

HIV ENV MEDIATES APOPTOSIS OF HUMAN HEPATOCYTES VIA CXCR4

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Abstract

Background: The course of HIV infection is frequently associated with hepatotoxicity. Currently, one of the leading causes of hospital associated HIV deaths is due to liver disease. In HIV infected patients, depletion of CD4 and CD8 T cells and neurons is mediated by the HIV envelope (env) signaling cellular apoptosis through the chemokine co-receptors. We, therefore, sought to determine if HIV env induces hepatocyte death and delineated the molecular mechanisms.

Methods: Human hepatocyte cell lines HUH7, Hep3B and HepG2 were stained with the anti-CXCR4 antibody 12G5 and analyzed by flow cytometry. HUH7 cells were incubated for 48 hours with soluble X4 env or SDF1 α at different time points and analyzed for cell viability by MTS reduction and confocal microscopy using TUNEL staining. HUH7 cells were also incubated for 48 hours with non-infected HIV-infected CD4 T cells fixed with 2% paraformaldehyde, and then analyzed for cell viability.

Results: The human hepatocyte cell lines expressed the HIV chemokine co-receptor CXCR4 as demonstrated by flow cytometry (HUH7 40%, Hep3B 30%, and HepG2 20%). When incubated with soluble X4 env, HUH7 cell viability decreased by 30% compared to untreated controls. Cell death peaked at 48 hours of incubation and was confirmed to be apoptosis by TUNEL. In contrast, SDF1 α , the natural ligand of CXCR4, did not induce hepatocyte death at the same time points. HUH7 cells were also incubated with fixed HIV-infected CD4 T cells, resulting in a 40% decrease in viability compared to cells incubated with non-infected CD4 T cells.

Conclusions: Our results demonstrate that human hepatocytes express the HIV chemokine receptor CXCR4 that has been implicated in HIV mediated death of primary T cells and neurons. In addition, soluble X4 HIV envelope and X4 HIV infected CD4 T cells induce hepatocyte apoptosis. These findings suggest that HIV env may play a key role in causing liver disease in HIV infected patients.

Introduction

Patients with HIV frequently suffer from liver dysfunction during the course of their infection. This can be a result of hepatotoxic drugs used to treat HIV, or possibly co-infection with a viral hepatitis. In fact, one of the leading causes of hospital associated HIV deaths currently is due to liver disease. Our group and several others have demonstrated that the HIV envelope (env) can signal cellular apoptosis to an uninfected bystander cell through the HIV chemokine co-receptors. This has been shown in CD4⁺T cells, CD8⁺T cells as well as neurons. We, therefore, questioned whether HIV could be directly toxic to hepatocytes as well, and if the HIV env would also induce hepatocyte death by signaling through CXCR4, the HIV X4 chemokine co-receptor.

Methods

Human hepatocyte cell lines HUH7, Hep3B and HepG2, cultured in EMEM 10%FBS, 10nM Insulin were stained with the anti-CXCR4 antibody 12G5 and analyzed by flow cytometry. HUH7 cells were incubated for 48 hours with 50 μ g of soluble X4 env, pre-incubated on ice for 30 minutes with 3 μ g of soluble CD4, or 200 nM of SDF1 α at different time points and analyzed for cell viability by MTS reduction and confocal microscopy using TUNEL staining. HUH7 cells were also incubated for 48 hours with mock or HIV (Lav-Bru)-infected CD4⁺T cells fixed with 2% paraformaldehyde, and then analyzed for cell viability by MTS reduction and TUNEL staining.

Results

Figure 1: Human Hepatocyte Cell Lines Express the X4 HIV Chemokine Co-receptor CXCR4

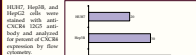


Figure 2A: X4 HIV env Directly Kills Human Hepatocytes

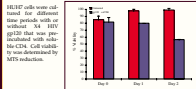


Figure 2B: X4 HIV env Kills Human Hepatocytes via Apoptosis

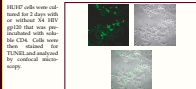


Figure 3: SDF1 α Signaling Through CXCR4 Does not Kill Human Hepatocytes

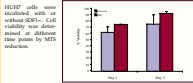


Figure 4A: HIV infected CD4⁺T cells Kill Human Hepatocytes

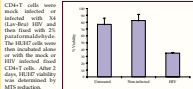
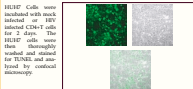


Figure 4B: HIV infected CD4⁺T cells Kill Hepatocytes via Apoptosis



Summary

- Human hepatocyte cell lines HUH7, Hep3B, and HepG2 express the HIV chemokine co-receptor CXCR4.
- X4 HIV env directly signals human hepatocytes to die via CXCR4.
- The HIV env mediated hepatocyte death is an apoptotic process.
- HIV infected CD4⁺T cells also trigger hepatocytes to die via apoptosis.

Conclusion

HIV mediated hepatocyte death, via the CXCR4 receptor, may be in part responsible for the liver disease that occurs in HIV infected patients.