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Eliciting neutralizing antibodies with gp120 outer domain constructs based on M-group consensus sequence

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ABSTRACT

One strategy being evaluated for HIV-1 vaccine development is focusing immune responses towards neutralizing epitopes on the gp120 outer domain (OD) by removing the immunodominant, but non-neutralizing, inner domain. Previous OD constructs have not elicited strong neutralizing antibodies (nAbs). We constructed two immunogens, a monomeric gp120-OD and a trimeric gp120-OD × 3, based on an M group consensus sequence (MCON6). Their biochemical and immunological properties were compared with intact gp120. Results indicated better preservation of critical neutralizing epitopes on gp120-OD × 3. In contrast to previous studies, our immunogens induced potent, cross-reactive nAbs in rabbits. Although nAbs primarily targeted Tier 1 viruses, they exhibited significant breadth. Epitope mapping analyses indicated that nAbs primarily targeted conserved V3 loop elements. Although the potency and breadth of nAbs were similar for all three immunogens, nAb induction kinetics indicated that gp120-OD × 3 was superior to gp120-OD, suggesting that gp120-OD × 3 is a promising prototype for further gp120 OD-based immunogen development.

Introduction

According to the 2013 UNAIDS report on the global Acquired Immunodeficiency Syndrome (AIDS) epidemic, an estimated 35.3 million people were infected with human immunodeficiency virus type 1 (HIV-1) in 2012. Despite the existence of highly effective anti-viral drugs, there were still 2.3 million new HIV-1 infections in 2012. A vaccine that could elicit broadly neutralizing antibodies (bnAbs) is hypothesized to be the most effective means to stop the AIDS pandemic (Haynes and Montefiori, 2006; Hoxie, 2010; Mascola and Montefiori, 2010). Unfortunately, eliciting bnAbs against HIV-1 has been a major scientific challenge (Ross et al., 2010). Several factors contribute to this difficulty, including the high mutation rate, extensive glycosylation (Wyatt et al., 1998), and significant conformational flexibility of the envelope glycoprotein (Karlsson Hedestam et al., 2008; Kwong et al., 2011), as well as low envelope spike density on the virion surface (Klein and Bjorkman, 2010; Klein et al., 2009).

The HIV-1 envelope glycoprotein is the sole known target of nAbs against the virus. It is comprised of two subunits; a surface glycoprotein, gp120, that contains the receptor (CD4) and co-receptor (*viz.* CCR5 or CXCR4) binding sites; and a transmembrane glycoprotein, gp41, that mediates fusion between the viral and the host cellular membranes (reviewed in LaBranche et al., 2001; Pantophlet and Burton, 2006; Wyatt and Sodroski, 1998). These two subunits form a trimeric heterodimer (Center et al., 2002, 2001; Zhu et al., 2003). Structurally, gp120 is divided into three domains: an inner domain (ID), an outer domain (OD), and a

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bridging sheet that connects the two (Kwong et al., 1998). In the native trimeric envelope spike, the ID is buried internally while the OD is exposed on the surface (Kwong et al., 2000). The envelope glycoprotein has four conserved regions that are targeted by potent bnAbs isolated from HIV-1 infected patients: (1) the CD4 binding site (*e.g.* b12, VRC01, NIH45-46, 3BNC117 (Burton et al., 1991; Diskin et al., 2011; Scheid et al., 2011;. Wu et al., 2010)), (2) glycans around N160 along with conserved elements on V1/V2 (*e.g.* PG9, PG16 (Walker et al., 2009)), (3) glycans and the base of V3 loop around N332 (*e.g.* 2G12 and PGT antibodies, including PGT128 (Buchacher et al., 1994; Walker et al., 2011)), and (4) the membrane-proximal external region (MPER) of gp41 (*e.g.* 2F5, Z13e1, 4E10 and 10E8 (Huang et al., 2012; Muster et al., 1994; Purtscher et al., 1994; Stiegler et al., 2001; Zwick et al., 2001; also reviewed in Kwong and Mascola, 2012; Kwong et al., 2013). A number of bnAbs that target other novel epitopes have also been isolated more recently: 3BC176 and 3BC315 that target a region near the V3 loop and the CD4i site (Klein et al., 2012), 8ANC195 that recognizes portions of gp41 and N-linked glycans adjacent to the CD4BS (Scharf et al., 2014), and PGT151 that targets a glycan-dependent epitope at the interface of gp120 and gp41 (Blattner et al., 2014; Falkowska et al., 2014).

Considering that many of the epitopes targeted by bnAbs are on the gp120 OD and that the highly immunodominant ID elicits non-neutralizing antibodies, it has been hypothesized that immunogens comprised of only the OD are better able to induce bnAbs than the intact gp120 (Kwong et al., 2011; Nabel et al., 2011; Zhou et al., 2007). Unfortunately, previous immunization studies using OD-based immunogens have largely been unsuccessful. Yang et al. (2004) expressed a clade B YU2 OD construct, termed OD1. The OD1 protein could be recognized by 2G12 and various anti-V3 human mAbs, but not by b12. In a later study, however, it was shown by surface plasmon resonance (SPR) that b12 could bind OD1, but with a high dissociation rate (Zhou et al., 2007). The protein also failed to bind CD4. Not surprisingly, rabbits immunized with OD1 failed to mount detectable levels of nAbs against HIV-1_{KB9} or HIV-1_{YU2}.

In another study, Chen et al. (2007, 2008) immunized mice with a clade C virus OD (CN54) fused to the human IgG1 Fc domain as an immune enhancer. However, there were no neutralizing activities in the mice sera. Bhattacharyya et al. (2010) designed an OD-based immunogen (OD_{EC}) from HIV-1 HxBc2. The OD_{EC} protein was expressed in *Escherichia coli* and, therefore, it was not glycosylated. It also lacked the variable loops V1/V2 and V3, and 11 mutations were introduced to increase binding affinity to CD4 and IgG1 b12. However, the sera from immunized rabbits showed only marginal neutralizing activity against four clade B viruses and one clade C virus. Wu et al. (2009) generated a membrane-anchored OD containing residues 252–482 from the HIV-1 clade B virus TAI-R3A. This OD variant had a truncated β20-β21 hairpin and a shortened V3 loop, fused to the transmembrane region of CD4. This optimized, membrane-anchored OD could be recognized by b12 and b13, but was not recognized by other CD4 binding site (CD4BS) antibodies. Joyce et al. (2013) recently reported an antigenically optimized OD4.2.2 from the clade A KER2018 strain, which could bind to VRC01 with nanomolar affinity. However, its immunogenic properties were not reported.

Despite these unsuccessful attempts to induce bnAbs using immunogens based on gp120 OD, it is our hypothesis that focusing immune responses to the OD of gp120 is still worth pursuing based on one or more of the following reasons: (1) the immunogens tested thus far have not been tested rigorously for their proper antigenic conformation, (2) the immunogens were based on a single HIV-1 isolate, and (3) the adjuvants used in these studies may not have been ideal to maintain correct antigenic structures. In this report, we describe our attempt to design and evaluate gp120 OD-based immunogens to induce bnAbs in rabbits. The results suggest that our immunogens and/or the antigen delivery system we used are better than others reported thus far in inducing nAbs against HIV-1.

Results

Construction, expression and purification of monomeric and trimeric forms of gp120 OD, and gp120

In past studies of generating immunogens based on gp120 OD, envelope protein sequences from a single HIV-1 isolate from a given clade were used. Considering that centralized HIV-1 antigens based on ancestral, consensus or a phylogenetic tree center sequences have been suggested to have the potential to increase the breadth of immune responses (Gao et al., 2004; Gaschen et al., 2002; Nickle, 2003), we have chosen to use an M group consensus sequence for generating our immunogens (MCON6, (Gao et al., 2005)). Antisera from guinea pigs immunized with recombinant MCON6 gp120 have previously been shown to neutralize several HIV-1 primary isolates, albeit weakly and somewhat sporadically (Gao et al., 2005).

First, we generated gp120-OD by cloning ^{249}STQLLL…RPGGGD^{477} (numbering based on MCON6) fused to the signal peptide (Fig. 1A). In addition to monomeric gp120-OD, we also constructed a trimeric form (gp120-OD × 3), which contains three tandem copies of OD covalently linked by a four amino acid linker (RVTG). The rationale for generating OD × 3 was as follows: when the ID is removed from gp120, the interface between the two domains would be exposed (Fig. 1B). The newly exposed surface could potentially be immunogenic, which might decrease immunogenicity of critical neutralizing epitopes on the OD. Because the N-terminal and the C-terminal ends of gp120-OD (S^{249} and D^{477}, respectively) are on the same face of the protein and in close proximity, we hypothesized that it might be possible to "mask" the newly exposed surface from the immune system by bringing the three tandem copies close together by covalently linking them with a short, four amino acid linker. One potential benefit of trimerizing gp120-OD was enhancement of immunogenicity by increasing the valency of epitopes. In addition to gp120-OD and gp120-OD × 3, we constructed the intact gp120 for comparing immunogenicity.

All three proteins were expressed efficiently in HEK293 cells, and all of them could be purified to near homogeneity using a combination of affinity chromatography (Con A and Ni-NTA) and ion-exchange chromatography using Q-sepharose (Fig. 1C). Approximate molecular weights of gp120, gp120-OD, and gp120-OD × 3 were 120 kD, 60 kD and 180 kD, respectively. Gp120-OD migrated as a broad smear, suggesting significant heterogeneity in glycosylation. Deglycosylation analyses with EndoH and PNGaseF showed that proteins are glycosylated with both high-mannose and complex carbohydrates (Fig. 1D). Fully deglycosylated gp120-OD, gp120-OD × 3 and gp120 migrated as expected theoretical molecular weights of 26 kD, 75 kD and 55 kD, respectively.

Antigenic properties of gp120, gp120-OD and gp120-OD × 3

To evaluate whether OD-based immunogens were conformationally intact, their antigenic properties were examined by immunoprecipitation using multiple nAbs and compared to that of gp120. First, the prototypic nAbs that target a variety of epitopes were tested: 17b (bridging sheet), b12 (CD4BS), 2G12 (carbohydrate) and 447-52D (V3 loop). As shown in Fig. 2A, gp120 was immunoprecipitated by all four nAbs. Among the four antibodies tested, b12 appeared the most efficient. Both gp120-OD