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ORIGINAL ARTICLE

Highly mutated monoclonal antibody 3F2 targets a conformational and strain-restricted epitope in human immunodeficiency virus gp41 with significant antibody-dependent cell cytotoxicity

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Abstract

Identifying epitope targets by studying the native antibody (Ab) response can identify potential novel vaccine constructs. Studies suggest that long-term non-progressor (LTNP) subjects have inherent immune mechanisms that help to control viremia and disease progression. To explore a role for antibodies (Abs) in LTNP progression, our lab has previously characterized a number of highly mutated Abs that target conformational epitopes of the human immunodeficiency virus (HIV) envelope protein from a single LTNP subject (10076). One Ab clone, 10076-Q3-2C6, had significant cross-clade Ab-dependent cell cytotoxicity. To assess if other LTNP subjects produced similar Abs, we expressed another highly mutated Ab from another subject; subject 10002, clone 10002-Q1-3F2 (variable heavy chain, 63.2% amino acid sequence identity to predicted germline). After expression with its native light chain, the recombinant Ab 3F2 bound to the trimeric envelope protein of HIV (trimer), as well as to the ectodomain of gp41. 3F2 binding to gp41 peptide libraries was consistent with non-linear epitope binding and showed possible overlap with the epitope of 2C6. Ab competition assays suggested that 3F2 may bind near the immunodominant epitope 1 loop region (ID1) of gp41. 2C6 blocked the binding of ID1-loop-binding Abs and 3F2 to the trimer, but 3F2 failed to block 2C6 binding. Together, these results suggest that 3F2 binds to a non-linear conformational epitope primarily localized between the epitope of 2C6 and the ID1. Since they are targeted by functional Abs, a more complete understanding of these ID1 and near-ID1 epitopes may be exploited in future immunization strategies.

Abbreviations

ADCC	Antibody-dependent cell cytotoxicity
Abs	Antibodies
ID1	Immunodominant 1 epitope
LTNPs	Long-term non-progressors
nnAbs	Non-neutralizing Abs

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Introduction

HIV vaccine development continues to be a daunting task [1]. To elucidate a role in disease progression, we have studied Abs cloned from long-term non-progressors (LTNPs). A number of these Abs target conformational epitopes in gp41 [2]. The recombinant Ab 10076-Q3-2C6 (referred herein as 2C6) is a highly mutated Ab (86% nucleotide sequence identity to germline variable heavy chain 1-69) cloned from an LTNP subject, 10076, with significant viremia at the time of blood sampling (~21,000 copies/mL) [2, 3]. 2C6 is particularly interesting, as it has cross-clade Ab-dependent cell cytotoxicity (ADCC) and can recognize SOSIP protein constructs and protein constructs from all major clades. Structural mapping of the amino acids that are targeted by 2C6 has suggested that 2C6 recognizes a pocket formed by two adjacent gp41 protomers in the intact HIV trimer [4].

Although most vaccine efforts have concentrated on developing broadly neutralizing Abs, neutralization is not the only useful function of anti-HIV Abs. ADCC is of particular interest, as this activity correlated with the protection seen in a recent RV144 vaccine trial [5–7]. Non-neutralizing

Abs (nnAbs) targeting conformational epitopes in gp41 with significant ADCC function are able to limit cell-to-cell spread of infection [8]. nnAbs that target the immunodominant 1 epitope (ID1) of gp41, such as F240 [9] and 7B2 [10], showed gave protection in macaques against vaginal challenge with simian-human immunodeficiency virus (SHIV) and had an effect on the viral set point [11]. Mutation of the Fc receptor, on which ADCC depends, reduced the protective effect of the neutralizing Ab B12 in a macaque SHIV challenge model [12]. These data suggest that ADCC is a desirable activity to target for vaccination strategies. Improved understanding of the epitope specificity of ADCC Abs is essential for developing effective immunization strategies that optimize protection by these Abs.

As the target of 2C6 in the major clades of HIV is known and it has robust ADCC activity, further identification and analysis of Abs similar to 2C6 should be considered for vaccine development. We originally sequenced other Abs (seven heavy chains with paired light chains) from LTNP subject 10002, whose samples were collected decades after the year of infection and who consistently maintained low to unmeasurable viral loads (65 at the time of blood draw). Two of these Abs (10002-Q1-1H5 and 10002-Q1-3F2, herein referred to as 1H5 and 3F2) formed a clonal group, utilized VH 1-69 (Fig. 1), and were highly mutated. As these utilized the same heavy chain and were even more highly mutated than 2C6, we pursued recombinant expression and functional characterization of the natively paired heavy and light chains from 3F2 to further our studies on highly mutated Abs from LTNPs in hopes of increasing our knowledge about the epitope of 2C6.

Materials and methods

Full-length antibody expression and antibody purification

Expression in Free Style 293-F cells (Thermo Fisher Scientific, R79007) and IG purification utilizing a HiTrap Protein G column (GE Healthcare 17-0404-01) on an AKTA FPLC System (GE Healthcare, Piscataway NJ) were done as described previously [4].

Direct-binding ELISA

For His-tagged SOSIP ELISA, SOSIP trimer creation was characterized previously in the respective references (version four (Zm197m v4.1, AMC008 v4.2, and B41 v4.2; [13]) and version five (BG505 v5.2; [14]). ELISA was performed using Ni-NTA plates from QIAGEN Inc. TRIS-buffered saline (TBS) buffer base, blocking with 2% milk, peroxidase-labeled secondary Ab, and 1-step Ultra-TMB (Thermo Fisher Scientific) substrate color development, as described previously [4]. Other direct protein ELISAs used Immulon 2-HB plates, sodium bicarbonate buffer, phosphate-buffered saline (PBS) buffer base, blocking with fetal bovine serum (FBS), peroxidase-labeled secondary Ab, and 1-step ultra-TMB (Thermo Fisher Scientific) color development as described previously [4].

Competition ELISA

ELISA plates were coated with 10 ng of the selected antigen per well in a 0.2 M bicarbonate buffer solution, pH 9.4, and allowed to incubate overnight at 4°C. The next day, the protein coating solution was removed and blocking with 200 µl of 10% FBS in PBS was carried out at 4°C for 2 h. After removing the blocking buffer, 100 µl of the respective Ab diluted in ELISA wash buffer (7.5% FBS in phosphate-buffered saline with Tween 20 [PBST]) was added and allowed to incubate at 37°C for 1 h. The antibody solution was then removed, and the ELISA plates were washed three times with ELISA wash buffer. Biotinylated Abs (performed as described previously [2]) diluted in 7.5% FBS in ELISA wash buffer were then added at 200 ng/well and allowed to incubate at 37°C for 1 h. The biotinylated Abs were then removed, and the plates were washed three times with ELISA wash buffer. Then, 100 µl of streptavidin-horseradish peroxidase (HRP) secondary Ab diluted 1:2000 in 7.5% FBS in ELISA wash buffer was added to the plates and allowed to incubate at 37°C for 1 h. After washing, plates were developed as described previously using 1-step ultra-TMB (Thermo Fisher Scientific) [4].

	FR1-IMGT (11-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)
VH1-69	EVKKPGSSVKVSCKAS	GGTF....SSYA	ISWVRQAPGQGLEWMGG	IIPI..FGTA	NYAQKFQ.GRVTITADEST STAYMELSSLRSEDTAVYYC
76-2c6	---------R------	--N-....LDFV	V---------------A	-V-R..V-A-	Q---R--.--A-F---V-- ----L-VT----------
76-6b8	---------R------	--S-....IDFX	V---------------A	-V-R..V-A-	Q--PR--.--A-F---V-- ----L-VP----------
02-1h5	---------R---Q--	---Y....-A--	-N----G----P----V	---S..L-VN	-L-PR--.--LS-N-VG-PGST-VL-Q--A-T-----T---
02-3f2	---R-----R---Q--	---Y....-A--	-N----G----P----V	---S..L-VT	-L-PR--.--LS-A-AG-PGST-VSLD--A-TPA---I---

Fig. 1 Heavy chain variable region of highly mutated Abs from subject 10002 (3F2 and 1H5) and 10076 (2C6 and 6B8). Dashes represent sequence identity to the germline VH1-69 variable segment, and the numbering is according to IMGT. The amino acid sequence identity values compared to predicted germline sequences for 2C6, 6B8, 1H5, and 3F2 are 77.0%, 75.9%, 67.8%, and 63.2%, respectively. During initial cloning, primers with overlapping nucleic acid sequences corresponding to amino acids 1-10 of framework (FR) 1 were used, and these were not included in the calculations.

Western blot analysis

Proteins were separated by native and non-native SDS-PAGE using a Bio-Rad Mini-Format 1-D Electrophoresis System (Bio-Rad Mini Protean gel, catalog no. 456-1094) using alkaline-phosphatase-conjugated goat-anti-human Ig (Southern Biotech, Birmingham, AL, catalog no. 2010-04) and then NBT/BCIP color developer (Thermo Fisher Scientific, catalog no. 34042) solution as described previously [4].

Antibody-dependent cellular cytotoxicity assay

A rapid and fluorometric ADCC (RFADCC) assay was performed as described previously [4]. The monoclonal Ab F16V3 influenza Ab was included as a negative control, and HIVIG was included as a positive control (both used at 500 ng/μL). For our analysis, we set the percent killing observed with HIVIG to 100% and normalized the rest of the samples to HIVIG. Relative ADCC activity was calculated by dividing the percent activity at that dilution minus background. The data were plotted with the relative ADCC activity on the *y*-axis and the Ab concentration on the *x*-axis. The 50% effective concentration (EC_{50}) was determined by fitting a sigmoidal dose-response curve to the data and identifying the concentration needed for 50% ADCC activity.

Peptide-binding ELISA

The following peptides were obtained from the NIH AIDS Reagents Program (ARP): Peptide Array, Human Immunodeficiency Virus Type 1 Potential T-Cell Epitopes (PTE) Env Region, ARP-11551, Group M consensus Env peptides, ARP-9487, Clade B group M Env peptides, ARP-6451, Clade A BG505 Env peptides, and ARP-13123. ELISA plates were coated with 10 μg of the respective peptide per well in 0.2 M bicarbonate buffer, pH 9.4, and allowed to incubate overnight at 4°C. The next day, plates were blocked with 10% FBS in PBS for 2 h at 4°C. After removing the blocking buffer, 100 μl of the respective Ab diluted to 1000 ng/mL in ELISA wash buffer (7.5% FBS in PBST) was added to each well and allowed to incubate at 37°C for 1 h. After 1 h, plates were washed three times with ELISA wash buffer. After washing, the plates were developed as described previously using 1-Step Ultra TMB solution (Thermo Fisher Scientific) [4].

Results

2C6 is a highly mutated Ab with 86.0% nucleotide sequence identity to the predicted variable heavy chain (VH1-69) germline and 81.6% nucleotide sequence identity to the predicted J heavy chain region (JH6) germline. The V gene prediction is kappa 1-39 with 95.3% predicted nucleotide sequence identity to the germline. The amino acid length of 2C6 HCDR3 is 21, and that of KCDR3 is 9. Similar mutations were seen in its clonal relative, 6B8 [2, 3]. From the heavy chains cloned from subject 10002, two sequences (1H5 and 3F2) formed a clonal group and were highly mutated. These are shown in comparison to 2C6 and 6B8 in Figure 1. 3F2 has 79.6% nucleotide sequence identity in the variable chain (VH1-69) and 65.8% identity in the J region (JH6) to the predicted germline. The V gene prediction is lambda 3-25 with 94.4% nucleotide sequence identity to the predicted germline. The amino acid length of 3F2 HCDR3 is 16, and that of LCDR3 is 11. 1H5 has similar mutations and a similar overall structure. These nucleotide changes lead to highly mutated Abs when compared to the germline with amino acid sequence identity to the predicted germline of 77.0%, 75.9%, 67.8%, and 63.2% for 2C6, 6B8, 1H5, and 3F2, respectively (Fig. 1). Both 3F2 and 1H5 have additional structural divergence, with a predicted insertion of a glycine (G) and a serine (S) at variable site 86 (IMGT numbering [15]). There were rare convergent amino acid changes from germline between these antibodies from the two individuals, with two lysine (K)-to-arginine (R) changes (at positions 20 and 70 according to IMGT numbering), which are both positively charged amino acids and likely to be of minimal overall significance.

After its expression as a recombinant antibody, we characterized the binding of 3F2 to a variety of HIV constructs. Our ELISA assays showed that 3F2 readily binds to both trimer and gp41 proteins, although with less efficiency than 2C6 (Fig. 2A and B). As 2C6 could recognize constructs from multiple clades [4], we also assessed 3F2 for possible multiclade recognition of various gp140 trimer constructs (Fig. 2C, open symbols). Compared to published data on 2C6, 3F2 is more restrictive, recognizing only clade B constructs (BaL trimer and JRFL gp140 protein). We next investigated whether 3F2 would bind to the BG505 SOSIP.664 gp140 trimer (herein referred to as SOSIP). The fact that the SOSIP constructs lack transmembrane and membrane-proximal regions [16] allows finer mapping within gp41 and allows advanced vaccine constructs to be assessed. In a western blot assay, 3F2 did not recognize BaL gp120, but it did recognize SOSIP and gp41 proteins under non-reducing conditions (Fig. 2D). Upon reduction, SOSIP bands (~ 25 kDa) corresponding to the gp41 protein showed binding. Unlike 2C6, 3F2 did not recognize SOSIP in the ELISA.

Since 2C6 and 3F2 both target gp41 and 2C6 has been shown to have robust ADCC activity against clade B and clade C Gp41 constructs, we next investigated whether 3F2 has ADCC activity. With clade C construct ZA1197 gp41, we saw significant ADCC activity, similar to that of 2C6 and the HIVIG control (Fig. 3). Representative LTNP-derived ID1-targeting Abs (10076-Q5-8F6 and 10076-Q5-5C2;

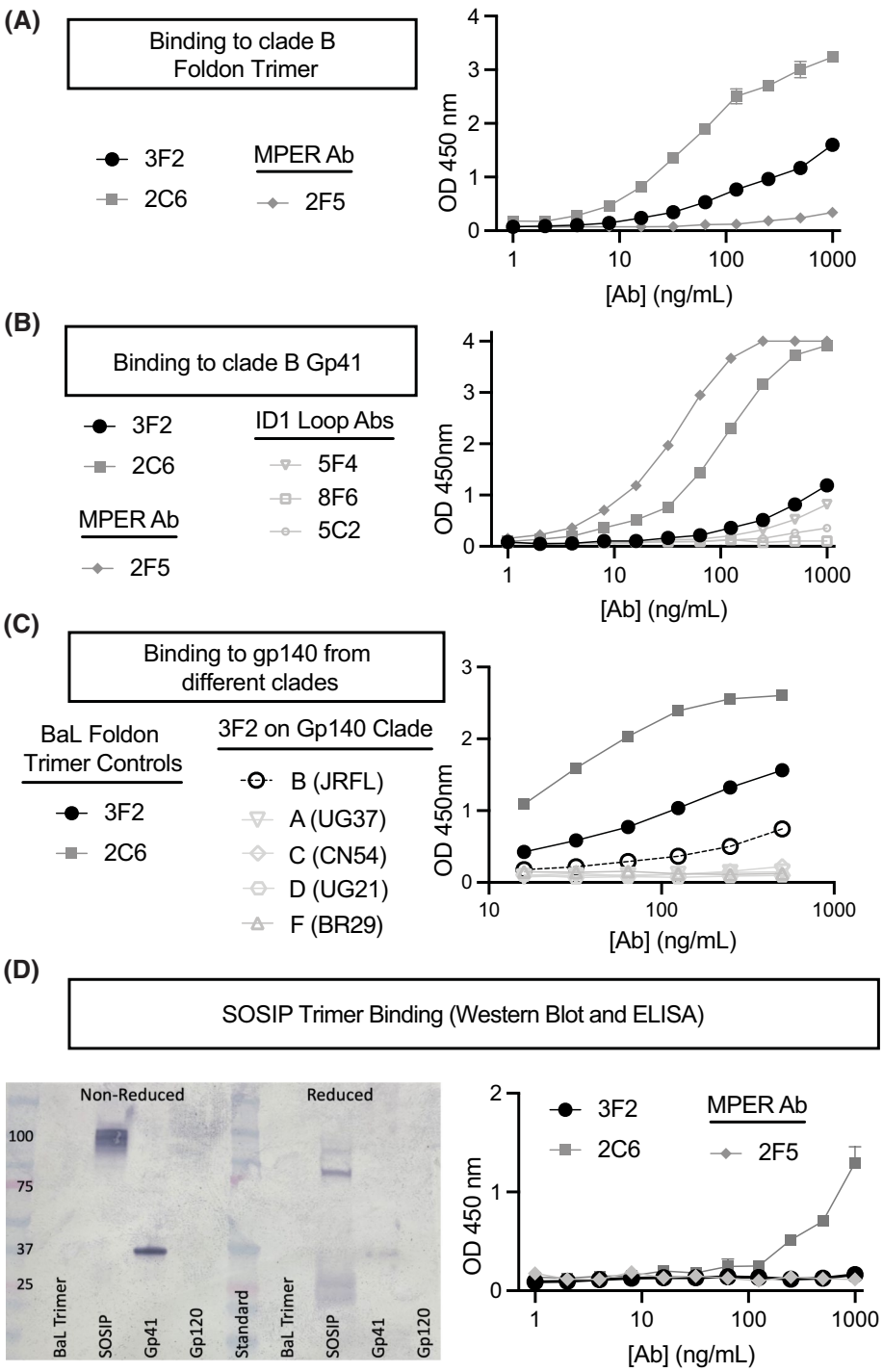

Fig. 2 3F2 targets an epitope in the gp41 region. ELISA plates were coated with antigen (commercially available and produced) and treated with Abs as indicated. 3F2 and controls were assessed by direct-binding ELISA with the (A) BaL foldon trimer and (B) clade B MN gp41. (C) 3F2 binding to various gp140 clades compared to the BaL foldon trimer and 2C6 binding to the BaL foldon trimer. (D) Western blot performed with 3F2 after protein transfer of SDS-PAGE-separated proteins BG505 SOSIP trimer, clade B MN Gp41, and clade B gp120. Prior to gel loading, proteins were reduced or not reduced (as indicated). Direct SOSIP ELISA was performed with 2C6 as a positive control. All assays were run twice, and representative results are shown.

herein referred to as 8F6 and 5C2) also had ADCC activity. These ID1-targeting Abs also presumably target conformational epitopes of the ID1 region, as they showed variable competition with other ID1-binding Abs (previously published with ID1 Abs 98-6 and 3D6 [2]).

To identify the specific binding site of 3F2, we assessed its binding to three overlapping gp41 peptide libraries of various clades. Each library consisted of 15-mer peptides with 11-amino-acid overlaps, and the results were compared to those obtained with the 2C6 and 2F5 controls. Within each set, mAb 2F5, which recognizes the linear epitope ELDKWA and shows binding to 2-3 overlapping peptides, representing linear epitope targeting, was used as an internal positive control. As shown in a previous publication [4], 2C6 bound specifically and repeatedly to a single peptide within each of the three peptide sets (Fig. 4).

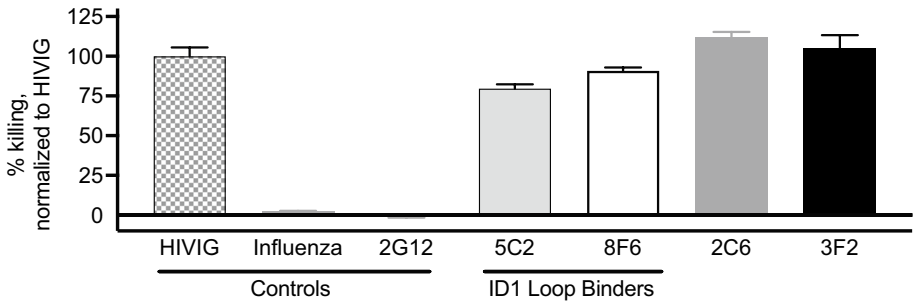

Fig. 3 ADCC activity of various Abs against gp41. ADCC activity of 3F2 was assessed to determine if it would be useful in possible vaccine constructs. As shown, 3F2 exhibited high ADCC activity against clade C ZA1197 gp41, which was comparable to that of the HIV immunoglobulin (HIVIG) positive control and similar to that of 2C6 and the ID1-binding Abs discovered in our laboratory. The normalized mean of two replicates with standard deviation is shown.

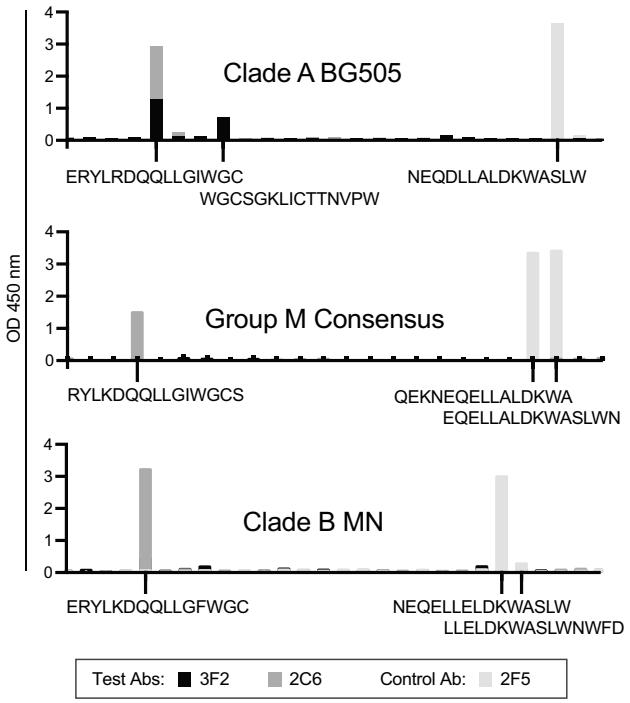

Fig. 4 Comparison of 2C6 and 3F2 binding to group M consensus, clade A, and clade B peptide sets. ELISA plates were coated with 10 µg of the respective antigen and were treated with Abs at 1000 ng/mL. 3F2 bound two separate peptides on clade A BG505 (A), and did not bind significantly to any peptides on group M (B) or clade B MN (C). 2F5, as the control, showed recognition of peptides that overlapped with the known ELDKWA motif.

In all three peptide sets, 2C6 failed to bind overlapping peptides within the ectodomain of gp41. Despite the lack of binding to clade A gp140 constructs, the best recognition of 3F2 was in the clade A BG505–derived peptide set. Recognition of WGCSGKLICTTNVPW is consistent with ID1 loop binding. However, 3F2 also recognized the peptide ERYLRDQQLLGIWGC, which is also recognized by 2C6.

Despite 3F2 being highly mutated, the antigen binding and peptide binding results are consistent with it being a relatively strain-specific Ab. To confirm the binding seen in peptide ELISA, other peptides were tested. We utilized a set of potential T-cell epitope (PTE) peptides, with numbering representing the internal numbers provided within the original peptide set. We compared binding of 3F2, 2C6, 8F6, and 5C2 against peptides that overlapped the epitopes of 2C6 and the ID1 loop region of gp41. A wide array of these peptides were screened for potential 3F2 binding, with pertinent positives and negatives shown (Fig. 5, Table 1). The pattern of binding mirrored that of the BG505 peptide, with two peptides with minimal overlap (105-VERYLRDQQLLGIWG and 101-CSGKLICTTTVPWNS) showing binding and no binding seen in the intervening peptides. 3F2 showed no binding to peptides that completely overlapped 105 and 101 but had sequence differences (399-VEKYLKDQQLLGLWG and 73-CSGKLICTTAVPWNS; differences underlined). The 101 peptide was also recognized by the ID1-loop-binding Abs 5C2 and 8F6, and intriguingly, at very low levels, 8F6 recognized peptides 105 and 399, but this was variable between assays. Taken together, these data further suggest that 3F2 binds in a conformation-dependent manner and is fairly strain-restricted. In our previous study on 2C6, we created a 25-mer peptide that incorporated the majority of the epitope identified by alanine scanning mutagenesis (Table 1). Consistent with prior data, 2C6 recognizes the 25-mer, and the R579A mutation only partially diminishes 2C6 binding, with other mutations ablating binding in this assay. None of these 25-mer peptides were recognized by 3F2. Notably, these 25-mer peptides had the substitution R588K when compared to the BG505 sequence that is recognized by 3F2.

To identify the binding site of 3F2 on gp41, competition ELISA was performed using various anti-gp41 monoclonal Abs with well-defined epitopes (Fig. 6). 3F2 binding can be competed by 2C6 and F240, but not 2F5. F240 binds to the ID1 loop region, while 2F5 binds to the membrane-proximal

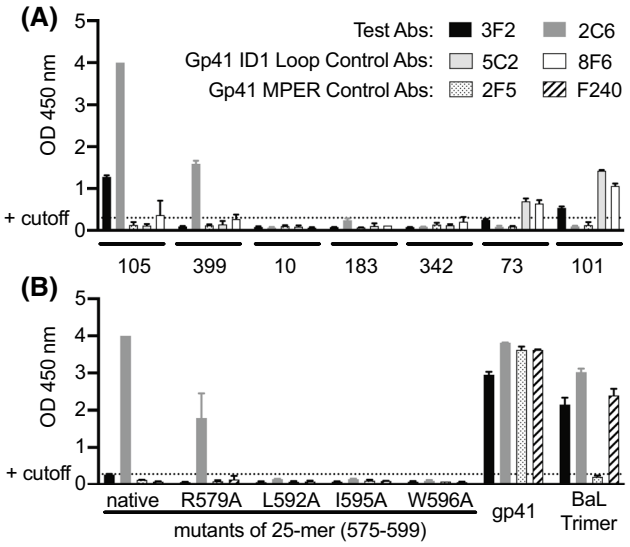

Fig. 5 3F2 recognizes distinct peptides not bound by 2C6. Selected peptides were coated at 10 μg per well and treated with the indicated Abs at a concentration of 1000 ng/mL. Positive binding was defined as three times above the background. (A) 3F2 binds selected peptides that are targeted by 2C6 and ID1-binding antibodies (PTE NIH AIDS reagents numbering is used) (B) Binding of 3F2 compared to 2C6 and controls of a previously described 25-mer peptide that incorporates the majority of amino acids involved in the 2C6 epitope identified by alanine scanning mutagenesis.

region (MPER) of gp41. This confirms that 3F2 targets a region in the ID1 loop area. Competition by 3F2 was used as an internal control. This experiment was repeated two times with representative data shown. Despite showing targeting of the same peptide (Figs. 3 and 4), 3F2 did not compete with 2C6. This confirms the previous findings that, although 2C6 can compete with ID1 loop binders, the 2C6 epitope is distinct and does not target the typical ID1 domain epitope [4].

Discussion

Here, we show that the epitope targeted by 3F2 is both conformation-dependent and generally clade-specific, predominantly recognizing clade BaL trimer and clade B JRFL gp140 constructs. Although the binding of 3F2 appears to be relatively specific for clade B proteins, we also observed consistent and quantitatively significant binding to clade A BG505 peptides and show ADCC with a clade C construct. We show that 3F2 binding to trimer was blocked by both 2C6 and F240. This suggests that 3F2 targets an epitope that is near the 2C6 region as well as the ID1 epitope. In the original characterization of these Abs, 2C6 could compete with ID1-binding Abs, but ID1-binding Abs could not compete with 2C6. 3F2 shows a similar pattern to the ID1-binding Abs, suggesting the 3F2 epitope is not centered on 2C6 or does not predominantly target that region, despite the recognition of the same peptide in the BG505 peptide set. This suggests that the Ab 3F2 targets a region between the 2C6 epitope and the ID1 loop area of gp41.

Due to similar variable chain usage, excessive mutation, and cloning from a LTNP subject, we initially hypothesized that 3F2 may also bind the epitope recognized by 2C6. Initial characterization failed to show cross-clade recognition, albeit using a limited dataset. By testing the group M consensus, group B MN, and clade A BG505 peptide sets, we were able to determine that 3F2 binds non-linearly near the epitope of 2C6, as targeting of the same peptide (ERYLRDQQLLGIWGC) was shown by 3F2 and 2C6 on clade A BG505. Targeting of this region was confirmed by assessment of 3F2 binding to PTE peptides, as 3F2 and 2C6 bound peptide 105 (VERYLRDQQLLGIWG). However, screening of 3F2 binding to the various PTE peptides also suggested possible targeting of the ID1 loop region, as 3F2 and our loop-binding Abs 8F6 and 5C2 bound to peptide 101 (CSGKLICTTTVPWNS). Interestingly, 8F6 and 5C2 both more readily recognized peptide 73 (CSGKLICTTAVPWNS), suggesting that these antibodies are less strain-constrained than 3F2. Both 8F6 and 5C2 also utilize VH1-69, with notable very low affinity over background of 8F6 for peptides 105 and 399. The angles of approach and epitope coverage in this region may be more similar with 8F6 and 3F2 than with 2C6 and 3F2. Crystallization studies and finer epitope mapping of these Abs are being pursued to address these subtle differences in targeting of these neighboring regions. Despite 3F2 being highly mutated, the bulk of these data suggest that 3F2 is similar to other loop-binding Abs that appear more strain-specific [17].

Functions other than neutralization, such as ADCC, are thought to be major contributors to the protection seen in the recent successful RV144 vaccine trial [5]. ID1-loop-binding Abs are known to have ADCC activity and generally have limited neutralization breadth [17]. nnAbs targeting structural epitopes in gp41 have been shown to have a significant ADCC function and to limit cell-to-cell spread of infection [8]. nnAbs targeting the ID1 loop epitope of gp41 can even protect macaques against vaginal challenge with SHIV, as was seen with F240 [9] and 7B2 [10]. The F_c portion of Abs, on which ADCC is dependent, is crucial for protection, even in Abs with significant neutralization activity [12].

As our Abs were cloned from an LTNP, it is intriguing to consider a role for specific epitope targeting contributing to this state. Both neutralizing [12, 18, 19] and non-neutralizing [9, 10] Abs have shown some level of protection from infection in challenge models, and there are other clues that these Abs may affect the immunologic state of infected individuals. Cervical IgA responses against gp41 [20] have been confirmed in cohorts of chronically exposed uninfected

Table 1 Summary of 3F2 binding studies: tabular summary of results of pertinent peptide binding experiments and previous pertinent data

Protein target	Region	Sequences from the near ID-1 loop	Ab Binding
Trimer binding	**HXB2 #**	**Sequences**	**3F2 targeting summary**
Group M	575-616	QLQARVLAVERYL**K**DQQLLGIWGCSGKLICTT**T**VPWN**S**SWSN	No peptides
Clade B, BaL	575-616	QLQARVLAVERYLRDQQLLGIWGCSGKLICTT**A**VPWNASWSN	Trimer binding
Clade B, JRFL	575-616	QLQARVLAVERYL**G**DQQLLGIWGCSGKLICTT**A**VPWNASWSN	Gp140, diminished
Clade B, MN	575-616	QLQARVLAVERYL**K**DQQLLG**F**WGCSGKLICTT**T**VPWNASWSN	No peptides
Clade A, Bg505	575-616	QLQARVLAVERYLRDQQLLGIWGCSGKLICTTNVPWN**S**SWSN	Two peptides
Clade C, ZA119	575-616	QLQ**T**RVLA**I**ERYL**K**DQQLLG**L**WGCSG**R**LICTT**A**VPWN**S**SWSN	ADCC
Peptide binding			**Qualitative binding Abs**
Clade A Bg505	584-598	*ERYLRDQQLLGIWGC*	3F2/2C6
Clade A Bg505	588-602	RDQQLLGIWGCSGKL	
Clade A Bg505	592-606	LLGIWGCSGKLICTT	
Clade A Bg505	596-610	*WGCSGKLICTTNVPW*	3F2
PTE 105	583-597	VERYLRDQQLLGIWG	3F2/2C6/8F6
PTE 399	583-597	VE**K**YL**K**DQQLLG**L**WG	2C6/8F6
PTE 10	589-603	DQQLLGIWGCSGKLI	
PTE 183	589-603	DQQLLG**L**WGCSGKLI	
PTE 342	593-607	LGIWGCSGK**H**ICTTN	
PTE 73	598-612	CSGKLICTT**A**VPWNS	5C2/8F6
PTE 101	598-612	CSGKLICTT**T**VPWNS	3F2/5C2/8F6
2C6 25-mer (from Sojar, et al, 2019)	575-599	QLQARVLAVERYL**K**DQQLLGIWGCS *(native)* QLQA**A**VLAVERYL**K**DQQLLGIWGCS *(R579A)*	2C6 2C6 (diminished)
Alanine Scanning data			
Sojar et al, 2019 Hicar et al, Plos One, 2016		R L IW W C G L C	2C6 5C2/8F6

Bolded amino acids are those that vary from BG505 peptides recognized by 3F2 (*) or consensus in regions outside these peptides. Decreased binding of the Ab is indicated by a grey font.

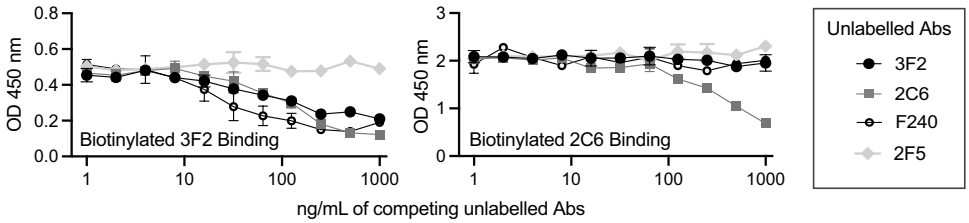

Fig. 6 Non-reciprocal competition distinguishes 2C6 from 3F2. Unlabeled monoclonal Abs diluted from 1000 ng/mL to 0 ng/mL were used in a competitive binding assay with 200 ng of biotinylated 3F2 (A) or 200 ng of biotinylated 2C6 (B) per mL. The data are representative of two experiments.

individuals [21]. Similar responses can be raised by gp41 vaccination and have shown protection of primates against mucosal challenge with SHIV [22]. Most Abs have no effect on viremia [23], but recent studies have suggested that Abs targeting specific epitopes may affect viremia. The broadly neutralizing Ab PGT121 showed suppression of viremia in an SHIV-infected rhesus model [24]. Suppression of viremia was also demonstrated in humans after infusion with the broadly neutralizing Abs 3BNC117 [25] and 10-1074 [26]. These Ab infusions have also been shown to enhance neutralization [27] and CD8 responses and provide long-lasting stabilization of CD4 levels [28]. The data support a role for immunotherapies as prophylaxis and provides proof of concept that Abs that target specific epitopes can lead to viremic control and may contribute to LTNP.

As the epitope recognized by 2C6 is present on a variety of clades and can support ADCC, further characterization of this epitope is warranted. For clade B studies, 3F2 might be a useful tool to discern binding and reactivity to the 2C6 epitope. Vaccine constructs that were designed to induce 2C6-like Abs are currently in development, and 3F2 Ab could be used to optimize constructs that would focus

responses on the 2C6 epitope and away from more strain-specific structures recognized by Abs such as 3F2.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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