Hyaluronan reduces HIV infection of CD4+ T cells and greatly enhances HIV inhibition by tenofovir

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Introduction

Better strategies are needed to prevent mucosal transmission of HIV. The major extracellular matrix (ECM) receptor CD44, present on virion and target cells, has been reported to strongly influence viral infectivity through as yet incompletely understood mechanisms. These interactions may be particularly relevant to transmucosal HIV transmission given the abundance of ECM components and CD44 expressing cells in mucosal tissues and genital secretions. Topical formulations of antiviral drugs like tenofovir disoproxil fumarate (TDF) are being studied in HIV prevention. We postulated that ECM-CD44 interactions might have particular relevance to TDF used in this setting and investigated how the natural ligand of CD44, hyaluronan (HA), modulates HIV infection in vitro when used alone or with TDF.
The goals of this study were:
1)To assess HA-CD44 interactions during HIV infection of CD4T cells.
2)To determine whether exogenous HA can reduce HIV infection.
3)To test whether exogenous HA can enhance inhibition of HIV infection by TDF.

Methods

1. Production of HIV virions that either contain CD44 or not: 293T cells (do not express appreciable levels of endogenous CD44) were transfected with NL43 plasmid together with either a CD44 expression vector or empty vector. These produced CD44 containing virions (HIV_{CD44}) or virions without appreciable CD44 (HIV_{mock}).

Production of HIV virions in PBMCs: stimulated PBMCs were inoculated with lab NL43(X4) or YKJRCSF(R5) virus or clinical isolates of 93BR011(R5) or 92HT599(X4)(both from NIH) and harvested at day 9 post infection.

2. M7-Lue cell assay:1x10^6 cells were washed with complete RPMI without FCS, then treated with study reagents, inoculated with 1000pg HIV-p24 of HIV for five hours, and washed to remove the inoculum. Three days post-infection, HIV infectivity in M7-lue cells was measured by the Bright-GloTM Luciferase assay system; luciferase activity was expressed as relative light units (RLU).

3. Jurkat (E6.1) cell assay: Jurkat cells (have no endogenous CD44 expression on surface of the cells) were transfected with CD44 expression vector or empty vector. Three days after transfection, the cells were infected with HIV (5ng of p24) for five hours and cultured for three additional days. Three days post infection, supernatant p24 was measured by ELISA and the percentage of infected cells was assessed by flow-cytometry after staining for intracellular p24 with mAB conjugated to PE.

4. Unstimulated CD4+T cell assay: 1x10^6 healthy donor CD4+ T cells were inoculated with HIV (1000pg of p24) for five hours and then washed. The cells were stimulated with 5µg of PHA-M and 20 unit/ml of hIL-2 for 24 hours. HIV infectivity was assessed by measurement of supernatant HIV-1 p24 at day seven post-infection by ELISA.

5. Co-immunoprecipitation with CD44 for PKCa: resting CD4+T cells were treated with HA, then infected with either HIV_{CD44} or HIV_{mock} for five hours. The cells were lysed and immunoprecipitated with anti-CD44. The immunoprecipitates were subjected to western blot with primary antibodies to CD44, PKCa and phosphoryated-PKCa(pPKCa). Detection was performed using goat anti-mouse IgG-conjugated HRP . The bands were analyzed using Quantity One 1-D analysis .

6. Inhibition of HIV infection by TDF and exogenous HA: The M7-lue cell assay was used to measure the infectivity in presence of TDF or TDF in combination with 100µg/ml of high molecular weight HA (HHA)(>950KDa). Two clinical virus isolates were tested: 93BR011(R5) and 92HT599(X4).

7. Statistical analyses. Data averages and standard deviations (SD) were calculated in Microsoft Excel and presented as means ± standard deviation (SD). Significance was assessed by a T-test, with p<0.05 considered significant.

Figure 1: Exogenous HA reduces infectivity of both R5 and X4 HIV virions containing CD44. A. Infection of M7-Lue cells with HIV_{CD44} or HIV_{mock}. B. Infection of CD4+T cells with NL4-3(X4) or YKJRCSF(R5).

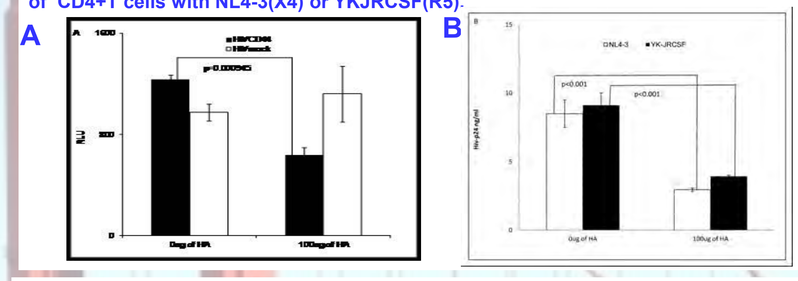


Figure 2: CD44 expression on recipient cells is required for the reduction of HIV infectivity by exogenous HA. Infection of Jurkat cells expressing or not expressing CD44 with HIV_{CD44} and HIV_{mock}, and the effect of exogenous HA.

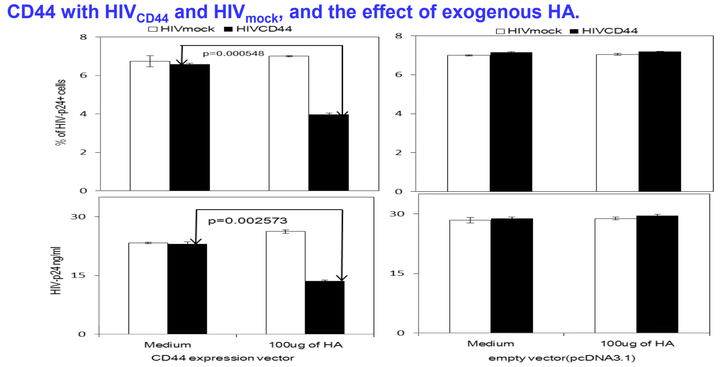


Figure 3: HA treatment reduces phosphorylation of CD44-associated PKCa. Western blot for phospho-PKCa after immunoprecipitation with anti-CD44 antibody.

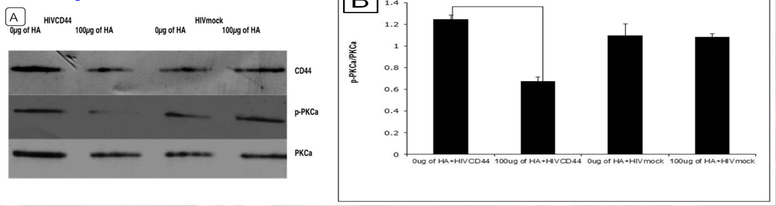
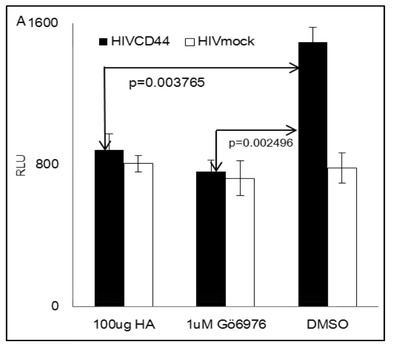


Figure 4: The selective PKCa inhibitor "Gö6976"[1,] reduces the infectivity of HIV_{CD44} (but not of HIV_{mock}) by a degree approximating treatment with HA in: A. M7-Lue cells; and B. unstimulated CD4+ T cells (infected at rest, washed, then stimulated).



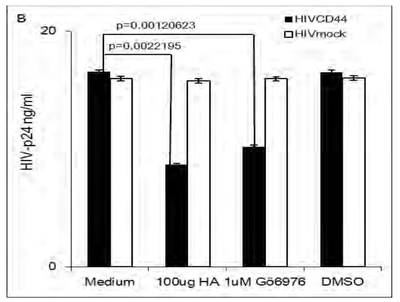
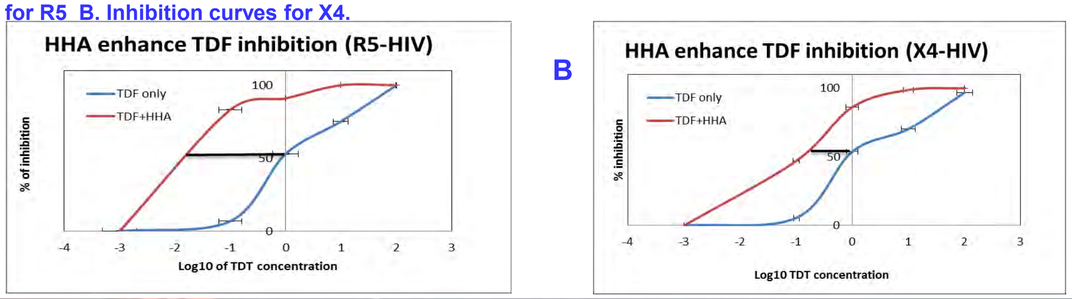


Figure 5: HHA enhances the ability of TDF to inhibit infection with clinical R5 and X4 virus isolates. A. Inhibition curves for R5 B. Inhibition curves for X4.



Results

1. Exogenous HA reduces infectivity of HIV virions containing CD44 (HIV_{CD44}) but does not reduce infectivity of HIV virions without CD44 (HIV_{mock}) (Fig1A). Exogenous HA reduces infectivity of both R5 and X4 virus produced in PBMCs (which express $CD44^2$) (Fig1B).

2. CD44 expression on recipient cells is required for the reduction of HIV infectivity by exogenous HA. Jurkat (E6.1) cells do not express endogenous CD44, and exogenous HA reduced HIV infectivity in CD44-expressing Jurkat cells but not in Jurkat cells transfected with empty vector (Fig2) .

3.PKCa signaling is important for the reduction of HIV_{CD44} infectivity in unstimulated CD4+T cells by exogenous HA. HA reduces levels of phospho-PKCa associated with CD44 (figure 3). The selective PKCa inhibitor "Gö6976" reduces HIV_{CD44} infectivity to the same degree as exogenous treatment with HA, but has no effect on HIV_{mock} infectivity (Fig4) .

4. HHA (>950kDa) and TDF synergistically inhibit infection by both R5 and X4 virus (Fig. 5). 50% Inhibition (IC_{50}) concentrations of TDF are 2 Log_{10} lower in the presence of HA compared with TDF alone for HIV R5, and one Log_{10} lower for HIV X4 virus. Inhibition of HHA plus TDF is significantly higher than TDF alone in both R5 and X4 virus, p<0.01.

Conclusions

1. **HA participates in HIV infection through its receptor CD44, and exogenous HA can reduce HIV infectivity only if the virions also contain CD44.**
2. **CD44 on recipient cells is required for HA interference with HIV infection.**
3. **Inhibition of PKCa signaling may be important for the reduction of HIV infectivity by HA.**
4. **High molecular weight HA synergistically enhances inhibition of HIV by TDF.**

Acknowledgments

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