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Increased surface expression of HIV-1 envelope is associated with improved antibody response in vaccinia prime/protein boost immunization

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ABSTRACT

HIV-1 envelope (Env)-based vaccines have so far largely failed to induce antibodies that prevent HIV-1 infection. One factor proposed to limit the immunogenicity of cell-associated Env is its low level of expression on the cell surface, restricting accessibility to antibodies. Using a vaccinia prime/protein boost protocol in mice, we explored the immunologic effects of mutations in the Env cytoplasmic tail (CT) that increased surface expression, including partial truncation and ablation of a tyrosine-dependent endocytosis motif. After vaccinia primes, CT-modified Envs induced up to 7-fold higher gp120-specific IgG, and after gp120 protein boosts, they elicited up to 16-fold greater Tier-1 HIV-1 neutralizing antibody titers, although results were variable between isolates. These data indicate that the immunogenicity of HIV-1 Env in a prime/boost vaccine can be enhanced in a strain-dependent manner by CT mutations that increase Env surface expression, thus highlighting the importance of the prime in this vaccine format.

1. Introduction

The HIV-1 pandemic remains a major threat to global public health, with 2.6 million new infections annually, and a safe and effective vaccine is urgently needed (Wang et al., 2015; Harmon et al., 2016). Passive immunity experiments have demonstrated that anti-HIV-1 neutralizing antibodies (NAbs) can confer protection from infection in nonhuman primate models (Gautam et al., 2016; Parren et al., 2001; Hessell et al., 2009; Emini et al., 1992; Baba et al., 2000; Mascola et al., 2000); as a result, such antibodies are a major target of ongoing vaccine efforts (Haynes and Burton, 2017). The sole target of neutralizing or other protective antibodies on HIV-1 is the envelope glycoprotein (Env), which assembles as a trimer of heterodimers composed of surface gp120 and transmembrane gp41 subunits. The HIV-1 Env has evolved a variety of mechanisms to evade host antibody responses, including its ability to tolerate escape mutations in immunogenic epitopes (Wei et al., 2003; Moody et al., 2015), extensive glycosylation (Behrens et al., 2016; McCoy et al., 2016; Stewart-Jones et al., 2016; Zhou et al., 2017), conformational masking (Kwong et al., 2002), mimicry of host proteins (Yang et al., 2013), and low expression of Env on virus-infected cells and virions (Zhu et al., 2003; Chertova et al., 2002). Together, these features create substantial barriers to the design of an effective Env-based vaccine.

Despite extensive pre-clinical studies and six phase 2 or 3 clinical trials of HIV-1 vaccines (Rerks-Ngarm et al., 2009; The rgp120 HIV Vaccine Study Group, 2005; Buchbinder et al., 2008; Pitisuttithum et al., 2006; Gray et al., 2011; Hammer et al., 2013), only the RV144 trial has shown any efficacy, with 31.2% protection in humans at 42 months post-vaccination (Rerks-Ngarm et al., 2009). The RV144 vaccine regimen included four intramuscular inoculations of replication-incompetent canarypox vector expressing HIV-1 Gag, protease, and Env antigens and two injections of purified gp120 protein in alum. The primary correlates of protection were non-neutralizing IgG antibodies in plasma that bound to variable loops 1 and 2 (V1/V2) of gp120 and

Abbreviations: CT, cytoplasmic tail; VACV, vaccinia virus; N7, N197Q

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low levels of anti-Env IgA (Haynes et al., 2012). Secondary correlates in a post-hoc analysis included modest NAb activity and the ability of antibodies to mediate antibody-dependent cellular cytotoxicity (ADCC) on HIV-1-infected target cells (Haynes et al., 2012; Chung et al., 2014). These results have generated renewed interest in the potential advantages of poxvirus prime/protein boost vaccine approaches, as well as the antiviral functions of non-neutralizing antibodies (Corey et al., 2015; Ackerman et al., 2016; Huang et al., 2016; Forthal et al., 2013). Studies have since attempted to improve on the efficacy of RV144 by optimizing various aspects of the vaccine regimen, including dose schedule, vector, adjuvant, and Env sequence (Teigler et al., 2014; Vaccari et al., 2016; Easterhoff et al., 2017; NCT02404311, NCT02968849). There is considerable biological diversity among Env variants that can be used in the prime or boost, including differences in glycan organization (Stewart-Jones et al., 2016), epitope exposure (Sanders et al., 2013; de Taeye et al., 2015), and cell surface expression (Ye et al., 2004; Wyatt et al., 2008), but the exact contributions of these attributes to immunogenicity remain poorly understood.

The low expression of HIV-1 Env on the cell surface has long been hypothesized to impede an effective antibody response to membrane-associated forms of Env, as in viral infection or gene-based vaccines (Marsh et al., 1997). This low expression has been attributed at least in part to the presence of multiple endocytosis motifs within the long cytoplasmic tail (CT) of Env (Boge et al., 1998; Rowell et al., 1995; Bowers et al., 2000; Egan et al., 1996; Berlioz-Torrent et al., 1999; Wyss et al., 2001; Byland et al., 2007). One well-described signal in the CT of HIV and SIV Envs is the membrane-proximal tyrosine (Tyr)-dependent endocytosis motif formed by the consensus amino acids GYxxΦ, where x is any amino acid and Φ is a bulky hydrophobic residue. This highly conserved motif binds to cellular adaptor protein complex 2 (AP-2) and recruits Env that is not incorporated into virions into clathrin-coated pits, thereby mediating internalization and clearance from the cell surface (Boge et al., 1998; Rowell et al., 1995; Bowers et al., 2000; Egan et al., 1996). GYxxΦ has also been shown to mediate directional budding of virus in polarized cell types (Lodge et al., 1997; Deschambeault et al., 1999) and to contribute to pathogenesis in SIV infection of pigtail macaques (Fultz et al., 2001; Breed et al., 2013, 2015). Additional but less well characterized internalization signals are present in the more distal CT (Wyss et al., 2001; Byland et al., 2007), consistent with the view that a low steady-state expression of Env on infected cells is an important and conserved viral property.

Surface expression of HIV and SIV Envs can be increased by mutations in the CT that ablate endocytosis signals. Our lab has previously described a variant of SIVmac251 termed CP-MAC that exhibited a marked increase in surface expression on infected cells. This increase was shown to be the result of a substitution of Tyr in the GYxxΦ motif and a premature stop codon immediately prior to the overlapping second exons of *tat* and *rev* (LaBranche et al., 1994, 1995; Sauter et al., 1996)—a truncation that arises commonly when SIVs are propagated in human cells (Kodama et al., 1989). It is unknown whether comparable mutations in the HIV-1 Env CT would confer a similar increase in surface expression, thereby making a potentially useful immunogen. At least two studies have directly compared the immunogenicity of HIV-1 Env CT mutants with increased surface expression to that of wild-type (WT) Envs (Ye et al., 2004; Wyatt et al., 2008), and both noted increases in gp120-binding IgG. However, the impact of high surface expression on IgG and especially NAb responses in the context of a viral prime/protein boost vaccine regimen has not yet been well defined.

In the present study, we used a clinically relevant vaccinia prime/gp120 protein boost protocol to evaluate the impact of mutations in the HIV-1 Env CT that ablate known endocytosis signals and increase expression on the cell surface. We hypothesized that the magnitude and particularly the quality of the antibody response would correlate with the level of Env cell surface expression driven by the vaccinia vector and thus the amount of native Env antigen available for interactions with B cells.

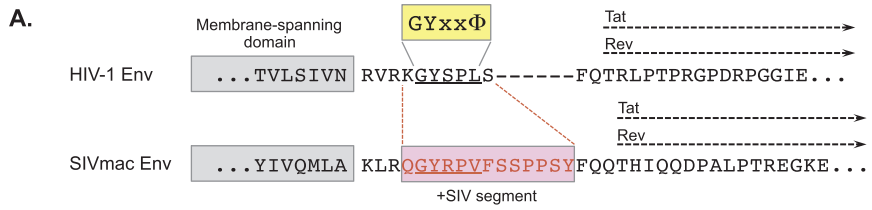
2. Materials and methods

2.1. Ethics statement

The investigators faithfully adhered to the "Guide for the Care and Use of Laboratory Animals" by the Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Commission on Life Sciences, National Research Council. The animal facilities at the University of Pennsylvania are fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All studies were conducted under protocols approved by University of Pennsylvania IACUCs.

2.2. Generation of CT-modified HIV-1 Env constructs

Mutant Env constructs were generated using HIV-1 R3A *env* (Meissner et al., 2004) (Genbank accession AY608577) in the pHSPG plasmid, HIV-1 89.6 *env* (Collman et al., 1992) (Genbank accession U39362) in the pCIneo plasmid (Promega), or JRFL *env* (Koyanagi et al., 1987) (Genbank accession U63632) in the pSVIII plasmid. The 89.6 and JRFL Envs each contain portions of sequence from strain HXB2



B.

HIV-1 cytoplasmic tail mutants

WT	RVRKGYSPLSF------QTRLPTPRGPDRPGGIE...
Δ147	RVR*
Δ139	RVRKGYSPLSF*
$Y_{712}I$	RVRKGISPLSF------QTRLPTPRGPDRPGGIE...
$Y_{712}I$,Δ139	RVRKGISPLSF*
+SIV	RVRQGYRPVFSSPPSYFQTRLPTPRGPDRPGGIE...
+SIV,$Y_{712}I$	RVRQGIRPVFSSPPSYFQTRLPTPRGPDRPGGIE...
+SIV,Δ139	RVRQGYRPVFSSPPSYF*
+SIV,$Y_{712}I$,Δ139 ("TM1")	RVRQGIRPVFSSPPSYF*

SIV CP-MAC cytoplasmic tail KLRQGCRPVFSSPPSYF*

Fig. 1. Schematic of HIV-1 Env cytoplasmic tail mutants. (A) Partial amino acid sequences of HIV-1 R3A and SIVmac239 Envs are shown, including part of the membrane-spanning domain and the highly conserved Tyr-dependent endocytosis motif (GYxxΦ). For both viruses, the positions overlapping the second exons of Tat and Rev in alternative reading frames are shown. The indicated segment from SIVmac (+SIV) was substituted into the HIV-1 Env CT to create Env constructs shown in Panel B. (B) HIV-1 Env CT mutants created to evaluate effects on Env surface expression. Substitutions included a $Y_{712}I$ substitution (HXB2 numbering) and/or a premature termination codon (*). Mutations were also made in the same positions in the Envs of HIV-1 89.6, 89.6 N7 ($N_{197}Q$), and JRFL. Dashes (–) are used to facilitate alignment and highlight SIV residues with no homology in HIV-1 (SSPPSY). The sequence of SIV CP-MAC, which exhibits high levels of Env surface expression (LaBranche et al., 1994, 1995; Sauter et al., 1996), is shown for reference.