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HSV-1-induced Enhancement of HIV-1 Replication is Dependent on Decrease in IFITM3 Levels

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Background

The IFITM3 restriction factor has been described as a protein that impairs HIV-1 replication upon IFN induction. Since herpesvirus infections are extremely common in HIV-1-infected individuals and some HSV-1 proteins inhibit the IFN cascade, we analyzed whether HSV-1 could enhance HIV-1 replication by diminishing the levels of IFITM3. For that, monocyte-derived macrophages were infected with HSV-1 or co-infected with HIV-1 upon different conditions. The expression of IFITM3 protein was analyzed by immunoblotting and knockdown of HSV-1 proteins was performed with siRNA against Us11 and VHS transcripts. Changes in IFITM3 expression occurred primarily at the late stages of HSV-1 infection. Treatment with ACV prevented reduction of IFITM3 by blocking HSV-1 DNA polymerase, indicating that viral infection during late phases of replication is critical for IFITM3 modulation. To confirm that HSV-1 proteins produced at the late phase of replication are indirectly reducing IFITM3 levels by inhibiting the IFN cascade, Us11 and VHS were silenced. This was sufficient to prevent HSV-1-induced IFITM3 reduction. siRNA-mediated silencing of US11 and VHS also consistently prevented HSV-1-mediated enhancement of HIV-1 production. Increased HIV-1 replication observed during HIV-1-HSV-1 co-infection is due to the production of Us11 and VHS by HSV-1. Upon inhibiting the IFN cascade, IFTM3 levels are reduced which contributes to an increased replication of HIV-1 in co-infected macrophages. Since co-infections are common in HIV-1 infected-individuals, our findings provide additional for exacerbation of HIV-1 replication in HSV-1/HIV-1 co-infected individuals.

Methods

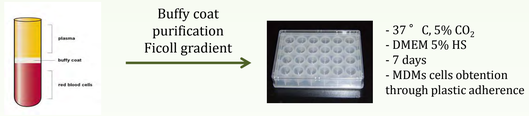


Figure 1. Purification of MDMs from buffy coats of healthy donors. After purification, monocytes were resuspended in DMEM 5% HS and seeded onto 24-wells plate. After 7 days incubation at 37 ° C, media was washed out and replaced for obtention of MDMs through plastic adherence.

Infection Assays

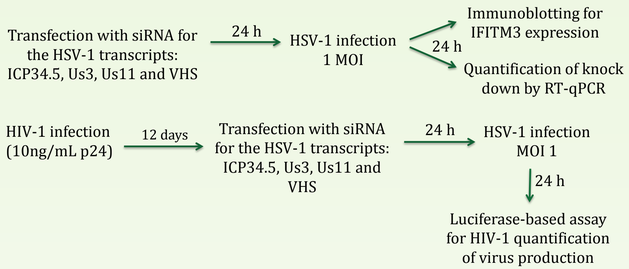
Co-infection: MDMs → HIV-1 (5-10 ng/mL p24) → 12 days incubation → co-infection with HSV-1 in different MOIs (0.1 and 1) → 48 h.p.i supernatants were harvested and cells lysed.

HSV-1 Infection: MDMs→ HSV-1 at 0.1 and 1 MOI for 24 h → monolayer was lysed.

Quantification of Virus Production:

Luciferase-based Assay: TZM-Bl cells were infected with supernatants from co-infection and fluorescence was measure accordingly to luciferase production.

Transfection Assay



Results

HIV-1 Production is Increased on Co-infection

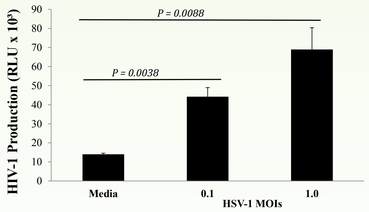


Figure 2 - Increased production of HIV-1 particles during HSV-1 co-infection. MDMs were infected with HIV-1 for 12 days and co-infected with HSV-1 at the indicated MOIs of HSV-1. After 2 days of co-infection the supernatants were tittered by luciferase-based assay (n = 3).

HSV-1-induced decrease on IFITM3 expression at 1 MOI

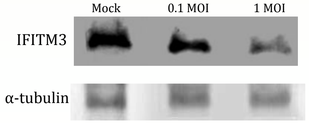


Figure 3 – IFITM3 expression during HSV-1 infection. MDMs were infected with HSV-1 at the indicated MOIs for 2 days and monolayer was lysed for IFITM3 expression analysis (n = 3).

HSV-1-induced decrease on IFITM3 levels is a late event on HSV-1 replication

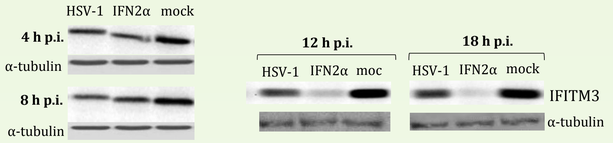


Figure 4 - Kinetic of IFITM3 expression on HSV-1 infection. MDMs were either infected with 1 MOI of HSV-1 or treated with IFN2α (10 ng/mL) for 24 h. Monolayers were lysed every 2 h for IFITM3 expression analysis.

IFITM3 levels were restored after treatment with Acyclovir

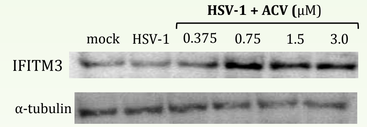


Figure 5 – IFITM3 expression after treatment with Acyclovir (ACV). MDMs were either infected with HSV-1 at 1 MOI or treated with increased concentrations of ACV. After 24 h incubation cells were lysed for IFITM3 expression analysis.

IFITM3 expression is rescued after silencing of HSV-1 late phase proteins

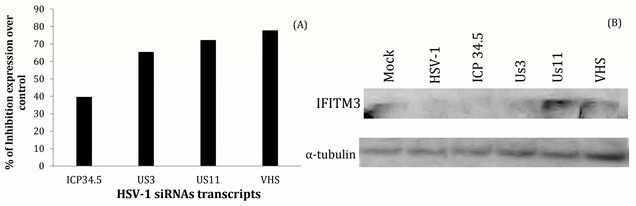


Figure 6 - Analysis of IFITM3 expression after HSV-1 late phase proteins silencing. MDMs were transfected with siRNA for the following HSV-1 transcripts: ICP35.5, Us3, Us11 and VHS and infected with HSV-1 at 1 MOI. After 24h infection, monolayers were lysed either for quantification of knock down by RT-qPCR (A) or for analysis of IFITM3 content by immunoblotting (B).

HSV-1 blocks IFITM3 expression by inhibiting phosphorylation of eIF2α and PKR

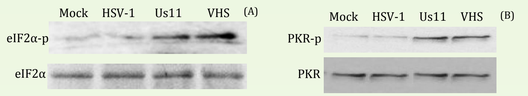


Figure 7 - Analysis of eIF2α and PKR total or phosphorylated after Us11 and VHS silencing. MDMs were transfected with siRNA for Us11 and VHS transcripts and infected with HSV at 1 MOI. After 24h infection, monolayers were lysed for quantification of either eIF2α (A) and PKR (B) total or phosphorylated.

HIV-1 infection is reduced after HSV-1 proteins Knock down

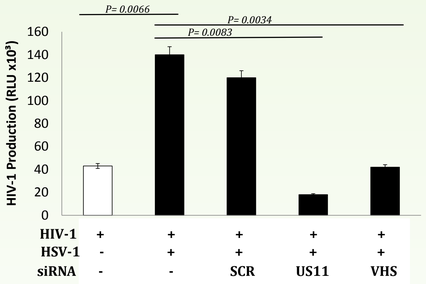


Figure 8 - MDMs were infected with HIV-1 for 12 days and then transfected with Us11 and VHS siRNAs. After 24 h post transfection cells were co-infected with HSV-1. After a total of 14 days of HIV-1 infection, supernatants were harvested and the infection was quantified by luciferase-based assay.

Conclusions

- Co-infection increases HIV-1 replication.
- IFITM3 expression decreases at late phase of HSV-1 infection.
- HSV-1 late phase proteins are responsible for IFITM3 reduced expression and thus by elevated HIV-1 titters.
- HSV-1 proteins Us11 and VHS are responsible for the high levels of HIV-1 replication during co-infection.
- The reduced IFITM3 level is due to a decrease on eIF2α and PKR phosphorylation.

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