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Identification and characterization of a naturally occurring, efficiently cleaved, membrane-bound, clade A HIV-1 Env, suitable for immunogen design, with properties comparable to membrane-bound BG505

Supratik Das[a], Saikat Boliar[a], Sweety Samal[a], Shubbir Ahmed[a], Tripti Shrivastava[a], Brihaspati N. Shukla[a], Sandeep Goswami[a], Manish Bansal[a], Bimal K. Chakrabarti[a,b,*]

[a] THSTI-IAVI HIV Vaccine Design Program, Translational Health Science and Technology Institute, 3rd Milestone, Faridabad-Gurgaon Expressway, PO box #04, Faridabad 121001, Haryana, India
[b] IAVI Neutralizing Antibody Center at TSRI, La Jolla, CA, USA

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ABSTRACT

Efficient cleavage of HIV-1 Env gp160 into its constituent subunits correlates with selective binding to neutralizing antibodies and are the closest mimetic of native, functional Envs. This was first demonstrated with the clade B Env, JRFL. The correlation between efficient cleavage and selective binding to neutralizing antibodies is the guiding principle for immunogen design for HIV vaccine. We have recently reported that Envs 4-2.J41 (clade C) and JRCSF (clade B) are also efficiently cleaved and show similar properties. However, an efficiently cleaved, membrane-bound clade A Env suitable for genetic vaccination has not been directly demonstrated. Here we report that BG505 and a new clade A Env, QB726.70M.ENV.C4 (or A5) are efficiently cleaved on cell membrane. A5 shows desirable antigenic properties comparable with BG505 on cell surface. A5SOSIP in supernatant displays majority of bNAb binding epitopes. Thus, both BG505 and A5 Envs can be used in DNA prime-protein boost vaccination studies.

1. Introduction

One of the critical areas of research currently, in HIV-1 vaccinology, is to develop immunogens that closely mimic the native Env spikes (Guenaga et al., 2015; Sanders et al., 2013) and activate the humoral immune system to elicit broadly neutralizing antibodies (bNAbs) upon vaccination (Crooks et al., 2015; Dosenovic et al., 2015; Sanders et al., 2015). The functional, trimeric Env spike is a trimer of a heterodimer of gp120 and gp41 formed by proteolytic processing of the gp160 polypeptide by endogenous furin (Hallenberger et al., 1992), and bind specifically to neutralizing antibodies (NAbs) but not to non-neutralizing antibodies (non-NAbs) (Guenaga et al., 2015; Ringe et al., 2013). We and others have previously shown that the efficient cleavage of Env gp160 into the gp120 and gp41 subunits co-relates with specific exposure of neutralizing antibody epitopes but not non-neutralizing epitopes on the cell surface and also neutralization capacities of viruses pseudotyped with such Envs (Boliar et al., 2015; Das et al., 2016; Pancera and Wyatt, 2005). Uncleaved Envs bind to both neutralizing and non-neutralizing antibodies when expressed on the cell surface (Boliar et al., 2015; Chakrabarti et al., 2011; Das et al., 2016; Haim et al., 2013). Thus, an essential property of Envs suitable for genetic vaccination studies is that the membrane-bound form is efficiently cleaved and therefore displays epitopes for NAbs only. Such Envs, that are naturally occurring, are relatively rare. The first reported naturally occurring, efficiently cleaved Env was the clade B Env, JRFL (Pancera and Wyatt, 2005). Subsequently, we have reported that the clade C Env, 4-2. J41 (Boliar et al., 2015) and clade B Env, JRCSF (Das et al., 2016) are also efficiently cleaved and bind preferentially to NAbs. Artificially converting 4-2. J41 and JRCSF to an uncleaved form by mutating the cleavage site, REKR to SEKS results in binding to both neutralizing and non-neutralizing antibodies (Boliar et al., 2015; Das et al., 2016; Pancera and Wyatt, 2005).

The native, functional Env immunogen can be presented to the immune system through DNA vaccination e.g. using viral vectors, apart from as soluble proteins or virus-like particles. Reports suggest that DNA prime-protein boost vaccination strategies elicit qualitatively superior antibodies as compared to vaccination with protein alone (Chakrabarti et al., 2013; Law et al., 2008; Vaine et al., 2008). Such strategies have been used in several clinical trials e.g. RV144 which has shown the best efficacy. Although, SOSIP (Klasse et al., 2013; Sanders

* Corresponding author at: THSTI-IAVI HIV Vaccine Design Program, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, PO box #04, Faridabad 121001, Haryana, India.
E-mail addresses: bchakrabarti@iavi.org, bimal.iavi@thsti.res.in (B.K. Chakrabarti).

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et al., 2013) and NFL (Sharma et al., 2015) versions of soluble clade A Envs have been reported, and the clade A Env BG505, whose soluble forms are well-characterized, binds specifically to neutralizing antibodies on the cell surface including the cleavage-specific bNAb PGT151; an efficiently cleaved, membrane-bound clade A Env with requisite antigenic properties, suitable for priming through genetic vaccination has not been directly demonstrated. In this report, we present data demonstrating that BG505 is efficiently cleaved on the plasma membrane and also the identification and characterization of a new, naturally occurring, efficiently cleaved clade A Env, QB726.70M.ENV.C4 or A5 with desirable antigenic properties that is suitable for genetic vaccination and also for developing soluble immunogens.

2. Materials and methods

2.1. Recombinant DNA, antibodies and cell lines

DNA clones of full-length clade A Envs were obtained from NIH AIDS Reagent Program. These clade A Env DNAs have been cloned into pCI-neo and express protein from the CMV promoter. Site-directed mutagenesis was carried out using mutagenic primers designed according to Quikchange site directed mutagenesis kit manual (Qiagen) and PCR was carried out with *Phusion* polymerase. Reaction mixtures were digested with Dpn1, transformed into competent cells and plated onto LB-Amp plates. Plasmid DNA was isolated from single colonies and mutation confirmed by sequencing. Codon optimization was done commercially by Gene Art (Life Technologies). The full-length A5 codon-optimized DNA fragment was cloned into the *Bam*H1-*Eco*R1 sites of pcDNA3.1 (+) and CD5 leader sequence was used for higher expression. Broadly NAbs (VRC01, b12, PGT121, PGT128, PGT145, PGT151, PG9, 10E8, 2G12), non-NAbs (F105, b6, 39F, 2.2B, 19b and 17b) were obtained from the IAVI Neutralizing Antibody Center (NAC) at TSRI, La Jolla, California. VRC34 antibody was obtained from Dr. John Mascola of VRC, NIH. Cell lines (TZM-bl and 293T) were obtained from NIH AIDS Reagent Program and ATCC and maintained as described before (Boliar et al., 2015).

2.2. Cell surface assay using FACS-based method

All flow cytometry-based cell surface assays were carried out as described previously (Boliar et al., 2015). Following transfection of 293T cells with plasmids expressing clade A subtype Envs, cells were harvested, washed three times with FACS buffer I (DMEM + 5% HI-FBS) and stained with increasing concentration of broadly neutralizing and non-neutralizing antibodies in 96 well U-bottomed plate wells for 1 h at room temperature (RT). Next, the cells were washed with FACS buffer I and incubated with PE-conjugated goat anti-human secondary antibody (1:200 dilutions, Jackson ImmunoResearch) for 1 h at RT. Following a second round of washing with FACS buffer II (PBS + 5% HI-FBS), the cells were fixed with 0.5% paraformaldehyde. The stained and fixed cells were analyzed in a FACS Canto analyzer (BD Biosciences) and MFI determined using the FlowJo software (version 10.0.6, Tree Star Inc).

2.3. gp120 shedding assay

Enhancement of gp120 shedding in the presence of soluble CD4 was determined as described previously (Boliar et al., 2015). Clade A Env A5 transfected cells were harvested and incubated in FACS buffer II with or without two-domain sCD4-183 (NIH AIDS Reagent) at a concentration of 50 μg/ml for 1 h at 4 °C with mixing every 5 min. Following incubation, cells were centrifuged and the supernatant used in ELISA assay as described previously (Boliar et al., 2015). Briefly, 96 well flat bottomed plates were incubated with 2.5 μg/ml of lectin O/N at 4 °C, washed three times with PBST (PBS + 0.1% Tween-20) and 100 μl of supernatant was added and incubated for 1 h at RT with shaking, washed again three times with PBST. Next, 100 μl of a rabbit anti-clade A gp120 antibody (ABLinc/Immunetech) (1:1000 dilution) was added and incubated for 1 h at RT with shaking, washed with PBST and incubated with goat anti-rabbit secondary antibody coupled to HRP (1:1000 dilution). Colorimetric analysis was carried out with TMB solution (Invitrogen) according to the manufacturer's protocol.

2.4. Plasma membrane isolation and immunoprecipitation assay

Plasma membrane fractions of A5 and JRCSF transfected cells were isolated as described previously (Boliar et al., 2015) using the Plasma Membrane Protein Isolation kit (Abcam) following the manufacturer's protocol. Proteins in plasma membrane fractions were solubilized in lysis buffer (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton-X, 1 mM DTT and protease inhibitors) and immunoprecipitated with respective antibodies O/N at 4 ^{0}C with rotation. Protein-antibody complexes were captured with immobilized Protein G resin (G Biosciences), washed three times with PBS + 1% Triton-X and bound proteins analyzed by western blot analysis using anti-clade A and anti-clade B Env antibodies (ABLinc) as probes.

3. Results

3.1. Preliminary screening of putative efficiently cleaved clade A Envs

The efficiency of cleavage of the Env gp160 polypeptide into its constituent subunits is correlated with preferential binding to bNAbs and not to non-NAbs (Boliar et al., 2015; Das et al., 2016; Pancera and Wyatt, 2005; Yasmeen et al., 2014). Thus, for immunogen design, efficient cleavage of candidate Envs is a desirable property. We and others have previously shown that determining the ratio of binding of membrane bound Env to NAbs and non-NAbs can be used as a measure of efficiency of cleavage (Boliar et al., 2015; Das et al., 2016; Pancera and Wyatt, 2005). This method has been used to identify three efficiently cleaved Envs namely the clade B Envs, JRFL (Pancera and Wyatt, 2005), JRCSF (Das et al., 2016) and a clade C Env, 4-2.J41 (Boliar et al., 2015). Recently, a cleavage-dependent, trimer-specific bNAb, PGT151 has been reported (Blattner et al., 2014). Given, its binding-specificity to cleaved, native trimers, we used this antibody for initial screening of cleaved, functional clade A Env expressed on the cell membrane. Seventeen clones expressing clade A Envs (Supplementary Table 1) were screened by a FACS-based cell surface staining assay using PGT151 and the CD4bs-directed non-NAb, F105 (Fig. 1A and Supplementary Fig. 1). The area under curve of each binding curve (Supplementary Fig. 1) and the ratio of binding to PGT151 versus F105 was determined as shown for some clones in Supplementary Table 2. Efficiently cleaved JRFL Env was used as a positive control. The Env QB726.70M.ENV.C4 (designated as A5) showed robust binding to PGT151 as compared to F105 (Fig. 1A) with highest binding ratio of PGT151 to F105 (Supplementary Table 2) of all the clones tested suggesting that this A5 Env is most efficiently cleaved. Other clones with modest to high binding ratios are shown in Supplementary Fig. 1. Binding ratios of clade A clones that were not significant have not been shown in Supplementary Fig. 1 and Supplementary Table 2.

3.2. Clade A Env, A5 binds preferentially to bNAbs

Previous reports demonstrated that efficiently cleaved HIV-1 Envs, JRFL, JRCSF and 4-2.J41 bind selectively to bNAbs (Boliar et al., 2015; Das et al., 2016; Pancera and Wyatt, 2005). Similarly, soluble Envs that mimic the native, cleaved trimer, e.g. BG505SOSIP.664 bind differentially to bNAbs and non-NAbs (Guenaga et al., 2015; Yasmeen et al., 2014). Although, soluble, NFL, single polypeptide versions of Envs have been isolated and characterized (Sharma et al., 2015) and