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Research Paper

HIV-1 Promotes the Degradation of Components of the Type 1 IFN JAK/STAT Pathway and Blocks Anti-viral ISG Induction

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ABSTRACT

Anti-retroviral therapy successfully suppresses HIV-1 infection, but fails to provide a cure. During infection Type 1 IFNs normally play an essential role in viral clearance, but in vivo IFN-α only has a modest impact on HIV-1 infection, suggesting its possible targeting by HIV. Here, we report that the HIV protein, Vif, inhibits effective IFN-α signalling via degradation of essential JAK/STAT pathway components. We found that STAT1 and STAT3 are specifically reduced in HEK293T cells expressing Vif and that full length, infectious HIV-1 IIIB strain promotes their degradation in a Vif-dependent manner. HIV-1 IIIB infection of myeloid ThP-1 cells also reduced the IFN-α-mediated induction of the anti-viral gene, ISG15, but not MxA, revealing a functional consequence of this HIV-1-mediated immune evasion strategy. Interestingly, while total STAT levels were not reduced upon in vitro IIIB infection of primary human PBMCs, IFN-α-mediated phosphorylation of STAT1 and STAT3 and ISG induction were starkly reduced, with removal of Vif (IIIBΔVif), partially restoring pSTATs, ISG15 and MxB induction. Similarly, pSTAT1 and pSTAT3 expression and IFN-α-induced ISG15 were reduced in PBMCs from HIV-infected patients, compared to healthy controls. Furthermore, IFN-α pre-treatment of a CEM T lymphoblast cells significantly inhibited HIV infection/replication (measured by cellular p24), only in the absence of Vif (IIIBΔVif), but was unable to suppress full length IIIB infection. When analysing the mechanism by which Vif might target the JAK/STAT pathway, we found Vif interacts with both STAT1 and STAT3, (but not STAT2), and its expression promotes ubiquitination and MG132-sensitive, proteosomal degradation of both proteins. Vif's Elongin-Cullin-SOCS-box binding motif enables the formation of an active E3 ligase complex, which we found to be required for Vif's degradation of STAT1 and STAT3. In fact, the E3 ligase scaffold proteins, Cul5 and Rbx2, were also found to be essential for Vif-mediated proteasomal degradation of STAT1 and STAT3. These results reveal a target for HIV-1-Vif and demonstrate how HIV-1 impairs the anti-viral activity of Type 1 IFNs, possibly explaining why both endogenous and therapeutic IFN-α fail to activate more effective control over HIV infection.

1. Introduction

Human immunodeficiency virus (HIV) remains a major global health challenge, with over 40 million people infected worldwide (Mayer and Beyrer, 2007). Anti-retroviral therapy (ART) suppresses HIV-1 replication (Potthoff and Brockmeyer, 2010), but a drug that fully clears infection remains undiscovered. Lifelong ART is costly and brings with it issues of adherence, side-effects and mutational resistance (Reust, 2011), highlighting the remaining need for new therapeutic strategies to cure HIV. Harnessing endogenous anti-viral immune responses provides a possible alternative for therapeutic discovery. Type 1 Interferons (IFNs), such as IFN-α, upregulate IFN Stimulated Genes (ISGs), that exert a broad range of anti-viral effector functions. The anti-viral mechanisms of several ISGs are well documented, in fact, by blocking the IFN-I receptor of Simian Immunodeficiency Virus (SIV)-infected rhesus macaques, Sandler et al., eloquently highlighted the importance of Type I IFNs for anti-viral gene expression, suppression of viral reservoir and control of CD4 T-cell depletion (Sandler et al., 2014). ISGs suppress HIV replication at multiple stages of the viral lifecycle (Doyle et al., 2015), but therapeutic trials of exogenous IFN-α have had surprisingly weak clinical response, with suppression of viral load poorly sustained (Lane, 1991, Bosinger and Utay, 2015, Asmuth et al., 2010). Therefore, we hypothesised that, as with other viruses (Elliott et al., 2007; Stevenson et al., 2013; Ulane et al., 2003), HIV may promote the degradation of Type 1 IFN Janus Kinase/Signal Transducers

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and Activators of Transcription (JAK/STAT) signalling pathway components, thus explaining the block in anti-viral ISG induction and HIV's in vivo resistance to IFN-α.

During infection viruses are detected by Pattern Recognition Receptors (PRRs) that induce Type I IFN production (Perry et al., 2005). Type I IFNs bind to the Type I IFN Receptor (IFNAR) and signal through the JAK/STAT pathway (Haller et al., 2006). IFN-α receptor engagement specifically results in tyrosine phosphorylation of JAK1 and Tyrosine Kinase (Tyk)2 (Colamonici et al., 1994; Shuai et al., 1993), leading to receptor phosphorylation and recruitment of STAT proteins (Platanias, 2005), which, when activated, dissociate from the receptor and form homo- or hetero-dimers that translocate to the nucleus. Type I IFNs classically signal via a trimer of STAT1, STAT2 and IFN Regulatory Factor (IRF)9, called the IFN-Stimulated Gene Factor (ISGF)3. Upon translocation to the nucleus, the ISGF3 complex binds IFN-Stimulated Response Elements (ISREs) (Levy et al., 1988) and promotes the induction of >500 ISGs (de Veer et al., 2001; Der et al., 1998a).

In this study we analysed the effects of HIV-1-Vif upon the expression of three well known, but functionally different, ISGs: ISG15, MxA and MxB. The ubiquitin like protein, ISG15, acts by targeting proteins for proteasomal degradation (Zhao et al., 2013) and has been shown to effectively inhibit the cellular release of the HIV-1 virion by inhibiting the endosomal trafficking pathway (Okumura et al., 2006). Furthermore, ISG15 can inhibit the release of HIV virions from the plasma membrane (Pincetic et al., 2010). Indeed, the expression of ISG15 is enhanced in CD4+ T cells of HIV-infected patients (Scagnolari et al., 2016) and HIV-1-Gag protein is post-translationally conjugated with ISG15, further highlighting its anti-viral importance (Woods et al., 2011). MxA shares structural homology with the dynamin family of GTPases and inhibits viral replication via physical formation of oligomeric rings around viral nucleocapsids (Haller and Kochs, 2011). It is well documented to inhibit a broad range of viruses, including influenza A virus, La Crosse encephalitis virus, bunyaviruses, phleboviruses, hantaviruses and Hepatitis C and B Viruses (HCV and HBV) (Pavlovic et al., 1990; Kochs et al., 2002; Frese et al., 1996; Gordien et al., 2001; Shi et al., 2017; Stevenson et al., 2011), but its direct anti-viral function against HIV-1 is not currently known. In contrast to MxA, MxB has been shown to inhibit HIV viral infection (Goujon et al., 2013). MxB prevents the nuclear import and integration of HIV-1 viral DNA and mutation of HIV capsid protein attenuates MxB's inhibition of HIV-1 (Kane et al., 2013; Liu et al., 2013).

Anti-viral ISGs provide Type I IFNs with an arsenal of weapons against viral infection, however, viral immune evasion strategies have evolved to block many of these immune mechanisms. We and others have discovered that several viruses, including HCV (Stevenson et al., 2013), Respiratory Syncytial Virus (RSV) (Elliott et al., 2007), Mumps (Ulane et al., 2003) Simian Virus 5 and Human Parainfluenza Virus Type 2 (Andrejeva et al., 2002), use a conserved mechanism to promote STAT proteasomal degradation, thus cutting the intracellular "lifeline" of ISG induction. Given the strong anti-viral effects of IFN-α, it is not surprising that diverse viral species have evolved this mechanism to inhibit its JAK/STAT signalling pathway. In fact, these viruses "hijack" an immunoregulatory mechanism commonly used by Suppressor Of Cytokine Signalling (SOCS) proteins to endogenously control JAK/STAT signalling (Yoshimura et al., 2007). SOCS all share a conserved "SOCS box" motif, which interacts with Elongin B, Elongin C, Cullin (Cul) and Ring box (RBX) proteins, forming a functional Elongin-Cul-SOCS box (ECS) E3 ligase complex (Kamura et al., 2004). This ECS enzymatic complex catalyses the poly-ubiquitination of target proteins, such as JAK2, thus marking them for degradation via the 26S proteasome (Kamizono et al., 2001). The RSV protein, NS1, interacts with Elongin C and Cul2, thereby forming an E3 ligase complex, which specifically targets STAT2 for proteasomal degradation (Elliott et al., 2007). Similarly, Paramyxovirus protein V complexes with DNA damage binding protein-1 (DDB1) and Cul4A, thus promoting STAT1 and STAT2 degradation (Ulane and Horvath, 2002); while Mumps protein V promotes degradation of STAT1, STAT2 and STAT3 via formation of a Cul4A-DDB1-dependent E3 complex (Ulane et al., 2003). Furthermore, we recently discovered that HCV also targets STAT1 and STAT3 for ubiquitination and proteasomal degradation, revealing yet another virus that suppresses anti-viral IFN-α signalling (Stevenson et al., 2013).

HIV-1 has evolved several mechanisms for disabling specific anti-viral ISGs. While the powerful immune evasion strategies HIV-1-Vif are clearly displayed by its ability to down-regulate APOBEC3 gene transcription (Anderson and Harris, 2015), it also contains SOCS box-like motifs that promote the formation of an ECS E3 ligase complex, which ubiquitinates and promotes proteasomal degradation of APOBEC3G protein (Sheehy et al., 2003; Kamura et al., 2004; Yu et al., 2004). Here, we report that the Vif-ECS also targets key components of the IFN-α JAK/STAT pathway. We demonstrate that HIV-1-Vif associates with STAT1 and STAT3 and promotes their ubiquitination and MG132-reversible proteasomal degradation, which is reliant on the SOCS box binding motifs of Vif and expression of the E3 ligase proteins, Cul5 and RBX2. Interestingly, while several HIV immune evasion processes are likely to be "at play" in vivo, compared to controls, we observed less IFN-α-mediated STAT1 and STAT3 phosphorylation and ISG induction in PBMCs infected in vitro with HIV-1 IIIB and in PBMCs from HIV patients, highlighting a functional signalling reduction in this anti-viral pathway. In fact, we also observed that T-lymphoblast cells pre-treated with IFN-α were less resistant to infection with IIIBΔVif (measured by p24 levels), than the WT IIIB clone, indicating that the presence of Vif functionally inhibits IFN-α's anti-viral protection. Together, these findings are an insight into the suppressive capabilities of HIV-1 on the IFN-α JAK/STAT pathway and may even provide some explanation for the failure of endogenous and therapeutic IFN-α to mediate control of HIV-1 replication in patients.

2. Materials and Methods

2.1. Cell Culture

HEK293T and Huh7 cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) or ThP-1 and CEM cells were cultured in (Roswelle Park Memorial Institute) RPMI supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified atmosphere. Cells were treated with 1000 U/ml human IFN-α (Roferon) and 1 μM MG132 (Sigma).

2.2. PBMC Isolation

PBMCs were isolated from 15 to 30 ml of blood by centrifugation (900g × 20 min) over Ficoll and washed ×2 in RPMI. Cells were treated with 1000 U/ml human IFN-α (Roferon).

2.3. Transfection

HEK293T cells were transfected with Mirus TransIT-2020 transfection reagent at a ratio of 1 μl transfection reagent: 1 μg DNA with HIV-1 Vif (a kind gift from Prof. Michael Malim, King's College London School of Medicine, UK) or the pCMV Empty Vector (EV) control for 48 h. HEK293T cells were also transfected with HIV-1 provirus pIIIB or HIV-1 provirus pIIIBΔVif (kind gifts from Prof. Reuben Harris, University of Minnesota), HIV-1 provirus YU-2 (a kind gift from Prof. Greg Towers, University College London) or an empty vector control. HEK293T cells were also transfected with HA-tagged STAT1 (a kind gift from Dr. Antonis Koromilas, McGill, Montreal), STAT2, myc-tagged STAT3 (a kind gift from Dr. Valeria Poli, University of Turin, Italy), HA-tagged Ubiquitin, or empty pcDNA3.1 using Mirus TransIT-2020 at the same ratio of transfection reagent: DNA. HEK293T cells were also transfected with constructs encoding hairpin sequences specific for human Cul5 (1637–1657), RBX2 (161–181), and EGFP (Clontech) mRNAs (kind gifts from Keiichi I. Nakayama, Kyushu University, Japan) for 48 h

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