Virology 489 (2016) 141–150

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Effects of modification of the HIV-1 Env cytoplasmic tail on immunogenicity of VLP vaccines

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ARTICLE INFO

Article history:
Received 9 July 2015
Returned to author for revisions
23 September 2015
Accepted 24 September 2015
Available online 4 January 2016

Keywords:
HIV-1
VLP vaccine
Env glycoprotein
Cytoplasmic tail
Trimer stability
Neutralizing antibodies
Antibody avidity
Immunogenicity
Protein modification
Cross-clade immunization

ABSTRACT

We investigated the effects on assembly and antigenic properties of specific modifications of the transmembrane spanning (TMS) and cytoplasmic tail (CT) domains of HIV-1 Env from a transmitted/founder (T/F) ZM53 Env glycoprotein. A construct containing a short version of the TMS domain derived from the mouse mammary tumor virus (MMTV) Env with or without a GCN4 trimerization sequence in the CT exhibited the highest levels of incorporation into VLPs and induced the highest titers of anti-Env IgG immune responses in a VLP context. Sera from guinea pigs immunized by VLPs with high Env content, and containing the CT trimerization sequence, had increased neutralization activity and antibody avidity. A cross-clade prime-boost regimen with clade B SF162 or clade C ZM53 Env DNA priming and boosting with VLPs containing modified ZM53 Env further enhanced these immune responses. The modified VLPs demonstrate improved potential as HIV-1 vaccine antigens.

Introduction

Induction of an antibody response capable of preventing infection by various HIV-1 isolates is a critical goal for vaccines that will protect against HIV-1 infection (Doria-Rose, 2010; Stamatatos et al., 2009). Broadly neutralizing antibodies can prevent HIV-1 infection via binding to envelope (Env) glycoprotein complexes on the virion surface (Burton et al., 2011, 2012), and the HIV-1 Env spike is the most important viral antigen for antibody-based vaccine design. However, the Env gp120 subunit contains a number of features that enable it to evade humoral immunity including variable loops, N-linked glycosylation, and conformational flexibility (Douek et al., 2006; Go et al., 2008). The natural viral membrane context may be important to maintain a fully native Env trimer conformation (Chen et al., 2015). VLP vaccines presenting native trimeric HIV-1 spikes have been explored, but none have yet demonstrated a high potential to induce neutralizing antibodies even against moderately sensitive (tier 2) strains of HIV-1 (Tong et al., 2014).

Previous studies have shown that truncation of the cytoplasmic tail of the SIV envelope glycoprotein increases Env incorporation into virions (Johnston et al., 1993; Zingler and Littman, 1993). Moreover, it was found that the truncated Env proteins have the ability to self-associate on the cell surface and are assembled into a more closely packed array than FL Env (Vzorov and Compans, 1996, 2000). Substitution of the HIV TMS-CT with sequences derived from other viral glycoproteins, such as the mouse mammary tumor virus envelope glycoprotein, was found to enhance Env incorporation into VLPs (Wang et al., 2007). Using a leucine zipper trimerization sequence from the yeast GCN4 transcription factor, trimeric forms of target proteins can be stably expressed (Vzorov and Compans, 2011; Waning et al., 2004; Weldon et al., 2011). Stabilized Env trimers may avoid “immune misdirection” and induction of non-neutralizing antibody responses resulting from exposure of non-native epitopes on monomeric and dimeric forms of Env (Moore et al., 2006; Tong et al., 2012). Based on these reports, we compared Env constructs with a modified leucine zipper GCN4 trimerization sequence to stabilize the trimeric Env structure, and with MMTV membrane-anchoring sequences to increase the incorporation of Env into VLPs containing ZM53 (clade C) Env. The results indicate that HIV VLPs incorporating high levels of conformation-stabilized Env exhibit enhanced immunogenicity and may be useful for further development of a prophylactic HIV vaccine.

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http://dx.doi.org/10.1016/j.virol.2015.09.015
0042-6822/

Results

Design of modified Env proteins

Previously, chimeric HIV-1 Env with TMS-CT domains derived from mouse mammary tumor virus (MMTV) exhibited enhanced Env incorporation into VLP and represent promising immunogen for developing an effective vaccine (Wang et al., 2007). In the present study CCR5-tropic clade B SF162 and a transmitted/founder (T/F) ZM53 Env glycoproteins were used as immunogens for DNA priming. For boosting, VLPs containing modified ZM53 Env were used. We compared different modifications of the MMTV TMS-CT domains and additional modifications of CT domain. Based on analysis of Env expression and incorporation into VLPs, constructs were selected for immunization studies and are shown schematically in Fig. 1.

Modifications of the TMS region

To develop more effective VLP immunogens, we compared TMS domain modifications for enhancement of Env incorporation into VLPs (Wang et al., 2007) and CT domain modifications for induction of conformational changes in the gp120 subunit (Vzorov and Compans, 2011; Vzorov et al., 2005). It was previously observed that a chimeric Env with substitution of the HIV TMS-CT with a sequence derived from MMTV Env exhibited enhanced incorporation into VLPs (Wang et al., 2007). We compared additional TMS modifications and determined their effects on the incorporation of trimeric Env into VLPs. We initially compared SIVmac239 Env constructs (Table 1) with replacements of the TMS with a heterologous TMS or with different modifications in the TMS region (Table 2). Modified env genes in plasmids containing sequences coding the external sequences of SIVmac239 Env proteins were expressed in Hep2 cells. Because SIVmac239 Env can exhibit naturally truncated forms, it is a convenient model to test effects of different structural modifications on Env functions. The effects of the TMS domain on total Env expression and cell surface expression levels were measured by radioactive metabolic labelling followed by surface labeling and immune precipitation. The surface expression level relative to wild type (WT) was calculated for each modified Env by phosphorimager analysis. Generally, the modified Env constructs exhibited higher levels of surface expression ranging from 114 to 170% of the FL SIVmac239 Env (Fig. 2a). Because the TMS domain has a conserved organization and length (22 aa) among HIV and SIV Env proteins, a shortened 22 aa version of the MMTV TMS-CT domain was compared and further modified by changing Met (M25) to a positively charged Arg (R+) which is conserved in HIV and SIV Envs. The 22 residues segment of 35 MMTV-derived amino acids was selected based on sequence comparison with the HIV TMS (Table 2). Modified Envs containing other sequence segments from the MMTV TMS exhibited lower levels of surface expression (not shown). The construct designated TMS22 with a shorter 22 aa version of the MMTV TMS domain, and containing a positively charged Arg, exhibited the highest level of surface expression. Env TMS22 also exhibited higher fusion activity than Env TMS35 with a full-length 35 aa MMTV TMS domain (Table 1a; Fig. 2b). Taken together the results indicate that the length and sequence of the TMS domain can be modified to improve cell surface expression of Env proteins without affecting fusion activity of the SU domain.

Modifications of the CT domain

The cytoplasmic domain is required to enhance Env protein surface stability (Vzorov and Compans, 2011; Ye et al., 2004). To examine the effects of modifications of the CT structure, we compared the SF162 Env TMS22-hb construct having an addition of a GCN4 sequence, which is able to form a three helix bundle (hb), and a construct TMS22-hb3a with a mutant GCN4 sequence, in which critical isoleucine residues required for formation of a three-helix structure were changed to alanine. A functional cell–cell fusion assay indicated that the TMS22-hb construct with the GCN4 sequence had impaired fusion activity, as also observed before with SIV Env constructs (Vzorov and Compans, 2011)

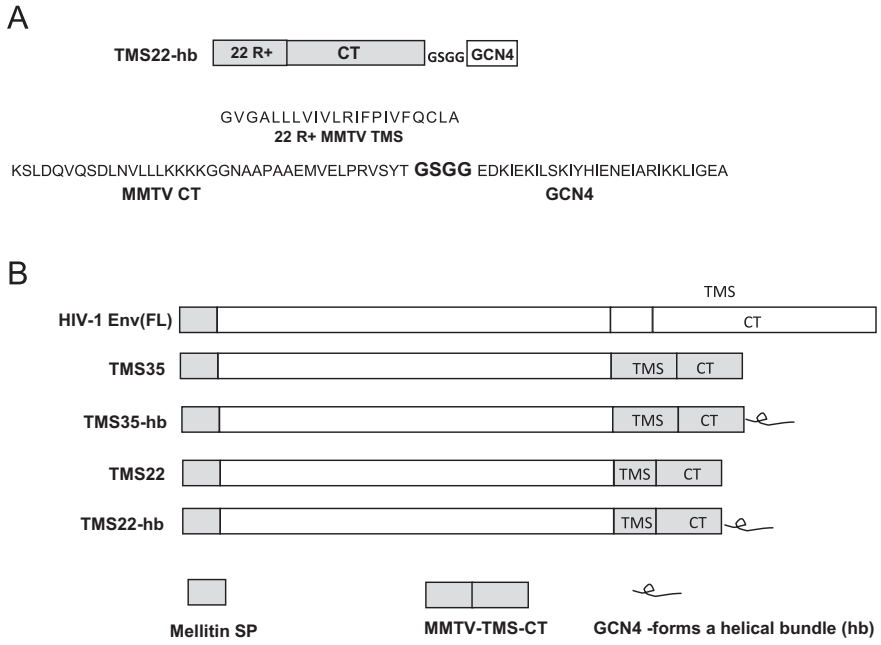

Fig. 1. Schematic diagrams and designations of modified Env. HIV-1 Envs were modified by replacement of TMS and CT domains of ZM53 HIV-1 Env. (A) Sequences of modified MMTV22 (TMS-CT region) in which Met (M25) was replaced by positively charged Arg and addition of aa GCN4 sequence to the CT domain with a GSGG linker. The construct was designated – TMS22-hb. (B) Schematic diagrams and designations of other constructs used for VLP production and immunization studies.