Roles for HIV strain, accessory proteins, and cytokines in macrophage HO-1 deficiency

Alexander J. Gill[1]. Patricia, J. Vance[1], Ronald G. Collman[2], Dennis L. Kolson[1]

Departments of Neurology[1] and Medicine[2], Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Contact
Alexander Gill
Perelman School of Medicine
University of Pennsylvania
Clinical Research Building, Room 280
Phone: 215-573-3504
agill@mail.med.upenn.edu

ABSTRACT

Background: Protein expression of the cytoprotective enzyme heme oxygenase-1 (HO-1) is reduced in the prefrontal cortex of HIV+ individuals with HAND and negatively correlates with CNS viral replication and neuroinflammation. HIV infection of monocyte-derived macrophages (MDM) reduces HO-1 protein expression and pharmacologic rescue of HO-1 expression ameliorates HIV-MDM neurotoxin production. We now present data describing the change in HO-1 expression and neurotoxicity in MDM infected with 13 distinct HIV strains and the role of HIV accessory proteins and cytokine signaling in HIV-mediated HO-1 loss.

Methods: Using 13 macrophage-tropic HIV-1 strains, we infected MDM and determined the association between viral replication, glutamate release, supernatant neurotoxicity, and HO-1 protein expression as measured by reverse transcriptase (RT) activity, enzymatic assay, neuron-based MAP2 ELISA, and Western blot, respectively, on day 12 post-infection. Data was analyzed by ANOVA with Holm-Sidak post-test or Pearson's correlation.

Results: Most HIV replication variability in MDM was accounted for by viral strain (75.3%, p<0.001), although a significant, but smaller, proportion of variability was attributed to the donor (9.49%, p <0.003; n=5). Among the 13 viral strains, 11 significantly reduced MDM HO-1, but not HO-2, protein expression (p<0.05). This reduced HO-1 protein expression correlated significantly with increased viral replication, supernatant neurotoxicity, and extracellular glutamate (p<0.001). The viral inoculum of a single viral strain (89.6) predictably correlated positively with viral replication and supernatant neurotoxicity and negatively with HO-1 protein expression. Despite altered replication kinetics, infection of MDM with mutant 89.6 HIV molecular clones lacking Nef, Vpr, or Vpu, nonetheless reduced HO-1 protein expression (p<0.05). Chronic exposure (9 days) to the cytokines TNFα, IL-1β, IL-18, IL-23, and GM-CSF also reduced HO-1 protein expression in uninfected MDM (p<0.05).

Conclusion: Infection with most, if not all, macrophage-tropic HIV-1 strains reduce HO-1 protein expression in human macrophages. This reduction of HO-1 protein expression correlates with viral replication and likely augments HIV-MDM neurotoxin production. The HIV accessory proteins Nef, Vpr, and Vpu are not required for HIV-mediated HO-1 deficiency in macrophages. However, chronic inflammatory signaling associated with HIV replication may play a role in HIV-mediated reduction of HO-1.

BACKGROUND

Heme oxygenase-1 (HO-1) is a sentinel, detoxifying enzyme that is induced in response to numerous insults including inflammation and oxidative stress. HO-1 is regulated through the NRF2-dependent antioxidant response element (ARE), which drives the induction of a coalition of antioxidant and detoxifying effector proteins in response to cellular stress and injury. Protective functions of HO-1 have been linked to the enzyme's degradation of heme, a strong pro-oxidant, and the subsequent generation of the anti-inflammatory and anti-oxidative products, carbon monoxide, biliverdin, and bilirubin, although non-enzymatic cytoprotective functions of HO-1 have also been proposed. The potent anti-inflammatory and cytoprotective actions of HO-1 have identified it as a potential therapeutic target in CNS inflammatory and neurodegenerative diseases. Among these diseases, HIV infection is associated with neurodegeneration that is thought to result from effects of persistent inflammation and oxidative stress in both systemic and CNS compartments, which persists in individuals on antiretroviral therapy (ART). Within the CNS, such effects are driven by HIV infection of macrophages and microglia and contribute to the continued prevalence of cognitive, motor, and behavioral deficits collectively known as HIV-associated neurocognitive disorders (HAND), which affect up to 50% of HIV-infected ART-treated individuals. This high prevalence of HAND strongly underscores the need for adjunctive therapies that target the neuropathological processes associated with persistent inflammation and oxidative stress in HIV-infected individuals.

Previously, we have demonstrated a deficiency of HO-1 within the dorsolateral prefrontal cortex of HIV-infected individuals, a deficiency that associated with elevated viral replication within the CSF and brain parenchyma, neuroinflammatory markers, as well as a clinical diagnosis of HAND. We further demonstrated that HIV-1 infection of monocyte-derived macrophages (HIV-MDM) markedly reduced HO-1 protein expression without reduction in the expression of other ARE-regulated proteins (e.g. NQO1, GPX1, FTH, TRXR1). This selective loss of HO-1 was associated with an increase in macrophage neurotoxin production, including glutamate, a HAND-associated neurotoxin. Moreover, pharmacologic induction or genetic derepression of HO-1 reduced HIV-MDM neurotoxin production. In contrast, pharmacologic inhibition or siRNA knockdown of HO-1 enhanced HIV-MDM neurotoxin production. This data suggests that HIV-infection drives HO-1 loss in vivo and in vitro and suggests that HIV-mediated reduction in HO-1 protein expression may be promoting macrophage-mediated neuronal injury in HIV-infected individuals. In this current study, we use our in vitro model of HIV-MDM neurotoxicity to further explore the relationship between HIV infection and replication, HO-1 loss, and neurotoxin production.

RESULTS

Variability of HIV-1 replication in macrophages depends upon viral strain and macrophage donor



Figure 1: Monocyte-derived macrophages (MDM) isolated from 5 healthy donors were infected with 15 HIV-1 viral strains. **A)** Day 12 supernatant reverse transcriptase (RT) activity stratified by strain. **B)** Representative Western blot of cell-associated HIV-1 p24 on day 12 post infection. **E)** Correlation between day 12 supernatant RT values and cell-associated HIV-1 p24. Error bars indicated mean ± SEM. Statistical comparisons to Mock-MDM were made by two-way ANOVA with Holm-Sidak post hoc test. Correlations were assessed by Pearson's correlation. * p <0.05, ** p <0.01, ***p<0.001

HIV-MDM have elevated supernatant neurotoxicity and glutamate that associate with higher viral replication

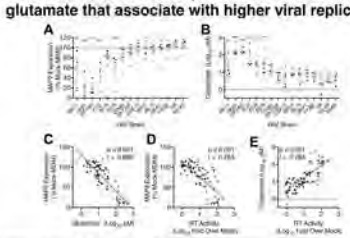

Figure 2: Neurotoxicity in MDM supernatants was assessed by quantification of total microtubule associated protein-2 (MAP2) expression in a cell-based ELISA where primary rat cortical neurons are exposed to HIV-MDM supernatants. Glutamate levels by Amplex Red Glutamate Assay. **A)** Supernatant neurotoxicity and **B)** glutamate levels stratified by strain. **C)** Correlation between supernatant MAP2 ELISA neurotoxicity and glutamate levels. Correlation between supernatant RT activity and supernatant **D)** MAP2 ELISA neurotoxicity and **E)** glutamate levels. Error bars indicate mean ± SEM. Statistical comparisons to Mock-MDM were made by two-way ANOVA with Holm-Sidak post hoc test. Correlations were assessed by Pearson's correlation ** p <0.01, ***p<0.001

Macrophage tropic HIV-1 strains consistently reduce HO-1 protein expression in HIV-MDM

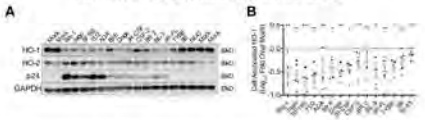

Figure 3: Protein expression was assessed in MDM lysates by Western blot on day 12 post HIV infection with cnb strains. **A)** Representative Western blot of HO-1, HO-2, HIV-1 p24, and GAPDH protein expression. **B)** Western blot densitometry analysis of HO-1 protein expression in all 5 donors stratified by HIV strain. Mock-MDM expression was set to 0 on a log scale (represented by dotted line). Error bars indicate mean ± SEM. Statistical comparisons to Mock-MDM were made by two-way ANOVA with Holm-Sidak post hoc test. * p <0.05, ** p <0.01, ***p<0.001

HO-1 protein deficiency correlates with HIV replication and supernatant glutamate and neurotoxicity

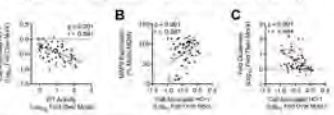

Figure 4: HIV-MDM lysates and supernatants were analyzed on day 12 post HIV infection in MDM from five donors infected with fifteen HIV strains. **A)** Correlation between MDM cell-associated HO-1 protein expression and viral replication. **B)** supernatant neurotoxicity, and **C)** extracellular glutamate. Correlations were assessed by Pearson's correlation.

For an individual HIV-1 strain, inoculum predicts replication, neurotoxicity, and HO-1 protein loss in HIV-MDM

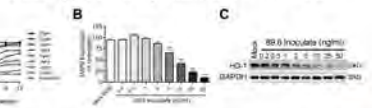

Figure 5: MDM were infected with a wide range of inoculum. Representative data from a single donor infection showing **A)** HIV replication curves as measured by supernatant RT activity , **B)** supernatant neurotoxicity as measured by MAP2 ELISA, and **C)** HO-1 and GAPDH protein expression as measured by Western blot. Statistical comparisons to Mock-MDM were made by one-way ANOVA with Holm-Sidak post hoc test. * p <0.05, ** p <0.01, *** p<0.001

HIV-1 accessory proteins Nef, Vpr, and Vpu are not required for HIV-mediated reduction of HO-1 protein in macrophages

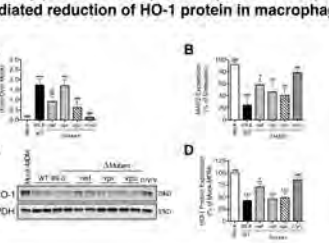

Figure 6: MDM from 5 independent donors were infected with wild-type (WT) 89.6 or molecular clones lacking expression (Δ) of nef, vpr, or vpu singularly or in combination (NVV). Supernatants from day 12 post infection were analyzed for A) HIV-replication as measured by supernatant RT activity and B) supernatant neurotoxicity as measured by MAP2 ELISA. Protein lysates from day 12 post infection were analyzed by HO-1 expression (normalized to GAPDH) as shown in a C) representative Western blot and D) summary densitometry analysis. Errors bars indicate mean ± SEM (n = 5). Statistical comparisons to Mock-MDM (*) or WT 89.6 (#) were made by One-Way ANOVA with Holm-Sidak post hoc test. * /# p <0.05, ** /## p <0.01, ***/### p<0.001

Chronic cytokine and chemokine exposure in macrophages may result in loss of HO-1 protein expression

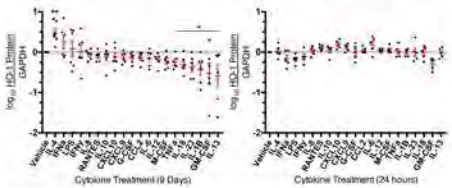

Figure 7: Uninfected MDM isolated from healthy donors were exposed to various cytokines, chemokines, or immune modulators for either 9 days (n=6) or 24 hours (n=5). For 9 day treatments, media was changed every three days and treatments were refreshed. At end of treatments, protein lysates were collected and HO-1 protein expression was analyzed by Western blot. Densitometry analysis ($\log_{10}$) of HO-1 protein expression normalized to GAPDH for **A)** 9-day and **B)** 24-hour treatments. Error bars indicate mean ± SEM. Vehicle HO-1 expression was set to 0 (dotted line). Screening statistical comparisons to vehicle-treated MDM were made by uncorrected paired student's t tests. * p <0.05, **p<0.01

SUMMARY & CONCLUSIONS

Summary

- Macrophage tropic HIV-1 strain replication in primary human macrophages consistently results in HO-1 protein deficiency
- Loss of HO-1 protein expression in HIV-infected macrophages associates with viral replication and elevated levels of supernatant glutamate and associated neurotoxicity
- HIV-1 accessory proteins Nef, Vpr, and Vpu are not required for the loss of HO-1 protein expression or associated neurotoxicity in HIV-infected macrophages.
- Chronic exposure of primary macrophages to particular cytokines and chemokines (TNFα, IL-1β, IL-18, IL-23, and GM-CSF) may induce loss of HO-1 protein expression.

Conclusion

Loss of HO-1 protein expression in HIV-MDM is a conserved feature of most, if not all, macrophage-tropic HIV-1 strains. While HO-1 loss associates with higher viral replication, even low level replicating strains result in macrophage HO-1 deficiency. HO-1 loss in HIV-MDM across multiple strains and donors associates with neurotoxin production, consistent with our previous findings that HO-1 modulates HIV-MDM neurotoxin production. While the HIV-1 accessory proteins Nef, Vpr, and Vpu are not required for loss of HO-1, our data suggests that chronic inflammatory signaling perhaps triggered by viral replication may play a role in the observed HO-1 deficiency.

ACKNOWLEDGEMENTS

We acknowledge the support the University of Pennsylvania Perelman School of Medicine Center for AIDS Research (CFAR) Virology Core and the Mahoney Institute of Neurological Sciences (MINS). This work was supported in part by National Institutes of Health R01 grants MH104134 (DLK) and MH095671 (DLK), T32 grant AI007632 (AJG), and F30 grant MH102120 (AJG).