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The Impact of HIV TAT and gp120 in Neuroinflammatory Response During HIV Active Infection

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Abstract: Despite the fact that acquired immune deficiency syndrome (AIDS) has been treated effectively by highly active anti-retroviral therapy (HAART), the incidence of HIV associated neurocognitive disorders is still high due to the inability of most HAART to pass through the blood-brain barrier (BBB) and target the virus. Neurocognitive disorder results from an inflammatory cascade in the brain. It is thought that the HIV-1 transactivating protein (TAT) alone or in combination with the major virus envelope glycoprotein gp120 trigger glial cells to secret chemokines that elicit the influx of more immunocytes and results in inflammatory amplification. To test this hypothesis, co-cultures of microglia and astrocytes were incubated with TAT, with gp120, and with TAT plus gp120. Chemokine expression then was estimated using an immunodot blot array. Macrophage chemotactic protein (MCP1) and TIMP metallopeptidase 1 (TIMP1), a tissue inhibitor of metalloproteinases, were present in the three coculture test groups. In addition to MCP1 and TIMP1, interleukin-8 (IL-8) was detected following coculture with TAT plus gp120. These results implicate the role of HIV- TAT and gp120 in activating brain glial cells to evoke an inflammatory response.

Keywords: HIV, gp120, HAART, TAT, TIMP1.

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Introduction:

HIV-associated neurocognitive disorders (HANDs) occur when the HIV-1 enters and infects the central nervous system (CNS). There are three cognitive conditions associated with HIV-1: Asymptomatic neurocognitive disorder, Mild neurocognitive disorder and HIV-associated dementia(1). Although Highly Active Anti-Retroviral Therapy (HAART) diminishes the viral load in patients to undetectable levels, HANDs are still the challenge since most components of HAART do not pass through the blood-brain barrier. Furthermore, low-level viremia persists in patients under HAART(2). This viremia results from latent HIV-1 reservoirs that formed early in viral infection(3). Mechanisms by which HIV-1 causes HANDs at the molecular level are undefined. Several studies have focused on explaining the role of microglial cells (resident macrophage-like cells in the CNS) and astrocytes in many brain disorders including HANDs(4,5). Microglial cells and perivascular macrophages are active during advanced stages of HANDs and appear as multi-nucleate giant cells and support HIV productive infection(6). It is hypothesized that HIV TAT (Trans-Activating Transcription protein) alone or in combination with gp120 triggers microglia cell and astrocyte signaling

pathways that lead to the production of chemokines and cytokines. These inflammatory factors, in turn, are secreted from glial cells, attract more immune cells from the peripheral circulation, and amplify the inflammatory response in CNS leading to expansive neuropathology(7,8).

Materials and methods:

SV40-immortalized human brain microglial cells (cat.No.T0251, Applied Biological Materials, Inc., Richmond, BC, Canada) and human brain astrocytes (SVGp12, ATCC CRL-8621, ATCC Manassas, VA) were cultured in 125 cm^2 tissue culture flasks in complete Dulbecco's Modified Eagle Medium (DMEM). Cells were examined microscopically to ensure their integrity. When cells reached 75% confluency, cocultures were split and passaged. Microglial cells and astrocytes (1×10^6) were cocultured at a 1:5 cell ratio to mimic the ratio identified in normal human brain. TAT (50nM) and gp120(20nM) were added to coculture of microglial cells and astrocytes as follows: (1) coculture with 0.01% ethanol was used as a vehicle control; (2) coculture in the presence of vehicle with TAT (50nM), with gp120 (20nM), or with TAT(50nM) plus gp120(20nM). After 24 hours incubation, cells were removed and the culture media were harvested to assess for the presence of chemokines or cytokines. A human membrane array (RayBiotech mouse cytokine antibody array III; RayBiotech, Inc., Norcross, GA) was used. Membranes were processed as recommended by the manufacturer and were exposed to X-ray film. Spot intensities were quantified by using The Quantity-1 computer software program.

Statistics: Density of each spot was calibrated against a standard included in each membrane array (individual membranes) then was subjected to comparison to asses four treatment variables. Fischer test was applied to identify significance between variables, grade of 2.1 or more consider significant.

Results:

1. Microglial cells and astrocytes were cultured, Image 1 after 24 hrs of incubation in 10% CO_2.

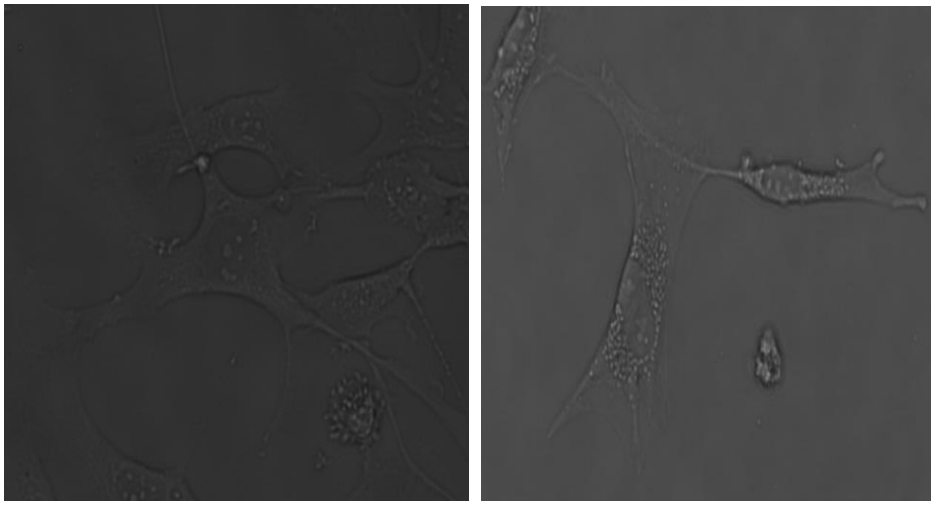


Figure (1): Microglial cells and astrocytes under light microscope 4X.

2. Control group: microglial cells and astrocytes are treated with 0.01% ethanol as a vehicle. Immunoblot array showed chemokines and cytokines that identified by numbers, (Figure 2). Spot densities were evaluated using Quantity-1 software. The density of each spot was calibrated against a standard included in each membrane array.

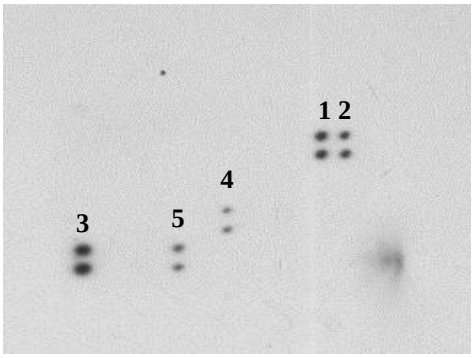


Figure (2): Control group consist of cells treated with 0.01% ethanol. 1and 2 are positive controls, 3= negative control, 4= TIMP1 and 5= MCP1.

3. Group1: Coculture of microglial cells and astrocytes were treated with HIV TAT (50 nM), (Figure 3).

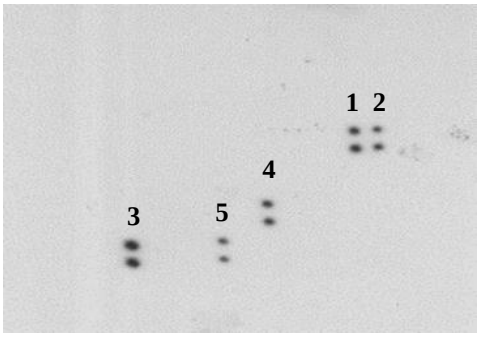


Figure (3): Group 1, Coculture of cells were mixed with TAT protein. 1and 2 are positive controls, 3= negative control, 4= TIMP1 and 5= MCP1. 4and 5 spots appeared more dense compare to control group.

4. Group 2: gp120 (20nM) was added to coculture of cells. Same chemokines were appeared as group 1, (Figure 4).

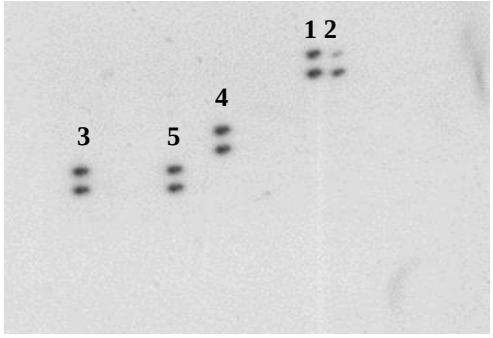


Figure (4): Group 2, 1and 2 are positive controls, 3= negative control, 4= TIMP1 and 5= MCP1. spots appeared denser than in group 1.

5. Group 3: Coculture of cells were treated with both TAT protein (50Nm) AND gp120 (20nM). Interleukin8 was expressed (spot 6), (Figure 5).

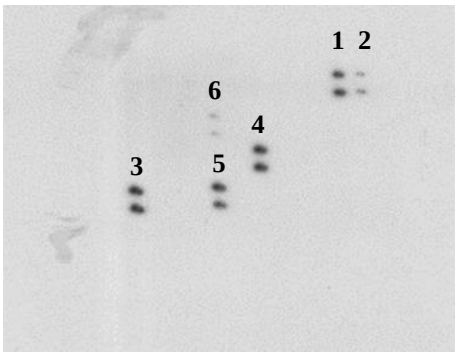


Figure (5): Additional chemokine was expressed upon cells treatment with both HIV proteins. 1and 2 are positive controls, 3= negative control, 4= TIMP1 and 5= MCP1.

6. Comparison of spots densities of group 1,2 and 3 with control group, fold increases of chemokines for each group was observed. * means significant increase. Image 6,7and 8.

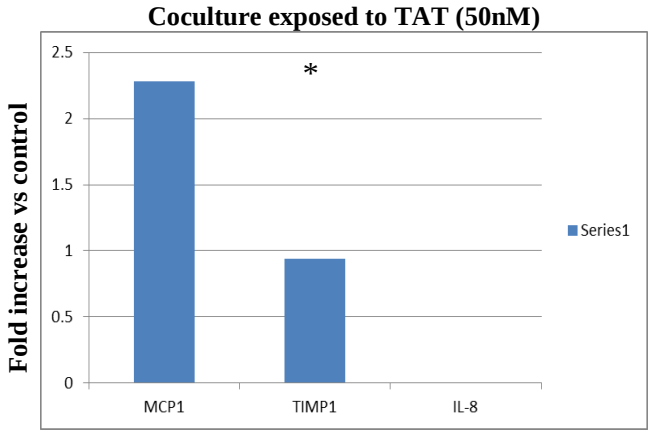
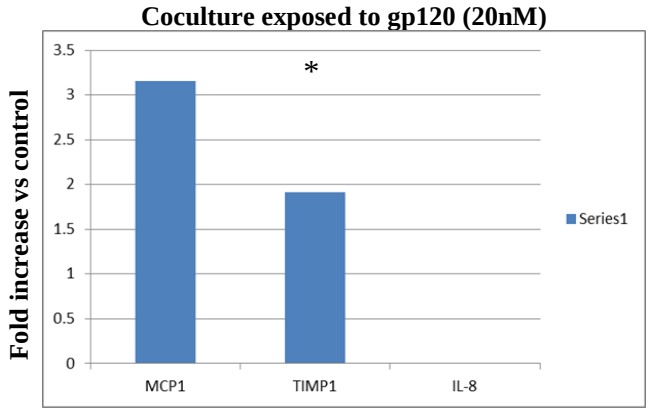
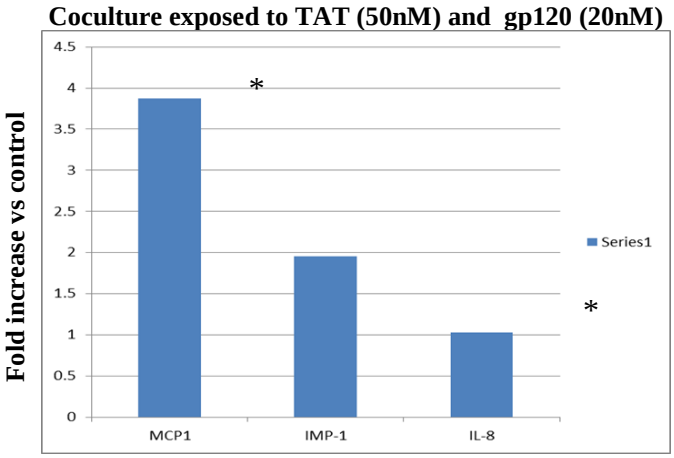


Figure (6): Coculture of microglial cell and astrocytes exposed to TAT (50nM).



Figure(7): Coculture of microglial cell and astrocytes exposed to TAT (20 nM).



Figure(8): Cocultured of microglial cell and astrocytes exposed to TAT (50 nM) and gp 120 (20 nM).

Discussion:

HIV dementia and encephalitis have been linked to the activation of microglia and infiltration of monocytes(9). Microglia are resident macrophage in the brain that maintain HIV productive infection while astrocytes constitute the majority of brain cells(10). Studies shows that both HIV TAT and gp120 proteins are contributed to neurons inflammation through induction of chemokines production(11). TAT is a transcription enhancer which increases HIV production by more than 100 folds. Transgenic mice which express TAT protein in their astrocytes have developed brain inflammation similar to those seen in people with AIDS(12,13). TAT can infect microglia, astrocytes and other cells including monocytes, promoting them to produce several chemokines and cytokines(14). Also, HIV TAT disrupt blood brain barriers (BBB) by affecting the tight junction of microvascular endothelial cells(15). In the current study, results showed that HIV TAT activate microglia and astrocytes to produce MCP1, a monocyte chemoattractant protein. MCP1 is a proinflammatory mediator that has been observed in many CNS diseases(16). Also, studies showed its ability to compromise the integrity of BBB by affecting proteins of tight junctions belong to microvascular endothelial cells(8). our study results proved that both HIV proteins activate brain cells to produce TIMP-1, TIMPS is a tissue inhibitors of metalloproteases (MMPs) including disintegrin-metalloproteinases, thus TIMPS are regulators of extracellular matrix (ECM) turnover and remodeling(17). Imbalance between plasma and CSF levels of TIMPS/MMPs has been associated with HIV-1 neurocognitive disorders(18). The role of gp120 in developing HANDS are contributed to its ability in increasing IL8 production(19). Gp120 is a glycoprotein which play a role in HIV attachment and entry, besides its role in astrocytes activation to produce IL8 through enhancing NF- kB pathway(20). IL8 is a chemokine that has been seen in brain injury, also its role in disrupting BBB(21). The findings of our study are strongly support the role of HIV TAT and gp120 in developing HANDs through activation an inflammatory cascade.

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