HIV-2 viral production and infectivity are affected by APO3 Host Factors

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Background

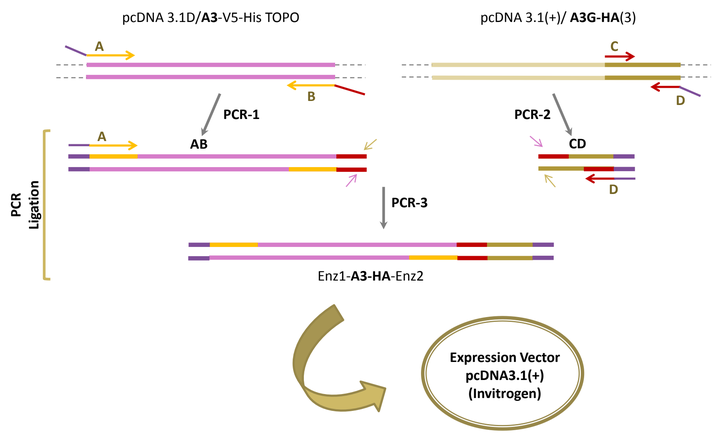
HIV type 2, closely HIV type 1 related retroviruses discovered few years later, exhibits in infected individuals significantly lower plasma viral loads, causes longer periods of asymptomatic infection in patients that survive, without treatment, for longer periods compared with HIV-1 patients. Determining why HIV-2 is much less pathogenic than HIV-1 is a current challenge that will increase further our understanding of HIV pathogenesis. Several studies indicate that host factors could play a role in these differences. One of the known host cell restriction factor is the deaminase APOBEC3G (A3G). This enzyme belongs to APOBEC3 family (A3) and all members have antiviral activity against HIV-1 suppressed by the viral protein Vif. Our previous studies have shown that A3G viral inhibition is less active against HIV-2 than HIV-1.[1] Moreover, the proteins HIV-2Vif and HIV-1Vif share only 30% of identity and these viruses showed differential replication and capacity for productive infection in cell lines, suggesting either different threshold requirements for the same cellular factor or the involvement of different factors to compensate for Vif1 and Vif2 functions.[2] In the last decades, in contrast with Vif1-A3 interactions, Vif2-A3 interactions have been very poorly explored. Recently, it was reported that HIV-1 and HIV-2 can both target A3 via their Vif proteins but each Vif protein has distinct recognition sites to specific targets, namely A3F and A3G. Moreover, this work indicate that Vif2 can bind to several members of A3 protein family and the sensitivity of A3 proteins to Vif2-induced degradation is different from the one previously observed with Vif1.[3] Comparative studies of both viruses and A3 host factors that may affect viral infectivity will be useful for understanding the determinants of HIV-2 pathogenesis and the differences between HIV-1 and HIV-2.

Objectives

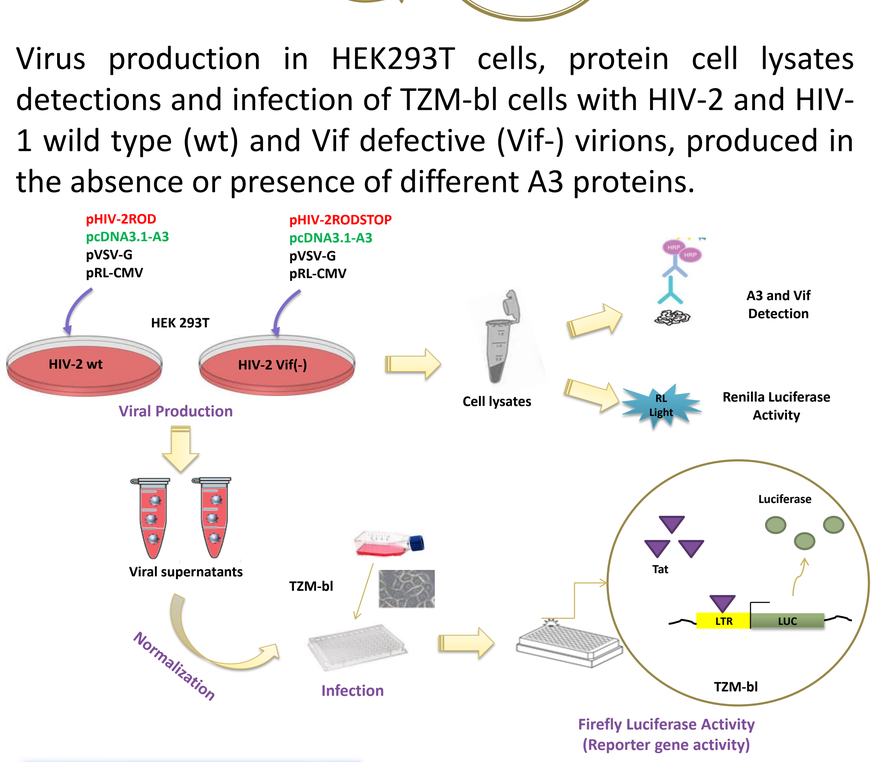
Our aim is to understand at molecular level what is the role of the A3 protein family members in HIV-2 infection and compare with HIV-1.

Methods

A3 members were cloned in the same expression vector system, expressing A3 in fusion with 3 HA-tags in C-terminal.



Virus production in HEK293T cells, protein cell lysates detections and infection of TZM-bl cells with HIV-2 and HIV-1 wild type (wt) and Vif defective (Vif-) virions, produced in the absence or presence of different A3 proteins.



Results

Viral production of HIV-1 is higher than HIV-2 production after 293T cells transfection using the same amounts of DNA and infectivity detection system used has a higher discriminatory power in HIV-1 infections.

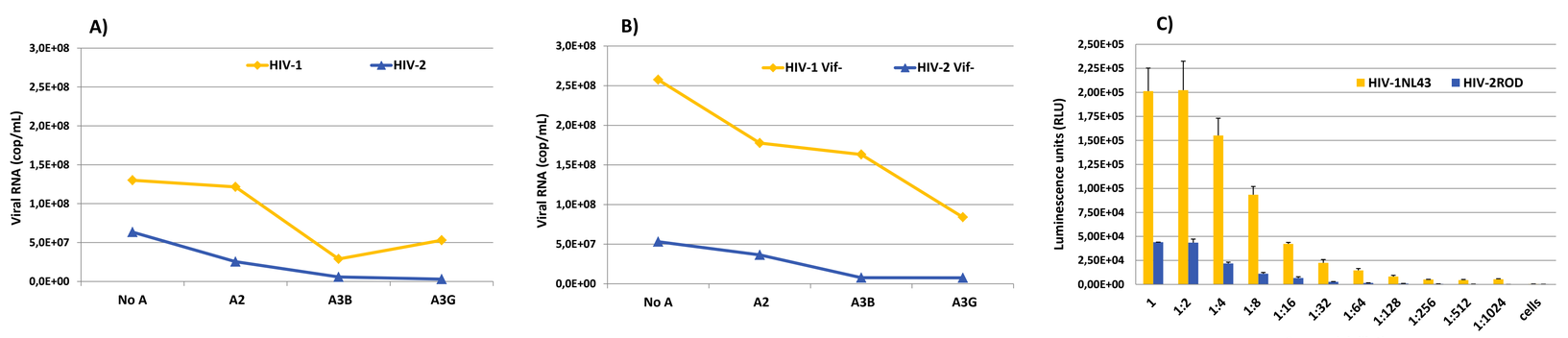


Fig. 1 Differences between production levels of HIV-2 and HIV-1 virions. A), B) 293T cells were transfected with HIV_{WT} and HIV Vif- molecular clones, in the presence or absence of several A3 expression vectors in ratio of 1:1 HIV:A3 (1µg of each DNA). Supernatant viral RNA levels was determined by RT-PCR in Virology Laboratory of Egas Moniz Hospital using specific primers for LTR region of HIV-2 and ENV region of HIV-1. **C)** TZM-bl Infectivity values using different dilutions of HIV-2 and $HIV\text{-}1_{WT}$.

Viral production is affected by the efficiency of transfection and the presence of A3 proteins. In the presence of A3 proteins the levels of HIV-2Vif- decrease drastically.

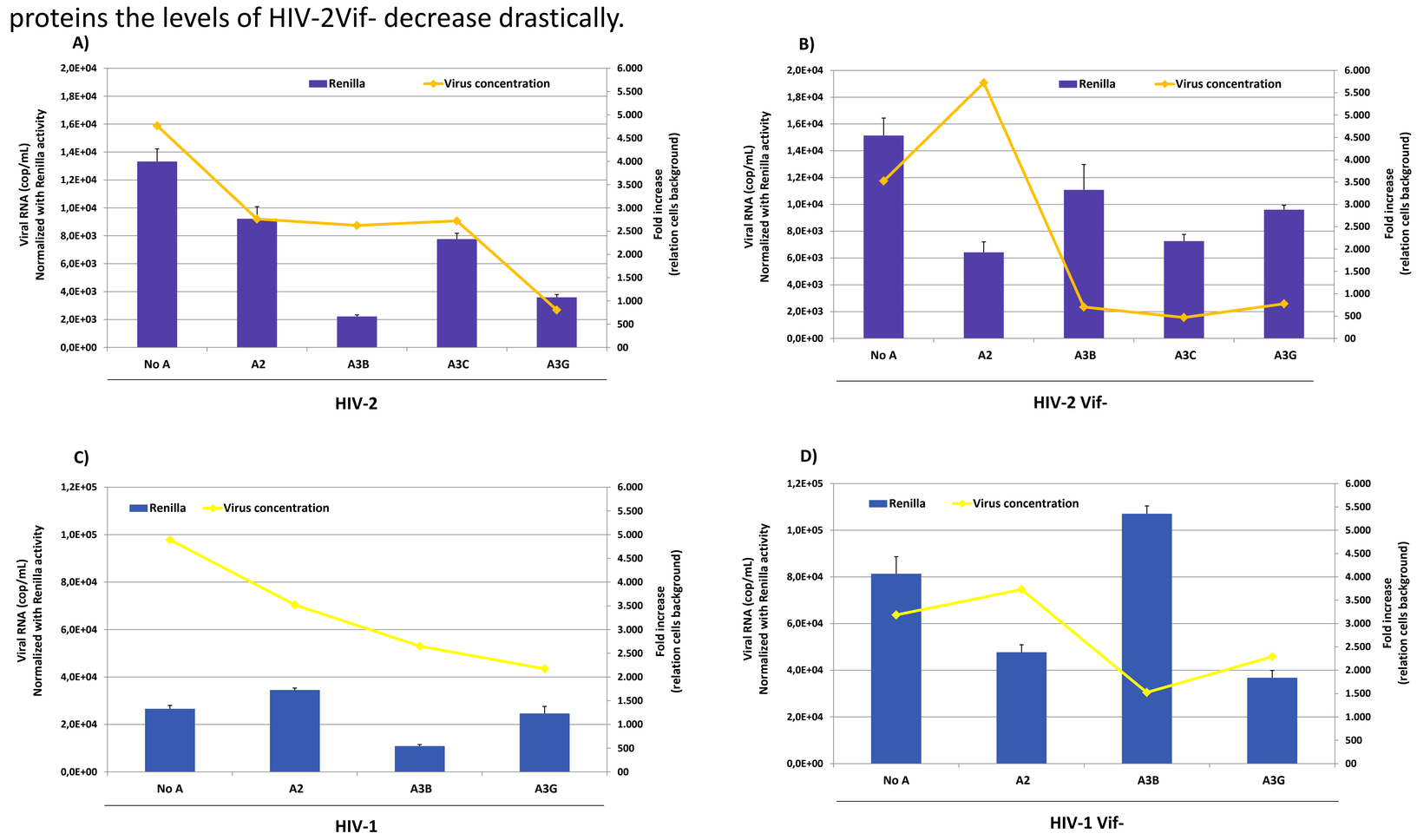


Fig. 2 A3 proteins inhibit viral production of HIV-2 (A)(B) and HIV-1 (C) (D) . pRL.CMV (Renilla expression vector) it wasused as a transfection efficiency control of 293T producing cells . Values of Renilla activity are presented as n fold in relation to cell background (secondary axis). Produced viral levels (cop/mL) were normalized with values of transfection efficiency control determined in the producing cell lysates for each condition.

The restriction factors A3B and A3G inhibit HIV-2 Vif- infections and they were strongly neutralized by Vif2. HIV-2-producing cells lysates showed sensitivity of A3B and A3G to Vif2-induced degradation.

Fig. 3 A) Viral infectivity of HIV-2wt and HIV-2Vif- produced in the presence of different APOBEC. After virus normalization, A3 antiviral effects were evaluated by one cycle infectivity assay in TZM-bl cells. Values are presented as % of infectivity relative to viruses produced in the absence of A3, defined as 100% (empty vector was used as control). Columns and error bars represent average ± SD, respectively (n=2); **B) Sensitivity of A3 proteins to Vif2.** Western blot of A3 proteins in virus-producing cells lysates.

Conclusions

1- Viral production of HIV-1 is higher than HIV-2 production.
2- Viral production is affected by the efficiency of transfection and the presence of A3.
3- This effect is more pronounced in HIV-2Vif- productions.
4- HIV-2 infectivity is strongly inhibited by A3B and A3G and this antiviral effect is efficiently suppressed by Vif2 protein.
In the future, we will compare HIV-2 and HIV-1 infectivity using viral stocks produced in the presence of lower amounts of A3, to avoid the effect on viral production.

References

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