Virology 513 (2018) 11–16

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

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

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ARTICLE INFO

Keywords:
HIV
HIV-2
Env
Envelope glycoprotein
NF-κB
BST-2
Tetherin
Vpu
Antagonism
Restriction factor

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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http://dx.doi.org/10.1016/j.virol.2017.09.024
Received 13 July 2017; Received in revised form 26 September 2017; Accepted 30 September 2017
Available online 11 October 2017

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.,

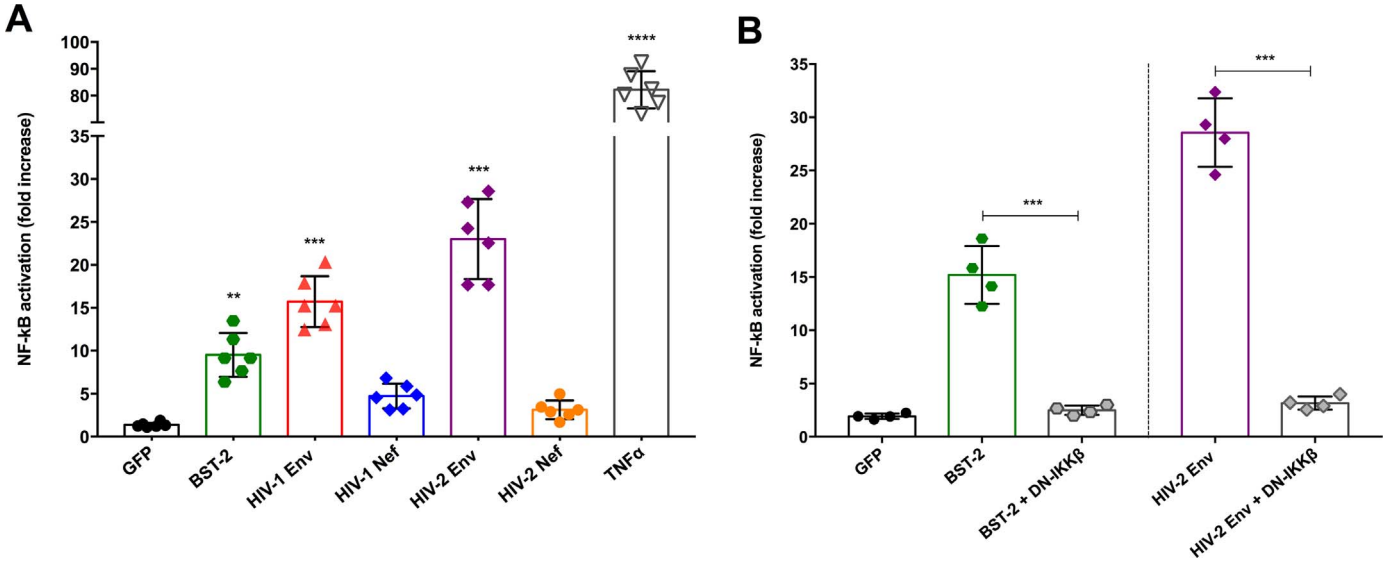

Fig. 1. Stimulation of NF-κB activity by human BST-2 and lentiviral proteins. (A) Transduced HEK293T cells were transfected with expression vectors encoding GFP (as a negative control), human BST-2 or viral proteins of HIV-1 and HIV-2 (Env or Nef proteins). HEK293T cells were lysed and luciferase activities 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).

2010; Goffinet et al., 2009; Neil et al., 2008; Van Damme et al., 2008). Vpu binds BST-2 through its membrane and cytoplasmic domain, causing subsequent proteasomal degradation of this restriction factor (Douglas et al., 2009; Goffinet et al., 2009; Kueck and Neil, 2012; Vigan and Neil, 2010). As previously reported (Bour et al., 2001; Galao et al., 2012; Sauter et al., 2015; Tokarev et al., 2013), co-expression of HIV-1 Vpu and human BST-2 nearly abolished the NF-κB activity (Fig. 2A and B).

However, the HIV-2 genome does not encode *vpu* gene and relies on the envelope glycoprotein to antagonize BST-2 (Hauser et al., 2010; Le Tortorec and Neil, 2009; Le Tortorec et al., 2011). As shown in the previous experiment, HIV-2 Env and BST-2 both activated the NF-κB signaling pathway when expressed separately (Fig. 1A). Thus, we sought to determine whether co-expression of both proteins might modulate the NF-κB signaling. Interestingly, co-expression of HIV-2 Env and BST-2, at 24 and 48 h post transfection, resulted in an incomplete inhibition of the NF-κB activation compared to the potent Vpu-mediated inhibition (Fig. 2A and B).

Furthermore, we showed in a previous study that N659D substitution in the extracellular domain of the HIV-2 Env protein, as well as a truncated cytoplasmic tail, impaired the anti-BST-2 ability (Dufrasne et al., 2016). Here we observed that the HIV-2 Env N659D activated NF-κB in a similar way to HIV-2 Env wild-type when expressed alone. Nevertheless, in the presence of BST-2, the resulting NF-κB activation was not reduced significantly (Fig. 2A and B).

2.3. Activation of the NF-κB signaling pathway by BST-2 upon HIV-2 assembly

Human BST-2 can act as a viral sensor of HIV-1 assembly and release by inducing a potent activation of NF-κB (Galao et al., 2012; Tokarev et al., 2013). Therefore, we next sought to determine whether

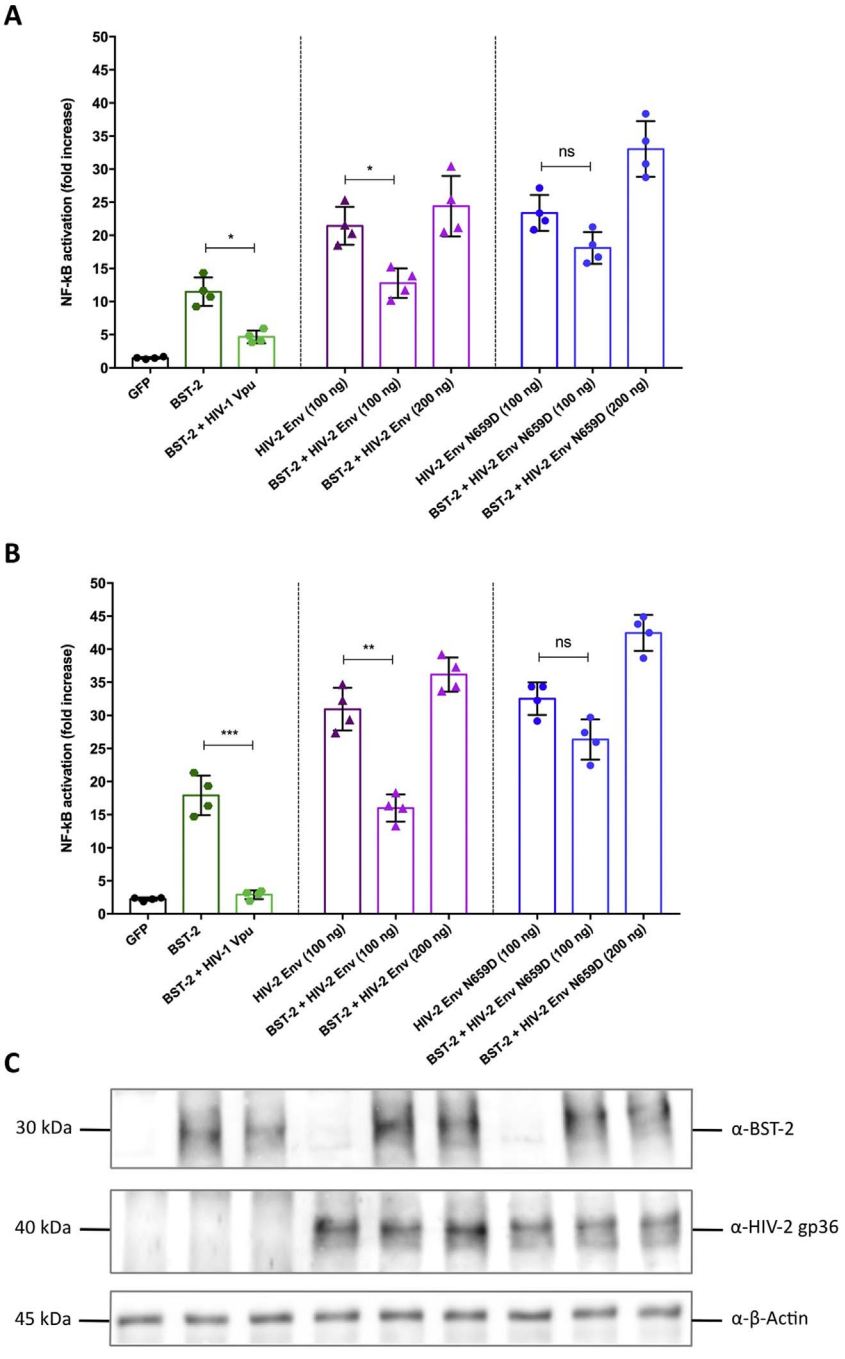

Fig. 2. Modulation of the NF-κB activity by the anti-BST-2 HIV-1 Vpu and HIV-2 Env.
Transduced HEK293T cells were transfected or co-transfected with expression vectors encoding GFP, BST-2, viral proteins of HIV-1 (Vpu) or HIV-2 (Env or Env N659D) as indicated. Co-transfections were carried out by varying Env and Env N659D plasmid amounts (100 ng or 200 ng) while maintaining constant the BST-2 plasmid amounts (70 ng). Transfected HEK293T cells were lysed and luciferase activities were determined at (A) 24 h and (B) 48 h post transfection. Results were derivated from four independent experiments (n = 4) and were analyzed by one-way ANOVA followed by a multiple comparison test (* p-value < 0.05, ** < 0.01, *** < 0.001, ns = non-significant). Error bars indicate mean ± SD. (C) Expression levels of BST-2 and HIV-2 Env proteins were evaluated by Western blots. The order of the samples is the same as that of the co-transfections in (B). Expression levels of HIV-2 Env and Env N659D proteins were equivalent and HIV-2 Env proteins did not degrade BST-2, in contrast to HIV-1 Vpu proteins.

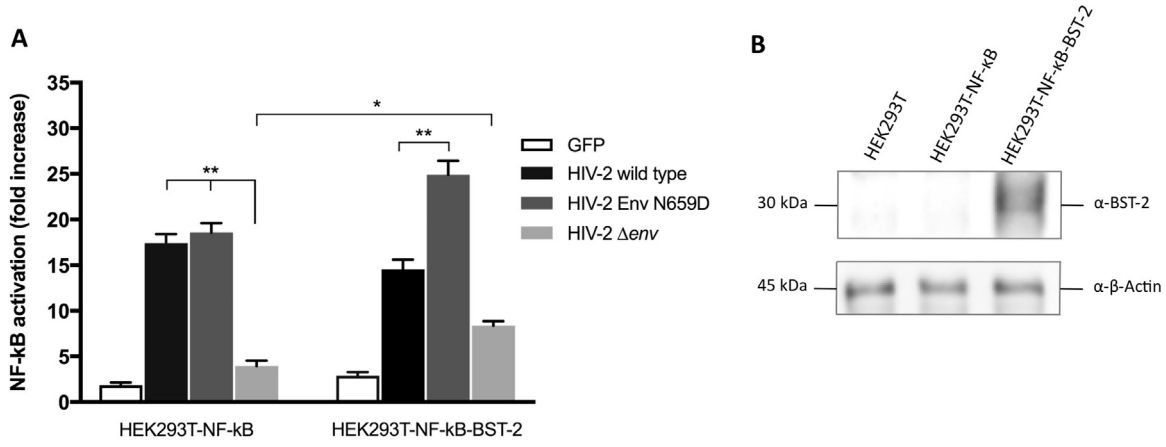

Fig. 3. NF-κB activation by BST-2 upon HIV-2 assembly. (A) HEK293T-NF-κB-BST-2 cells were generated by stable transfection and used to perform transfections of HIV-2 wild type, HIV-2 Env N659D or HIV-2 Δ*env* proviral clones (300 ng). Thirty hours post transfection, luciferase assays were performed as previously described. Results were derivated from three independent experiments (n = 3) and were analyzed by one-way ANOVA followed by a multiple comparison test (* p-value < 0.05, ** < 0.01). Error bars indicate mean ± SD. (B) Expression of BST-2 was verified in the HEK293T-NF-κB-BST-2 cells by Western blots.

assembly of HIV-2 virions stimulates BST-2 to activate NF-κB, and whether the HIV-2 Env-mediated anti-BST-2 antagonism modulates this additional activation of NF-κB. We generated a HEK293T cell population that encoded the NF-κB binding sequence while constitutively expressing BST-2 (called HEK293T-NF-κB-BST-2 cells) to prevent the NF-κB activation due to transient expression of BST-2 (Galao et al., 2012, 2014). We used these cells to perform transfections of HIV-2 wild type, HIV-2 Env N659D or HIV-2 Δ*env* proviral clones and we compared levels of NF-κB activation obtained from these cells with those from the HEK293T-NF-κB cells that did not express BST-2. First, the NF-κB activation induced by the expression of HIV-2 ΔEnv virions was significantly reduced compared to expression of HIV-2 wild type or HIV-2 Env N659D in the HEK293T-NF-κB cells (Fig. 3A). To evaluate the activation of NF-κB resulting only from the viral sensing by BST-2, we also transfected HEK293T-NF-κB-BST-2 cells with the HIV-2 Δ*env* proviral clone. As shown in the Fig. 3A, levels of NF-κB activation by BST-2 were slightly but significantly increased upon sensing of the assembly of HIV-2 ΔEnv virions. Secondly, the transfection of HEK293T-NF-κB-BST-2 cells with the HIV-2 Env N659D proviral clone, unable to antagonize BST-2, induced important NF-κB activity, probably due to BST-2 viral sensing and additional NF-κB stimulation by HIV-2 Env. In addition, transfections of these cells with the HIV-2 wild type proviral clone demonstrated a significant but incomplete inhibition of the NF-κB activity when compared to the results of HIV-2 Env N659D transfections.

3. Discussion

HIV-2-infected individuals develop immunodeficiency with a substantial delay and a majority do not progress to the AIDS disease state compared to HIV-1-infected persons. These are called long-term non progressors (de Silva et al., 2008; Rowland-Jones and Whittle, 2007; Thiébaut et al., 2011). HIV-2-infected patients present a lower viral load as well as a slower decline in CD4+ T cell counts observed during asymptomatic infection. The contributing factors of these differences are attributed to the immune responsiveness of HIV-1- and HIV-2-infected persons, which can explain the differences in pathogenesis between the two types of infection. It has been proposed that better immune control and lower replication capacity of HIV-2 could account for these discrepancies (Damond et al., 2002; de Silva et al., 2013a, 2013b; Leligdowicz et al., 2010, 2007; Makvandi-Nejad and Rowland-Jones, 2015; Michel et al., 2000; Nyamweya et al., 2013; Ozkaya Sahin et al., 2013; Rowland-Jones and Whittle, 2007).

The host cellular factor NF-κB is a key factor in the immune antiviral responses but can also be manipulated by primate lentiviruses to promote proviral genome transcription. In this context, study of the interplay between HIV-2 and NF-κB could provide new insights to better define, at least partly, molecular mechanisms that underpin the particular HIV-2 pathogenesis. In this study, we demonstrated that the HIV-2 Env was capable of inducing NF-κB activation, similarly to the HIV-1 Env protein. Secondly, we confirmed that human BST-2 also primed stimulation of NF-κB and that co-expression with HIV-1 Vpu nearly abolished this activation, essentially through the potent Vpu-mediated anti-BST-2 activity (Cocka and Bates, 2012; Galao et al., 2012; Tokarev et al., 2013) and stabilization of IκB proteins (Akari et al., 2001; Bour et al., 2001; Sauter et al., 2015). Importantly, HIV-2 does not encode the *vpu* gene and relies on its envelope glycoprotein to overcome the BST-2 host restriction factor but contrary to HIV-1 Vpu protein, this does not lead to BST-2 degradation (Hauser et al., 2010; Le Tortorec and Neil, 2009; Le Tortorec et al., 2011; Neil, 2013). Thus, we tested the effectiveness of HIV-2 Env anti-BST-2 activity to inhibit the activation of NF-κB. In presence of BST-2, HIV-2 Env protein failed to thoroughly inhibit NF-κB activation, contrary to the multifunctional HIV-1 Vpu protein.

In this study, we only used HEK293T cells. Reproduction of our experiments using CD4+ T cells single-round infections could strengthen these conclusions.

Overall, our results suggest that the HIV-2 Env-mediated BST-2 antagonism was not sufficient to completely inhibit NF-κB activity, in contrast to HIV-1 Vpu antagonism. In our experimental design, although the cells triggered a strong NF-κB activation in response to the HIV-2 Env, this late protein failed to completely restrict antiviral gene expression, as revealed by the experiments of NF-κB activation upon HIV-2 assembly. Furthermore, our results would also suggest that HIV-2 does not express a potent inhibitor of the NF-κB activity during its replication cycle, in contrast to HIV-1. Moreover, it has not yet been demonstrated whether the HIV-2 Env protein was capable of sequestering β-TrCP proteins, or whether this protein prevented the nuclear translocation of NF-κB.

This study highlights the importance of an efficient anti-BST-2 activity in the regulation of NF-κB throughout the viral cycle and could provide new insights in the understanding of the enhanced immune antiviral responses in HIV-2 disease as compared to HIV-1.

4. Materials and methods

4.1. Cell lines and viral transduction

HEK293T cells were obtained from the ATCC and maintained in Dulbecco's modified Eagles's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA). This medium was supplemented with

10% fetal bovine serum (FBS, HyClone™ FetalClone™ from GE Healthcare Life Sciences, Fairfield, CT, USA) and 1% gentamicin (Thermo Fisher Scientific).

To establish HEK293T reporter cells stably expressing luciferase gene under the control of NF-κB binding sequence (HEK293T-NF-κB), we generated VSV-pseudotyped viruses upon transfection of three lentivectors into 3.5×10^5 HEK293T cells: the pHAGE NFkB-TA-LUC-UBC-GFP-W plasmid (750 ng) (Wilson et al., 2013), the packaging psPAX2 plasmid (400 ng) and the pMD2.G plasmid encoding vesicular stomatitis virus envelope protein (200 ng) (all obtained from Addgene; #49343, #12260 and #12259 respectively). Fourty-eight hours post transfection, supernatant was collected and precleared by centrifugation 10 min at $800 \times g$. Cell-free supernatant containing viral particles was centrifuged at $32,000 \times g$ at 4 °c for 2 h. Pseudotyped viral particles were then used to infect and transduce HEK293T cells. The pHAGE NFkB-TA-LUC-UBC-GFP-W plasmid encodes four copies of the NF-κB consensus sequence. NF-κB activation and binding trigger promoter activity and drive expression of the firefly luciferase reporter gene. The green fluorescent protein (GFP) is constitutively expressed by the ubiquitin C (UBC) promoter, enabling sorting of transduced cells by flow using a BD FACSAria™ III Cell Sorter (BD Biosciences, San Jose, CA, USA) (Wilson et al., 2013).

To obtain a cell line encoding NF-κB binding sequence while constitutively expressing BST-2 (HEK293T-NF-κB-BST-2), HEK293T-NF-κB cells were stably transfected with pcDNA-BST-2 and pSF-MinCMV-Puro (OG579) Minimal CMV Puromycin vector (Oxford Genetics, Oxford, UK), using a ratio 10:1. Fourty-eight hours post transfection, cells were incubated in culture medium supplemented with puromycin (1 μg/ml) to select, after several passages, resistant cells that stably expressed human BST-2 protein.

All cell lines were tested for mycoplasma contamination using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Basel, Switzerland).

4.2. Plasmids and transfections

HIV-1 *nef* and *env* coding sequences were obtained by subcloning PCR technique from the HIV-1 NL4-3 molecular clone (pNL4-3 plasmid obtained from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH) (Adachi et al., 1986). Amplified fragments were purified and inserted into the mammalian expression vector pcDNA3.1(+) (Invitrogen™; Thermo Fisher Scientific) with the *Hind*III-*Apa*I restriction sites for *nef* and with the *Eco*RI-*Apa*I for *env* sequence. The codon-optimized *vpu* sequence cloned into pcDNA3.1(-) expression plasmid was also obtained from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH (pcDNA-Vphu) (Nguyen et al., 2004).

HIV-2 *nef* and *env* sequences were amplified using the proviral expression clone pKP59 HIV-2 ROD (molecular clone obtained from Dr. Isabel Barahona, Instituto Superior de Ciências da Saúde-Sul, Portugal) (Ribeiro, 2005). This infectious clone encodes the whole genome of HIV-2 ROD reference strain (GenBank: M15390.1) and an Env protein harboring a complete cytoplasmic tail (158 amino acids) (Dufrasne et al., 2016). DNA fragments were inserted into the pcDNA3.1(+) expression vector.

The HIV-2 Δ*env* proviral clone was generated using a site-directed mutagenesis reaction from the pKP59 HIV-2 ROD clone (QuickChange II Site-Directed Mutagenesis kit from Agilent, Santa Clara, CA, USA).

The human *bst-2* sequence was amplified from cDNA obtained by reverse transcription of H9 cell mRNAs using the Transcriptor First Strand cDNA Synthesis Kit from Roche (Mannheim, Germany). Amplicons were also inserted in the pcDNA3.1(+) plasmid.

All plasmid constructs were verified by nucleotide sequencing using an ABI 3500 genetic analyser (Applied Biosystems™, Thermo Fisher Scientific).

All HEK293T cell lines were transfected using TurboFect Transfection Reagents (Thermo Fisher Scientific). For luciferase reporter gene assays, 2×10^5 cells were harvested and seeded in a 24-well plate twenty-four hours before transfection and transfected with 100 ng pcDNA-Env, 150 ng pcDNA-Nef, 150 ng pcDNA-Vphu (HIV-1), or 100 ng pcDNA-Env, 150 ng pcDNA-Nef (HIV-2), or 70 ng pcDNA-BST-2. Luciferase activities were measured 24 h after transfections (and 48 h for co-transfections), as indicated in figure legend.

The dominant negative IKKβ (K44M) plasmid was obtained from Addgene (plasmid #11104). This plasmid expresses an inactive kinase of IκB (Mercurio et al., 1997).

4.3. Reporter gene assay

Transduced HEK293T cells were used to assess the NF-κB activation by expression of different HIV-1, HIV-2 or human proteins. Twenty-four hours (or 48 h) post transfection, cells were washed once with DPBS (Gibco™; Thermo Fisher Scientific) and lysed with 1x passive lysis buffer (Promega, Madison, WI, USA) for 15 min. Cell lysates were centrifuged 5 min at $15,000 \times g$ and 20 μl of cell-free lysates were added to 100 μl of Luciferase Assay Reagent II (LAR II) containing luciferin. Bioluminescent signals were measured with a GloMax™ 20/20 luminometer (Promega).

4.4. Western blots and antibodies

Expression levels of HIV-2 Env and BST-2 proteins were determined from cell lysates used to perform the luciferase assays. Samples were added to 2x Laemmli sample buffer, boiled for 5 min, separated by electrophoresis on 12% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. The membranes were blocked with 5% BSA in TBS containing 0.1% Tween-20 for 1 h, and probed overnight at 4 °C with a rabbit monoclonal anti-BST-2 antibody (Abcam, cat #ab134061, dilution 1:5000) or a mouse monoclonal anti-HIV-2 gp36 antibody (OriGene Europe-Acris antibodies, cat #BM3335, dilution 1:1000). Endogenous β-Actin was detected with the rabbit monoclonal anti-β-Actin antibody (Cell Signaling, cat #4970, dilution 1:1000). Membranes were rinsed three times for 5 min in TBS 0.1% Tween-20 and the blots were probed with either HRP-conjugated anti-rabbit or anti-mouse secondary antibody (Cell Signaling, cat #7074 and #7076, respectively) at a dilution of 1:2000 for 1 h at room temperature. Membranes were then rinsed three times and treated with the Amersham™ ECL™ Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences), and blots were analyzed with the ChemiDoc™ XRS+ System (Bio-Rad).

Acknowledgments

We thank Philippe de Sany and Armelle Duquenne for the cell lines maintenance. We acknowledge Dr. Darrell Kotton for the pHAGE NFkB-TA-LUC-UBC-GFP-W plasmid, Dr. Didier Trono for the psPAX2 and pMD2.G plasmids, and Dr. Anjana Rao for the IKKβ (K44M) plasmid.

Author contributions

F.E.D. P.G. and J.R. conceived and designed the experiments; F.E.D., M.L. performed the experiments and analyzed the data; F.E.D., M.L., P.G. and J.R. wrote the paper. A.M., E.A., G.D. and B.K. revised the manuscript and suggested or made modifications.

Conflicts of interest

The authors declare no conflict of interest.

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