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A stably expressed llama single-domain intrabody targeting Rev displays broad-spectrum anti-HIV activity

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ABSTRACT

The HIV Rev protein mediates the transport of partially and unspliced HIV mRNA from the nucleus to the cytoplasm. Rev multimerizes on a secondary stem-loop structure present in the viral intron-containing mRNA species and recruits the cellular karyopherin CRM1 to export viral mRNAs from the nucleus to the cytoplasm. Previously we have identified a single-domain intrabody (Nb_{190}), derived from a llama heavy-chain antibody, which efficiently inhibits Rev multimerization and suppresses the production of infectious virus. We recently mapped the epitope of this nanobody and demonstrated that Rev residues K20 and Y23 are crucial for interaction while residues V16, H53 and L60 are important to a lesser extent.

Here, we generated cell lines stably expressing Nb_{190} and assessed the capacity of these cell lines to suppress the replication of different HIV-1 subtypes. These cells stably expressing the single-domain antibody are protected from virus-induced cytopathogenic effect even in the context of high multiplicity of infection. In addition, the replication of different subtypes of group M and one strain of group O is significantly suppressed in these cell lines. Next, we analysed the natural variations of Rev amino acids in sequence samples from HIV-1 infected patients worldwide and assessed the effect of Nb_{190} on the most prevalent polymorphisms occurring at the key epitope positions (K20 and Y23) in Rev. We found that Nb_{190} was able to suppress the function of these Rev variants except for the K20N mutant, which was present in only 0.7% of HIV-1 sequence populations ($n = 4632$).

Cells stably expressing the single-domain intrabody Nb_{190} are protected against virus-induced cytopathogenic effect and display a selective survival advantage upon infection. In addition, Nb_{190} suppresses the replication of a wide range of different HIV-1 subtypes. Large-scale sequence analysis reveals that the Nb_{190} epitope positions in Rev are well conserved across major HIV-1 subtypes and groups. Altogether, our results indicate that Nb_{190} may have broad potential as a gene therapeutic agent against HIV-1.

1. Introduction

HIV expresses three main RNA species, fully spliced, partially spliced and unspliced RNA, which encode for the regulatory, structural and viral enzymatic proteins. Various viral proteins are thus encoded by intron-containing mRNAs, which are naturally retained in the nucleus and degraded by host cell mechanisms. However, the nuclear export of these intron-containing viral mRNAs is crucial for viral replication (Pollard and Malim, 1998). To circumvent the nuclear retention and the degradation of these RNAs, HIV has developed a sophisticated mechanism. Through expression of the regulatory protein Rev, viral RNAs are hooked up to the cellular CRM1-mediated nuclear export pathway. Rev multimerizes on a secondary stem-loop structure, called Rev Responsive Element (RRE), present in all intron-containing viral RNAs (Fischer et al., 1999; Malim et al., 1989; Sodroski et al., 1986). This ribonucleoprotein complex is then exported to the cytoplasm via the CRM1-mediated nuclear export pathway (Neville et al., 1997). In the cytoplasm these RNAs serve as

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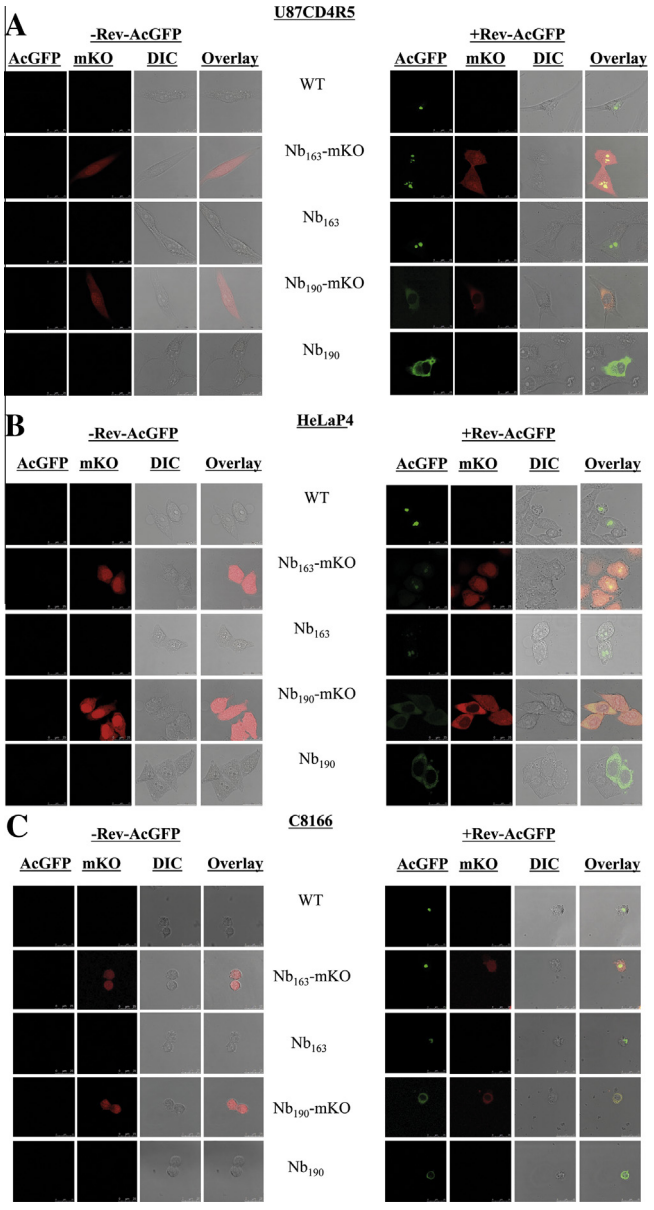

Fig. 1. Co-expression of Rev and Nb_{190} results in a relocalization of both Nb_{190} and Rev (A) U87CD4CCR5 or (B) HeLaP4 cells stably expressing the negative control Nb_{163}(mKO) or the active Nb_{190}(mKO) were transfected with Rev-AcGFP. GFP (green) and mKO (red) were visualized using confocal fluorescence microscopy. The third column shows differential interference contrast (DIC). (C) C8166 cells stably expressing the negative control Nb_{163}(mKO) or the active Nb_{190}(mKO) were transduced with a vector expressing Rev-AcGFP. GFP (green) and mKO (red) were visualized using confocal fluorescence microscopy. The third column shows differential interference contrast (DIC).

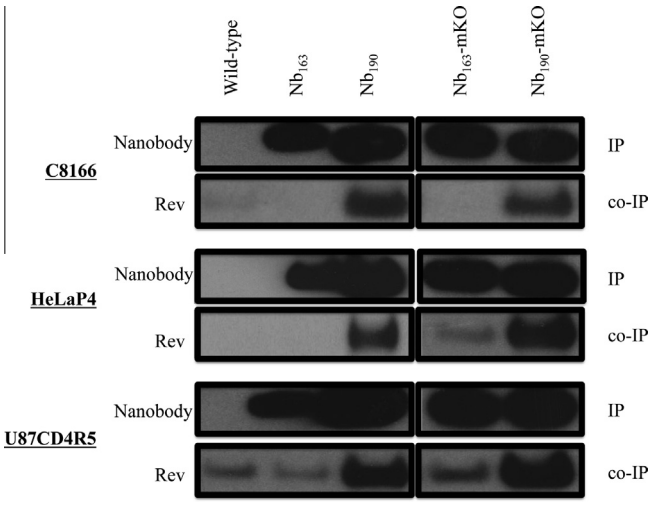

Fig. 2. Nb_{190} interacts with Rev in Nb_{190} expressing stable cell lines as demonstrated by co-immunoprecipitation. Lysate of HeLa cells transfected with Rev-AcGFP was mixed with lysate of wild-type (C8166, HeLaP4, U78CD4R5) cells or cells stably expressing Nb_{190}(mKO) or the negative control Nb_{163}(mKO). Nanobodies (bait, IP) with the bound Rev (prey, co-IP) were pulled-down out of the mixture using coated magnetic beads. Presence of nanobody (IP) and/or Rev (co-IP) in the pull-down was visualized by Western blotting.

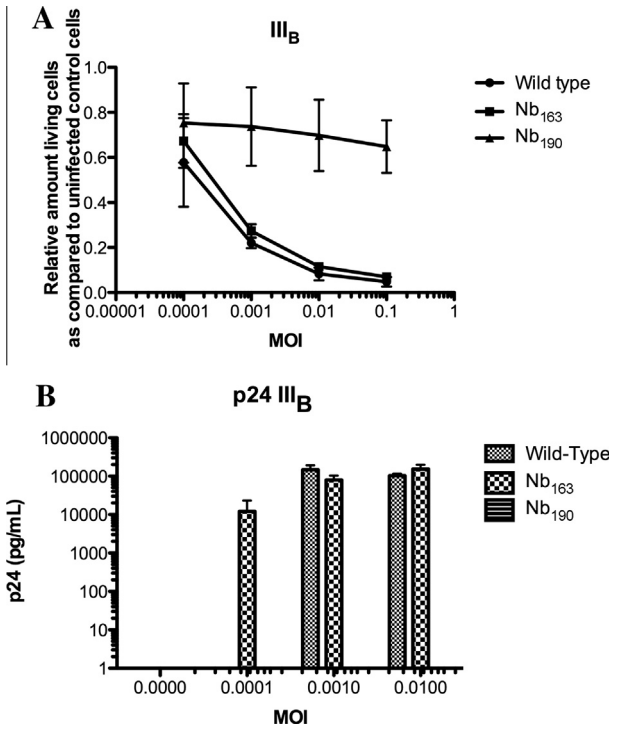

Fig. 3. Stably expressing Nb_{190} cell lines are resistant to HIV-1 III_B replication and are protected from CPE. C8166 stable cell lines were infected with HIV-1 (III_B) at different multiplicities of infection (MOI). Five days post infection, cell viability was assessed using MTT (A) and virus replication was assessed by quantification of the virus-associated p24 in the supernatant of infected cell cultures (B). Average of 3 independent experiments with standard deviations (SD) are shown.

templates for translation into viral proteins and/or as genome for packaging into newly formed viral particles.

Structural analysis revealed that Rev consists of three major functional domains, (i) a nuclear localization signal (NLS) that also serves as RNA-binding domain and ensures binding of Rev to the RRE, (ii) a nuclear export signal (NES) that is essential for Rev to interact with the exportin CRM1 (Daelemans et al., 2005; Fischer et al., 1995; Neville et al., 1997) and (iii) two alpha helical multimerization domains that join together to form a head and tail multimerization surface, which is essential for the multimerization on the viral RNA (Jain and Belasco, 2001). During HIV replication, one Rev protein first binds to the RRE (stem IIB), facilitating dimerization via tail–tail interactions and further multimerization via both head-head and tail–tail interactions (Daly et al., 1989; Daugherty et al., 2008; Heaphy et al., 1990; Malim and Cullen, 1991; Vercruysse and Daelemans, 2014; Zapp et al., 1991). Subsequently, the exportin CRM1 is recruited to this ribonucleoprotein complex, which exports viral RNA to the cytoplasm.

In previous research we have discovered a single-domain llama antibody (Nb_{190}) that interacts with the head multimerization