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Modulation of the NF-κB signaling pathway by the HIV-2 envelope glycoprotein and its incomplete BST-2 antagonism

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ABSTRACT

The HIVs have evolved by selecting means to hijack numerous host cellular factors. HIVs exploit the transcription factor NF-κB to ensure efficient LTR-driven gene transcription. However, NF-κB is primarily known to act as a key regulator of the proinflammatory and antiviral responses. Interestingly, retroviruses activate NF-κB during early stages of infection to initiate proviral genome expression while suppressing it at later stages to restrain expression of antiviral genes. During HIV-1 infection, diverse viral proteins such as Env, Nef and Vpr have been proposed to activate NF-κB activity, whereas Vpu has been shown to inhibit NF-κB activation. It is still unclear how HIV-2 regulates NF-κB signaling pathway during its replication cycle. Here we confirm that human BST-2 and HIV-1 Env proteins can trigger potent activation of NF-κB. Importantly, we demonstrate for the first time that the HIV-2 Env induces NF-κB activation in HEK293T cells. Furthermore, the anti-BST-2 activity of the HIV-2 Env is not sufficient to completely inhibit NF-κB activity.

1. Introduction

The transcription factor NF-κB plays pivotal roles in the innate immune and antiviral responses by inducing expression of proinflammatory cytokines, type I interferons (IFN-α and IFN-β), interferon-stimulated genes (ISGs) as well as expression of genes involved in cell growth and survival (Chan and Greene, 2012; Gosh and Hayden, 2012; Sen and Baltimore, 1986). In absence of stimuli, NF-κB is maintained in an inactive form in the cell cytoplasm by the inhibitors of κB (IκBs). A variety of stimuli promote degradation of IκB proteins: viral antigens, cytokines and specific cell surface receptors such as interleukin 1 receptor, IFN receptors, T cell TCR-CD3 complex, Toll-like receptors (TLRs) or tumor necrosis factor (TNF) receptors (Chan and Greene, 2012; Pfeffer, 2011; Vallabhapurapu and Karin, 2009). Furthermore, human BST-2/Tetherin acts as an innate sensor of viral assembly and budding. A dityrosine motif (Y_6xY_8) in the BST-2 cytoplasmic tail recruits Syk kinase to prime the NF-κB signaling pathway (Arias and Evans, 2014; Cocka and Bates, 2012; Galao et al., 2012, 2014; Tokarev et al., 2013). The induction of the NF-κB signaling pathway requires activation of the IKK complex (IKKα, IKKβ and NEMO subunits). This complex phosphorylates IκB regulatory domains, thus allowing its ubiquitination and degradation. Consequently, NF-κB is translocated into the cell nucleus and binds to specific DNA sequences located in the promoters of NF-κB-dependent target genes (Chan and Greene, 2012; Heusinger and Kirchhoff, 2017; Kanarek and Ben-Neriah, 2012; Pfeffer, 2011; Vallabhapurapu and Karin, 2009). Interestingly, most of the HIV long terminal repeats (LTRs) include κB binding sequences, typically one in HIV-2 and two or three in HIV-1 (Bachu et al., 2012; Chen-Park et al., 2002; Hiebenthal-Millow et al., 2004; Jeeninga et al., 2000; Stroud et al., 2009). Although NF-κB is a key regulator of the host immune and antiviral responses, it is manipulated by retroviruses for promoting effective transcription of viral genes (Chan and Greene, 2012; Hiscott et al., 2001). However, even though NF-κB activation is beneficial for initial viral replication, this leads to rapid antiviral immune responses against the retroviruses. HIVs overcome this challenge by activating NF-κB at early stages of the viral cycle while inhibiting this transcription factor at later stages, restraining therefore expression of various antiviral genes (Heusinger and Kirchhoff, 2017; Hiscott et al., 2001).

While many studies attempted to identify the proteins used by HIV

Abbreviations: HIV, human immunodeficiency virus; Env, envelope glycoprotein; BST-2, Bone Marrow Stromal Cell Antigen 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκBs, inhibitors of κB proteins; IKKs, IκB kinases

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to fine-tune NF-κB activity throughout the replication cycle, it is still unclear and controversial if the HIV-1 envelope glycoprotein (Postler and Desrosiers, 2012), the HIV-1 and HIV-2 Nef proteins (Mangino et al., 2011; Sauter et al., 2015) or the Vpr proteins (Liang et al., 2015; Liu et al., 2013; Roux et al., 2000) activate the NF-κB signaling pathway. At later stages of infection, it is well defined that the HIV-1 Vpu protein - which is not encoded by HIV-2 - inhibits the NF-κB activity by three means: internalization and degradation of BST-2/Tetherin (Galao et al., 2012; Tokarev et al., 2013), impeding the IκB degradation through sequestration of β-TrCP proteins (Akari et al., 2001; Bour et al., 2001) and blocking the NF-κB nuclear translocation (Sauter et al., 2015).

To date, no study investigated neither the effects of the HIV-2 Env protein in the NF-κB activation, nor the impacts of the Env-mediated anti-BST-2 antagonism in the modulation of the NF-κB signaling pathway. In the present study, we tested the potential impacts of HIV-2 Env in the NF-κB activity.

2. Results

2.1. The HIV-2 envelope glycoprotein induces NF-κB activation

Since it has been described that the cytoplasmic domain of the HIV-1 glycoprotein gp41 enhances NF-κB activation (Postler and Desrosiers, 2012), we first sought to determine whether the HIV-2 envelope glycoprotein was capable of inducing the NF-κB pathway. To test this hypothesis, we generated a derivative of HEK293T cells stably expressing firefly luciferase gene under the control of an NF-κB-dependent promoter (HEK293T-NF-κB cells). Transfections of the transduced cells with plasmids encoding viral Env or Nef proteins of either HIV-1 or HIV-2, followed by a luciferase reporter assay allowed to assess the effect of these proteins on NF-κB activity. We observed that HIV-1 Env activated NF-κB ~ 12- to 20-fold compared to the negative control (Fig. 1A). These results confirmed that the HIV-1 Env protein stimulates NF-κB activity in HEK293T cells, in agreement with published data (Postler and Desrosiers, 2012). Interestingly, expression of HIV-2 Env protein potently enhanced NF-κB activation with a ~ 17- to 28-fold increase compared to the negative control (Fig. 1A). Therefore, we showed for the first time that the HIV-2 envelope glycoprotein may activate the NF-κB signaling pathway in HEK293T cells.

A recent study conducted by Sauter et al. (2015) assigned the NF-κB activation ability to the viral accessory Nef protein in both HIV-1 and HIV-2. We partly reproduced these experiments and we tested the potential activation of NF-κB pathway by the Nef proteins. Transfections with plasmids encoding HIV-1 or HIV-2 Nef both showed a weak and non-significant induction of NF-κB activity, as previously reported (Fig. 1A) (Heusinger and Kirchhoff, 2017; Sauter et al., 2015).

As previously mentioned, human BST-2 can activate the NF-κB pathway, triggering thereby proinflammatory responses. This restriction factor acts as an innate immune sensor of the assembly and budding of mammalian enveloped virions (Cocka and Bates, 2012; Galao et al., 2012, 2014; Tokarev et al., 2009, 2013). In our experiments, overexpression of BST-2 indicated, as expected, that this protein induced the NF-κB signaling pathway (Fig. 1A).

To validate the specificity of the NF-κB pathway activation by BST-2 and HIV-2 Env proteins, we used a dominant negative form of IKKβ. Dominant negative IKKβ (DN-IKKβ) is unable to phosphorylate the IκB protein, therefore preventing its proteasomal degradation and nuclear translocation of NF-κB subunits. Thus, NF-κB is consistently maintained in its inactive form in the cytoplasmic compartment (Cocka and Bates, 2012). Firstly, co-transfection of expression vectors encoding BST-2 and DN-IKKβ revealed an extensive and significant inhibition of NF-κB activity initially induced by BST-2. Secondly, co-transfection of plasmids encoding HIV-2 Env and DN-IKKβ showed a similar inhibition (Fig. 1B). These results demonstrated that the HIV-2 Env-mediated NF-κB activation observed was specific of the NF-κB pathway and ruled out the potential involvement of another transcription factor.

2.2. Differential modulation of the NF-κB activation by the anti-BST-2 HIV-1 Vpu and HIV-2 Env

HIV-1 utilizes the accessory Vpu protein to overcome BST-2 host restriction factor by removing BST-2 from the cell surface and facilitates therefore the budding of nascent virions from infected cells (Dube et al.,

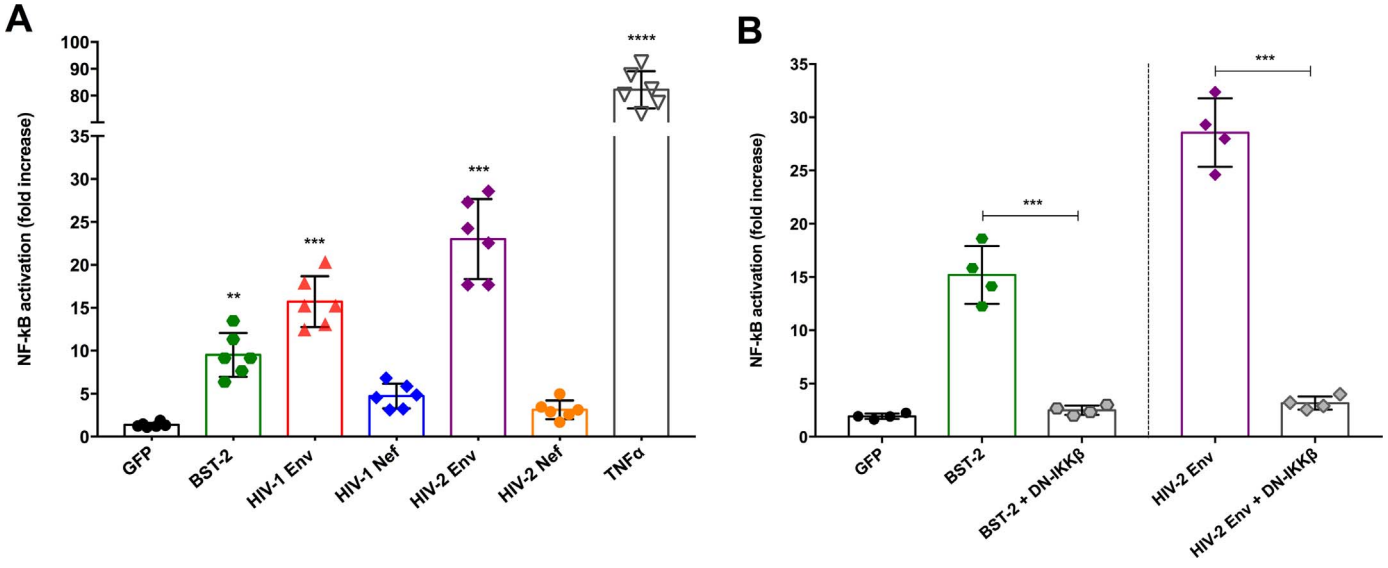

Fig. 1. Stimulation of NF-κB activity by human BST-2 and lentiviral proteins. (A) Transduced HEK293T cells were transfected with expression vectors encoding GFP (as a negative control), human BST-2 or viral proteins of HIV-1 and HIV-2 (Env or Nef proteins). HEK293T cells were lysed and luciferase activities were determined 24 h post transfection. As a positive control, TNFα was added into the cell culture medium (25 ng/ml) to induce a potent activation of NF-κB pathway (Chan and Greene, 2012). (B) Specificity of the NF-κB pathway activation by BST-2 and HIV-2 Env proteins. Transduced HEK293T cells were co-transfected with BST-2 or HIV-2 Env plasmids alongside with a dominant negative form of IKKβ (DN-IKKβ). NF-κB activity is represented as fold increase, being the ratio between the luciferase activity (RLU) of the corresponding measure and that of transduced but non-transfected HEK293T cells. These results were derivated from six independent experiments (n = 6) in (A) and four independent experiments (n = 4) in (B). Data were analyzed by one-way ANOVA statistical test followed by a multiple comparison test which compares each mean with that of the negative control (GFP) in (A) or compares each mean with all other means in (B) (** *p*-value < 0.01, *** < 0.001 and **** < 0.0001). Error bars indicate mean ± standard deviation (SD).