**Abstract**

Background: Monocyte-derived macrophages (MDM) are professional phagocytes that contribute to viral production and antiviral immunity. STAT5 inhibition reduces HIV-1 infection and TLR7/8 stimulation. STAT5 inhibition also primes MDMs rendering the cells sensitive to subsequent TLR stimulation. This paper presents 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 regulates the function and response to TLR7/8 stimuli.

**Methods:** Human MDMs or TZM-bl cells were treated with a STAT5 specific inhibitor prior to infection with replication competent, CCR5 using, HIV-1_AΔ or single cycle, luciferase-tagged HIV-1 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 intracellular 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 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 HIV RNA and IL-6 production. The sustained STAT5 inhibitor treatment suppressed TNF production independent of LTR ligand albeit to different extents. HIV-1 infection partially overcome STAT5 inhibitor mediated TNF reduction. However, total TNF production in STAT5 inhibitor treated cells 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 effect on steps in viral life cycle like 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. Suppression 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.

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