STAT5 Inhibition Reduces HIV-1 Infection and TLR7/8 Responses in Human Macrophages

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Abstract

Background: Monocyte-derived macrophages (MDM) are significant contributors to persistent viral production and chronic inflammation, which are major challenges in the elimination of infection and in the health of HIV-1 infected individuals. HIV-1 infection of MDMs activate STAT5. Infection also primes MDMs rendering the cells hypersensitive to subsequent TLR stimulation. STAT5 has two functional binding sites in the HIV-1 long terminal repeat (LTR), however the effects of STAT5 activation on HIV-1 infection and immune responses in MDMs are unknown. We tested the hypothesis that STAT5 activation supports HIV-1 infection and priming of MDMs.

Methods: Human MDMs or TZM-bl cells were treated with a STAT5 specific inhibitor prior to infection with replication competent, CCR5 using, HIV-1_{AD} or single cycle, luciferase-tagged HIV-1_{JRFL} envelope pseudotyped virus. Inhibition of STAT5 activation was verified by western blot. Infection was monitored by measuring supernatant p24 levels, luciferase enzyme activity, non-integrated *gag* and integrated HIV-1 copies. Flow cytometry was utilized to examine cell surface expression of CD4 and CCR5 and intracellular p24 and TNF. Supernatant TNF and IL-6 were determined by ELISA.

Results: STAT5 inhibitor treatment significantly reduced HIV-1 infection of MDMs compared to control without affecting cell viability. Suppression of *de novo* infection by STAT5 inhibition was independent of changes in CD4 or CCR5 surface expression or number of non-integrated *gag* or integrated HIV-1 copies, but associated with a significant reduction in HIV-1 LTR activity. Twenty-four hour STAT5 inhibitor treatment dramatically reduced TLR7/8 mediated TNF and IL-6 production, while sustained STAT5 inhibitor treatment suppressed TNF production independent of TLR ligand albeit to different extents. HIV-1 infection partially overcame STAT5 inhibitor mediated TNF reduction. However, total TNF production in STAT5 inhibitor treated, HIV-1 infected cultures was significantly reduced compared to TNF in control cultures.

Conclusions: Our novel findings show both direct and indirect roles for STAT5 in modulating HIV-1 infection in MDMs. STAT5 directly supports transcription of the viral genome, without effecting steps in the viral life cycle from entry to integration. STAT5 is involved in pro-inflammatory cytokine production specifically induced by TLR7/8 receptors, which sense ssRNA and viral infections, indicating a possible indirect role in HIV-1 infection. Disruption of TLR7/8 signaling mediated by STAT5 inhibition provides no apparent advantage for the virus. Thus, STAT5 is a key factor for HIV-1 replication and a novel anti-viral, anti-inflammatory drug candidate.

Results

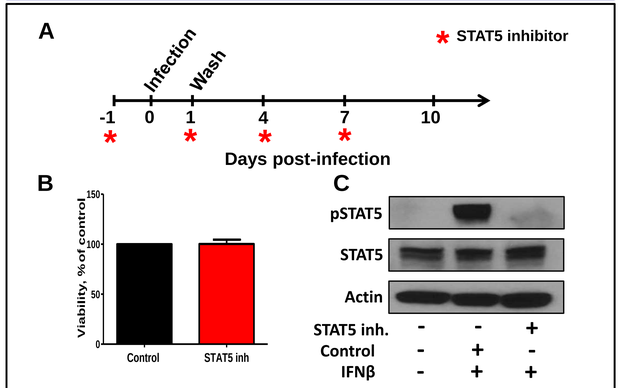

Figure 1: STAT5 inhibitor treatment reduces STAT5 phosphorylation while cell viability is unaltered. (A) Human monocyte-derived macrophages (MDMs) were treated with the STAT5 inhibitor 573108, (EMD Millipore Chemicals, Gibbstown, NJ) or DMSO control 24 hours prior to infection with HIV-1_{AD}. On PID 1, cells were washed and new media containing STAT5 inhibitor or DMSO added. Cells also received STAT5 inhibitor or DMSO on PID 4 and 7, concomitant with supernatant collection. (B) Viability of cells treated with 80 μM STAT5 inhibitor was measured on PID 10 using MTS assay. Viability of STAT5 inhibitor treated cells ranged between 93.5% - 108.2% of control cells. (C) MDMs were treated with 80 μM STAT5 inhibitor for 24 hours prior to 30 min stimulation with 10 ng/ml IFNβ. Whole cell lysate was collected and western blotting confirmed that STAT5 inhibitor treatment suppressed IFNβ induced phosphorylation of STAT5 without affecting expression of total STAT5.

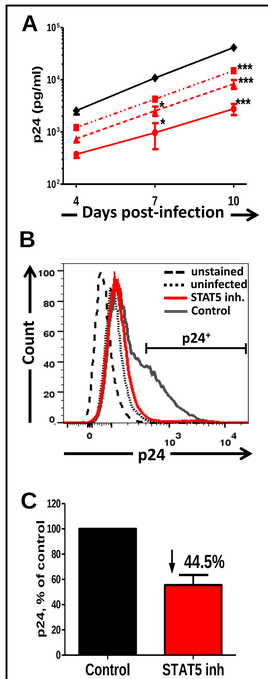

Figure 2: STAT5 inhibitor treatment suppresses HIV-1 infection of human macrophages. MDMs were treated with 80 μM (●), 40 μM (▲) or 20 μM (■) STAT5 inhibitor or DMSO control (◆) and infected with HIV-1_{AD} as indicated in Figure 1A. (A) Supernatant p24 on PID 4, 7 and 10 measured by ELISA showed a dose dependent reduction due to STAT5 inhibitor treatment. *p<0.05, ***p<0.001 vs. control using Bonferroni's post-test. (B) On PID 10, number of infected ($p24^+$) cells in 80 μM STAT5 inhibitor and control treated cultures was determined by intracellular p24 staining and flow cytometry. Significant reduction in $p24^+$ cells was observed in STAT5 inhibitor treated cultures compared to control. (C) Average p24 production by infected cells in STAT5 and control treated cultures was calculated by dividing supernatant p24 levels with the total number of infected cells for each treatment. Infected cells in STAT5 inhibitor treated cultures produced 44.5% less p24 per infected cell compared to infected cells in control cultures. The reduction in p24 production indicates that infected cells in STAT5 inhibitor treated cultures produces less virus compared to infected cells in control cultures.

Results

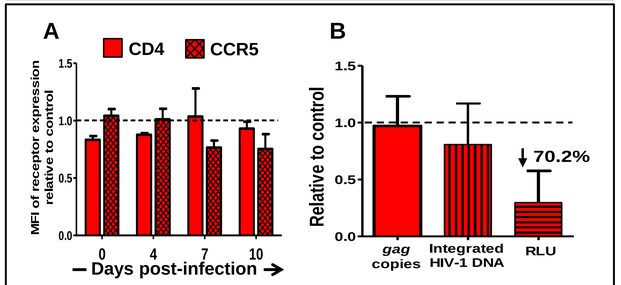

Figure 3: Inhibition of STAT5 suppresses HIV-1 gene expression independent of entry, reverse transcription or integration. MDMs were treated with 80 μM STAT5 inhibitor or DMSO control and infected with HIV-1_{AD} as indicated in Figure 1A. (A) Flow cytometry on PID 0, 4, 7 and 10 showed no difference in CD4 (■) or CCR5 (▩) expression due to STAT5 inhibitor treatment. Data are expressed as mean fluorescence intensity (MFI) of receptor expression ± SD relative to control for 3 donors. (B) DNA or whole cell lysate were collected on PID 1 and non-integrated viral *gag* and integrated HIV-1 DNA were measured using real-time qPCR. After normalization to *ApoB* copies, no significant reduction in viral *gag* or integrated DNA due to STAT5 inhibitor treatment compared to control was seen. In addition, MDMs were treated with 80 μM STAT5 inhibitor 24 hrs prior- and post-infection with single cycle HIV-1_{JRFL}. Relative light units (RLU) were measured on PID 4 and normalized to RLU from control treated cells. Based on 3 donors, STAT5 inhibitor treatment reduced the mean RLU 70.2% compared to control.

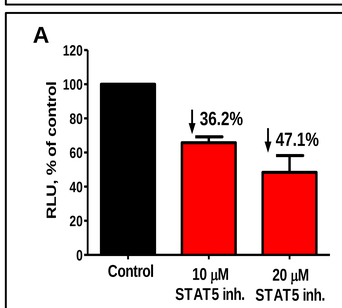

Figure 4: STAT5 inhibition reduces HIV-1 LTR activity. (A) TZM-bl cells with an integrated luciferase gene under the control of the HIV-1 LTR were treated with 10 or 20 μM STAT5 inhibitor or DMSO control for 24 hours prior to infection with HIV-1_{AD}. RLU observed on PID 2 showed a dose dependent reduction due to STAT5 inhibitor treatment. Data are based on 3 experiments and expressed as mean percentage RLU ± SD.

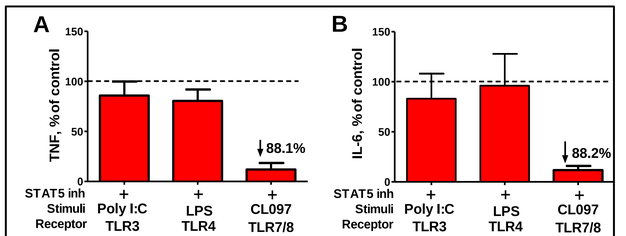

Figure 5: Twenty-four hour STAT5 inhibitor treatment specifically suppresses TLR7/8 induced pro-inflammatory cytokine production. MDMs were treated with 80 μM STAT5 inhibitor 24 hours prior to stimulation with 100 μg/ml Poly I:C (TLR3), 10 ng/ml LPS (TLR4) or 1 μg/ml CL097 (TLR7/8) for 6 hrs. (A) TNF or (B) IL-6 levels in supernatant were measured using ELISA. Twenty-four hours STAT5 inhibitor treatment suppressed TLR7/8 induced TNF and IL-6, but not TLR3 or TLR4 induced cytokine production. Data are based on at least 3 donors.

Results

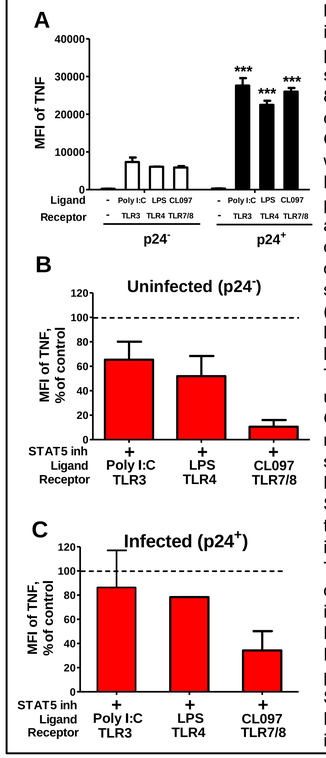

Figure 6: STAT5 inhibition indirectly suppresses HIV-1 priming by modulating TLR signaling. MDMs were treated with 80 μM STAT5 inhibitor or DMSO control as indicated in Figure 1A. On PID 10 cells were stimulated with 100 μg/ml Poly I:C, 10 ng/ml LPS or 1 μg/ml CL097 for 6 hours prior to staining for intracellular p24 and TNF. Uninfected and infected cells were identified by the absence or presence of intracellular p24 staining using flow cytometry. (A) HIV-1 infected MDMs (black bars) produce significantly higher levels of TNF due to subsequent TLR stimulation compared to uninfected MDMs (white bars). Graph show MFI of TNF and is a representative of 5 donors with similar results. *** p<0.001 using Bonferroni's post-test. (B) Sustained STAT5 inhibitor treatment reduces TNF production in uninfected MDMs independent of TLR stimulation. (C) HIV-1 priming of MDMs overcame the STAT5 inhibitor mediated suppression of Poly I:C and LPS induced TNF. However, TLR7/8 induced TNF production is still reduced (>65%) in STAT5 inhibitor, HIV-1 infected MDMs compared to control treated, infected cells.

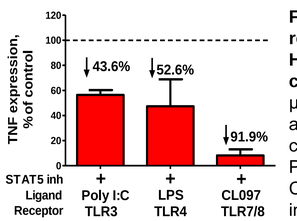

Figure 7: STAT5 inhibitor treatment reduces total TNF production in HIV-1 exposed macrophage cultures. MDMs were treated with 80 μM STAT5 inhibitor or DMSO control as indicated in Figure 1A. On PID 10 cells were stimulated with 100 μg/ml Poly I:C, 10 ng/ml LPS or 1 μg/ml CL097 for 6 hrs prior to staining for intracellular p24 and TNF. Total TNF expression in HIV-1 exposed, STAT5 inhibitor or DMSO control treated cultures was calculated using the following formula: (number of uninfected cells x MFI of uninfected cells) + (number of infected cells x MFI of infected cells). Due to STAT5 inhibitor mediated reduction of TNF production in both infected and uninfected cells, expression of total TNF in STAT5 treated cultures was reduced by 43.6% (Poly I:C), 52.6% (LPS) and 91.9% (CL097) compared to control. Data are expressed as mean percentage TNF ± SD in STAT5 inhibitor treated cultures compared to control.

Acknowledgments

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