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Conditionally replicating HIV and SIV variants

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ABSTRACT

Conditionally replicating human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) variants that can be switched on and off at will are attractive tools for HIV and SIV research. We constructed HIV and SIV variants in which the natural transcription control mechanism was replaced by the doxycycline (dox)-inducible Tet-On gene expression mechanism. These HIV-rtTA and SIV-rtTA variants are fully replication-competent, but replication is critically dependent on dox administration. We here describe how the dox-dependent virus variants may improve the safety of live-attenuated virus vaccines and how they can be used to study the immune responses that correlate with vaccine-induced protection. Furthermore, we review how these variants were initially designed and subsequently optimized by spontaneous viral evolution. These efforts yielded efficiently replicating and tightly dox-controlled HIV-rtTA and SIV-rtTA variants that replicate in a variety of cell and tissue culture systems, and in human immune system (HIS) mice and macaques, respectively. These viruses can be used as a tool in HIV and SIV biology studies and in vaccine research. We review how HIV-rtTA and SIV-rtTA were used to study the role of the viral TAR and Tat elements in virus replication.

1. Novel approach toward a safe live-attenuated HIV vaccine

The development of effective antiviral drugs has significantly improved the life expectancy of HIV-infected patients, but these therapies are not available to everyone and do not provide a permanent cure. As a consequence, an estimated 1.5 million people died of acquired immunodeficiency syndrome (AIDS)-related illnesses worldwide in 2013 (http://www.who.int/gho/hiv/en/). Therefore, the development of an effective prophylactic vaccine remains a crucial goal in the battle against HIV-AIDS. However, whilst huge financial resources have been invested in the development of such a vaccine – resulting in several Phase 2 and 3 clinical trials – these efforts have proved largely unsuccessful and it is obvious that new strategies are required.

Live-attenuated viruses have proven to be very effective in inducing protective immunity against several pathogenic viruses, including smallpox, polio and measles virus. HIV variants attenuated through deletion of accessory functions like the nef gene, have therefore been considered as prophylactic HIV-AIDS vaccine. In vaccination studies in macaques, in which vaccinated animals were challenged with a pathogenic SIV strain, live-attenuated SIV vaccines have proven to be more effective than other SIV-AIDS vaccine approaches (Desrosiers, 1998; Johnson, 1999; Mills et al., 2000). For example, 95% of the Indian rhesus macaques vaccinated with live-attenuated SIV demonstrated a viral load suppression of more than 3 logs (compared to unvaccinated animals) upon challenge with wild-type SIV, whereas such protection was observed in only 7% of macaques immunized by other vaccine strategies (Koff et al., 2006). However, in vivo and in vitro studies with live-attenuated HIV and SIV variants questioned the safety of such vaccines. In a minority of the macaques vaccinated with live-attenuated SIV, the attenuated vaccine virus reverted to a more virulent variant that caused AIDS-like symptoms (Baba et al., 1995, 1999; Chakrabarti et al., 2003; Whatmore et al., 1995). Similarly, humans infected with a natural Nef-deficient HIV-1 did eventually progress to AIDS (Churchill et al., 2006). Furthermore, long-term culturing of an HIV-1 with deletions in the nef, vpr and LTR sequences resulted in the selection of a more virulent variant that had acquired compensatory changes elsewhere in the viral genome (Berkhout et al., 1999). These results demonstrate that attenuated HIV and SIV strains are not genetically stable and may thus evolve to better replicating variants, which raises serious concerns about the safety of a live-attenuated HIV vaccine in humans.

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The safety problem of a live-attenuated HIV/SIV vaccine is due to persistence of the vaccine strain. Continuous low-level replication in combination with the error-prone replication machinery spurs virus evolution. These characteristics may eventually result in the appearance of virus variants with increased fitness that are pathogenic. In principle, the safety of an attenuated vaccine strain can be improved by deletion of additional genes or regulatory elements, but a reduced replication capacity will decrease vaccine efficacy (Lohman et al., 1994; Wyand et al., 1996). Single-cycle virus variants that can complete only a single round of replication have been developed as an alternative vaccine approach, but such vaccines do also not provide sufficient protection (Baliga et al., 2006; Evans et al., 2004, 2005; Kuate et al., 2003). Replication and evolution of the vaccine virus should thus be limited in another way. The vaccine strain should be allowed to replicate sufficiently and induce strong protective immune responses, after which replication should be stopped to prevent evolution of the attenuated virus toward a pathogenic variant. Replication of the vaccine virus can be halted by administration of antiviral drugs (Lifson et al., 2001), but this approach will require long-term drug administration and incomplete adherence may result in the appearance of drug-resistant virus variants. In this setting, we considered the design of a conditionally replicating HIV-1 virus that replicates exclusively when a non-toxic exogenous drug is administered.

2. Conditionally replicating HIV-1 variant

We and others previously presented a conditionally replicating HIV-1 as a novel vaccine strategy. This approach uses an HIV-1 variant that replicates exclusively in the presence of doxycycline (dox), a member of the tetracycline antibiotic group (Berkhout et al., 2002; Das et al., 2004a, 2002; Smith et al., 2001; Verhoef et al., 2001). Upon vaccination with this virus, replication can be temporarily induced to the extent needed for activation of the immune system by transient dox administration. Subsequent dox-withdrawal will switch of viral gene expression and replication, thus preventing continuous viral replication and evolution toward a pathogenic variant. We realize that before a conditionally replicating HIV-1 can be used as vaccine in humans, major hurdles need to be overcome. Obviously, safety of the vaccine virus is an important issue. Nevertheless, as we will describe in this review, dox-controlled HIV-1 and SIV variants can be used in vaccination studies in laboratory animals to learn what protective correlates are induced by an effective vaccine and to study HIV-1 and SIV biology.

2.1. Construction of a dox-dependent HIV-1

To turn the HIV-1 LAI strain into a dox-controlled HIV-1 variant, named HIV-rtTA (Verhoef et al., 2001), we functionally replaced the Tat-TAR regulatory mechanism that controls viral gene expression by the Tet-On system for inducible gene expression (Fig. 1) (Baron and Bujard, 2000).

2.1.1. Inactivation of Tat/TAR mediated activation of viral gene expression

HIV-1 transcription is normally activated by the binding of the viral Tat protein to the TAR RNA hairpin that is present at the 5′ end of nascent transcripts (Fig. 1A). Tat subsequently recruits positive transcription elongation factor b (pTEFb), TATA box binding protein and chromatin-modifying proteins to the LTR promoter, which leads to phosphorylation of RNA polymerase II, assembly of new transcription complexes, and remodeling of the chromatin (see Das et al., 2011 for references). As a result, a processive transcription complex is formed that produces full-length viral RNAs. In the absence of Tat, only short RNAs resulting from non-processive transcription are produced. For the construction of the dox-controlled

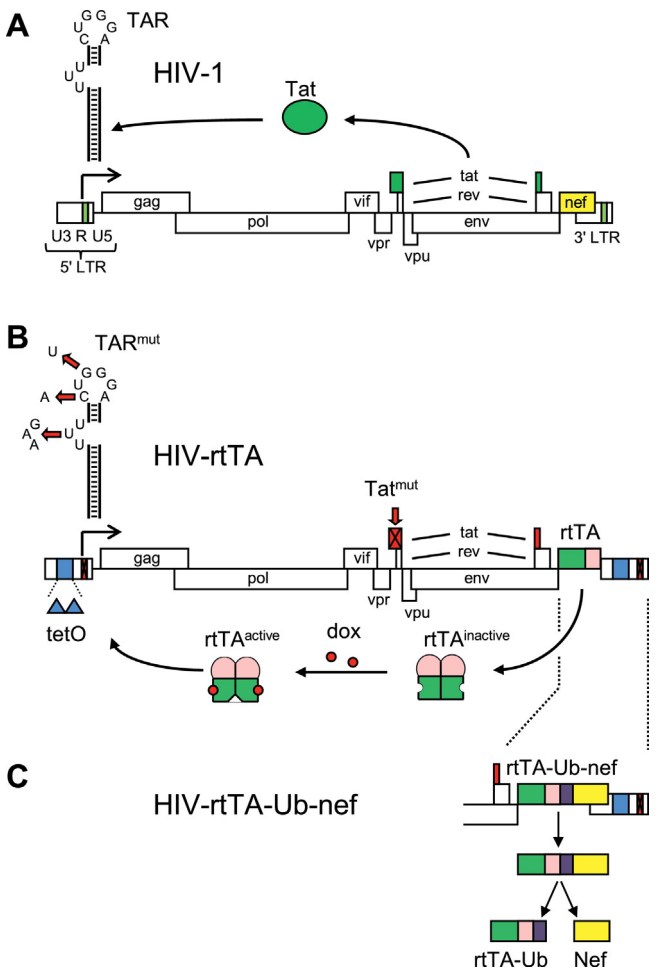

Fig. 1. Construction of a conditionally replicating HIV-1 variant. (A) Schematic of the HIV-1 proviral DNA genome with the LTR region subdivided in U3, R and U5 domains. Transcription from the 5′ LTR promoter is activated by the binding of the viral Tat protein to the TAR hairpin that is formed at the 5′ end of nascent transcripts. The 3-nt bulge and 6-nt loop are critical for this transactivation. (B) In HIV-rtTA, the Tat-TAR mechanism was inactivated through a tyrosine to alanine substitution at Tat position 26 (Tat^{mut}) and multiple nucleotide substitutions in the bulge and loop domains of TAR (TAR^{mut}). The tetO and rtTA components of the dox-inducible Tet-On system were integrated into the viral genome to make transcription and replication dox-dependent. The tetO elements were introduced in the U3 promoter region and the accessory nef gene was replaced by the rtTA gene. (C) In HIV-rtTA-Ub-nef, the Nef function was restored using the Ub fusion protein system (Varshavsky, 2005). The rtTA and Nef ORFs are placed in frame and connected by the 76-amino acid Ub sequence. Translation of the spliced RNA results in the production of the rtTA-Ub-Nef fusion protein. Cotranslational cleavage between the C-terminal amino acid of Ub and the N-terminal amino acid of Nef by Ub-specific proteases results in rtTA-Ub and Nef proteins.

HIV-rtTA, the Tat-TAR axis was disabled through mutations in both Tat and TAR. Tat was inactivated through a tyrosine-to-alanine substitution at position 26 (Y26A; Tat^{mut} in Fig. 1B) that hampers activation of the HIV-1 LTR promoter, and TAR was inactivated through several nucleotide changes in the bulge and loop elements (TAR^{mut} in Fig. 1B) that prevent binding of Tat and subsequent transcription activation.

2.1.2. Integration of the dox-inducible Tet-On gene expression system

The components of the Tet-On system, the reverse tetracycline-controlled transactivator (rtTA) protein and the tet operator (tetO) DNA sequence element, are based on the control elements of the Tn10 tetracycline-resistance operon of *Escherichia coli*. The Tet repressor protein (TetR) forms a dimer that binds to the tetO