Our data is complicated by the addition of AMPT IP which exaggerated the gp120 effect while not affecting corticosterone

when administered with saline ICV. However, α–msh and AMPT elevated corticosterone to the same extent as gp120 and AMPT.

As a neurotransmitter, α–msh is involved in normal waking mechanisms and attention. It enhances learning and memory, actions which implicate projections to the locus ceruleus. Damage to α–msh/ACTH neurons in the arcuate nucleus of the newborn rat is associated with a permanent reduction in wakefulness (Opp, OBal, and Krueger, 1988). It has been suggested that gp120 may antagonize endogenous neurotropic peptides such as VIP and that this antagonism is responsible for the early effects on attention and learning (Buzy, Brenneman, Pert, Martin, Salazar and Ruff, 1992). Our results indicated that gp120 attenuated the α–msh induced increase in splenic NE turnover. This suggests that the early gp120 effect on attention and memory which occures before AIDs and obvious brain damage, may be a result of antagonism of α–msh, a peptide clearly associated with this function. In addition, α–msh has been recently shown to be a neural growth factor and to increase dendritic aborization (Lipton, 1990), whereas gp120 reduces dendritic arborization (Buzy et al., 1992). Further studies will have to be undertaken to confirm both the α–msh induced increase in splenic NE turnover and the apparent gp120 attenuation of this increase. Confirmation of these results should encourage elucidation of the mechanism of IL-1 signalling of α–msh neurons and the transmission of the signal to the sympathetic nervous system.

If gp120 does in fact attenuate the increase in splenic sympathetic activity ordinarily induced by cytokine induction in the brain or serves to lessen the tonic sympathetic induced suppression of splenic macrophage production of IL-1 or TNF-

$\alpha$, there are several implications for the development of an ongoing HIV infection and subsequent progression to AIDs. First, gp120 ICV does appear to reliably increase plasma corticosterone which would result in a generalized immune suppression (Besedovsky et al., 1979) as well as enhancement of productive infection (Solomon, Kemeny, and Temoshok, 1991). The attenuation of sympathetic mediated suppression is consistent with the observed enhancement of splenic TNF-$\alpha$ production at 2 hours post injection. Further studies should be undertaken to determine if a similar enhancement of IL-1 production occures. The results of an NK assay from the same experiment suggest that NK activity as well was not suppressed but may have been in the IL-1 ICV animals. The animals were not primed for an NK response with poly I:C and as a result NK activity was minimal. The observed effects were not biologically significant and so were not included in this thesis. However, the experiment should be repeated with primed animals.

Confirmation of a gp120 ICV enhancement of splenic IL-1 similar to that of TNF-$\alpha$ could shed some light on the dynamics of the early immune response to HIV and specifically the presence of two distinctly different exposed HIV populations, both asymptomatic. One population shows evidence of an earlier cellular mediated immune response, no HIV antibody (AB) response, and resulted in viral clearance. The other population is HIV AB positive, probably with latent infection and faces inevitable development of AIDs. It has been observed recently that a limited infection develops when a pathogen induces a stable cell mediated

immunity and that chronic, progressive and fatal disease occures if AB are produced and cell mediated immunity declines (Salk, Bretscher, Salk, Cleric and Shearer, 1993). The course of disease is determined early in infection and largely depends upon the ambient balance of cytokine production in lymphoid tissue. Two lymphocyte phenotypes, TH1 and TH2 have been demonstrated in both mice and humans. The TH1 response is associated with cellular mediated immunity and the TH2 response is associated with a robust AB response. Typically, viral and parasitic infections induce a TH1 response when the pathogen dose is low, the pathogen is cleared and no AB response develops. At higher pathogen doses, a transient TH1 response is followed by a shift to a TH2 response and production of AB. Most infections induce both responses to varying degrees and it may be that the balance between the two populations is crucial because the two responses are mutually inhibitory. The early course of HIV in humans is characterized by a virus specific cellular mediated response of the TH1 type, a sharp drop in the amount of virus and the presence of circulating peripheral lymphocytes which respond to HIV antigens with a TH1 response. This should happen early in the immune response since lymphokines are produced in the first few hours of T-cell activation, whereas AB effects influencing a shift to TH2 response would not occur for several days (Mossman and Coffman, 1989). In the HIV exposed population the shift can go either way and may result in the two types of HIV exposed populations. In humans who go on to become seropositive, circulating lymphocytes display a shift to the TH2 phenotype (Mossman and Coffman, 1989). In nature, bacterial infection results in an early shift to TH2 response but viral and parasite infection usually

maintain a bias toward TH1. If gp120 in the brain prevents the tonic sympathetic suppression of macrophage production of IL-1 in the spleen as it appears to with TNF-α, a shift in the T cell balance from TH1 to TH2 might occur early in the first stages of viral exposure. Whether the TH1 cell population or the TH2 population undergoes clonal expansion depends upon the cytokine environment in the lymphoid tissue at the time of initial immune activation with the presence of IL-1 being associated with TH2 expansion (Teale and Estes, 1990). High levels of IL-1 resulting from inhibition of tonic IL-1 suppression by gp120 at the same time as elevated glucocorticoid levels would serve to upregulate IL-1 receptor expression and mitigate against a downregulation ordinarily expected with high levels of IL-1 (Neta, Sayers and Oppenheim, 1992). This would be expected to favor the clonal expansion of TH2 cells and may preclude any significant TH1 response. Although these ideas are highly speculative, it is at this juncture of the immune response that immunotherapy, psychotherapy or pharmacotherapy is most likely to effect the results of HIV exposure. Unlike other viruses, no therapy exists which can alter the ultimately fatal course of infection in the HIV seropositive individual.

Recently, elevated levels of plasma TNF-α have been documented in asymptomatic HIV positive patients and still higher in AIDs patients in Ethiopia (Ayehunie, Sonnerborg, Yemane-Berhan, Zewdie, Fritton and Stannegard, 1993). TNF-α was formerly referred to as cachectin and is responsible for the emaciation of serious disease such as HIV or slimming disease as it is referred to in Africa. As an activator of HIV replication in low doses, elevated levels early in disease may be indicative of increased viral replication at the same time that elevated IL-1 would

contribute to AB production . In a manner similar to the complex neural-macrophage/microglial interactions in the brain, complex cytokine networks are functioning in the steady or ambient state as well as during immune reactions, and regulate HIV expression in macrophage and T-cells while also regulating the balance between the TH1 and TH2 clonal expansion in lymphoid tissue (Poli and Fauci, 1992). For instance TNF-α is known to stimulate viral transcription in T-cells and macrophages while IFNγ, a TH1 product, can enhance or suppress viral expression depending on the stage of viral replication. Interestingly, IFNγ remains low in Ethiopian HIV patients (Ayehuni et al., 1993). TGF-ß also has both effects depending on the stage of viral production while IFNα appears to uniformly suppress all stages of viral spread (Poli and Fauci, 1993). Increased sympathetic activity is associated with IFNα in the brain.

The effects of stress on the splenic cytokine network may also be of critical importance at the point of HIV exposure. It has been reported that exposure of rats to a single session of footshock at the point of immunization resulted in enhanced cellular and humoral response to keyhole limpet hemocyanin when challanged with antigen 14 days later (Wood, Karol, Kusnecov and Rabin, 1993). This agrees with previous reports of an enhanced primary response to sheep red blood cells with exposure to restraint stress or a novel environment as well as work in our lab which found increased levels of macrophage TNF-α and IL-1 secretion after footshock (Nance, 1993, unpublished raw data). Thus it appears that acute stress at the point of exposure may also favor TH2 clonal expansion and a strong AB response at the

expense of the TH1 response. These studies do not address the effects of chronic stress on immune function or ambient cytokine environment in lymphoid tissue. However, they do suggest that pharmacological or psychotherapeutic manipulation of sympathetic outflow to the immune tissues should be examined.

In any event, further accumulation of gp120 in the brain as a result of productive systemic or brain infection would eventually result in macrophages that are refractory to cytokine induction (Merrill, 1992). At that stage of progressive infection, other factors may become involved in the CNS damage associated with HIV. These would include such factors as the proposed gp120 neurotoxin which binds to NMDA receptors or the more recently isolated protease resistant and heat stable small (<2kDa) toxin which is thought to be a gene product of infected macrophages rather than from the viral genome and which causes vacuolization and neuronal death (Spencer and Price, 1992). Further studies will have to be undertaken to explore the effects of low doses of gp120 in the brain on the splenic cytokine environment as well as the cytokine environment in other lymphoid tissues and in the brain itself.

In conclusion, previous work suggested that central injection of gp120 induced IL-1 in the brain and resulted in suppression of peripheral immunity. We have demonstrated that IL-1 injected centrally increases NE turnover in the spleen while suppressing splenic immune response. The hypothesis of the present study predicted that as a result of gp120 induction of IL-1 in the brain, the rate of NE turnover in the spleen would increase, plasma corticosterone would be elevated and macrophage activation, as measured by TNF-$\alpha$ production, would be suppressed.

Contrary to the hypothesis, central injection of gp120 did not increase the rate of NE turnover in the spleen, and enhanced rather than suppressed TNF-α production. However, gp120 did elevate plasma corticosterone in a manner similar to central IL-1 injection.

In the expectation that gp120 would increase NE turnover rates in the spleen, the IL-1 antagonist α-msh was coinjected with gp120 and with saline in a control group. Surprisingly, α-msh with saline increased the NE turnover rate in the spleens of control animals, and this increase appeared to be attenuated by gp120 in those animals injected with both gp120 and α-msh. These results suggest that central injection of gp120 does not have the same effect on the splenic cellular environment as IL-1 despite evidence that it induces IL-1 production in the brain. Furthermore, endogenous α-msh may play a role in the central IL-1 downregulatory mechanism.

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