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Development of a DNA vaccine expressing a secreted HIV-1 gp41 ectodomain that includes the membrane-proximal external region

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ABSTRACT

A limited number of sites on the HIV-1 Envelope protein are vulnerable to broadly neutralizing antibodies (bnAbs). One of these sites, the membrane proximal external region (MPER), is located at the C-terminus of the gp41 ectodomain ($gp41_{ecto}$). This highly conserved sequence is bound by several well-characterized bnAbs. Efforts to produce a gp41 immunogen are in part hampered by the MPER's hydrophobicity and propensity to induce aggregation. We sought to produce a DNA vaccine expressing a $gp41_{ecto}$ that is both secreted from mammalian cells and maintains binding by bnAbs to the MPER. Through *in silico* analysis, we predicted regions of $gp41_{ecto}$ that could induce aggregation and possibly hinder secretion. We generated deletion mutants of $gp41_{ecto}$ and tested their ability to be secreted by mammalian cells. Upon deletion of regions in either the fusion peptide (FP) or MPER, secretion of the $gp41_{ecto}$ increased. In an effort to both augment secretion and maintain binding by bnAbs, we developed constructs with the FP deletion and introduced point mutations in the MPER. Two constructs (gp41 ΔFP and gp41 ΔFP+I682E) maintained binding by gp41 MPER-specific bnAbs (4E10, Z13e1 and 10E8). These were evaluated as DNA vaccines in a mouse model. Both vaccines proved to be immunogenic and appeared to elicit some MPER-specific antibodies that bound gp41 ectodomain-derived proteins but not short peptides spanning the MPER. No neutralizing capacity was detected against a clade C virus containing a homologous MPER.

1. Introduction

Over 30 years have elapsed since the discovery of the causative agent of AIDS, yet the development of an effective vaccine against HIV-1 remains elusive. The main target of an antibody-mediated vaccine is the Envelope protein (Env), a trimeric heterodimer of the viral glycoproteins gp120 and gp41. Env initiates CD4 receptor binding through gp120 and gp41 mediates membrane fusion between the virus and host cell [1]. Inducing sterilizing immunity against HIV-1 through vaccination has been a daunting challenge due in part to the complexity and diversity of the virus [2]. However, despite this failure, between 10 and 30% of infected individuals are able to produce broadly neutralizing antibodies (bnAbs) several years after infection [3,4]. A growing number of these bnAbs have been cloned and characterized. They have been shown to target conserved regions on gp120 and gp41 including the CD4 binding site, V1-V2 glycans, V3 glycans and the membrane proximal external region (MPER) of gp41 [5–10]. Being able to correctly display these regions will be critical for an HIV-1 vaccine.

Attempting to reproduce a bnAb response towards HIV-1 through vaccination has proven to be a formidable challenge. Several issues that plague this effort are HIV-1's rapid mutation rate, extensive glycosylation that shields protein surfaces, and immunodominant regions that elicit antibodies to non-neutralizing epitopes [11]. In addition, there are several characteristics of bnAbs themselves that are exceptional and contribute to our inability to produce bnAb by vaccination [12]. These features include hypermutated V(D)J regions and longer HCDR3 sequences as compared to other antibodies [12].

Abbreviations: MPER, membrane proximal external region; FP, fusion peptide; bnAb, broadly neutralizing antibody.

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The gp41 MPER represents both a promising and challenging target for HIV-1 vaccine development. The MPER is an attractive vaccine target as it is well conserved and targeted by several bnAbs, including 4E10, 2F5 and 10E8. Indeed, these antibodies have been shown to prevent infection through passive immunization [13,14]. Moreover, 10E8 has remarkable potency and breadth with an ability to neutralize 98% of a diverse panel of viruses that is representative of most circulating strains [8]. Nevertheless, a myriad of issues hinders the development of gp41 MPER as a vaccine target. Analysis of sera from HIV-1 infected individuals has shown that the MPER is weakly immunogenic due in part to steric hindrance and lack of accessibility [15,16]. The production of anti-MPER antibodies in infected individuals is also thwarted by the immunodominant region of gp41, which does not contain any neutralizing epitopes [17,18]. The broadly neutralizing antibodies, 2F5, 4E10 and 10E8, have all also displayed varying levels of reactivity towards lipids, which may further impede the elicitation of these types of antibodies by vaccination [19,20]. Numerous animal studies have failed to elicit robust neutralizing antibody responses using MPER peptides or fusion proteins that are soluble or presented in the context of a lipid membrane [21–28]. This may suggest that the MPER must be presented in the context of gp41 to be able to induce neutralizing antibodies. This represents a significant challenge since gp41 contains hydrophobic regions and is prone to aggregate in solution [29]. Moreover, to date, a mammalian expression vector producing a secreted and soluble gp41 that contains a full length MPER sequence has not been described.

DNA vaccination has been used in over 160 human trials (http://clinicaltrials.gov), including HIV-1 [30,31]. Recent advances in DNA vaccine technology are making DNA vaccines a viable alternative to traditional vaccination methods. DNA vaccines offer several advantages over traditional vaccination methods including safety, stability and ease of production [32]. These vaccines generate both a cellular and humoral immune response towards the target antigen [33,34]. Most importantly, host production of antigens encoded by DNA vaccines allows for antigens to undergo mammalian post-translational modifications and to more closely resemble their native structure. Finally, modification of DNA sequences is a relatively facile process, thus allowing researchers to test multiple antigens without the need for costly and resource intensive purification procedures [35].

In this study, we developed and optimized a clade C gp41 DNA vaccine that produces a secreted gp41 ectodomain protein. Through sequential deletion of gp41 regions, we mapped the regions that abrogate or hinder secretion of gp41. We subsequently used deletions and point mutations to generate a secretable gp41 ectodomain DNA vaccine and evaluated immunogenicity in a small animal model.

2. Materials and methods

2.1. HIV-1 gp41 DNA vaccine design and development

We generated a Clade C gp41 consensus sequence based on 450 Clade C amino acid sequences derived from Sub-Saharan Africa that were available from the Los Alamos Sequence Database (http://www.hiv.lanl.gov/) in 2012. Sequences were aligned and gaps were squeezed. This gave us the following sequence: AVGI GAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLRAIEAQQH MLQLTVWGIKQLQARVLAIERYLKDQQLLGIWGCSGKLICTTAVPWNS SWSNKSQEDIWDNMTWMQWDREISNYTDTIYRLLEESQNQQEKNEK DLLALDSWKNLWNWFDITNWLWYIK. This sequence corresponds to the gp41 ectodomain spanning amino acids 512 to 683 in gp160 according to HxB2 numbering. A tPA signal sequence [36] was added to the N-terminus and a GGGS linker, 3XFLAG tag and a Twin-Strep-tag were added to the C-terminus synthetically. This sequence was codon optimized and synthesized using the GENEART online platform (http://www.lifetechnologies.com/ca/en/home/life-science/cloning/gene-synthesis/geneart-gene-synthesis/geneoptimizer.html) (Life Technologies, Carlsbad, CA). This gene was cloned into the pVAX plasmid using BamHI and NotI enzymes. Deletion and point mutants were generated using the Seamless Cloning Kit (Life Technologies, Carlsbad, CA). Insert sequences containing deletions and point mutations were generated by PCR. 5′ and 3′ overhangs with homology to the pVAX backbone were added by PCR to inserts so that the seamless cloning reaction could occur. The following deletions (depicted in Figs. 1A and 2A), based on the gp41 ectodomain ($gp41_{ecto}$) and according to HxB2 numbering, correspond to the constructs used in this paper: gp41 ΔFP (Δ512-525), gp41 ΔHR1 (Δ512-607), gp41 ΔIDR (Δ512-618), gp41 ΔHR2 (Δ618-683), gp41 ΔMPER (Δ672-683), gp41 ΔFP+ΔMPER (Δ512-525 and Δ672-683), gp41 ΔFP+ΔWYIK (Δ512-525 and Δ680-683) and gp41 ΔFP+ΔIK (Δ512-525 and Δ682-683). Constructs which include a mutation at position 682 are described in the results section and Fig. 3A.

2.2. Cell culture and transfection

HEK 293T cells (CRL-3216; ATCC, Manassas, VA) were cultured in Dulbecco's Minimal Essential Media (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 0.1 M HEPES, penicillin and streptomycin (all from Corning, Tewksbury, MA). Cells were cultured at 37 °C and 5% CO_2 in a humidified incubator. 293T cells were seeded at 3×10^6 cells/well in a 6 well plate 24 h prior to transfection. JetPEI (Polyplus, Illkirch, France) was used for transient transfection of 293T cells according to the manufacturer's protocol. Media was changed 24 h after transfection to DMEM supplemented with 1% FBS, 0.1 M HEPES, penicillin and streptomycin. Cell supernatants and lysates were collected 48 h after transfection. Cell supernatants were clarified by centrifugation for 15 min at 16,000*g*. Clarified supernatants were recovered and subsequently stored at −20 °C until analyzed. Cells were washed twice with phosphate-buffered saline (PBS) and spun down at 300*g* for 10 min. Cells were lysed in NP40 cell lysis buffer (Life Technologies, Carlsbad, CA) containing a protease inhibitor cocktail (Sigma, St Louis, MO). Cell lysates were stored at −20 °C until analyzed.

2.3. SDS-PAGE, immunoblotting and deglycosylation studies

Unless otherwise stated, all SDS-PAGE and immunoblotting reagents and devices were from the same manufacturer (Life Technologies, Carlsbad, CA). Cell lysates and supernatants were incubated at 70 °C for 10 min after the addition of 4X LDS Loading Buffer and Reducing Agent. Samples were run on NuPAGE 4–12% Bis-Tris gels at 150 V under reducing conditions. Gels were transferred to PVDF membranes using the iBlot transfer device. Membranes were blocked for 20 min in 10% non-fat dry milk dissolved in Tris-buffered saline containing 0.05% Tween (TBS-T). Resolved proteins were detected by blotting with a mouse anti-FLAG antibody, clone M2 (Sigma, St. Louis, MO) diluted to 0.5 μg/ml in 3% milk TBS-T or a mouse anti-Tubulin antibody, clone B3 (Thermo Scientific, Waltham, MA) diluted to 1:2500 in 1% milk TBS-T. A secondary goat anti-mouse IgG antibody conjugated to alkaline phosphatase (AP) was diluted to 1:5000 in TBS-T and used to detect the primary antibody. CDP-star substrate was used to generate a chemiluminescent signal, which was detected using X-ray film (VWR, Radnor, PA) and an X-ray imager (Kodak, Rochester, NY). In deglycosylation studies we used PNGase F (NEB, Ipswich, MA) to remove N-glycans from gp41 secreted into cell supernatants. Cell supernatants at a volume of 13.5 μl were incubated with 1.5 μl of glycoprotein denaturing buffer (NEB,