Poster #0217

EXTRACELLULAR VESICLE-ASSOCIATED CYTOKINES IN HIV-INFECTED HUMAN EX VIVO TONSILS

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Abstract

Background: Cytokines play an important role in HIV infection. Some of these cytokines are present on the surface or encapsulated in extracellular vesicles (EVs). We investigated the modulation of EV-associated cytokines during HIV infection and antiretroviral therapy (ART) in human *ex vivo* tonsils.

Methods: *Ex vivo* tonsils were infected with HIV-1 strains, $X4_{LAI04}$ or $R5_{SF162}$. HIV was either allowed to replicate, or tissues were treated with 3TC and AZT. 33 cytokines in soluble or EV-associated forms were evaluated.

Results: Infection with HIV-1 led to increases in many soluble cytokines, with the highest increases in IL-2, IFN-g, MIP-1a, MIP-1b, and RANTES. Certain cytokines were also elevated in EV-associated form, including some that were also increased in soluble form. Likewise, soluble cytokines unaffected by HIV infection tended to also be unchanged in EVs. ART treatment halted HIV-1 replication, but many cytokines increased by HIV infection remained elevated. EV-associated cytokines were less likely to be restored: 13 days after ART, all cytokines that remained elevated with HIV infection were also still elevated in ART conditions.

Conclusions: Cytokine levels increased during HIV infection in both soluble and EV-associated forms; the same cytokine is often upregulated in both forms. Many soluble cytokines upregulated by HIV did not decrease even after 13 days of ART, and EV-associated cytokines were even less likely to decrease. X4 induced increases were less likely to return to control levels. ART-treated infected human tissues provide a new model to study tissue activation after HIV replication is suppressed, in particular, the role of EVs in this phenomenon.

Materials and Methods

- **Sample preparation and storage**

Tonsillar tissue (n = 7) was obtained from routine tonsillectomies performed at the Children's National Medical Center in Washington DC. Medium was collected and changed at day 2, 5, 8, 12 and 15.

- **HIV infection and antiretroviral treatment**

Tonsils were infected with HIV-1 strains, $X4_{LAI04}$ or $R5_{SF162}$ at the beginning of culture. HIV was allowed to replicate, or tissues were treated with nucleoside reverse transcriptase inhibitors: 3TC (lamivudine) and AZT (zidovudine).

- **Preparation of EV fractions**

EVs were extracted from 200 µl of supernatant using ExoQuick reagent.

- **Evaluation of HIV-1 replication**

We evaluated HIV-1 replication in tissue by measurement of HIV-1 $p24_{gag}$ antigen in the culture medium, using a dynamic immunofluorescent cytometric bead assay.

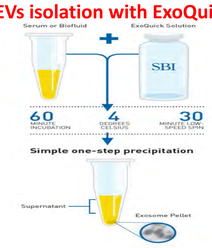
- **Cytokine measurement**

We previously developed an in-house multiplexed bead-based assay for measurement of 33 cytokines (Luminex) in soluble and EV form. Intact EV samples and lysed EV samples, to which Triton X was added at final concentration of 1%, were run in separate wells. Standard curves were performed with and without Triton X.

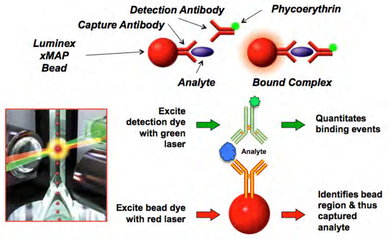
Human *ex vivo* tonsil culture



EVs isolation with ExoQuick



Luminex workflow



Results

HIV Replication

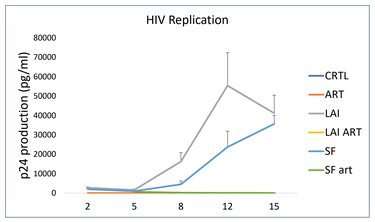


Fig 1: p24 evaluation shows that tonsil tissues were permissive to HIV-1 infection, and treatment with ART was able to stop infection (n=7).

RANTES: Changes in Distribution in Early HIV Infection

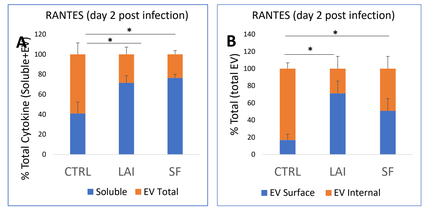
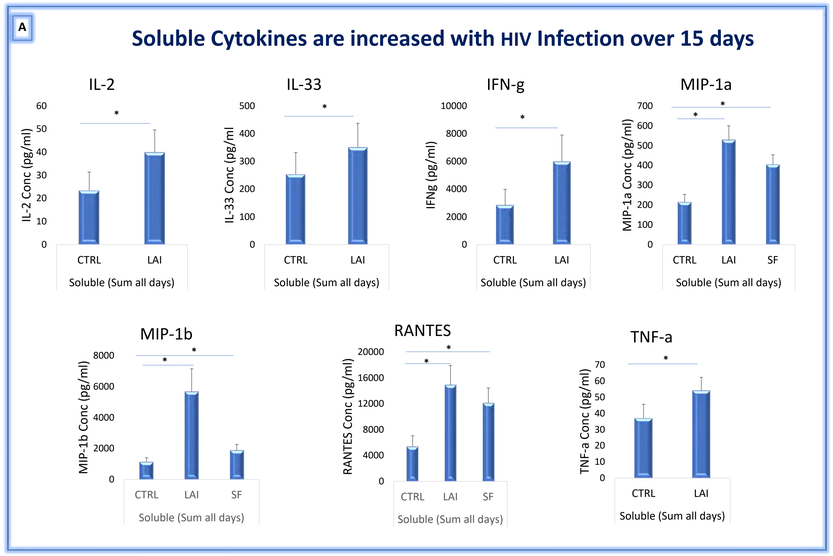


Fig 2: HIV infection led to changes in distribution of RANTES with respect to the control: RANTES shifts to higher percent of soluble cytokine compared to EV-associated (**A**); and it shifts to more EV surface compared to EV internal (**B**). *p<0.05, n=7



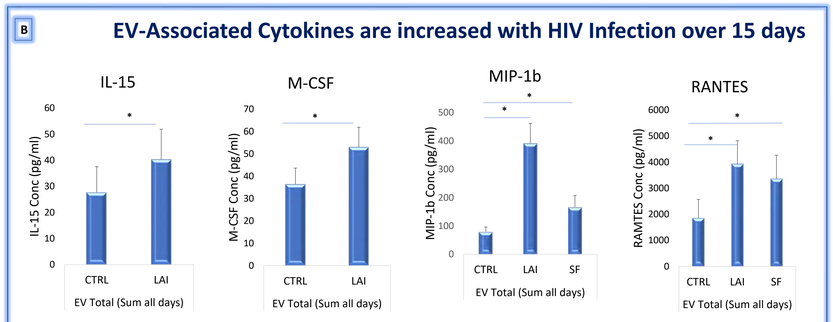
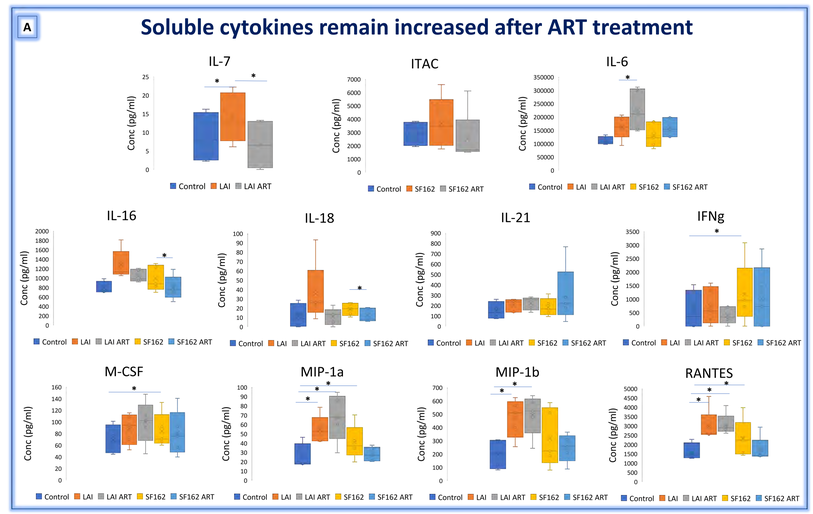


Fig 3: We evaluated cytokine release by uninfected and infected tissues as cumulative production of cytokines from day 2 to day 15. We evaluated total cytokine release in soluble form (**A**) and EV-associated (**B**). ***p<0.05, n=7.**



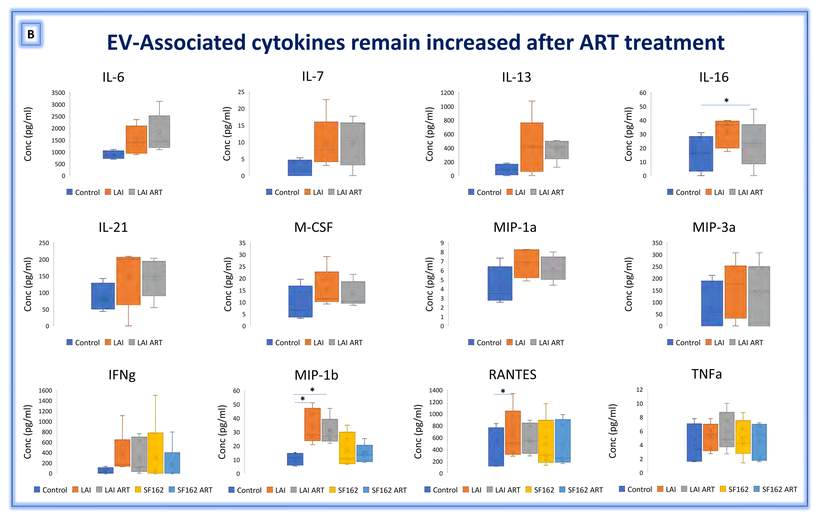


Fig 4: We investigated whether suppression of HIV replication by ART, after 13 days of treatment, reverses the immune activation, and restores cytokine levels back to baseline production. We evaluated total cytokine release in soluble form (**A**) and EV-associated form (**B**). ***p<0.05, n=5**

Conclusions

- ✓ Cytokines which increased during HIV infection tend to increase in both soluble and EV associated form.
- ✓ Cytokines upregulated by HIV did not decrease even after 13 days of ART, demonstrating that despite control of viral replication by ART, lymphoid tissue cells remain activated.
- ✓ HIV induced elevation of EV-associated cytokines were less likely to be restored by ART, compared to soluble forms.
- ✓ These studies will assist in deciphering mechanisms of pathologies that develop in ART-treated patients.

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