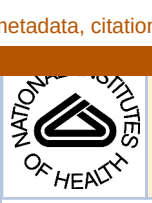

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Broadly Reactive Monoclonal Antibodies to Multiple HIV-1 Subtype and SIVcpz Envelope Glycoproteins

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Abstract

The extraordinarily high level of genetic variation of HIV-1 *env* genes poses a challenge to obtain antibodies that cross-react with multiple subtype Env glycoproteins. To determine if cross-reactive monoclonal antibodies (mAbs) to highly conserved epitopes in HIV-1 envelope glycoproteins can be induced, we immunized mice with wild-type or consensus HIV-1 Env proteins and characterized a panel of ten mAbs that reacted with varying breadth to subtypes A, B, C, D, F, G, CRF01_AE and a highly divergent SIVcpzUS Env proteins by ELISA and Western blot analysis. Two mAbs (3B3 and 16H3) cross–reacted with all tested Env proteins, including SIVcpzUS Env. Surface plasmon resonance analyses showed both 3B3 and 16H3 bound Env proteins with high affinity. However, neither neutralized primary HIV-1 pseudoviruses. These data indicate that broadly-reactive non-neutralizing monoclonal antibodies can be elicited, but that the conserved epitopes that they recognize are not present on functional virion trimers. Nonetheless, such mAbs represent valuable reagents to study the biochemistry and structural biology of Env protein oligomers.

Keywords

cross-reactivity; monoclonal antibody; subtype; envelope glycoprotein; HIV-1

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Introduction

One of the hallmarks of HIV-1 is its extraordinary variability. Based on phylogenetic analysis, the most prevalent group M viruses are classified into nine subtypes (Kuiken et al., 2008; Robertson et al., 2000). Some of these subtypes have recombined and generated circulating recombinant forms (CRF) (Robertson et al., 2000). Some CRFs have become predominant in certain geographic areas, e.g., CRF01_AE in South and south-East Asia and CRF02_AG in West Africa (Hemelaar et al., 2006; Osmanov et al., 2002). Although considerable efforts have been devoted to the development of diagnostic tests that can detect all HIV-1 subtypes, the high level of genetic variability renders the generation of reagents that reliably detect all divergent viruses challenging (Lal, Chakrabarti, and Yang, 2005; Peeters, Toure-Kane, and Nkengasong, 2003; Taylor et al., 2008).

The *env* gene is one of the most divergent genes in the HIV-1 genome (Gaschen et al., 2002). The Env glycoprotein mediates virion attachment and fusion to host cells and is the only viral protein that can elicit neutralizing antibodies. It is important to identify mAbs that recognize conserved regions in the HIV envelope to generate reagents for virion detection. Moreover, the development of mAbs that bind all HIV-1 subtypes would facilitate the development of diagnostic tests targeted at body fluid soluble Env detection. Cross-reactivity of HIV-1-infected patient sera to Env proteins from different subtypes has been observed, suggesting recognition of conserved epitopes (Gao et al., 2003; Gao et al., 2005; Gilljam et al., 1999; Moore et al., 1996; Moore et al., 1994). Thus far, only a handful of broadly reactive monoclonal antibodies (mAbs) have been isolated, some of which (b12, 2G12, 2F5, and 4E10/z13) are also capable of neutralizing a broad spectrum of viruses (Binley et al., 2004; Lin and Nara, 2007; Moore et al., 1994; Trkola et al., 1995; Zwick et al., 2001). These rare neutralizing human mAbs either target glycans on the surface of gp120, bind conformation sensitive CD4 binding or induced sites, or bind Env regions close to the lipid membrane on gp41 (Burton et al., 1994; Moore et al., 1994; Moulard et al., 2002; Sanders et al., 2002; Trkola et al., 1996; Zwick et al., 2001). Nonetheless, identification and characterization of additional cross-reactive mAbs, regardless of their neutralizing capacity, would be helpful to understand the structure and biology of HIV-1 envelope glycoproteins as immunogens. In this report, we have produced and characterized ten novel murine mAbs, two of which cross-react with HIV-1 envelope glycoproteins from all HIV-1 envelope subtypes as well as SIVcpz.

Materials and Methods

Proteins

HIV-1 Env gp140CFI proteins (C, cleavage deficient; F, fusion deficient; I, immunodominant region deletion) were purified using *galanthus nivalis* lection column from supernatant of 293T cells infected with recombinant vaccinia viruses expressing Env proteins or CHO cell lines from the Programme EVA Centre for AIDS Reagents, NIBSC, UK as described before (Gao et al., 2005; Liao et al., 2006). Other recombinant HIV-1 Env proteins were obtained from NIH AIDS Reference Reagent Repository Program (NIH, USA). Their ability to bind soluble CD4 and various mAbs was determined by surface plasmon resonance (SPR) and their oligomer conformation was confirmed by blue native gel analysis as previously described. The following Env proteins were used in ELISA and Western blot assays to determine the cross reactivity: 92UG037 gp140 and 92RW020 gp140CFI (subtype A); HXB2/Bal gp140CFI, 89.6 gp120, IIIB gp120, SF162 gp120 and JRFL gp120 or gp140CF (subtype B); 97ZA012 gp140CFI, DU123 gp140CF and 96ZM651 gp120 (subtype C); 92UG021 gp140 (subtype D); 93TH975 gp120 and CM235 gp120 (CRF01_AE); 93BR029 gp140 (subtype F); A1.con, B.con and C.con gp140CF (subtype consensus); CON6 gp140CFI, CON-S gp140CF and CON-T gp140CF (group M consensus based on 1999, 2000 and 2003 database, respectively); and SIVcpzUS1 gp140CF.

Immunization and generation of hybridoma cell lines

BALB/c mice were immunized with 25 μg of purified HIV-1 Env proteins (CON6 gp140CFI or C.97ZA012 gp140CFI) in Emulsigen (MVP Laboratories, Omaha, NE) and oCpGs (Midland Certified Reagent Company, Inc., Midland, Texas). Immunization was carried out four times at a two-week interval. Animals were housed in the Duke University Animal Facility under AALAC guidelines with animal use protocols approved by the Duke University Animal Use Committee. HIV-1 specific antibodies in mouse sera were monitored 10 days after each immunization. Three days after the fourth immunization, splenocytes were harvested. Hybridoma fusions were performed as previously described using HAT (hypoxanthine, aminopterin, thymidine) sensitive mouse myeloma cells, P3X63 Ag8 or NS-1 (Palker et al., 1984). The hybridomas that secreted anti-HIV-1 Env antibodies were identified by ELISA with autologous Env proteins after cultured for 14 days. Positive cell lines were then expanded in large scale culture and mAbs were purified from cell culture supernatants for further analysis.

Western blot analysis

A panel of HIV-1 Env proteins from five subtypes (A, B, C, D and F), CRF01_AE and SIVcpzUS1 was used to determine the cross reactivity of mAbs. Env proteins (100 ng) were separated on a reducing 10% SDS-polyacrylamide gel and blotted onto a nitrocellulose membrane. After blocking overnight in PBS containing 1% casein at 4° C, the membranes were washed and probed with mAbs in blocking buffer (1μg/ml). After 2 hr incubation at room temperature, the membranes were washed and probed with the Alexa-Flour 680 labeled goat anti-mouse IgG (H+L) antibody (1μg/ml) in the blocking buffer. The membranes were washed thrice with PBS containing Tween-20 (0.05%) and the images were acquired on an Odyssey Infrared imager.

Binding kinetics of mAbs to HIV-1 Env proteins

HIV-1 Env gp140 proteins were immobilized to about 1000 RU using amine coupling chemistry and as described before (Alam et al., 2007; Alam et al., 2008). Positive control V3 mAb F39F and gp41 mAb 4E10 and 7B2 were used to assess activity of gp140 proteins following immobilization. For comparative analyses of binding to each Env protein, mAbs were injected at 50 μg/mL for 2 min at 30 μl/min. For binding kinetics analyses, mAbs were injected at varying concentrations ranging from 1.2 mg/ml to 1.5 μg/ml. Following each cycle of binding, mAb bound surfaces were regenerated by injecting glycine pH 2.0 for 12 sec at 50 μl/min. Due to the bivalency of the mAbs used, binding rate constants and K_d values were derived from global curve fitting using the “Bivalent Analyte” model and BIAevaluation 3.2 (BIAcore/GE Healthcare, Inc) as described before (Alam et al., 2008). For comparative analyses, binding K_d was calculated from faster rate constants (ka1 and kd1) derived from curve fitting analyses.

Virus capture analysis

MAbs were diluted in 0.1 M sodium bicarbonate (5 μg/ml) and used to coat high-binding 96-well plates (100 μl/well) in triplicates. The plates were kept at 4°C overnight. After the plates were washed three times with PBS containing 0.1% Tween-20 and blocked (150 μl/well) with PBS containing 4% whey protein, 15% goat serum, 0.5% Tween-20 and 0.05% NaN_3 for 1 hr at room temperature, the plates were washed twice. SF162 or BG1168 Env pseudovirus with or without soluble CD4 was added and incubated at 37° C for 1 hr. After three washes, 100 μl of 0.5% Triton X-100 was added to each well to lyse virus particles. The released P24 protein was measured with P24 detection kit (Beckman Coulter, Miami, FL).

Peptides

A number of peptides from different region of the Env protein were used for epitope mapping. DP178: YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (gp41 MPER); SP400-BaL: RVLAVERYLRDQQLLGIWGCSGKLICTTAVPWNASWSNKSLNKI (gp41 immunodominant region); Bcon V3: TRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAH (gp120 V3); C.con env03 V3: TRKSIRIGPGQTFYATGDIIGDIRQAH (gp120 V3); SP62: QQEKNEQELLELDKWASLWN (gp41 MPER containing the 2F5 epitope); P-4E10: SLWNWFNITNWLWYIK (gp41 MPER containing the 4E10 epitope) DP107: NNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQ (gp41 heptad repeat-1 region).

Full length *env* gene cloning

Full length *env* genes were amplified from genomic DNA of HIV-1 infected individuals (15313 and 17779) by nested PCR. The *env* genes of a lab adapted HIV-1 MN strain (subtype B) as well as subtypes A1 and C consensus were synthesized using codon optimized sequences (DNA 2.0, Menlo Park, CA). All *env* gene fragments were cloned into pcDNA3.1/V5-His (Invitrogen, Carlsbad, CA). Positive clones were identified by PCR.

Flow Cytometry

293T/17 cells seeded in a 6-well plate were transfected using Fugene HD (Roche Diagnostics, Indianapolis, IN) at a ratio of 12:3 (Fugene:plasmid) with the gp160 plasmids. Transfected cells were washed 48 hrs post transfection and resuspended in PBS buffer (Mediatech, Manassus, VA). The live/dead fixable violet dead cell stain kit was used per manufacturer's protocol (Invitrogen Molecular Probes, Eugene, OR). Cells were then washed and resuspended in XVivo15 medium (Lonza, Basel, Switzerland). Monoclonal antibodies 3B3, 16H3, 13D5 and 2G12 (Polymun, Vienna, Austria) were added. All antibodies had been conjugated to PE at BD Biosciences. Cells were incubated with antibodies for 30 minutes at room temperature in the dark. After washing, cells were resuspended in 1% paraformaldehyde (pfa; Electron Microscopy Sciences, Hatfield, PA). Cells were washed and resuspended in 1% pfa and stored at 4°C until data acquisition. All samples were analyzed within 24 hrs of staining and run on the FACS Canto II or FACs Calibur (BD Biosciences, San Jose, CA).

Results

Identification of broadly reactive mouse monoclonal antibodies

Hybridoma culture supernatants were screened by ELISA to identify mAbs that reacted with HIV-1 Env glycoproteins from multiple subtypes. A total of 12 Env proteins were used as coating antigens for ELISA screening: 92RW020 (subtype A); HXB2, 89.6 and JRFL (subtype B); 97ZA012, DU123 and 96ZM651 (subtype C); A1.con, B.con and C.con (subtype consensus), CON-S (group M consensus); and SIVcpzUS. Seventeen hybridoma lines were found to secret mAbs reacting with autologous Env proteins in mice immunized with C_97ZA012 gp140. Among eight cloned mAbs, three (15E9, 16H3 and 3B3) reacted with all Env proteins tested, while the five others exhibited varying levels of cross-reactivity (Table 1). In CON6 gp140 immunized mice, 21 hybridoma lines were found to produce mAbs reacting with CON6 gp140 and two (13D5 and 13D7) were cloned. While 13D7 reacted to all Env proteins, 13D5 did not react with A_92RW020 and A1.con. This suggested that the 13D5 epitope was not present on subtype A Env glycoproteins. Among all cloned mAbs, 3B3 and 16H3 showed the strongest ELISA responses to all proteins tested (Table 1).

Determination of breadth of mAb reactivity by Western blot analysis

To further confirm the cross-reactivity of these mAbs, a panel of wild type Env proteins from more Env subtypes was used for Western blot analysis. Ten mAbs that reacted with multiple

subtype Env proteins in ELISA assays were tested against 14 Env proteins from subtype A (92UG037 and 92RW020), subtype B (JRFL, IIIB and SF162), subtype C (96ZM651, DU123 and CN5), subtype D (92UG021), CRF01_AE (93TH975 and CM235), subtype F (93BR029), the group M consensus (CON6) and SIVcpzUS (Figure 1). For direct comparison, equal amounts of Env protein (100 ng) were loaded in each gel. As observed in the ELISA analysis, both 3B3 and 16H3 mAbs showed the strongest response to all wild-type, group M consensus and SIVcpzUS Env glycoproteins. The intensities of Env bands for subtype A, subtype F, group M consensus and SIVcpzUS were stronger than those of other Env proteins. 13D7 also recognized all Env proteins, but reacted only weakly with 6 of the 14 Env proteins tested. 13D5 showed good cross-reactivity but did not react with subtype A and F Env proteins. This finding was consistent with the ELISA results and suggested that 13D5 epitope was not present in subtype A and F envelope glycoproteins.

Five other mouse mAbs (17G12, 18A7, 18F11, 8H3 and 19C12) reacted either weakly with all Env proteins or recognized only a subset in the panel, again consistent with the ELISA results. 15E9 mAb was reactive to all Env proteins in ELISA, but only recognized A_92RW020 in Western blot analysis, suggesting 15E9 react predominantly with a conformational determinant that was not preserved in most Env proteins in their denatured state. Similar results were observed with mAbs 2G12 and 1b12 which recognize conformation dependent binding sites (Fig. 1B). Both 2F5 and 4E10 recognize linear epitopes near the membrane-proximal external region, but they only reacted with a few gp140 Env proteins in the panel due to the amino acid substitutions in the epitopes (Fig. 1B and 1C). Interestingly, six mAbs (3B3, 16H3, 13D5, 13D7, 17G12 and 13D5) strongly reacted with the highly divergent SIVcpzUS Env protein. These data indicate conservation of some epitopes following cross-species transmission from chimpanzees to man (Gao et al., 1999;Hahn et al., 2000). However, 3B3 and 16H3 mAbs did not react with SIVmac251 gp120 or SIVmac259 gp130 proteins (not shown).

Characterization of binding kinetics of mAbs to HIV-1 Env gp140

To determine mAb binding kinetics and avidity, each mAb was characterized by surface plasmon resonance (SPR) with three distinct oligomeric gp140 proteins: a wild type subtype B (JRFL), a group M consensus (CON-S) and a subtype B consensus (B.con). Marked differences in binding kinetics were observed (Figure 2). Based on the relative binding kinetics and Kd (affinity) values, the mAbs could be classified into three groups: i), moderate affinity with fast binding kinetics (fast on and off-rates); ii) high affinity with medium binding kinetics (fast on- and slow off-rates); and (iii) low affinity with slow binding kinetics (slow on- and off-rates) (Table 2). Representatives of each group are shown in Figure 2. The first group of mAbs (17G12 and 8H3) displayed fast binding kinetics (on- and off-rates, ka and kd) and bound more strongly to CON-S (Kd = 105 and 710 nM, respectively). While both 8H3 and 17G12 also bound to B.con with μM Kd, only 8H3 bound to B_JRFL (K_d = 3.2 μM).

Among the mAbs with slow binding dissociation rate constants (off-rates, kd), the highest binding affinity (lower K_d) was measured for 3B3, 16H3, and 15E9. MAbs in this group were high-affinity binders with slow k_d values. 3B3, 16H3 and 15E9 bound Env proteins with much slower (about 10-fold when compared to 8H3 and 17G12) k_d and with the resulting K_d values ranging from 72-384 nM (Table 2 and Fig. 2). More breadth in reactivity was observed with 3B3 and 16H3, both of which bound to all three Env proteins strongly. 15E9 was more selective and bound strongly to CON-S and B.con (K_d = 384 nM and 317 nM, respectively) while binding to B_JRFL gp140 was too weak to be measured.

The third group of mAbs included those that bound weakly to Env proteins with K_d in μM range. The weaker binding Kd of these mAbs was largely due to slower on-rates (Table 2 and Fig. 2). For the majority of mAbs in this group, binding was preferentially observed with CON-

S and B.con. However, 13D5 and 18F11 also bound to JRFL (K_d = 3.5 μM and 10.9 μM, respectively). Binding of 18F11 to all three Env proteins was relatively weaker (K_d= 21.8 μM, 10.9 μM and 11.7 μM to CON-S, B_JRFL and B.con, respectively).

Overall, this panel of mAbs bound better with lower binding K_d to CON-S and B.con when compared to JRFL (Fig. 2 and Table 2). Among all mAbs, 3B3 bound to Env proteins with higher affinity (Kd = 72 nM, 275 nM and 238 nM for CON-S, JRFL and B.con, respectively). Ability of mAbs to bind B_JRFL Env was restricted and the highest affinity binding was observed with 16H3 and 3B3. Thus among three Env proteins, both consensus Env proteins (CON-S and B.con) showed broad reactivity to all tested mAbs, while JRFL was relatively restricted to only 5 out of 10 mAbs.

Detection of cell surface Env proteins

To determine whether mAbs 3B3, 16H3 and 13D5 could bind Env proteins on the cell surface, we transfected 293T cells with subtype A1 and C consensus, B_MN (subtype B), and two clinical isolate (subtype B) *env* genes (B_15313, B_17779). 293T cells were treated 48 hrs after transfection and stained with PE-conjugated cross-reactive mAbs (3B3, 16H3 and13D5) and one positive control mAb (2G12). Compared to mock transfected cells, positive reactivity was observed in 16H3 and 3B3 mAb treated cells, each expressing one of five different *env* genes (Fig. 3). When labeled with 13D5 mAb, cells expressing three subtype B Env and a subtype C consensus Env were reactive compared to mock transfected cells, but as in the ELISA and Western blot analyses, 13D5 did not recognize the subtype A consensus Env (Table 1 and Fig. 1). Cells expressing A1.con, B.con and B_17779 Env proteins also reacted with the glycan specific mAb, 2G12. Although the expression levels of Env proteins were not normalized, our results clearly showed that both 3B3 and 16H3 were reactive with Env proteins on cells transfected with the subtype A, B or C *env* genes. When tested with H9 cells infected with B_MN, none of the three mAbs bound Env on the surface of HIV-1 infected T cells, while the control 2G12 bound well (Fig. 3).

Ability of mAbs to neutralize HIV-1

To determine if mAbs in the cross-reactive panel could neutralize primary HIV-1 strains, all mAbs (3B3, 16H3, 13D7, 13D5, 17G12, 18A7, 18F11, 8H3, 19C12 and 15E9), select other mAbs (2B9, 7B9, 7G9, 15B10 and 15D6), and two control mAbs (2F5 and 4E10) were tested in single round pseudovirus neutralization (TZM-bl) assays (Wei et al., 2002). A total of 15 purified mAbs were assayed against a panel of 11 tier 1 and tier 2 subtype B and C pseudoviruses (Li et al., 2005; Li et al., 2006a). None of the cross-reactive mAbs neutralized any pseudoviruses at 50 μg/ml, suggesting that the conserved epitopes that they targeted were not accessible on HIV-1 functional Env proteins.

We then determined whether these mAbs could bind free virus particles in an ELISA based capture assay. Of 13 tested mAbs, only a previously described human anti-gp41 immunodominant region mAb 7B2 (Binley et al., 2000) was able to capture SF162 or BG1168 pseudovirus particles (data not shown). Three previously characterized control mAbs (17b, F39F and 2G12) were also able to capture virions, and the capture was significantly increased when soluble CD4 was added. Taken together, these data suggested that mouse mAbs to the most cross-reactive HIV-1 Env epitopes did not efficiently bind to native envelope spikes on virions.

Epitope mapping of mAbs

All mAbs in Table 1 were tested against a panel of peptides from the MPER region, variable loops, and the conserved gp41 immunodominant region. All mAbs were non-reactive with all

peptides studied. In competitive inhibition assays, none of the mAbs blocked the binding of mAbs 2F5, 2G12, or 1b12 to B_JRFL Env oligomer (data not shown).

Discussion

Well-characterized mAbs can play an important role in understanding of the structure, biology and neutralization epitopes of HIV-1 glycoproteins (Binley et al., 2004; Lin and Nara, 2007; Moore et al., 1994; Wyatt et al., 1998). In this study, we identified two broadly reactive mAbs (3B3 and 16H3) from mice immunized with HIV-1 Env proteins. They were both cross-reactive at a high affinity to all tested HIV-1 Env proteins from five subtypes (A, B, C, D and F), one CRF01_AE and one highly divergent SIVcpzUS - thus, demonstrating these epitopes were conserved among HIV-1 subtypes as well as SIVcpz (Gao et al., 1999; Hahn et al., 2000).

A number of mouse mAbs have been obtained from HIV-1 Env immunized mice (Abacioglu et al., 1994; Derby et al., 2007; Reeves et al., 1995; Sugiura et al., 1999). Although many of those were found to target conserved regions (C1, C2 and C3) or the CD4 binding site in gp120, cross-clade reactivities were not evaluated. Some were able to weakly neutralize sensitive tier 1 HIV-1 strains (Derby et al., 2007; Sugiura et al., 1999). Mabs 2F5, 4E10, 2G12, IgG1b12 (neutralizing) and A32 (non-neutralizing) are broadly reactive gp120 human mAbs that have been characterized. Since mAbs 2G12, 1b12, and A32 bind to highly conformation-sensitive epitopes, they decrease or lose their ability to bind gp120 under denaturing conditions. In contrast, both 3B3 and 16H3 mAbs can bind gp120 from subtypes B, C and CRF01 as well as gp140 from other subtypes under denaturing conditions by Western blot analysis, indicating that they likely target linear epitopes. It is striking that antibodies that were so broadly reactive and conserved among HIV-1 subtypes and SIVcpz, did not bind to Env on virions nor neutralize HIV-1.

The recent identification of antibodies from HIV-1 infected subjects that do not bind to recombinant envelope yet broadly neutralize HIV-1 strains demonstrate that native Env expresses epitopes not on HIV-1 recombinant oligomers (Chan-Hui et al., 2009). A strain specific human mAb 2909 was also found to unable to react with gp120 monomeric or gp140 oligomeric forms of SF162 Env glycoptotein, but bind to virions and potently neutralized SF162 (Gorny et al., 2005). Theses studies suggest that some mAb target quaternary structures present only on the surfaces of intact virions. Data presented here, our work (Liao et al., 2006) and other's work (Beddows et al., 2007; Chakrabarti et al., 2002; Crooks et al., 2007; Kwong et al., 1998; Li et al., 2006b; Lin and Nara, 2007; Srivastava et al., 2003; Tobin et al., 2008; Wyatt et al., 1998) in animals, as well as immunizations in man (Gilbert et al., 2005; Lee et al., 2001) all demonstrate that recombinant Envs though antigenic and binding broadly neutralizing antibodies are, as yet, do not induce sufficiently broad-reactive neutralizing antibodies in standard TZMbL cell neutralization assays. Moreover, that these specificities of antibodies induced in mice do not capture virions demonstrates they will not block virions traversing mucosal surfaces. Our mAbs do bind Env expressed on the surface of transfected T cells, but do not bind Env on the surface of HIV-1 infected T cells, implying that these antibodies will also not mediate ADCC or other binding anti-HIV-1 activities. It is not known precisely how modification of the cleavage site and fusion domain alters the recombinant Envs leading to the induction of the antibodies in this study. Nonetheless, all in the field would agree that these recombinant Envs are not native. However, the 92RW020, JRFL, 97ZA012, CONS and CON6 Envs in this study have been previously characterized extensively for their ability to bind sCD4, and undergo CD4 Induced conformational changes to expose the chemokine receptor binding site, and thus shared antigenic similarity to native Envs to the point of being able to bind some ligands to which native Envs bind (Liao et al., 2006). DU123, 89.6, A,Con, B.Con, C.Con and US-1 SIVcpz Envs have been similarly characterized (Alam, M. and Haynes, B. unpublished).

In summary, it is well known that antigenic regions are present on HIV-1 Env and conserved on SIVcpz Env, yet these regions, although immunogenic, are not exposed on the surface of the native Env trimers. Nevertheless, mAbs raised against these regions can be used as reagents to purify and characterize expressed Env proteins used in research. Thus, combinations of these Env mAbs may be able to be used to identify, qualify and purify soluble Env in plasma and mucosal fluids. Finally, the ability of these antibodies to bind to recombinant HIV-1 Envs with high affinity suggest they may be of use in co-crystallization studies of soluble Env trimers.

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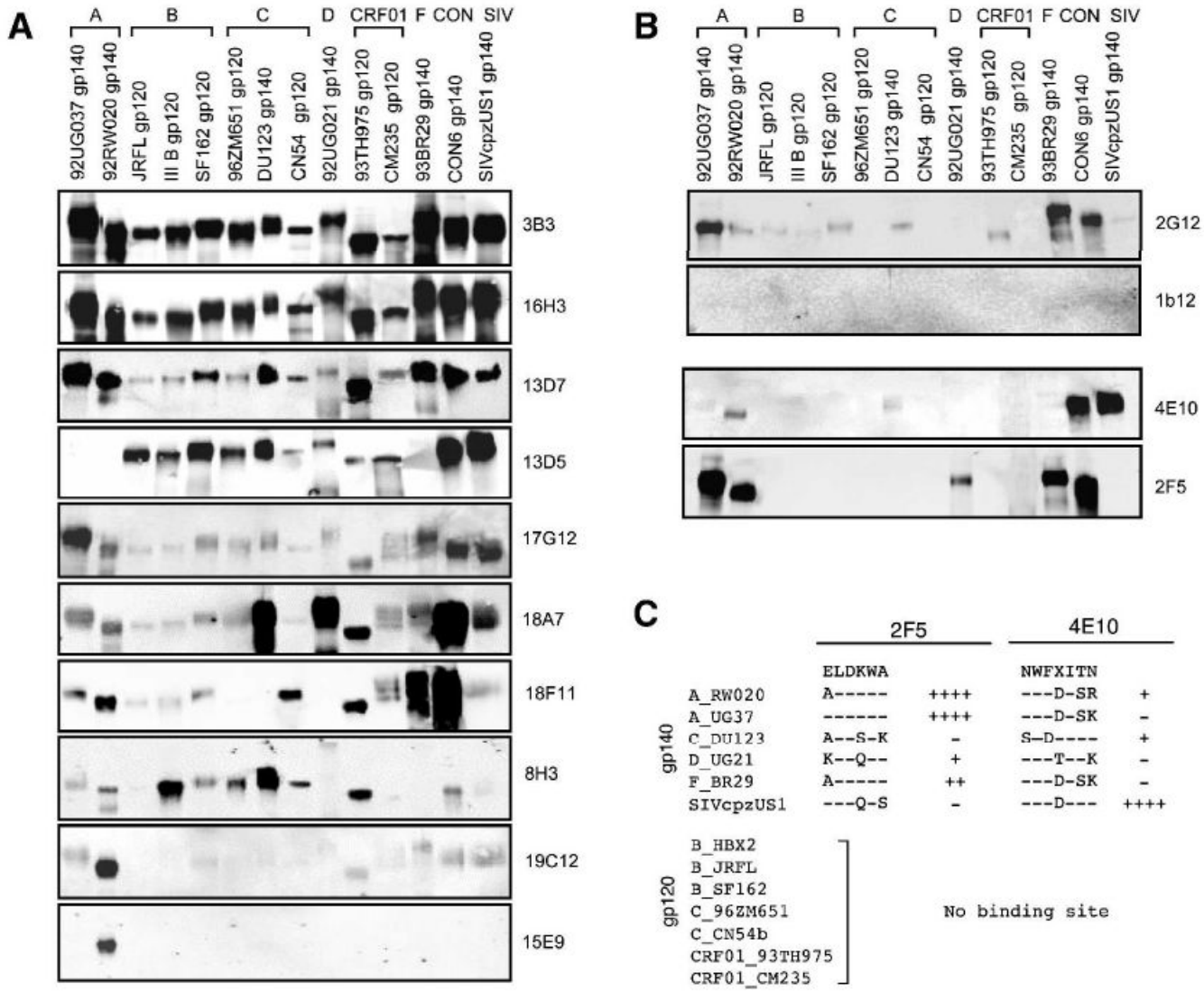

Figure 1.

Cross clade reactivity of mAbs to a panel of divers HIV-1 envelope proteins. HIV-1 Env proteins (100 ng/each) from five subtypes (A, B, C, D and F), a circulating recombinant (CRF01_AE), a group M consensus (CON), and an SIVcpzUS1 (SIV) was tested with newly characterized mAbs (A) and broadly neutralizing mAbs (B). The names of mAbs are shown at the right of the blots. The Env proteins were separated on a reducing 10% SDS-polyacrylamide gel, blotted onto a nitrocellulose membrane, reacted to mAbs (1μg/ml), and probed with fluorescent labeled secondary antibodies (1μg/ml). The 2F5 and 4E10 epitopes in gp140 and their reactivity to mAbs are shown (C).

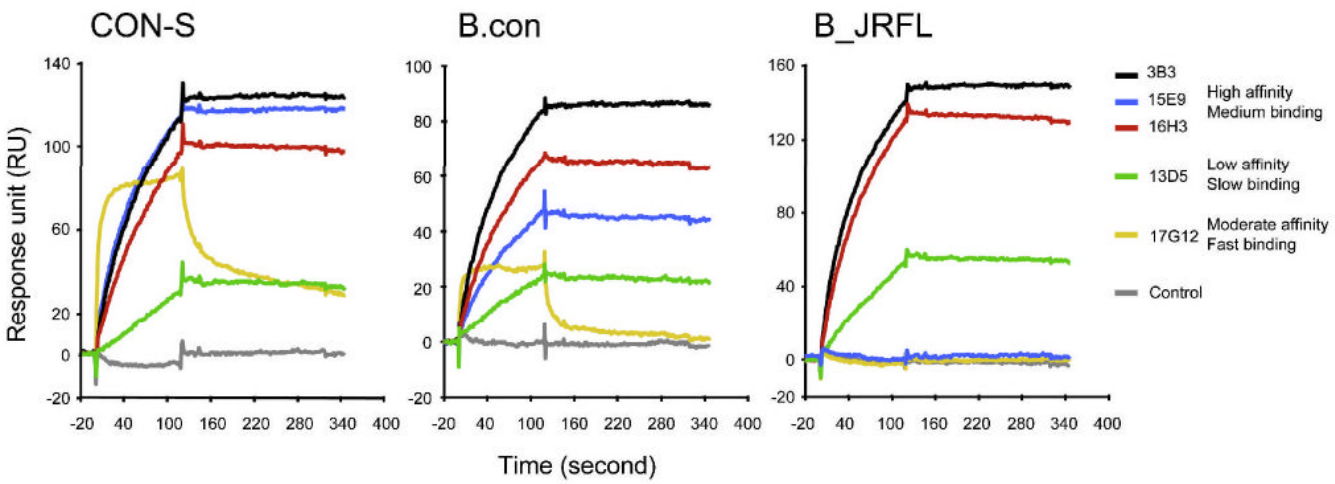

Figure 2.

Binding kinetics analysis of mAbs to HIV-1 Env proteins by Surface Plasmon Resonance. HIV-1 Env gp140 proteins were immobilized to about 1000 RU using amine coupling chemistry. Positive control V3 mAb F39F was used to assess activity of gp140 proteins following immobilization. Monoclonal Abs were injected at 50 μg/mL for 2 min at 30 ul/min. For binding kinetics analyses, mAbs were injected at varying concentrations ranging from 1.2 mg/mL to 1.5 μg/ml. Due to the bivalency of the mAbs used, binding rate constants and Kd values were derived from global curve fitting using the "Bivalent Analyte" model and binding K_d was calculated from faster rate constants (ka and kd).

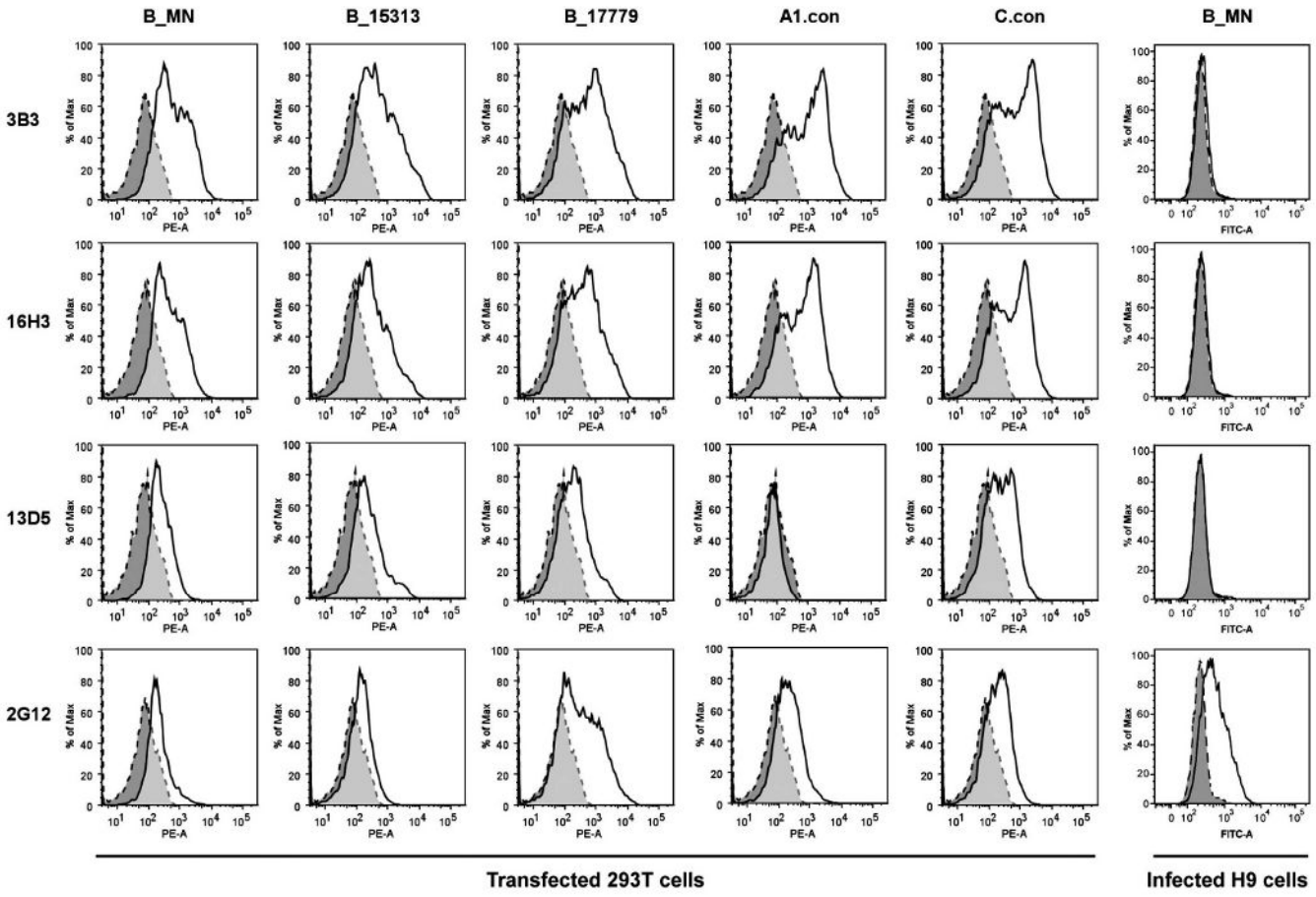

Figure 3.

Detection of Env proteins on cell membrane by flow cytometry. The first five columns show transfected 293T cells labeled with PE-conjugated mAbs to HIV-1 envelope (16H3, 3B3, 13D5, and 2G12). White histograms correspond to expression on transfected 293T cells (A1.con, B.con, B_MN, B_15313 and B_17779). Grey shaded histogram represents the empty vector (control) transfected cells. The rightmost column show B_MN infected H9 cells labeled with unconjugated mAbs and detected with FITC-conjugated secondary antibody. White histograms represent binding to the infected cells, while grey shaded histogram represents binding of the secondary antibody in the absence of primary antibody. All plots are representative of triplicate samples.

Table 1

Cross reactivity of mouse monoclonal antibodies to multiple subtype envelope glycoproteins

Immunogen	mAb	Subtype A	Subtype B			Subtype C			Subtype concensus			Group M concensus	SIVcpz
		92RW020	HXB2/Bal	89.6	JRFL	97ZA012	DU123	95ZM651	A1.con	B.con	C.con	CON-S	US-1
C_97ZA012	18A7	-	-	-	+	+	+	-	-	-	-	+	-
	19C12	+	-	-	+	++	-	-	-	+	-	+	-
	15E9	++	+	+	++	++++	+	+	+	+	+	++	+
	16H3	+++	++++	++++	+++	++++	+++	+++	+++	++++	+++	+++	+++
	17G12	+	+	+	+	+++	+	-	+	+	+	+	+
	18F11	-	-	-	+	+	-	-	-	-	+	+	-
	3B3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8H3	+++	-	++	++	++++	++++	++++	+	++	++++	+++	-
CON6	13D5	-	++++	++++	++++	+++	+++	++++	-	+++	+++	+++	++++
	13D7	++	+	++	+	+	+	+	++	+	+	+++	+

Results were scored as following OD values: -, between 0.0 and <0.25 and < 3 fold of background; +, between 0.25 and < 1; ++, between 1 and < 1.5; +++, between 1.5 and < 2; ++++, >2

Table 2

Surface plasmon resonance binding kinetics of mAbs to oligomeric HIV-1 Env gp140 proteins

Group	mAb	JRFL			CON-S			B.con		
		k_a $M^{-1}s^{-1}$ ($\times 10^3$)	k_d s^{-1} ($\times 10^{-2}$)	K_d M ($\times 10^{-9}$)	k_a $M^{-1}s^{-1}$ ($\times 10^3$)	k_d s^{-1} ($\times 10^{-2}$)	K_d M ($\times 10^{-9}$)	k_a $M^{-1}s^{-1}$ ($\times 10^3$)	k_d s^{-1} ($\times 10^{-2}$)	K_d M ($\times 10^{-9}$)
Moderate affinity fast	17G12	-	-	NM	95	1	105	16.4	5.4	3,305
binding	8H3	20	6.4	3,200	14	1.2	710	10	3	2,600
High affinity medium	3B3	28	0.77	275	24	0.16	72	26	0.63	238
binding	16H3	15	0.19	129	11	0.29	260	11.2	0.29	256
	15E9	-	-	NM	7.4	0.28	384	9.2	0.29	317
Low affinity slow	19C12	-	-	NM	1	2.4	2,450	2	1.4	7,000
binding	13D5	1.3	4.5	3,460	0.8	1.9	2,310	0.8	7	8,750
	18A7	-	-	NM	1.2	4.4	3,550	-	-	NM
	13D7	-	-	NM	5.6	3.2	5,754	6.6	4	6,050
	18F11	0.68	0.74	10,870	0.8	1.8	21,770	0.56	6.5	11,650
Positive control	F39F	6.3	0.002	380	13	0.15	115	6.5	0.045	69.2
Negative control	7G9*	-	-	-	-	-	-	-	-	-

NM: binding too weak to be measured;

*7G9 mAb did not bind to Env gp140