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Immune Modulation by Estrogens

Role in CNS HIV-1 Infection

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Experimental and epidemiological data suggest that estrogen can be protective in both brain injury and infection. While estrogens can act directly on neurons to promote neuronal survival, estrogen also has anti-inflammatory properties that may contribute to overall neuroprotection. Accordingly, estrogens may have particular relevance in chronic neuroimmune disorders such as HIV dementia. As AIDS is now a leading cause of death among women in their reproductive years, understanding the role that female sex hormones might play in the physiology of HIV-1 infection is especially critical. Indeed, there is accumulating evidence that many manifestations of HIV differ in women. For instance, it is now well established that women present with a lower viral titer at the time of seroconversion, have lower HIV viral loads compared to men at similar stages of disease, and may have altered disease progression during pregnancy. Conversely, while epidemiological studies suggest that women may be more vulnerable to certain late-stage AIDS-related illnesses including HIV dementia, there is accumulating data that strongly suggest an estrogen-deficient state is associated with long-term HIV infection in some women. Evaluated as a whole, existing evidence indicates that estrogen can directly protect neurons from damage, can modulate brain inflammation, and could act to maintain low titers of the HIV-1 virus. Accordingly, it can be hypothesized that maintenance of serum estradiol levels could decrease the incidence of HIV dementia and other AIDS-related neurological syndromes in HIV-1 positive women. In this article, we both summarize current understanding and present new data related to the potential mechanisms whereby estrogen could modulate the mechanics and the consequences of HIV-1 infection in the brain and thereby thwart the development of HIV dementia.

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Estrogen and Estrogen Receptors in the Brain

Estrogen and Neuroprotection

Estrogen is a sex steroid hormone produced primarily by the ovary, and is responsible for reproductive cyclicity and the maintenance of secondary sex characteristics in women. It has long been understood that estrogen exerts trophic effects in reproductive target tissues such as the uterus, mammary gland, and ovary. It is becoming appreciated, however, that estrogen also has important trophic and protective roles in other tissues including those of the skeletal, cardiovascular, immune, and nervous systems *(1–3)*. For example, while the role of circulating estrogens in skeletal health in women has been well known for years *(4,5)*, clinical studies suggest that estrogen can delay the onset of Alzheimer's disease and improve aspects of cognitive function associated with normal aging *(6–10)*. These observations demonstrate the importance of estrogen in suppressing age-related physiological declines, and underscore the relative risk incurred by women during menopause when levels of all sex steroids, including estrogens, drop precipitously.

There is considerable evidence supporting a role for estrogen as a trophic and protective factor in the central nervous system (CNS). During development, estrogen modulates the growth and sexual differentiation of the brain *(11)*, while in the adult, hippocampal synaptic density is highest at the point in the estrous cycle when circulating estrogen levels are elevated *(12)*. Furthermore, synaptic spine density of hippocampal neurons decreases following ovariectomy and can be restored to levels comparable to those observed during the estrous cycle by estrogen replacement *(13)*. Additionally, physiological levels of estrogen can prevent or attenuate neuronal cell death following a variety of neurotoxic insults including glutamate excitotoxicity and stroke *(14,15)*. Importantly, in a stroke model of injury, estrogen priming of 1 wk was required for protection where estrogen administration at the time of injury did not prevent cell death *(14)*. This suggests that estrogen acts in a genomic

fashion as a long-term trophic factor that promotes neuronal cell survival. Additional in vitro studies also document the trophic and protective effects of estrogen. For instance, in organotypic explant cultures of cortical neurons, estrogen increases neurite outgrowth *(16)*, suggesting a role for estrogen in regulating neuronal growth responses. In other studies, pharmacological doses of estrogen are shown to protect isolated neurons against oxidative damage and beta-amyloid toxicity *(17)*, with the high levels of estrogen needed for these effects suggesting a free-radical-scavenger role for estrogen. Lower, more physiologically relevant doses of estrogen have been shown to protect isolated primary neurons against glutamate excitotoxicity in a receptor-dependent fashion *(18)*. The in vitro data correlate well with the growth-promoting and protective actions of estrogen observed in vivo and begin to shed light on the mechanisms of estrogen action in the brain.

Estrogen Receptors and Signaling

The estrogen receptor (ER) is a member of the steroid hormone receptor superfamily. Steroid hormone receptors are intracellular receptors, which, when activated by ligand binding, translocate to the nucleus and act as transcription factors by binding to DNA in the promoter regions of target genes. There are currently two predominant forms of the estrogen receptor, termed ER-alpha and ER-beta *(19,20)*, both of which are expressed in the CNS. ER-alpha is predominantly found in areas of the brain responsible for reproduction, including the hypothalamus *(21)*, while ER-beta is the predominant subtype in areas of the brain associated with emotion and cognition, including the hippocampus and cortex *(21)*. While there are areas of overlapping expression, the differential patterns of expression suggest distinctive functions of the two receptors. Estrogen receptors are found in neurons and glia, indicating that estrogen's protective actions on the brain may involve effects on multiple cell types *(22–24)*.

Both ER-alpha and ER-beta bind 17β estradiol (the primary biologically active estrogen) with similar affinity (0.1–1 n*M*) and act as transcription factors to regulate gene expression *(20,25)*. ER-alpha and ER-beta bind to the same DNA response element, but significant differences exist between the alpha and beta subtypes in the transactivating domain of the protein *(26)*. This suggests that these two receptor subtypes interact differently with other factors of the transcriptional machinery. More specifically, it has been suggested that ER-alpha and ER-beta likely activate different genes under different physiological conditions, based on specific promoter context and the presence/absence of other transactivating factors and co-factors *(27)*. In addition to the "classical" genomic actions of estrogen receptors, rapid effects of estrogen can be triggered by estrogen receptors at the plasma membrane or in the cytoplasm *(28)*. For example, estradiol has been shown to increase cAMP levels in breast cancer cells *(29)*, modulate neuronal calcium and kainate *(30)* currents, and increase phosphorylation of the cAMP response element in rat brain *(31)*. Indeed, published data from our labs has documented that estrogen can alter neuronal anti-apoptotic signaling pathways in response to toxic stimuli in a receptor-dependent manner *(32, 33)*. Additional studies demonstrate the important role of activated p42/44 MAP kinase pathways in estrogen-mediated neuroprotection *(34–36)*. Specifically, activation of ERK1/2 has been shown in neurons following 5 min of estrogen treatment, and this activation of MAPK is required for neuroprotection *(37)*. While activation of the ERK pathway is thought to be ER-dependent, ERK1/2 phosphorylation can be mediated by a newly identified third estrogen receptor subtype, ER-X *(38)*. Both ER-alpha and ER-X have been identified in complexes at the cell surface *(38,39)*. Although the exact mechanism(s) whereby estrogen receptors trigger intracellular signal-transduction pathways remains to be determined; taken as a whole, available data emphasize the potentially very important role of estrogen in maintenance of CNS homeostasis.

HIV Infection and NeuroAIDS

HIV-1 Infection and Replication

The human immunodeficiency virus (HIV) is a lentivirus belonging to the retrovirus family. HIV is identified as the primary etiologic agent of acquired immunodeficiency syndrome (AIDS), which is characterized by a profound decrease in the number of CD4+ T cells, severe immune dysfunction, opportunistic infections, and proliferative diseases *(40)*. The HIV type 1 (HIV-1) life cycle begins with attachment of the virus to cell surface receptors (CD4 and CCR5/CXCR4), followed by fusion with the target cell membrane. Following cell entry, viral RNA is reverse transcribed into DNA and then usually stably integrated into the host genome, although episomal infections can also occur. Replication of HIV-1 is largely controlled transcriptionally, regulated by viral and host proteins at the long terminal repeat (LTR) region of the HIV promoter *(41–43)*. The LTR, present at both ends of the viral genome, contains *cis*-acting elements necessary for transcription initiation from the 5' LTR and for polyadenylation of the viral transcripts in the 3' LTR. The 5' LTR is divided into three regions—U3, R and U5—and numerous host and viral transcription factor binding sites in these regions have been identified, including multiple sites for NFκB, Sp-1, and the ligand-binding protein 1 (LBP-1) *(44)*. The R region interacts with the transactivation response element (TAR), a prominent RNA structure present at the 5' end of all viral transcripts that recruits and binds the viral activator Tat, a non-structural, non-glycosylated viral protein. Tat promotes efficient transcriptional elongation via LTR "*trans*-activation," resulting in a several hundred–fold increase in transcription *(42)*. Because Tat is one of the earliest proteins released during the HIV-1 life cycle, and is such a potent stimulator the LTR, the

suppression of Tat's effects on viral replication could be a significant mechanism to control HIV-1 infection.

In addition to viral Tat proteins, transcriptional regulation of HIV-1 gene expression is controlled by cooperative and cell-specific interactions between Tat and several host-cell transcription factors, including AP-1, NFκB, NF-AT, NF-IL-6, CREB, IRF, Sp1, LEF-1/TCF-1a, Ets-1, and USF *(41–43)*. Studies using siRNA to block pertinent host transcription factors, such as the NFκB p65 subunit, document a decrease of HIV-1 replication in MAGI and Jurkat cells *(45)*. However, NFκB proteins, like many of the transcription factors listed above, are also critical for the regulation of host cellular functions and therefore siRNA-therapies directed at host factor-based HIV-1 transcriptional regulation are inappropriate clinical interventions. Interestingly, estrogen can regulate several of the above transcription factors and ERs have been shown to interact with several of the others, suggesting that estrogen may be able to alter the transcriptional activity of HIV-1 (see below).

NeuroAIDS and HIV-1 Dementia

The CNS is susceptible to infection by various retroviruses and lentiviruses, in particular. Before the widespread use of highly active antiretroviral therapy (HAART), 20–30% of HIV-1 positive individuals developed a slowly progressing, dementing illness associated with cognitive and motor symptoms, including impaired short-term memory, reduced concentration, and ataxia *(46)*. Collectively known as HIV-associated dementia (HIVD), these symptoms were also often associated with behavioral personality abnormalities, including apathy, social isolation, decreased job performance, and limited self-care dependence *(47,48)*. HIVD generally occurs in the context of advanced immunosupression, but may present as the initial clinical manifestation of AIDS, particularly in pediatric cases, where it causes developmental delay and loss of motor and intellectual milestones *(49,50)*.

The gross pathology of HIVD generally includes sulcal widening and ventricular dilation *(51)*, while histopathologically, the lesions are characterized by microglial nodules with multinucleated giant cells, myelin pallor, astrocytosis, and neuronal loss *(52,53)*. Neuropathological changes have been reported in the basal ganglia and also in the hippocampus, in which neuronal losses of 50–90% are observed in interneuron *(53)* as well as the pyramidal and non-pyramidal cell populations *(54,55)*. Additionally, Golgi analysis of the frontal cortex demonstrates a 40% loss of dendrites and a 40–60% loss of spine density along apical dendrites of large pyramidal neurons *(56,57)*. The neuropathological changes in the brain correlate loosely with the variable distribution of viral load, which is greater in the hippocampus and basal ganglia as compared to other regions of the brain *(58)*. The mechanisms, however, by which the virus leads to dementia are still not understood.

Despite such profound changes in neuronal morphology and function found in many AIDS brains, the HIV-1 virus does not infect neurons. In the perivascular region of the brain, several cell types including endothelial cells, astrocytes, and microglia come into direct contact with virus-infected cells that have entered the CNS from the blood. Of these, microglia may be the most important in CNS HIV infection as these are the predominant resident cell type permissive for HIV-1 infection, although astrocytes are also thought to carry a non-productive infection. Microglia cells are members of the monocyte/macrophage family and are the brain-resident tissue macrophage *(59)*. While the function of resting microglia is largely unknown, these cells rapidly transform to an activated state in response to brain infection or injury, and extensive microglial activation is both a characteristic feature of HIVD brains and a good correlate of dementia in AIDS patients *(60,61)*. Activated microglia are potent sources of cytokines, reactive oxygen and nitrogen intermediates, and excitotoxins *(62)*, and hence one can hypothesize that aberrant microglial activation in response to the presence of HIV-1 could cause release of free radicals and other neurotoxic substances, initiating a cascade of events leading to the behavioral neuropathological features of HIV dementia. Indeed, there is wide support for the theory that HIVD is caused by aberrant microglial activation, and, accordingly, endogenous or exogenous agents that modulate cell-mediated immunity in the brain could alter the development of HIVD in AIDS patients (see below). While the role of astrocytes in CNS HIV-1 infections is not as clear, chronic infection of astrocytes could contribute to HIVD via changes in the support environment for neurons or through the release of inflammatory and neurotoxic viral proteins such as Tat and/or gp120.

HIV Infection and HIVD in Women

HIV infection/AIDS in the United States was initially a disease of gay men, and hence most early research cohorts in the United States were primarily composed of males. However, epidemiologic patterns have shifted and HIV infection is now a major cause of death for reproductive-age women. As more women have been infected with HIV, the concern that women may be subject to different disease progression has grown. While very few studies have had adequate numbers of women to sufficiently address the unique characteristics of HIV in women, key differences between genders are known. For instance, women with HIV infection have a lower viral load titer, a higher CD4 cell count at seroconversion, altered progression during pregnancy, and may be more prone to develop lipodystrophy *(63)*. Women also have about a 60% higher risk of developing AIDS with the same viral load and CD4+ count than men *(64)*, and also have a higher CD4+ count at seroconversion and at death *(65,66)*. These observations are important not only because treatment and prognosis are based on viral titers and CD4 count at seroconversion, but these findings also suggest that HIV-1 infection in women must be regulated by host factors differently than in men. It is possible that hormonal

changes in women play a role in HIV susceptibility and their immune response to the infection *(67)*.

The role of gender in the development of AIDS-related diseases, including HIVD, is still not clear. There is some evidence suggesting that HIV-positive women are more susceptible to HIVD than HIV-positive men. For instance, in a multicenter European study of AIDS dementia complex, the records of 6548 adult patients (male = 5973; female = 626) with AIDS (diagnosed between 1979 and 1989) were analyzed, and it was noted that twice the numbers of women (8.9%) developed HIV dementia compared to men (4.1%) *(68)*. Additional studies have also supported the notion that women are more likely to develop neurological complications of AIDS than are men *(69–72)*. There is, however, some controversy over the role of gender and hormones in HIV/AIDS in that some studies report no gender differences in illnesses such as HIV dementia *(73,74)*. Interestingly, in one study of HIV-infected women, those >40 yr of age had a higher incidence of dementia *(75)* and none of the women diagnosed with dementia were on hormonal replacement therapy, indicating that HIV dementia is associated with low estrogen levels. Despite the retrospective nature of these studies, they suggest that if women are at greater risk for developing HIV dementia, endocrine abnormalities or gonadal failure may be a contributing factor. In support of this scenario, there are data suggesting HIV can adversely affect endocrine function in women. For instance, HIV-infected women are more likely to experience intervals > 6 wk without menstrual bleeding and amenorrhea > 3 mo. Premenstrual breast swelling, tenderness, and dysmenorrhea are less common in HIV-infected women *(76–78)*. Furthermore, in one study that failed to show a difference in circulating hormone levels between HIV-seropositive and high-risk seronegative women, only HIV-positive women with *self-reported regular menstrual cycles* were included *(79)*, suggesting that perhaps this particular population of women was relatively insensitive to the endocrine-manipulating effects of HIV. Thus, it is not surprising that in another study, estradiol levels were lower among HIV subjects with amenorrhea (17.6 ± 21.8 pg/mL) compared to eumenorrheic HIV-positive (48.9 ± 33.6 pg/mL) or control (68.3 ± 47.6 pg/mL) subjects *(80)*. Therefore, it appears that the HIV-1 virus does not affect all women equally, and hence one can hypothesize that in the subset of HIV-infected females with gonadal dysfunction, there may be an increased risk of HIVD caused by the loss of estrogen-mediated protective effects in the brain.

Potential Protective Mechanisms of Estrogen Against CNS HIV-1 Infection

Effects of Estrogen on HIV Replication/viral Load

As mentioned above, many reports document that HIV-1 infected women have significantly lower viral titers than men, especially at early stages of the disease *(64,81–86)*. While the mechanisms of this sex difference are not clear, there is evidence that sex hormones can directly modulate HIV-1 transmission and replication. Many nuclear hormone receptors have been shown to interact with the LTR *(87,88)*, and published reports have demonstrated that estrogen can prevent HIV-1 synthesis and replication in human monocytes *(89,90)*. Additionally, estrogen can block vaginal transmission of SIV in female macaques *(91)*. Therefore, one can speculate that estrogen might attenuate the progression of HIVD by decreasing brain viral load. While the molecular mechanisms of estrogen's actions on HIV synthesis and replication are not known, recent data from our labs suggest that estrogen can suppress Tat-enhanced transcription from the HIVIIIB LTR in astrocyte cell lines *(92)*. Specifically, our data show that physiological doses of estrogen can significantly block LTR activity induced by Tat, but not by PMA, in an ER-alpha-dependent manner. These studies highlight a novel and potentially very important mechanism whereby estrogen could modulate the development of HIVD. Indeed, as HAART therapies have only limited access to the brain, the potential ability of estrogen to decrease CNS viral load is made all the more critical clinically.

Effects of Estrogen on Neuronal Injury

Chronic HIV infection in the CNS is in many cases associated with widespread neuronal injury and synaptic loss (see above). Although the mechanisms of HIV-related neuronal loss are not completely clear, the HIV proteins Tat and gp120 have been shown to directly induce injury in neurons *(93–96)*. Estrogen is generally considered neuroprotective, and indeed, several studies from our labs and others have since shown that estrogen can specifically attenuate or prevent neurotoxicity induced by HIV viral proteins. For instance, estrogen has been shown to protect isolated human fetal neurons from toxicity caused by Tat in a receptor-dependent fashion *(97,98)*. Estrogen has also been shown to prevent the neurotoxicity induced by gp120 *(99,100)*. The mechanism whereby estrogen prevents Tat- and gp120-induced neurotoxicity is not known, but is thought to perhaps involve the antioxidant properties of estrogen, as well as modulation of microglial activation *(101,102)*.

As most published data on estrogen and HIV-related neurotoxicity were based on acute estrogen exposure in primary cell culture models, we sought to investigate the potential mechanisms whereby estrogen suppresses apoptosis in neurons in a more physiological setting. Specifically, we measured the effects of chronic estrogen treatment using organotypic explant cultures. Organotypic cortical slice cultures allow for long-term (weeks to months) preservation of intrinsic anatomical connections, neuronal cytoarchitecture, electrophysiological properties, and appropriate neuron/glia interactions *(103–105)*. Using this system, we measured the effects of long-term estrogen exposure on the dynamics of the MAPK pathway activation following ischemic injury. Data show that a 7-d treatment with physiological levels of

estrogen (1 n*M* 17β-estradiol) alters the activation patterns of ERK1/2, JNK, and p38 following injury (Fig. 1). Total cellular extracts were collected in uninjured controls and at various times following injury, and phosphorylation of the MAP kinases, ERK, JNK, and p38, were monitored by Western blot analysis. ERK phosphorylation was not altered by estrogen in the absence of injury (Fig. 1A). ERK phosphorylation was significantly greater in estrogen-treated explants than vehicle at 0 and 30 min ($p < 0.05$) suggesting a more rapid response following the initiation of injury. The same protein extracts were also used to examine JNK and p38 activation. Again, estrogen did not have any effect on JNK activation in the absence of injury (Fig. 1B). Although there is a trend toward a suppression of pJNK at 30 min by estrogen, the interaction did not reach significance ($p = 0.085$). Finally, activated p38 was increased by estrogen treatment alone ($p < 0.05$) (Fig. 1C). Estrogen treatment significantly elevated p38 levels at 0 h and suppressed at 0.5 h ($p < 0.05$). Furthermore, blocking the activation of ERK and p38 with specific kinase inhibitors PD98059 and SB203580, respectively, prevented the neuroprotection by estrogen, suggesting that regulation of MAP kinase signaling is necessary for the protective effects of estrogen.

To extend these studies of estrogen-mediated neuroprotection to include models of HIVD, we investigated estrogen and Tat interactions in the same organotypic explant culture model. Cortical explants were incubated with increasing concentrations of recombinant Tat protein, and cell death was monitored by propidium iodide (PI) uptake following 48 h of exposure. Quantification of neuronal injury (PI-positive cells) was achieved by fluorescent microscopy, and revealed that Tat exposure induced neuronal cell death in a dose-dependent manner (Fig. 2A). To assess the ability of estrogen to inhibit cell death induced by Tat administration, cortical explant cultures were incubated for 7 d with physiological levels (1 or 10 n*M*) of 17β-estradiol prior to administration of Tat. Quantification of PI-uptake in cortical explants demonstrated that 17β-estradiol pretreatment significantly attenuated Tat-induced neuronal injury in organotypic cortical slices (Fig. 2B). Thus, these data support a therapeutic role for estrogen in preventing neuronal injury and loss in HIVD. Importantly, plant estrogens have similar neuroprotective effects against HIV proteins *(106)*, which may represent a more practical alternative in individuals in which estradiol may be contraindicated.

Effects of Estrogen on Brain Inflammation

While estrogen can act directly on neurons to promote their survival and function, it is also likely that the neuroprotective properties of estrogen in brain injury are mediated in part through modulation of injury-induced immune responses. As discussed above, aberrant brain inflammation is thought to participate in HIVD, and, accordingly, the effects of estrogen on microglial activation may be especially relevant to HIVD. Estrogen modulation of immune cells

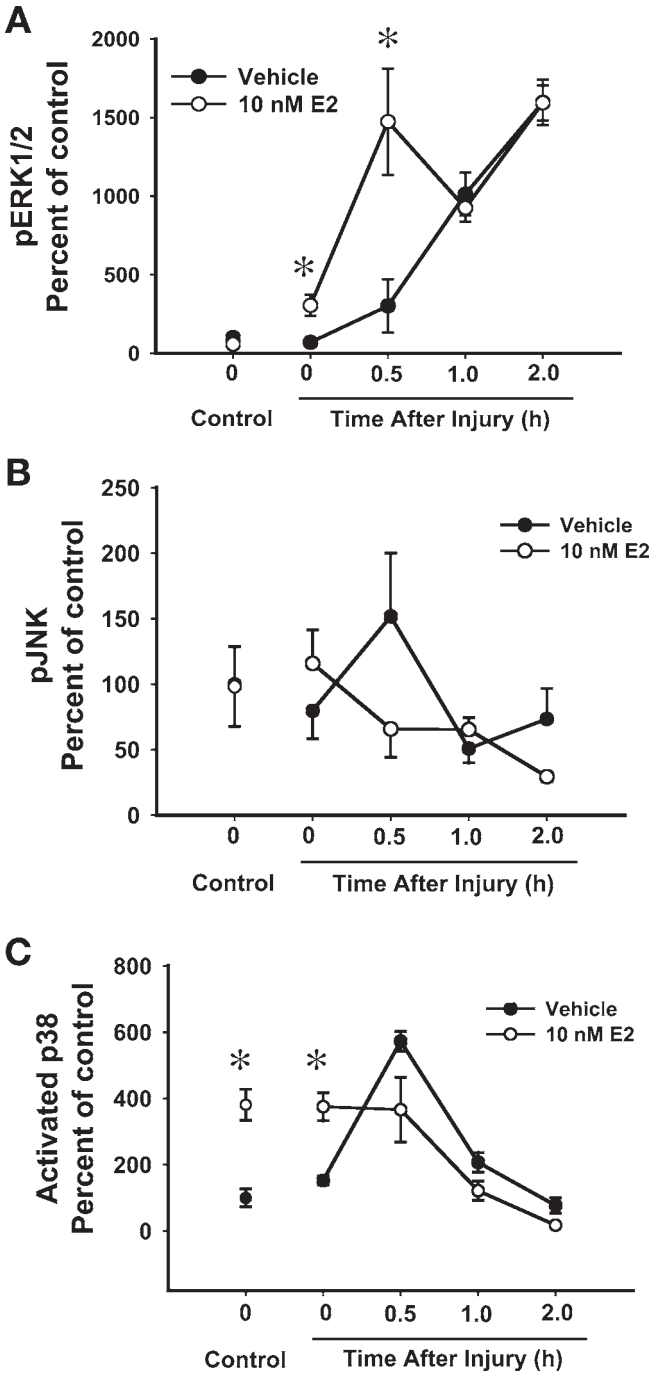

Fig. 1. Chronic 17β-estradiol (E2) treatment alters MAPK responses to neurotoxic insults. Organotypic cortical slice cultures were prepared from 5-d-old rats and then treated physiological levels of estrogen (1 n*M* 17β-estradiol) or vehicle (0.01% ethanol) for 7 d. Explants were then exposed to ischemic conditions (2 m*M* 2-deoxyglucose/1 m*M* potassium cyanide) for 2 h, after which MAPK activation was evaluated by Western blot analysis at various times up to 2 h after the end of the ischemic episode. The optical density of bands specific for phosphorylated ERK1/2 (**A**), pJNK (**B**), and activated p38 (**C**) was quantified with NIH Image v. 6.1. Data represent the means ± SEM, $n = 3$, and * indicates a significant increases ($p < 0.05$) in MAPK phosphorylation in estrogen-treated slices.

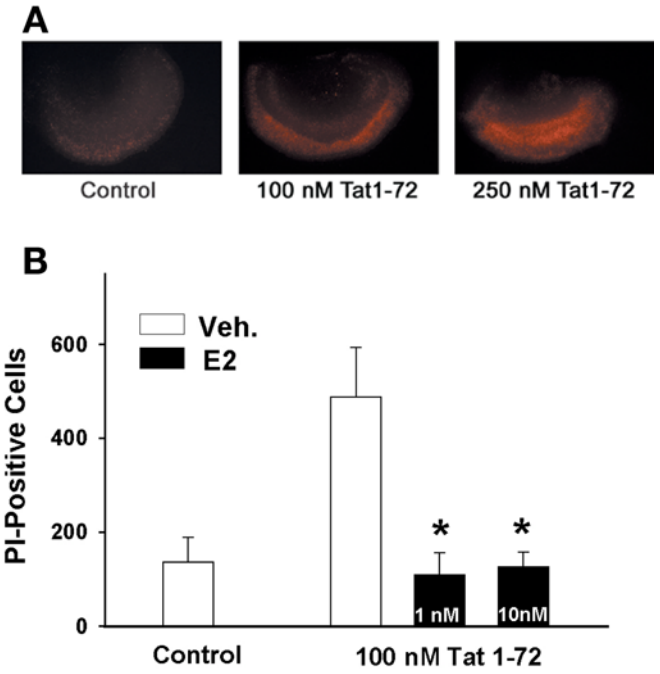

Fig. 2. 17β-estradiol (E2) pretreatment prevents cell death induced by $Tat_{1–72}$. Organotypic cortical explants were prepared from 5-d-old rats, and allowed to recover for 7 d before treatment. (**A**) Slices were then incubated with increasing concentrations of recombinant Tat protein, and cell death was monitored by propidium iodide uptake following 48 h of exposure. (**B**) Cortical explants were treated with 1 n*M* estrogen for 7 d prior to exposure to 100 n*M* $Tat_{1–72}$ for 48 h. Quantification of neuronal injury (PI-positive cells) was achieved by counting PI-positive cells by a researcher blinded to the experiment. Data represent the means ± SEM, $n = 3$, and * indicates = significantly attenuated PI uptake in slices pretreated with estrogen.

and its influence on the immune response has been investigated and described both in vitro and in vivo. In experimental studies in animals, estrogen has been shown to influence many different types of cells, including B cells, T cells, macrophages, NK cells, eosinophils, stromal cells, and endothelial cells (reviewed in ref. *107*). The in vivo effects of estrogen on immune parameters include altered cytokine production, cell differentiation, and expression of adhesion molecules, and there is evidence that estrogen increases Th2 type cytokines *(108,109)*, and accordingly decreases cell-based inflammation. In terms of local brain inflammation, this pattern would suggest a decrease in the expression of pro-inflammatory cytokines and a decrease in cell-mediated immunity, i.e., decreased microglial activation. Indeed, cell culture studies from our labs and others have shown that physiological levels of 17β-estradiol significantly decrease in vitro microglial activation in response to immune stimuli *(110–112)*. With specific relation to HIVD, we have previously shown that estrogen treatment significantly decreases several neurotoxic parameters of microglial activation induced by the HIV protein Tat, including free-radical release, phagocytic activity, and cytokine release *(113)*. While all of these parameters could participate in neuronal injury, cytokine release may be especially important, as many investigators have hypothesized that the cytokine TNF plays a critical role in HIV-related brain inflammation. Specifically, TNF levels in the CSF and brain correlate with the severity of HIV dementia *(114–116)*, and postmenopausal women have elevated levels of IL-1β and TNF, which can be reversed by hormone replacement therapy.

In addition to direct effects on microglial activation, estrogen could also affect brain inflammation via modulation of leukocyte recruitment and/or microglia/leukocyte interactions. Indeed, recent data from our labs and others have documented that estrogen can significantly decrease the expression of a variety of components of microglial/leukocyte dialog, including MHC class I and II, CD40, and CD152 *(117–119)*. In addition to modifying microglia/leukocyte interactions, estrogen could also affect the infiltration of peripheral immune cells into the CNS, as recent data have demonstrated that estrogen decreases Tat-induced expression of the chemokine MCP-1 in endothelial cells *(120)*. While some trafficking of leukocytes into the CNS occurs in the absence of pathology, changes in the integrity of the blood–brain barrier (BBB) are thought to greatly facilitate this process. For example, in experimental allergic encephalomyelitis, a correlation among inflammatory cells in the CNS, BBB breakdown, and clinical course has been observed *(121,122)*. In specific relation to HIVD, histological evidence suggests that the BBB is deranged in association with HIV and/or HIV dementia *(123,124)*. For instance, immunoreactivity for BBB proteins, including laminin and collagen type IV, is reduced in association with HIV infection, as is the mean thickness of the capillary endothelial cell basal lamina *(125,126)*. Furthermore, CSF to serum albumin ratios increase with the duration of HIV infection and become abnormally high in patients with HIVD *(127)*.

To determine if estrogen might modulate HIV-related alterations to the BBB, we investigated the interactions of estrogen and Tat on the expression of matrix metalloproteinase (MMP) proteins. MMPs belong to a family of structurally similar, zinc-containing endopeptidases and typically act to degrade specific components of the extracellular matrix (ECM) including collagen, entactin, and laminin. These ECM proteins are found in the CNS and their function is critical to proper maintenance of the BBB. While expression of MMP in the brain is generally very low, MMP-2, -3, and -9 have been detected in the CNS. Importantly, it has been reported that levels of MMP-2, -7, and -9 are elevated in the CSF of HIVD patients, MMPs are responsive to Tat treatment in vitro, and antibodies against MMP-2 and MMP-7 have been shown prevent Tat toxicity in vitro *(128–131)*. While estrogen has been shown to regulate MMP expression in a variety of non-CNS cell types *(132–134)*, the effects of estrogen on MMP release from glial cells has received only limited attention *(110)*. To determine if anti-inflammatory regimens of estrogen could modulate Tat-induced MMP

Fig. 3. 17β-estradiol decreases MMP-9 release from glial cells. Cultured human glial cells were treated with 1 n*M* 17β-estradiol or vehicle for 24 h, and then exposed to 200 n*M* $Tat_{1–72}$ or saline (Ctrl) for an additional 24 h, after which the medium was harvested and probed for MMP activity by zymography. Comparison of bands with MMP standards (Std) indicated specific production of MMP-9 zymogen (105 kDa). Data are representative of two separate experiments.

production in CNS immunocompetent cells, mixed human glial (astrocyte and microglial) cultures were treated with 1 n*M* 17β-estradiol overnight, and then exposed to 100 n*M* Tat for an additional 24 h. The medium was then collected, and MMP activity in the medium was determined by zymography as described in previous reports *(135)*. Images of zymograms were digitized, and examination of images revealed that pro-inflammatory doses of Tat clearly increased MMP-9 release into the medium, and that estrogen treatment markedly reduced MMP release (Fig. 3). Collectively, these data indicate that estrogen could affect multiple aspects of local brain inflammation. Thus, it is reasonable to hypothesize that maintenance of serum estradiol levels in AIDS patients could attenuate the development of HIVD through a variety of mechanisms, including direct actions on CNS HIV-1 replication and viral load, as well as indirect actions aimed at bolstering neuronal resistance to injury and attenuating HIV-related brain inflammation.

Conclusions

Unfortunately, the complex and likely reciprocal relationships between female sex steroids and the progression of AIDS are not at all understood. With specific reference to HIVD, while there appear to be only limited observable gender differences in the development of HIVD, there exist both epidemiological and research data supporting a protective role for estrogens in this process. Specifically, the published associations of HIV/AIDS with gonadal dysfunction *(76–78)*, and the inverse relationship found between estrogen levels and HIVD in HIV-1-positive women *(75)* clearly suggest a link between low estrogen levels and HIVD in female AIDS patients. In support of this scenario, basic research data have repeatedly shown that estrogen can provide trophic support for neurons and glial cells (reviewed in refs. *1* and *136*), and can also play an essential role in modulating brain inflammatory responses (reviewed in refs. *2* and *137*), both of which are likely to play key roles in the development/progression of HIVD.

It is important to note, however, that the clinical benefits of estrogen's actions in CNS infection might vary based on the type of infection. For instance, in diseases such as HIVD in which aberrant and excessive microglial activation is thought to play a critical role, the overall anti-inflammatory properties of estrogen may be of considerable clinical benefit. Accordingly, there is evidence that estrogen can be of clinical benefit in other neuroinflammatory diseases such as Alzheimer's disease and multiple sclerosis *(6–8,138–140)*. These potential benefits are in keeping with the known anti-inflammatory properties of estrogen, which are understood as decreasing cell-mediated immunity and attenuating Th1 cytokine responses *(2,137)*. However, estrogen-mediated alterations in innate immune responses have also been shown paradoxically to be important in neurological conditions typified by the presence of invading pathogens. For instance, animal studies have shown that ablation of circulating hormones through ovariectomy renders mice unable to respond to intraparencymal LPS injections with appropriate Th1 cytokine responses *(141)*. Furthermore, when such mice were inoculated intranasally with the neurotrophic virus HSV-1, they were subject to widespread viral replication and neurodegeneration that could be completely prevented by estrogen supplementation *(141)*. In other studies, estrogen manipulation has been shown to alter the anatomical course of pseudorabies infection in the CNS of rats *(142)*. Taken together, these data strongly suggest that estrogen may be required for the proper immune response to bacterial and viral pathogens in the brain and support a very important role for estrogen in the physiologically appropriate transfer from innate to adaptive immunity in the CNS. Thus, available data clearly support continued research into the potentially therapeutic role for estrogen and estrogen-like compounds in attenuating brain inflammation and neuronal dysfunction in chronic diseases and/or infections of the CNS.

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