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Membrane-bound SIV envelope trimers are immunogenic in ferrets after intranasal vaccination with a replication-competent canine distemper virus vector

Xinsheng Zhang [a,b,*], Olivia Wallace [a], Kevin J. Wright [a], Martin Backer [a], John W. Coleman [a], Rebecca Koehnke [a], Esther Frenk [a,1], Arban Domi [a,b], Maria J. Chiuchiolo [a,1], Joanne DeStefano [a], Sandeep Narpala [a,2], Rebecca Powell [a], Gavin Morrow [a,b], Cesar Boggiano [a,2], Timothy J. Zamb [a], C. Richter King [a,b], Christopher L. Parks [a,b]

[a] AIDS Vaccine Design and Development Laboratory, International AIDS Vaccine Initiative (IAVI), Brooklyn, NY, USA
[b] Molecular and Cellular Biology Program, State University of New York, Brooklyn, NY, USA

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ABSTRACT

We are investigating canine distemper virus (CDV) as a vaccine vector for the delivery of HIV envelope (Env) that closely resembles the native trimeric spike. We selected CDV because it will promote vaccine delivery to lymphoid tissues, and because human exposure is infrequent, reducing potential effects of pre-existing immunity. Using SIV Env as a model, we tested a number of vector and gene insert designs. Vectors containing a gene inserted between the CDV H and L genes, which encoded Env lacking most of its cytoplasmic tail, propagated efficiently in Vero cells, expressed the immunogen on the cell surface, and incorporated the SIV glycoprotein into progeny virus particles. When ferrets were vaccinated intranasally, there were no signs of distress, vector replication was observed in the gut-associated lymphoid tissues, and the animals produced anti-SIV Env antibodies. These data show that live CDV-SIV Env vectors can safely induce anti-Env immune responses following intranasal vaccination.

Introduction

Live-attenuated viral vaccines are used to effectively fight important human diseases, including measles and polio, demonstrating that this vaccination strategy safely controls highly infectious viral pathogens. Extending this vaccine approach to live-attenuated HIV vaccines has been investigated using the rhesus macaque SIV AIDS model. Vaccination of macaques with attenuated strains of SIVmac239, such as SIVmac239Δnef, protected a significant number of animals from disease by subsequent challenge with pathogenic virus administered via mucosal or intravenous routes (Johnson et al., 1999; Koff et al., 2006; Wyand et al., 1999). Unfortunately, translation of this approach to an HIV vaccine is not practical as illustrated by preclinical studies in macaques, which showed that even some highly attenuated SIV strains retained the ability to cause disease (Baba et al., 1999). An alternative approach, which still exposes the immune system to SIV/HIV proteins in the context of a viral infection, is to deliver HIV immunogens using live replicating viral vectors. Replicating vectors based on viruses including measles virus (MeV), yellow fever virus (YFV), and poxviruses have entered preclinical testing and clinical trials not only for vaccine development but also for cancer therapy (Cattaneo et al., 2008; Draper and Heeney, 2010; Liniger et al., 2007; Parks et al., in press). It is also important to note that replicating viral vectors are used safely in a number of veterinary vaccines (e.g. Newcastle disease viral vectors encoding avian influenza vaccine for poultry and chimeric YFV and West Nile Virus vaccine for horses), demonstrating the feasibility of the approach (Draper and Heeney, 2010).

Unique biological characteristics and availability of safe and effective attenuated vaccines make morbilliviruses attractive candidates for HIV vaccine vector development. For example, members of morbillivirus genus (e.g. MeV and CDV) are lymphotropic because they use the signaling lymphocyte activation molecule (SLAM) as a receptor (Tatsuo and Yanagi, 2002). Live attenuated MeV and CDV vaccines also have a long-standing record of safety

* Correspondence to: Brooklyn Army Terminal 140 58th Street, Building A, 8th Floor, Unit 8J, Brooklyn, NY 11220, USA. Fax: +1 718 765 1870.
E-mail address: xzhang@iavi.org (X. Zhang).
[1] Current address: Department of Genetic Medicine, Weill Cornell Medical College, NY, USA.
[2] Current address: National Institute of Health, Bethesda, MD, USA.

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and efficacy, and both elicit antibodies that confer long-lived protection (Dine et al., 2004). Because live attenuated MeV vaccines are safe and immunogenic, they have been investigated as vaccine delivery vectors for a number of infectious diseases including dengue virus, West Nile virus, and HIV (Brandler et al., 2007; Brandler and Tangy, 2008; Despres et al., 2005; Guerbois et al., 2009; Wang et al., 2001) producing promising preclinical data. However, use of MeV vectors for human vaccines might be constrained by prevalent anti-MeV immunity induced by universal MeV vaccination programs and outbreaks of natural infection in some areas.

Pre-existing anti-MeV humoral immunity is directed against the only MeV serotype. This precludes use of rare serotype viruses as vectors, which has been a strategy applied to other viral vector systems (Mingozzi et al., 2013; Santra et al., 2009; Stephenson et al., 2012). Thus, to minimize the effect of pre-existing anti-MeV immunity, we are investigating attenuated CDV as a replicating vector. CDV and MeV are antigenically related, but MeV antiserum has limited neutralization activity against CDV (Miest et al., 2011).

Other CDV characteristics also are advantageous for vector development. For example, live CDV vaccines have been used safely to vaccinate dogs for decades and the attenuated stain can be used as the basis for a vector. Also, no human diseases are linked convincingly to CDV exposure (Rima and Duprex, 2006). In fact, some early clinical investigation of experimental MeV vaccines was conducted with live attenuated CDV, and the results showed that CDV could be injected into non-human primates and humans without causing diseases (Hoekenga et al., 1960; Schwarz and Anderson, 1965). Although not known to cause disease in humans, CDV recently was associated with several instances of disease in nonhuman primates (Qiu et al., 2011; Sakai et al., 2013; Sun et al., 2010). It is important to note that a range of primates cells are permissive for CDV infection *in vitro,* demonstrating that it can be used as a delivery vector (Seki et al., 2003). CDV also has the potential to be used as a mucosal vaccine delivery vector, although subcutaneous or intramuscular injections are conventional routes for live attenuated CDV vaccination. The virus naturally infects through aerosol transmission and swiftly spreads to gut-associated lymphoid tissues (GALT) (von Messling et al., 2004a) thus favoring induction of immune responses in lymphatic cells underlying the gut mucosal surface. Such immune responses could be important for a vaccine to prevent depletion of gut CD4+ cells during acute HIV infection (Trono et al., 2010).

CDV is a paramyxovirus, and like all members of this family it has non-segmented RNA genome of negative polarity. The genome contains 6 transcription units arranged in order 3′ N–P–M–F–H–L 5′ (McIlhatton et al., 1997; Sidhu et al., 1993) that are separated by intergenic regions, which range in length from 100 to 500 nucleotides (nt) and contain *cis*-acting signals that control mRNA synthesis and processing (Anderson and von Messling, 2008). Using reverse genetics systems similar to those described before (Gassen et al., 2000; Parks et al., 2002), we developed a recombinant CDV from an attenuated Onderstepoort strain. Since the 1940s, live attenuated Onderstepoort CDV has been widely used in canine vaccines to safely and effectively control distemper in dogs (Ehmer et al., 1948; Haig, 1948) and other domestic and exotic carnivore animal species (Wimsatt et al., 2001).

Our goal is to develop a live vaccine vector capable of safely delivering trimeric HIV envelope (Env) immunogens. We are pursuing this objective because the Env spike is the only known target of antibodies that neutralize HIV (Kwong and Mascola, 2012) and it follows that a vaccine should deliver an immunogens that imitates the oligomeric structure on the HIV particle (Forsell et al., 2009). Accordingly, we have developed prototype CDV vectors that express SIV Env, which can be advanced for future testing in the rhesus macaque SIV challenge protection model (Koff et al., 2006). Env is a type I membrane glycoprotein that is synthesized as a precursor protein (gp160), which is cleaved by furin protease to produce noncovalently linked gp120–gp41 heterodimers, which are assembled into a functional trimeric spike (Checkley et al., 2011). Studies have shown that mature trimeric Env exposes fewer structures recognized by non-neutralizing antibodies suggesting that a correctly configured glycoprotein spike might be a more effective immunogen (Chakrabarti et al., 2011; Moore et al., 2006). Unfortunately, developing vectors that express full-length gp160 Env generally is difficult because the protein has cytotoxic properties (Wyatt et al., 2008), thus we have tested a number of vector and immunogen designs to identify a combination that balances expression of a membrane-bound trimer with genetic stability needed to propagate the vector. Our results show that CDV encoding a functional SIV spike can be produced if the Env cytoplasmic tail (CT) is truncated and protein is expressed from a transcription unit inserted between the H and L genes. Finally, we found that a CDV vector encoding Env could be safely administered to ferrets by intranasal administration and that a single dose elicited Env-specific antibody responses.

Results

Expression of membrane-anchored Env immunogen by CDV vectors

Our objective was to develop a CDV vector that efficiently expressed Env spikes on the surface of infected cells. The first vector we constructed was designed to encode full-length SIVmac239 Env from a gene inserted into the CDV genome between M and F genes (4th position, Fig. 1A). After multiple unsuccessful attempts to rescue this vector, we removed 159 amino acid codons from the SIV Env gene so it encoded just a 5-amino acid CT (Fig. 1B). After this modification, the rCDV-SIVEnvΔct4 vector was rescued, but was found to propagate poorly on Vero cells.

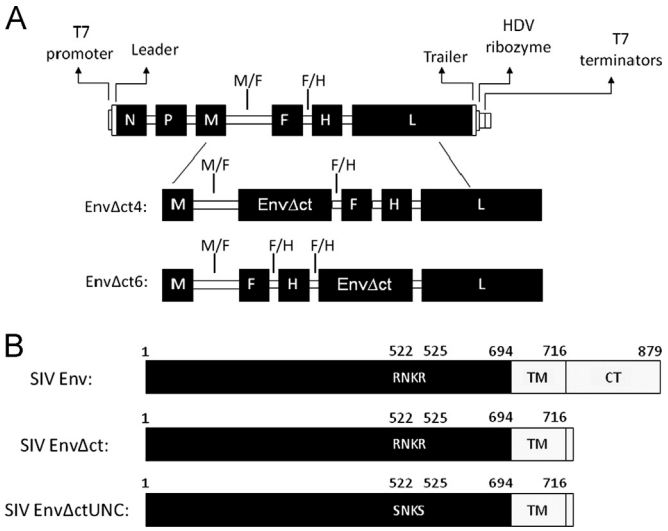

Fig. 1. (A) Schematics of the CDV genomic clone and rCDV-SIVEnvΔct4 and rCDV-SIVEnvΔct6 vectors. The CDV genomic clone contains six structural protein genes that are separated by untranslated regions (UTR). Length of UTR ranges from 100 to 200 nucleotides except for the M/F UTR that is 495 nucleotides. The genomic cDNA was cloned in pBluescript II SK(+/−) with flanking T7 promoter, hepatitis D virus (HDV) ribozyme, and T7 polymerase terminator sequences. The SIVmac239 EnvΔct gene was inserted before F in rCDV-SIVEnvΔct4 or after H in rCDV-SIVEnvΔct6 and rCDV-SIVEnvΔct6UNC vectors. In each of the Env vectors, an extra F/H UTR was introduced. (B) Map of full-length and truncated SIVmac239 Env. The 879 amino acid Env protein consists of a surface unit (amino acids 1–693), a TM domain (amino acids 694–715), and CT (amino acids 716–879). The EnvΔct lacks the C-terminal residues 721–879. In the EnvΔctUNC, the furin cleavage signal R N K R was mutated to S N K S.