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Mini review

Should accessory proteins be structural components of lentiviral vaccines? Lessons learned from the accessory ORF-A protein of FIV

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Abstract

The FIV regulatory protein Rev and accessory proteins Vif and ORF-A are essential for efficient viral replication and full-blown pathogenesis. Expressed at very low level during viral replication, they are nevertheless processed for recognition by cytotoxic T-lymphocytes (CTLs) and trigger cellular immune responses in FIV-infected cats. The observation that the accessory ORF-A protein of FIV is continuously expressed during viral replication and targeted by cellular immune responses in natural FIV infection, prompted us to investigate the protective potential of this protein. To this aim cats were immunized with three different strategies (protein alone in alum adjuvant, DNA alone, or DNA prime-protein boost) and generated clearly detectable immune responses. Upon challenge with ex vivo homologous FIV, ORF-A immunized cats showed distinct enhancement of acute-phase infection possibly due to an increased expression of the FIV receptor CD134. However, at subsequent sampling points plasma viremia was reduced and CD4+ T-lymphocytes in the circulation declined more slowly in ORF-A immunized than in control animals. These findings support the contention that a multicomponent vaccine, with the inclusion of both accessory and structural proteins, can not only improve the host's ability to control lentivirus replication and slow down disease progression but also draw attention to the fact that even simple immunogens that eventually contribute to protective activity can transiently exacerbate subsequent lentiviral infections.

Keywords: Feline immunodeficiency virus; FIV; Vaccine; Viral accessory proteins; Cat

Inactivated vaccines against infectious agents usually contain the whole fixed pathogen or one or more structural proteins. These strategies were the first to be pursued in the quest for a human immunodeficiency virus (HIV) vaccine (Girard et al., 2006). Structural antigens have been produced using countless technologies, formulated with either conventional or novel adjuvants, and delivered with a myriad of different systems. Unfortunately, after decades of research and experimental and clinical trials, an effective HIV vaccine is not yet available (Duerr et al., 2006; McMichael, 2006). The HIV infection, spreading at an alarming pace in developing countries, has therefore prompted the research community to explore new avenues. One of the most pursued is the exploitation of vaccinal antigens derived from HIV

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regulatory and accessory proteins, i.e. proteins which are not structural components of viral particles but promote and coordinate viral replication.

Like other lentiviruses, the HIV genome encodes the structural Gag, Pol and Env, and several other proteins having important functions during the early stages of the viral life cycle. HIV type-1 (HIV-1) has two regulatory proteins, designated Tat and Rev, that are essential for viral replication. Tat is a potent transcriptional activator of the long terminal repeat element and is necessary for efficient transcription of the viral genome. Rev acts as a nuclear export factor of unspliced and singly spliced viral messenger RNAs (mRNAs), which are otherwise retained in the nucleus (Emerman and Malim, 1998). Predictably, Rev, Tat and the accessory protein Nef are the only viral genes encoded by fully spliced mRNAs and are translocated from the nucleus to the cytoplasm like any cellular mRNA. Nef, together with Vpu, Vif and Vpr complete the array of proteins coded by the HIV-1 genome. These four small proteins are grouped as accessory proteins. As the adjective suggests, these proteins are dispensable for viral replication itself, yet they are fundamental for viral persistence and pathogenicity both at the cellular and host level (Steffens and Hope, 2001). One of the chief functions of Nef is the down-regulation of major histocompatibility complex (MHC) class I molecules on the cell surface of infected cells (Geyer et al., 2001). Vpu facilitates HIV-1 virion assembly and release by conveying the cellular receptor (CD4)/viral surface glycoprotein (gp160) complex to the endoplasmic reticulum where it is rapidly degraded and, thereby, allows the newly synthesized gp160 to be transported to the cell surface where virion assembly takes place (Bour and Strebel, 2003). Vpr is a pleiotropic protein involved in the very early and late steps of viral infections. Among other functions, Vpr blocks cell cycle replication and down-regulates CD4 on the cell surface. Because of the diminished expression of MHC class I and CD4, the infected cells are not readily recognized and cleared away by the immune system effectors (Le Rouzic and Benichou, 2005). Finally, Vif contributes to viral persistence by blocking potent antiviral cellular factors in both producer and target cells and by increasing efficiency of viral assembly and stability of intermediate complexes (Navarro and Landau, 2004). Mounting an effective immune response against regulatory and accessory proteins may therefore ultimately curb or block HIV-1 replication. Several additional observations further qualify these proteins for vaccine components: (1) they contain many cytotoxic T-lymphocyte (CTL) epitopes; (2) they trigger a feeble humoral response (if any) yet are actively targeted by CTL in natural infection; (3) Tat, Rev and Nef are expressed in the very early stage of viral replication and, therefore, cell-mediated responses against these proteins could eliminate infected cells before the virus has completed its life cycle and the viral progeny have been released (Gruters et al., 2002); (4) whereas deletion of Tat and Rev is incompatible with viral replication, pioneering studies by Desrosiers and colleagues demonstrated that Nef-deleted simian immunodeficiency virus (SIV) mutants are greatly attenuated (Girard et al., 2006), suggesting that an effective anti-Nef immune response, if not sufficient to prevent viral infection *per se*, may reduce the pathogenic potential and delay disease progression.

Several studies carried out in non-human primates have shown that vaccination with HIV-Tat alone or in combination with Rev effectively reduces viral replication and disease progression (Yu et al., 2005; Ensoli et al., 2006) but may also lead to the rapid emergence of viral variants containing escape mutations in Tat and Rev CTL epitopes (Goulder and Watkins, 2004) thus shadowing the attractiveness of these targets as candidate vaccines. Additional hurdles for the use of HIV-1 regulatory and accessory proteins in vaccine designs stem from the large intra- and inter-clade sequence diversity within these genes (Brander et al., 2006) and the apparent lack of class-recognition by HIV-1-specific T-cells (Allen et al., 2005). These features are further complicated by the small size of these proteins and the consequent lower number of CTL epitopes compared to structural proteins.

If the rationale to include HIV regulatory and accessory proteins in vaccines has been thoroughly worked out, the practical benefits in preventive vaccinal strategies have not been fully appreciated.

Feline immunodeficiency virus (FIV) with the low cost and easy manageability of its natural host may help to provide an answer to this issue. FIV has fewer regulatory and accessory proteins compared to HIV and non-human primate lentiviruses. The non-structural genes for which mRNAs and proteins have been unequivocally identified are *rev*, *vif*, and ORF-A, or ORF-2 (Fig. 1), other open codon reading frames have been predicted in some isolates but their products have not been found. The Rev and Vif functions overlap those observed in primate lentiviruses whereas the role and importance of ORF-A for FIV replication and full-blown pathogenesis is still debated (Sparger, 2006). Expressed at very low level during viral replication, FIV Rev, Vif and ORF-A are nevertheless processed for CTL recognition and trigger cellular immune responses in