HIV-1 Consensus Envelope-Induced Broadly Binding Antibodies

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Abstract

Antibodies that cross-react with multiple HIV-1 envelopes (Envs) are useful reagents for characterizing Env proteins and for soluble Env capture and purification assays. We previously reported 10 murine monoclonal antibodies induced by group M consensus Env, CON-6 immunization. Each demonstrated broad cross-reactivity to recombinant Envs. Here we characterized the Env epitopes to which they bind. Seven mapped to linear epitopes in gp120, five at the Env N-terminus, and two at the Env C-terminus. One antibody, 13D7, bound at the gp120 N-terminus (aa 30–42), reacted with HIV-1-infected CD4⁺ T cells, and when expressed in a human IgG1 backbone, mediated antibody-dependent cellular cytotoxicity. Antibody 18F11 bound at the gp120 C-terminus (aa 445–459) and reactivity was glycan dependent. Antibodies 13D7, 3B3, and 16H3 bound to 100 percent of HIV-1 Envs tested in ELISA and sodium dodecyl sulfate/polyacrylamide gel electrophoresis/western blot analysis. These data define the epitopes of monoclonal antibody reagents for characterization of recombinant Envs, one epitope of which is also expressed on the surface of HIV-1-infected CD4⁺ T cells.

Keywords: antibody-mediated immunity, HIV, monoclonal, vaccine design, ADCC

Introduction

RECOMBINANT HIV-1 ENVELOPES (ENVS) are currently used for serologic assays in HIV-1 infection and vaccination, and for immunizations with experimental HIV-1 vaccines.^{1–8} A number of antibodies have been found that bind to Env glycans on the surface of virus-infected cells and virions and neutralize HIV-1.^{9–22} Antibodies that can decorate the surface of the native Env on infectious virions have the potential to neutralize HIV-1.²³ Env antibodies may either bind to virions and be neutralizing,²⁴ or in the case of virus-infected cells, bind to infected cells and may have the capacity to mediate antibody-dependent cellular cytotoxicity (ADCC).^{25–27}

In this study, we have characterized murine antibodies induced by a group M consensus envelope gp140, CON-6,²⁸ three of which bind to all of 100% of recombinant Envs tested in sodium dodecyl sulfate/polyacrylamide gel electrophore-

sis (SDS-PAGE) and ELISA, and are important QC reagents for producing recombinant Envs. One mAb, 13D7, was found to be non-neutralizing, yet bound to the surface of tier 2 (difficult to neutralize) virus-infected cells and mediated ADCC.

Materials and Methods

Recombinant proteins

HIV-1 Envelopes were expressed in 293T cells and lectin column purified, with a subsequent purification step for monomeric Envs by size-exclusion chromatography SEC on a Superdex 200 FPLC (GE Healthcare) and purified to homogeneity for monomeric gp120. Envelopes denoted by Δ11 contained an 11 aa N-terminal deletion. Natively deglycosylated JR-FL gp140 Envelope was produced using PNGaseF as previously described.²⁹

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Immunization of mice and generation of hybridoma cell lines

BALB/c mice were immunized with 25 μ g 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, TX) as previously described.²⁸ Splenocytes were harvested 3 days following the fourth immunization, and hybridoma fusions were performed using HAT (hypoxanthine, aminopterin, thymidine)-sensitive mouse myeloma cells, P3X63 Ag8 or NS-1, as previously described.³⁰ Hybridomas secreting HIV-1 Env-specific antibodies after 14 days of culture were identified by ELISA with autologous Env proteins. Positive cell lines were expanded in large-scale culture. Monoclonal antibodies were then purified from cell culture supernatants for further analysis.

Epitope mapping

Epitope mapping was detected via ELISA, utilizing a peptide array of 15-mer peptides overlapping by 11 amino acids derived from HIV-1 gp140 Con6 Env. Antibodies were diluted to 10 μ g/ml. Binding was detected by goat anti-mouse conjugated to alkaline phosphatase and substrate. Binding was considered positive when the optical density (OD) at 405 nm was threefold over background.

Competition assays

Competition and cross-competition of purified mAb binding to HIV-1 Env were carried out via ELISA. For cross-competition assays, purified 13D5, 16H3, and 3B3 were titrated against plate-bound HIV-1 ConS gp140 and incubated at 37°C for 1 hour and washed, before addition of limiting dilutions of biotinylated 13D5, 16H3, and 3B3. Binding was detected by streptavidin-conjugated horseradish peroxidase and substrate, and OD was measured at 450 nm. Percent inhibition was measured as reduction in OD compared to uninhibited antibody after subtracting background. To measure inhibition of A32 binding, mouse mAbs were titrated against the target HIV-1 Env before addition of biotinylated mAb A32 at a limiting concentration. Binding of biotinylated A32 was detected with streptavidin-conjugated horseradish peroxidase (HRP) and substrate. Percent inhibition was calculated as described above.

Polyreactivity of mouse monoclonal antibodies

Indirect immunofluorescence binding of mouse mAbs to HEp-2 cells (Inverness Medical Professional Diagnostics, Princeton, NJ) was performed as described previously.³¹ Images were taken on an Olympus AX70 with SpotFlex FX1520 charge-coupled device with a UPlanFL 40 $\times$ (numerical aperture, 0.75) objective at 25°C in the FITC channel using SPOT software. All images were acquired for the time according to the figure legend. Image layout and scaling were performed in Adobe Photoshop without image manipulation.

Prediction of N-linked glycosylation sites

To predict N-linked glycosylation sites, HIV-1 Env amino acid sequences were submitted to the N-GlycoSite server at the Los Alamos National Laboratory HIV Database.

SDS-PAGE and western blot

A panel of 66 unique HIV-1 gp120 or gp140 Env recombinant proteins from six clades (A, B, C, AE, BC, M) were used to determine cross-reactivity of mAbs. Env proteins (500 ng) were separated on a reducing 4%–12% gradient Bis-Tris SDS-PAGE gel (Invitrogen, Carlsbad, CA) and blotted on to a nitrocellulose membrane using the iBlot dry blot system (Life Technologies, Grand Island, NY). Membranes were blocked overnight in phosphate-buffered saline (PBS) containing 1% casein at 4°C. Following blocking, membranes were washed, probed with 1 μ g/ml of mAb, and allowed to incubate at room temperature for 2 h. Membranes were again washed and then probed with goat-anti mouse IgG-AP antibody (5 μ g/ml) in blocking buffer. Membranes were washed thrice with PBS containing Tween-20 (0.05%), and binding of mAbs detected using Novex AP Chromogenic Substrate (Life Technologies, Grand Island, NY). Images were acquired on an Odyssey Infrared Imager.

Cell surface staining of HIV-infected CD4 T cells

Binding to HIV-1 subtype B IMC SF162 (accession number EU123924)-infected primary CD4⁺ target cells was determined by indirect surface immunofluorescence analysis as described.²⁶

Construction of humanized 13D7

V(D)J sequences for Con6 13D7 CL3-1 hybridoma were obtained as described³² with modifications. Briefly, total RNA was extracted from the hybridoma using TRIzol reagents (Invitrogen). cDNA was synthesized from DNase I-treated RNA using Superscript III with oligo (dT)₂₀ primers. One-twentieth (volume) of the cDNA was then subjected to polymerase chain reaction (PCR) using Herculase II Fusion DNA polymerase (Agilent Technologies) with established primers.^{33,34} PCR: 95°C for 4 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 45 s, and then 72°C for 10 min. V(D)J amplicands were gel purified, ligated into vectors, and transformed into bacteria. DNA sequences were obtained at the Duke DNA sequencing facility. The rearranged V, D, and J gene segments were identified using IMGT/V-QUEST (www.imgt.org/). We confirmed that V(D)J usage of Con6 13D7 CL3-1 hybridoma (IGHV5-6 or IGHV5-6-1/IGHD1-2/IGHJ3, IGKV1-117/IGKJ1) was different from that of fusion partner, P3X63 (IGHV5-17/IGHD5-7/IGHJ4, IGKV6-20/IGKJ2).

Antibody-dependent cellular cytotoxicity

ADCC activity was determined by a luciferase-based assay using HIV-1 IMC-infected cell targets as previously reported.²⁷ CEM.NKRCR5 cells were infected with IMC of HIV-1 encoding the subtype A Q23.17 (accession number AF004885), subtype AE CM235 (AF259954) and CM244 (KC822429), subtype B BaL (AY426110) and WITO (JN944948), and subtype C MW96.5 (U08455), 1086.c (FJ444395), TV-1 (HM215437), DU151 (DQ411851), DU422 (DQ411854), and CH505 (KC247577) env genes within an NL4-3 backbone that also expresses the Renilla luciferase reporter gene.³⁵ ADCC activity was determined as the change in the relative luciferase unit (RLU) resulting from the loss of intact targets in test wells (containing effector

the positive cutoff at $\geq 15\%$ specific killing. ADCC activities are reported as the endpoint concentration, defined as the mAb concentration that intersects the positive cutoff of 15% specific killing.

Neutralizing antibody assays in TZM-bl cells were performed as described previously.³⁶ Antibodies tested were

started at concentrations of 50 µg/ml and diluted serially using threefold dilutions for a total of eight concentrations tested. Diluted antibodies were added to Env-pseudotyped viruses grown in 293T cells at a predetermined titer and incubated for 1 h. TZM-bl cells were added and allowed to incubate for 48 h. Firefly luciferase (Luc) activity was quantified using RLU using the Britelite Luminescence Reporter Gene Assay System as described by the supplier (PerkinElmer Life Sciences, Waltham, MA). Percent neutralization was calculated as a reduction in RLU in test wells compared with control wells following subtraction of background RLU in control wells. Results are reported.

Results

Consensus HIV-1 Env gp140 induced antibodies with cross-clade binding reactivity to conserved linear epitopes

We previously isolated a panel of 10 cross-reactive monoclonal antibodies from mice immunized with oligomeric, lectin column-purified consensus HIV-1 gp140 Env produced in 293T cells.²⁸ To identify mAb epitopes, we generated 15-mer overlapping peptides derived from the HIV-1 Con6 gp140 Env, and tested each of these mAbs for peptide binding in ELISA. Seven mAbs, 13D7, 8H3, 13D5, 3B3, 16H3, 18A7, and 18F11, bound to linear epitopes in the peptide array (Fig. 1a, Supplementary Fig. S1, and Supplementary Table S1;

Supplementary Data are available online at www.liebertpub.com/aid). Five of which (13D7, 8H3, 13D5, 3B3, 16H3) bound to the first constant (C1) region and two (18A7, 18F11) bound to the C4 region (Supplementary Fig. S1). MAbs 13D5, 3B3, and 16H3 bound overlapping epitopes (Supplementary Fig. S1 and Supplementary Table S1). This was confirmed with cross-competitive inhibition assays demonstrating that these antibodies block binding of each other to HIV-1 gp140 Env in a dose-dependent manner (Supplementary Fig. S2). Conservation of epitopes bound by these antibodies is shown as sequence logos generated using WebLogo3 (Fig. 1a).³⁷ In addition, epitopes bound by these mAbs were mapped on the crystal structure of the HIV-1 HXBc2 gp120 core monomer³⁸ (Fig. 1b). MAbs 13D7, 8H3, 13D5, 3B3, and 16H3 bound to epitopes located within gp120 Env inner domain, while 18A7 and 18F11 bound to outer domain epitopes. Entropy of sequence variability observed within HIV-1 gp120 Env quaspecies was calculated and represented on the HIV HXBc2 gp120 core as a heat map (Fig. 1b). Each of these mAbs bound to regions of low viral sequence entropy (Fig. 1b). When mapped to the BG505 SOSIP gp140 trimer,³⁹ mAbs 13D5, 3B3, and 16H3 bound to epitopes located within the prefusion trimer core and were not accessible on the trimer surface (Fig. 1b).

Binding breadth was assessed via ELISA and reducing SDS-PAGE/western blot against a multiclade panel of 66 recombinant HIV gp120 and 32 gp140 Envs (Fig. 1C, Supplementary Table S2B–H), demonstrating binding breadth to

TABLE 1. SUMMARY OF MAb BINDING BREADTH TO HIV-1 ENV BY ELISA

<i>HIV-1 gp140 Env binding</i>							
HIV-1 mAb	Clade A (6 Env)	Clade B (10 Env)	Clade C (11 Env)	CRF_01 AE (4 Env)	CRF_0X BC (0 Env)	Group M consensus (1 Env)	Total (32 Env)
13D7 ^a (%)	6 (100)	10 (100)	11 (100)	4 (100)	NT	1 (100)	32 (100)
<i>HIV-1 gp120 or gp140 Env binding</i>							
HIV-1 mAb	Clade A (12 Env)	Clade B (19 Env)	Clade C (17 Env)	CRF_01 AE (13 Env)	CRF_0X BC (4 Env)	Group M Consensus (1 Env)	Total (66 Env)
8H3 (%)	11 (92)	19 (100)	17 (100)	13 (100)	4 (100)	1 (100)	65 (98)
13D5 (%)	5 (42)	19 (100)	16 (94)	13 (100)	4 (100)	1 (100)	58 (87)
3B3 (%)	12 (100)	19 (100)	17 (100)	13 (100)	4 (100)	1 (100)	66 (100)
16H3 (%)	12 (100)	19 (100)	17 (100)	13 (100)	4 (100)	1 (100)	66 (100)
18A7 (%)	1 (8)	0 (0)	4 (36)	11 (86)	0 (0)	1 (100)	14 (26)
18F11 (%)	10 (83)	16 (84)	10 (59)	11 (86)	3 (75)	1 (100)	51 (77)

Cross-reactivity of linear epitope-binding mAbs against a panel of HIV-1 Envs as determined by ELISA. The number of Envs tested versus those bound are listed, and cross-reactivity is expressed as percentage of HIV-1 Envs bound (in parentheses). See also Supplementary Table S2a–h.

^aRecombinant HIV-1 gp120 Env with N-terminal 11 amino deletion will not bind 13D7 due to deletion of epitope. Therefore these were not tested for binding to 13D7 mAb.

Binding breadth (%)

90–100
75–89
50–74
25–49
<25

Env, envelope.

range from 26% to 100% of HIV-1 Envs tested (Table 1). Of note, mAbs 13D7, 3B3, and 16H3 bound 100% of Envs tested.

Consensus HIV-1 Env immunization induced a non-neutralizing glycan-dependent antibody

HIV-1 gp140 envelope is extensively glycosylated⁴⁰ and is an important mechanism in masking antibody accessibility to the envelope trimer.⁴¹ However, several glycan-dependent broadly neutralizing antibodies that arose through natural infection have been described.^{9,12,14,20,42–46} Since recognition of Env glycans may, in part, confer cross-clade reactivity of HIV-1-specific antibodies, we sought to determine if any of these antibodies can recognize HIV-1 Env glycans.

We natively deglycosylated JRFL gp140 Env using PNGaseF as previously described.²⁹ Antibodies were then tested for binding via ELISA. We found that native deglycosylation of JRFL enhanced mAb 8H3 binding and decreased 18F11 binding (Fig. 2a). The binding of 13D7, 13D5, 3B3, 16H3, and 18A7 mAb remained unchanged with native PNGaseF treatment (data not shown). The epitopes bound by 8H3 and 18F11 mAbs contain predicted N-linked glycosylation sites at aa 97 and 448, respectively, relative to the reference HIV-1 HXB2 sequence. 18F11 mAb was tested for binding to a panel of Clade C HIV Envs, of which 9 Envs lacked the glycan site at position 448 within the 18F11 epitope due to naturally occurring mutations. 18F11 failed to bind to these Envs (Fig. 2b, Supplementary Table 2I). To directly define glycan reactivity of 18F11, we used biolayer interferometry to test binding of this mAb to chemically synthesized high-mannose glycans Man₉, Man₅, and Man₃, each of which is found on HIV-1 Env.⁴⁷ 18F11 mAb did not bind to free glycan (data not shown).

Cross-reactive antibodies induced by Con6 gp120 immunization are polyreactive

It has been shown that acute and chronic HIV-1 infection induces polyreactive antibodies that also bind HIV-1 envelope.^{48–51} Similarly, polyreactive anti-HIV gp120 antibodies can be induced through vaccination with gp120 protein, as observed in the GSK PRO-HIV-002 HIV-1 vaccine trial that used a Clade B w61d gp120 Env w61d as an immunogen.³¹ To determine if any of the antibodies described in this article were polyreactive, we used an indirect immunofluorescent assay where antibodies were incubated with human epithelial (HEp-2) cells and binding was detected with an FITC-conjugated secondary antibody (Supplementary Fig. S3). We observed binding to HEp-2 cells with antibodies 18A7, 8H3, 13D5, 13D7, 16H3, and 3B3 (Fig. 3). Although weakly positive, 18A7 showed a diffuse speckled pattern. 8H3 showed a cytoplasmic speckled pattern, while 13D7 brightly reacted with dividing cells. For mAbs 3B3 and 16H3, a similar staining pattern that resembles centrosomes is seen.

Env cross-reactive mAbs are non-neutralizing

It is known that antibodies that decorate either glycans or protein sequences on the native trimer on the HIV-1 virion are capable of neutralization. None of the seven mAbs that bound linear epitopes were capable of neutralization against either tier 1 or tier 2 HIV-1 Env pseudoviruses (Table 2-A), consistent with previous observations.²⁸ Since binding of

mAb 18F11 was sensitive to loss of the N448 glycan, we determined if treatment of virions with kifunensine could enable viruses to be sensitive to neutralization by mAb 18F11. Kifunensine is an endoplasmic reticulum mannosidase I inhibitor that, when used to treat cells producing HIV-1 virions, will promote the density of N-linked high-mannose

FIG. 2. 18F11 binds HIV-1 Env in a glycan-dependent manner. Mabs 8H3 and 18F11 have a predicted N-linked glycosylation site (N*S/T; *any amino acid) at position X and Y, respectively. (A) Mabs 8H3 and 18F11 were tested for binding to JRFL gp140 before and after native enzymatic deglycosylation by PNGaseF. (B) Mab 18F11 was tested for binding to HIV-1 Envelopes with naturally occurring mutations that delete the predicted N-linked glycan site at NXX. Binding of mAb 18F11 depended on the presence of this N-linked glycan. See also Supplementary Table S2c,h.

FIG. 3. A32 binding is competitively inhibited by C1 antibodies. C1-specific antibodies 8H3, 13D5, 3B3, and 16H3 block biotinylated A32 binding to A244 gp120 monomeric Env, consistent with the binding profile of A32 for C1 Env region. The C4 region antibody 18A7 minimally blocks A32 binding.

residues of Env spikes expressed on the surface of virions.^{42,45,52} As controls, we used the V3-glycan broadly neutralizing antibody (bnAb) PGT128 that recognizes Man₉ glycans and the V1V2-glycan bnAb PG9 that recognizes shorter glycans such as Man₃ and Man₅, and cannot accom-

modate longer glycans such as Man₉.^{20,53} As expected, viruses grown in the presence of kifunensine were more sensitive to neutralization by PGT128, but less sensitive to neutralization by PG9. However, despite the increased density of high-mannose glycans on the virion surface following kifunensine

TABLE 2. CROSS-REACTIVE HIV-1 MABS FAIL TO NEUTRALIZE HIV-1 PSEUDOVIRIONS

A. Cross-reactive murine mAbs induced by consensus Env gp140 were tested for their ability to neutralize HIV-1 pseudoviruses in the TZM-bl assay

HIV-1 isolate	Clade	Tier	Antibody							Pos. controls	
			13D7	8H3	13D5	3B3	16H3	18A7	18F11	PG9	19b
SF162	B	1	>50	>50	>50	>50	>50	>50	>50	>50	0.318
TV1.21	C	1	>50	>50	>50	>50	>50	>50	>50	<0.023	>50
CH505 w4.3	C	1	>50	>50	>50	>50	>50	>50	>50	0.203	0.090
CH505 TF	C	2	>50	>50	>50	>50	>50	>50	>50	0.477	>50
DU123	C	2	>50	>50	>50	>50	>50	>50	>50	0.124	>50
SVA-MLV	NA	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50

B. The glycan-dependent mAb 18F11 was tested for neutralization against pseudoviruses grown in the presence of kifunensine to increase the density of high-mannose glycans on the surface of the HIV-1 Env viral spike

HIV-1 isolate	Q23.17		BG505		JR-FL		MW965.26		CH505 w4.3		CH505s	
(±) KIF	–	+	–	+	–	+	–	+	–	+	–	+
18F11	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
PGT128 (+Ctl)	0.021	<0.002	0.205	0.038	0.03	0.005	>5	0.688	>5	>5	>5	>5
PG9 (+Ctl)	<0.005	0.104	0.022	1.117	>10	>10	>10	>10	0.305	>10	0.403	>10

Pseudoviruses remained resistant to neutralization by 18F11 despite kifunensine treatment.

IC50 (µg/ml)

<0.05
0.05–1
1.0–10.0
10–50
>50

treatment, 18F11 failed to neutralize kifunensine-treated HIV-1 pseudovirions (Table 2-B).

HIV-1 mAbs induced by consensus Env competitively inhibit A32, an ADCC-mediating antibody

CD4-inducible epitopes within the HIV-1 gp140 Env C1 region are bound by ADCC-mediating mAbs such as A32, which also makes contacts in the C4 region. To investigate if this panel of C1 and C4 region-specific antibodies could inhibit binding of A32, we measured inhibition of biotinylated A32 mAb via ELISA. We found that the C1-specific mAbs, 13D5, 3B3, and 16H3 inhibit binding of A32 to HIV-1 Env in

a dose-dependent manner, in addition to the C4-specific mAb, 18A7 (Fig. 3).

13D7 binds the surface of HIV-1-infected CD4⁺ T cells and mediates ADCC

To mediate ADCC effector activity, antibodies must bind the surface of HIV-1-infected cells.^{2,25–27} Since we found that several of these mAbs inhibit binding of A32 to recombinant Env oligomers (13D5, 3B3, 16H3, and 18A7), we sought to determine if any could bind the surface of HIV-1 Clade B SF162-infected primary human CD4⁺ T cells. None of the A32-blocking mouse mAbs bound to the surface of

FIG. 4. The 13D7 mAb epitope is expressed on the surface of HIV-infected cells. Scatter plot and histograms of non-neutralizing mouse mAb 13D7 binding to clade B SF162 HIV-infected human CD4⁺ T cells. A32-blocking mAbs T8, 13D5, 3B3, and 16H3 did not show observable binding (not shown). A32, a human mAb known to bind the surface of HIV-infected cells and mediate ADCC, and CH31, a broadly neutralizing CD4 binding site antibody (5), are shown for comparison. Non-neutralizing Abs 13D7 and A32 and broadly neutralizing Ab CH31 bind Env epitopes on the HIV-1-infected cell surface. ADCC, antibody-dependent cellular cytotoxicity.

HIV-1-infected cells. Rather, we found that mAb 13D7 did bind to the surface of HIV-1-infected CD4⁺ T cells, as did the CD4 binding site bnAb, CH31, used as a positive control (Fig. 4). We produced a humanized version of 13D7 comprising a mouse variable region and human IgG₁ constant and Fc region optimized to bind to FcγRIIIa on NK cells. We found that humanized 13D7 mediated ADCC in a dose-dependent manner against CD4⁺ T cells infected with 9 out of 11 HIV-1 infectious molecular clones (IMC) tested (Fig. 5).

Discussion

HIV-1 monoclonal antibodies can be important for understanding the structural dynamics of HIV-1 Env both in recombinant forms and when expressed on the surface of virions or infected cells. In this study, we show the characterization of a panel of highly cross-reactive murine antibodies induced with consensus HIV-1 Env immunization, and identified the epitopes bound by these antibodies. We identified monoclonal antibodies that will be useful for the characterization of recombinant gp120 monomer and gp140 oligomer Envs during good manufacturing practices (GMP) for human clinical trials (16H3 and 3B3). In addition, the epitope bound by mAb 13D7 represents a novel ADCC epitope and thus may be a worthwhile target in vaccination for the induction of ADCC-mediating antibodies. However, the reagents will not be useful for the study of stabilized recombinant native-like trimeric Envs (such as SOSIP trimers) unless they are open trimers and express non-neutralizing Env epitopes.⁵⁴

In this study, we found that cross-reactive anti-HIV gp120 murine mAbs bound to conserved epitopes in the HIV-1 Env C1 region, except for 18A7 and 18F11 that bound to conserved epitopes in the C4 region. Each of the C1-reactive mAbs bound to HEP-2 cells, suggesting that the C1 region of HIV-1 gp120 contains multiple epitopes that are polyreactive with self-antigen. Moreover, we identified one mAb (18F11) whose binding was sensitive to enzymatic native deglycosylation by PNGase and naturally occurring absence of the N448 glycan on HIV-1 Envs. Thus, either 18F11 binds to N-linked glycans

located within the HIV-1 Env C4 region or binding depends on the presence of the asparagine amino acid at 448.

Despite binding with high affinity to conserved epitopes, none of these mAbs was capable of neutralizing HIV-1 virions, including the C4 reactive mAb 18F11, whose binding depended on the presence of N448. Thus, these antibodies likely do not recognize epitopes expressed on the native trimer on the surface of virions nor on the surface of virus-infected CD4⁺ T cells. However, due to their broad cross-reactivity, this panel of antibodies can prove to be useful for the characterization of recombinant Envs during GMP production. In particular, 16H3 and 3B3 bound strongly to all of the recombinant Envs tested and have been important reagents for the characterization and quality control of GMP-produced recombinant Envs for vaccine studies (Haynes BF, unpublished).

HIV-1 neutralization may not be the only antibody-mediated mechanism to prevent HIV-1 acquisition since ADCC can also be an important mechanism for HIV-1 protection.^{2,25–27} In the RV144 vaccine efficacy trial, ADCC responses correlated with a reduced risk of infection, suggesting that non-neutralizing epitopes may be relevant for eliciting protective antibodies.^{2,25,55} In this study, we identified an antibody (13D7) that bound to the N-terminus of HIV-1 gp140 and bound to the surface of HIV-1-infected human CD4⁺ T cells. When expressed as a humanized recombinant monoclonal antibody, 13D7 was capable of mediating ADCC against 82% of IMCs tested. In contrast to A32- or A32-like antibodies that bind to a conformational epitope in the C1 and C4 regions of Env, 13D7 bound to a linear epitope.^{56–58} As such, it may be possible to selectively induce 13D7-like ADCC responses using a minimal peptide immunogen. Thus, the epitope bound by 13D7 may be a feasible vaccine target for the induction of anti-HIV-1-infected ADCC responses.

In summary, we have isolated a panel of cross-reactive mouse monoclonal antibodies induced with consensus Env gp140 immunization. Although non-neutralizing, these mAbs cross-react with a diverse panel of Envs, suggesting their utility for the characterization of recombinant HIV-1

FIG. 5. Humanized mAb 13D7 mediates ADCC. The non-neutralizing murine mAb 13D7 was humanized and tested for ADCC using primary human CD4⁺ T cells infected with a panel of infectious molecular clones (IMCs) of tier 1 and tier 2 viruses. Humanized 13D7 mediated ADCC in 9 out of 11 (82%) IMCs tested (left panel) in a dose-dependent manner with endpoint titers ranging from ~1 to 0.01 μg/ml (right panel).

Envs. Finally, we identified mAb 13D7 that not only binds to a highly conserved epitope but also mediates ADCC, and thus represents a new vaccine target for the induction of ADCC responses.

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Author Disclosure Statement

No competing financial interests exist.

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