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Membrane embedded HIV-1 envelope on the surface of a virus-like particle elicits broader immune responses than soluble envelopes

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Abstract

Virally regulated HIV-1 particles were expressed from DNA plasmids encoding Gag, protease, reverse transcriptase, Vpu, Tat, Rev, and Env. The sequences for integrase, Vpr, Vif, Nef, and the long terminal repeats (LTRs) were deleted. Mutations were engineered into the VLP genome to produce particles deficient in activities associated with viral reverse transcriptase, RNase H, and RNA packaging. Each plasmid efficiently secreted particles from primate cells in vitro and particles were purified from the supernatants and used as immunogens. Mice (BALB/c) were vaccinated intranasally (day 1 and weeks 3 and 6) with purified VLPs and the elicited immunity was compared to particles without Env (Gag_{p55}), to soluble monomeric Env_{gp120}, or to soluble trimerized Env_{gp140}. Only mice vaccinated with VLPs had robust anti-Env cellular immunity. In contrast, all mice had high titer anti-Env serum antibody (IgG). However, VLP-vaccinated mice had antisera that detected a broader number of linear Env peptides, had anti-Env mucosal IgA and IgG, as well as higher titers of serum neutralizing antibodies. VLPs elicited high titer antibodies that recognized linear regions in V4-C5 and the ectodomain of gp41, but did not recognize V3. These lentiviral VLPs are effective mucosal immunogens that elicit broader immunity against Env determinants in both the systemic and mucosal immune compartments than soluble forms of Env.

Keywords: HIV; VLP; Env; Mucosal vaccination; Vaccine

Introduction

The ultimate goal of an AIDS vaccine is to elicit potent cellular and humoral immune responses that will result in enduring, broadly protective immunity (reviewed in Slobod et al., 2005; Spearman, 2003). While much effort has focused on elucidating the mechanisms and specificity of cellular immune responses, less is known about virus-specific antibody responses. It has been well established that cellular immune responses mediate viral control during the primary infection and maintain the viral set point in chronic infection (Hamer, 2004). However, administration of human monoclonal antibodies passively protects against virus exposure in the HIV-1/chimpanzee (Conley et al., 1996; Murthy et al., 1998), SIV/monkey (Gardner et al., 1995; Haigwood et al., 1996) and SHIV/monkey models (Foresman et al., 1998; Li et al., 1997; Mascola et al., 1999, 2000; Shibata et al., 1999), demonstrating the ability of antibody alone to mediate protection against pathogenic virus infection. Thus, one goal of an AIDS vaccine should be to elicit robust and broadly reactive cellular and humoral immunity in both systemic and mucosal immune compartments, thereby maximizing the potential for protection from variant HIV-1 strains by different routes of exposure.

A particle-based immunogen, such as a non-infectious virus-like particle (VLP), is a promising candidate for a safe and effective AIDS vaccine. VLPs are defined as self-assembling, non-replicating, non-pathogenic, and preferably genomeless

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particles that are similar in size and conformation to intact infectious virions. There are multiple combinations of viral proteins that may be used to generate VLPs, however, they must contain Gag gene products in order to assemble and bud from cells. Env_{gp160} may also be processed and incorporated as trimeric spikes protruding from the surface of these particles. The VLP used in this study is expressed from a virally regulated, multi-gene DNA plasmid. However, unlike many other VLPs currently being tested in AIDS vaccine research, our VLP-DNA plasmids can express multiple viral gene products from the same cell intracellularly and secrete these VLPs extracellularly from primate cells from a single DNA plasmid *in vivo* (Young and Ross, 2005; Young et al., 2004).

Other multi-gene-based VLP expression systems have been developed to express VLPs from DNA plasmids (Ellenberger et al., 2004, 2005; Smith et al., 2004; Hammonds et al., 2003). While these approaches successfully elicited cellular immunity against VLP antigens, these VLPs elicited limited binding antibody and little, if any, neutralizing antibodies, even after multiple vaccinations (Amara et al., 2001; Ellenberger et al., 2004, 2005; Smith et al., 2004; Wyatt et al., 2004). In addition, low to undetectable cellular or humoral immune responses were observed at mucosal sites. The lack of a robust antibody responses may be due, in part, to the *in vivo* production of particles that were primarily retained intracellularly and therefore these viral antigens were processed for MHC class I presentation. Lastly, the lack of neutralizing antibodies in these studies may be a result of (1) the elicitation of low antibody titers or (2) the specific envelope chosen for incorporation into these particles.

Over the past 20 years, multiple AIDS vaccine strategies have been developed, but each has failed to protect against disease (for reviews, see Egan, 2004; Haigwood, 2004; Sauter et al., 2005; Slobod et al., 2005; Spearman, 2003). Early trials using monomeric HIV-1 envelopes, while eliciting high titer antibodies, failed to elicit antibodies capable of neutralizing HIV-1 *in vitro*. However, the role of Env appears critical for the induction of protective immunity in recent AIDS vaccines (Amara et al., 2002; Letvin et al., 2004), which may point to other antibody and cellular immune mechanisms, such as antibody-dependent cytotoxicity (ADCC) (Forthal et al., 2001; Forthal et al., 1999; Gomez-Roman et al., 2005; Peng et al., 2005), as important mediators of protection. Inclusion of Env in a multi-component AIDS vaccine results in lower viral set points and higher CD4 counts following challenge compared to the same vaccines lacking Env (Amara et al., 2002; Letvin et al., 2004).

Envelope on the native virion is predicted to form a trimer (Farzan et al., 1998; Kwong et al., 1998; Wyatt et al., 1998). Thus, while neutralizing antibody responses appear to be an important component to elicit in an AIDS vaccine, they tend to recognize highly conformational epitopes on Env that may not be immunogenic *in vivo*. The inability of monomeric envelopes to elicit neutralizing antibodies is most likely due to differences in structure between monomeric forms of gp120 and oligomeric forms of envelope as they are expressed on the surface of a virus particle. Several vaccine strategies have incorporated an oligomeric/trimeric form of Env in order to elicit cross-reactive immunity that neutralizes viral infection (Beddows et al., 2005; Bower et al., 2004, 2005; Pancera et al., 2005; Pancera and Wyatt, 2005; Sanders et al., 2002; Yang et al., 2000, 2002). The expression of trimeric Env on the surface of a particle does appear to elicit higher titers of neutralizing antibodies than soluble gp120 following intramuscular injection (Hammonds et al., 2005), even though much of the neutralizing antibodies were directed at membrane embedded cellular proteins.

To overcome this problem, soluble trimers of Env have been developed. Soluble, stabilized Env trimers are hypothesized to mimic the trimeric structure of the envelope on the native virion and possibly induce conformationally dependent antibodies that recognize epitopes present only on native virion-associated envelopes. Several of these trimeric Env immunogens do elicit slightly higher titers of neutralizing antibodies than monomeric Env_{gp120} (Barnett et al., 2001; Bower et al., 2004; VanCott et al., 1997; Yang et al., 2001). Often, these oligomeric Env proteins are produced by eliminating the natural cleavage site recognized by cellular proteases (Chakrabarti et al., 2005; Srivastava et al., 2002; Yang et al., 2002; Zhang et al., 2001). The lack of elicited high titer, broadly reactive neutralizing antibodies by these immunogens is associated with the elicitation of primarily non-neutralizing antibodies (Pancera et al., 2005; Schulke et al., 2002; Si et al., 2003), because these uncleaved envelopes are in non-native forms or are processed through different cellular pathways than cleaved forms of Env (Adams and Scheid, 2001; McCune et al., 1988; Pancera et al., 2005; Pancera and Wyatt, 2005). Therefore, in this study, mice were vaccinated with soluble envelopes (monomeric Env_{gp120} or trimeric Env_{gp140}) or trimeric envelopes that were membrane retained on an HIV-1 VLP to determine if the form(s) and presentation of envelope to the immune system influence the elicited immunity.

Results

In vitro expression and particle purification of virus-like particles

An HIV-1 virus-like particle expressing plasmid was constructed from a proviral genome, as previously described (Young et al., 2004). The full-length HIV-1 VLP genome expressed Gag, protease (PR), reverse transcriptase (RT), Vpu, Tat, Rev, and Env gene products to produce a PR-processed, but immature particle. A second VLP was expressed from a Gag only codon-optimized sequence that produced an unprocessed, immature particle. Plasmids expressing monomeric Env_{gp120} or trimeric Env_{gp140} envelopes have been previously described (Bower et al., 2004).

Expression of VLPs was determined *in vitro* following transient transfection of primate (COS) cells with each DNA plasmid. Cell culture supernatants and lysates containing Gag gene products were detected following SDS-PAGE and Western blot (Young et al., 2004). Particles were purified from the supernatants of transiently transfected cells by ultracentrifugation (20–60% sucrose gradient), and collected fractions were analyzed for particle composition and stability. VLPs banded in