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Inflammasome regulated cytokine IL-37 inhibits HIV-1 replication in primary human CD4 T-cells

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Abstract

Background: Interleukin (IL)-37 is a recently characterized member of the IL-1 family of cytokines that regulate innate immune responses. Like other inflammasome-regulated cytokines, its maturation and release is dependent on inflammasome activity and caspase-1 activation. HIV-1 infected individuals have elevated levels of circulating inflammasome cytokines.

Methods: We assessed the effects of IL-37 and other inflammasome-regulated cytokines on HIV-1 replication in human primary CD4+ T-cells.

Results: We find that activated T-cells express receptors for all inflammasome cytokines; however only IL-37 exerts an antiviral effect in HIV-1 infection. IL-37 induces expression of antiviral factors including IFITM proteins and APOBEC3A in CD4+ T-cells. IL-37 directly inhibits HIV-1 replication in infected primary human CD4+ T-cells by inhibiting life cycle steps prior to integration of the pro-viral genome.

Conclusions: IL-37 exerts a suppressive effect on HIV-1 by inducing expression of well-described innate antiviral factors.

Introduction & Aims

Introduction

1. Persons with HIV-1 infection have elevated levels of IL-1β and IL-18 in circulation.
2. It has been proposed that non-productive infection of activated T-cells leads to production of IL-1β and pyroptosis, while productive infection leads to IFNα production and transmission/generation of an antiviral state in uninfected T-cells.
3. It remains unclear whether the inflammasome cytokines themselves generate an antiviral state in newly infected T-cells.

Aims

1. To identify whether T-cells express receptors for inflammasome associated cytokines.
2. To determine whether inflammasome associated cytokines affect replication of HIV-1 in infected cells.
3. Identify mechanisms of HIV-1 suppression by inflammasome cytokines.

Methods

Isolation of and activation of human T-cells: Identity unlinked human whole blood Leukopaks were obtained from the Johns Hopkins Hemapheresis and Transfusion Services center. PBMCs were isolated by a Ficoll-Hypaque gradient. T-cells were isolated by magnetic sorting using CD3+ or CD4+ negative separation beads following the manufacturer's protocol (Miltenyi Biotec, Auburn CA) and cultured in RPMI 1640 media (Invitrogen, Grand Island NY), supplemented with 10% heat inactivated Fetal Bovine Serum (Sigma-Aldrich, St. Louis MO) and 2 mM L-Glutamine (Corning, Manassas VA), referred to as R10. Purity of separation was determined by flow cytometry using anti-CD3-FITC (R&D, Clone: UCHT1), anti-CD4-PerCP (Biolegend, Clone: RPA-T4), anti-CD8-PerCP (Biolegend, Clone: SK1). Purified T-cells were activated with 5 microg/mL phytohemagglutinin (PHA, Fisher Scientific, Waltham MA) or anti-CD3/CD28 microbeads (Life Technologies, Carlsbad CA). Stimulation was confirmed via CFSE staining (Invitrogen, Grand Island NY).

Antibodies: Anti-human IL-18Ra/IL-1R5- PE (Clone 70625, R&D, Boston MA); Anti-Human IL-18 Rb/ IL-1R7-FITC (clone 132029, R&D, Boston MA); anti-Human SIGIRR-APC (clone 162201, R&D, Boston MA); anti-Human ILRAP2/ IL-1R9-AF647 (Polyclonal, Bioss, Woburn MA); anti-Human CD4-PerCP (clone OKT4, Biolegend, San Diego CA); anti-Human CD8-PerCP (Biolegend, Clone: SK1) were used to identify expression of the respective proteins on the surface of T-cells.

Cytokines and drugs: Recombinant human cytokines IL-37, IL-18, IL-33, and IL-1α were purchased from PeproTech, Chennai India. Recombinant B18R was purchased from R&D Systems, Minneapolis, MN (ED50 0.3-1.8 ng/mL).

Preparation of HIV-1 virions: HIV-1_{IIIB} and HIV-1_{RF} were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. HIV-1_{IIIB} and HIV-1_{RF} were spinoculated (1200*g*, 25 ºC, 2 h) onto CD4+ T-cells cultured for 72 h with PHA at 2 ng/mL of HIV-1 p24 per 1 x 10^6 cells. Infected cells were cultured in R10 for 4-7 days and supernatants harvested, passed through a 200 μm exclusion filter and pelleted through a 20% sucrose cushion (106,000*g*, 20ºC, 2 h).

Cytokine co-culture of activated T-cells with HIV-1: T-cells sorted by negative selection were stimulated (PHA or anti-CD3/28 beads, 72 h), distributed at 5 x 10^5 cells/well in a 96-well U-bottom plate, and re-suspended in 40 μL HIV-1IIIB (5 ng/mL). Cells were then spinoculated (1200*g*, 25ºC, 2 h) and washed four times with 1x PBS to remove any free virus. Cytokines were then added to the culture in concentrations ranging 10 -100 ng/mL and replaced every 24 h. Supernatants were stored every 24 h for p24 analysis.

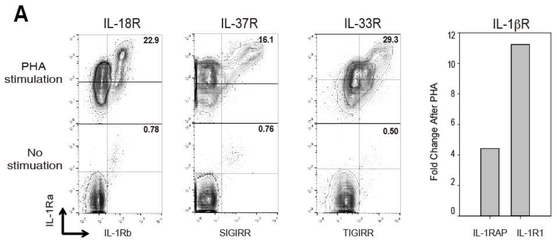
p24 ELISA: Culture supernatants (neat -1:800 dil.) from infected cultures (0, 24, 48, 72 h post infection) were assessed for HIV-1 using the p24 ELISA (PerkinElmer, Waltham MA). OD 490 nm were acquired using a SpectraMax M5 (Molecular Devices, Sunnyvale CA). The LLOD of p24 was 12.5 pg/mL in culture supernatant.

Quantitative real-time PCR: RNA was isolated with the RNeasy Kit (Qiagen) and used to synthesize cDNA with Superscript III Reverse Transcriptase system (Invitrogen). Quantitative PCR was performed with IDT pre-designed primers/probe mixes using Light Cycler 480 Probes Master mix (Roche). GAPDH and RPL13A were used as housekeeping controls. The ratio of target gene mRNA copies relative to the average mRNA copies from housekeeping controls was defined as Delta. Ratio of normalized mRNA copies among experimental conditions is defined as Delta/Delta and used to calculate the relative effect of IL-37 on T-cells.

Statistical Analysis: One-way Anova tests were used to evaluate statistically significant differences between groups. Differences considered statistically significant when the p value was less than 0.05.

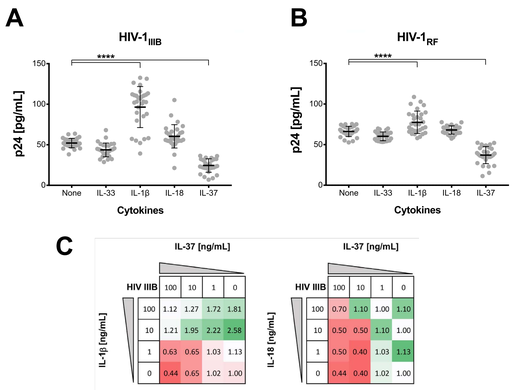
1. Activated primary human CD4+ T-cells express receptors for inflammasome cytokines

(**A**) T-cells were activated with PHA then labeled with fluorophore-conjugated antibodies against IL-1Ra, SIGIRR and IL-1Rb. Expression of the cytokine receptor chains on gated CD4+ T cells was assessed by flow-cytometry at 72 h. Activated T-cells increase expression of the IL18 (IL-1Rb + IL-1Ra+) and IL-37 receptors (SIGIRR + IL-1Ra +). Activated T-cells also co express the IL-33 receptor (IL-1Ra+ TIGIRR+). Abundance of mRNA for the component chains of the IL-1 receptor (IL-1RAP and IL-1R1) measured by qRT-PCR in activated T-cells.



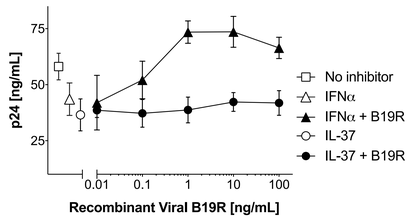
2. Inflammasome products modulate HIV-1 replication in T-cells

(**A**) HIV-1_{IIIB} or (**B**) HIV-1_{RF} was spinoculated onto activated T-cells and the culture maintained in the inflammasome cytokines IL-37, IL-33, IL1β and IL-18 for 72 h at a concentration of 100 ng/mL. HIV-1 replication was assessed by p24 ELISA at 72 h. The influence of each cytokine is shown as the fold change of p24 produced by the culture relative to the culture with no exogenous cytokine added. Horizontal lines indicate comparisons in which $p < 0.05$. (**C**) Activated T-cells were cultured with mixtures of IL-1β and IL-37 and viral replication assessed by p24 production relative to no cytokine condition



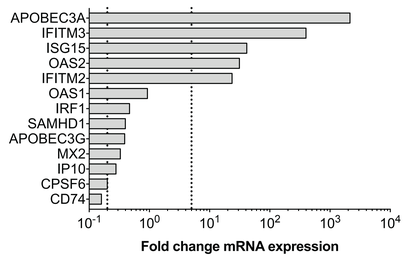
3. IL-37 restriction on HIV is independent of type I interferon

HIV-1IIIB was spinoculated onto activated T-cells and IL-37 (100 ng/mL) added as previously described. Immediately after spinoculation recombinant viral B19R was added to cultures as previously determined effective ranges. HIV-1 replication was assessed by p24 ELISA at 72 h.

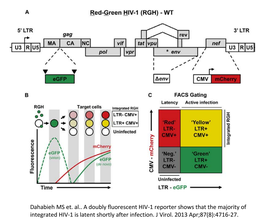


4. IL-37 induce expression of HIV restriction factors

Quantitative RT-PCR of genes known to inhibit HIV-1 replication was performed on activated T-cells treated with IL-37. Data is expressed as fold change compared to activated, untreated cells.

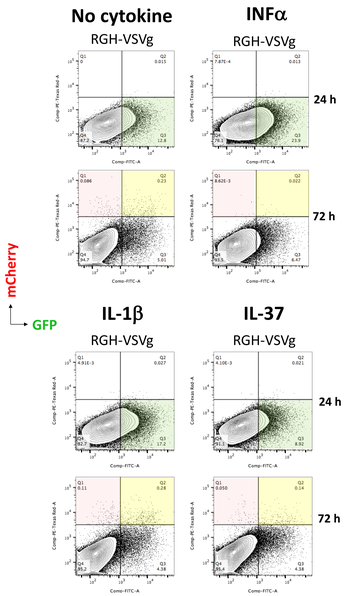


5. IL-37 inhibits early steps HIV-1 infection



Dahabieh MS et. al., A doubly fluorescent HIV-1 reporter shows that the majority of integrated HIV-1 is latent shortly after infection. J Virol. 2013 Apr;87(8):4716-27.

Doubly fluorescent HIV-1 virions complemented with the VSVg envelope was produced using the method of Dahanieh et. al. The virions carry eGFP and thus allow detection of the first round infection by the acquisition of eGFP. Integration of viral genome is detected by production of mCherry. Transcription from integrated genome is detected by new eGFP.



Activated T-cells were spinoculated with the replication incompetent HIV-1 RHG complemented with VSVg envelope. After spinoculation cells with cultured with INFα for 24 or 72 h. While IFNα suppresses the production of progeny virus from replication competent viruses more cells become infected in the presence of IFNα (green). Despite this at later time-points few T-cells allow HIV-1 integration (red + yellow). Among T-cells in which HIV-1 integration occurred production of new virions was unaffected by IFNα (red vs. yellow). Of note this assay does not detect viral egress.

Activated T-cells were spinoculated with the replication incompetent HIV-1 RHG complemented with VSVg envelope. After spinoculation cells with cultured with IL-37 or IL-1β for 24 or 72 h. IL-37 decreases the number of T-cells which become infected (green), however does not significantly affect transcription of integrated virus. By contrast IL-1β inhibits viral entry but does not significantly affect integration or viral production.

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Conclusions

- **Activated human CD4 T-cells express receptor chains for IL-18, IL-1α, IL-33 and IL-37 receptors.**
- **IL-1β and supports HIV-1 replication in activated T-cells, IL-37 and IFNα inhibits HIV-1 replication.**
- **IL-37 decreases HIV-1 infection events in activated T-cells.**
- **IL-37 upregulates expression of known HIV-1 restriction factors.**