Broad and potent neutralization of HIV-1 by a gp41-specific human antibody

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Characterization of human monoclonal antibodies is providing considerable insight into mechanisms of broad HIV-1 neutralization. Here we report an HIV-1 gp41 membrane-proximal external region (MPER)-specific antibody, named 10E8, which neutralizes ~98% of tested viruses. An analysis of sera from 78 healthy HIV-1-infected donors demonstrated that 27% contained MPER-specific antibodies and 8% contained 10E8-like specificities. In contrast to other neutralizing MPER antibodies, 10E8 did not bind phospholipids, was not autoreactive, and bound cell-surface envelope. The structure of 10E8 in complex with the complete MPER revealed a site of vulnerability comprising a narrow stretch of highly conserved gp41–hydrophobic residues and a critical arginine or lysine just before the transmembrane region. Analysis of resistant HIV-1 variants confirmed the importance of these residues for neutralization. The highly conserved MPER is a target of potent, non-self-reactive neutralizing antibodies, suggesting that HIV-1 vaccines should aim to induce antibodies to this region of HIV-1 envelope glycoprotein.

Induction of an antibody response capable of neutralizing diverse HIV-1 isolates is a critical goal for vaccines that protect against HIV-1 infection. Potentially the greatest obstacle to achieving this goal is the extraordinary diversity that develops in the target of neutralizing antibodies, the envelope glycoprotein (Env). Although vaccines have thus far failed to induce broadly neutralizing antibody responses, there are examples of chronically infected patients with sera that neutralize highly diverse HIV-1 isolates1–8. These individuals provide evidence that it is possible for the human antibody response to neutralize highly diverse strains of HIV-1, although the mechanisms by which such responses are induced or mediated remain incompletely understood9,10.

Recently, isolation and characterization of human monoclonal antibodies from cells of chronically infected patients have provided considerable advances in understanding the specificities and mechanisms underlying broadly neutralizing antibody responses to HIV-1. Env exists on the virion and infected-cell surface as a trimer of heterodimers made up of gp120 and gp41 subunits. For some time, only a small number of broadly neutralizing monoclonal antibodies had been isolated, consisting of one antibody that binds the CD4-binding site on gp120 (b12), one that binds a glycan configuration on the outer domain of gp120 (2G12), and three that bind the membrane-proximal external region (MPER) on gp41 (2F5, Z13e1 and 4E10)11–13. More recently, considerably more broad and potent antibodies have been discovered that target the CD4-binding site of the envelope protein14–17 (for which VRC01 is a prototype) and glycan-containing regions of the variable 1 (V1)/V2 and V3 regions of gp12018–20 (for which PG9 and PGT128 are prototypes). The specificities of these new antibodies are providing important information regarding antigen targets on Env to which the humoral immune response might be directed to mediate broad and potent neutralization. However, evidence for these specificities in many chronically infected patients within our HIV-1-infected cohort1 is lacking, suggesting that broad and potent neutralization may be mediated by other specificities.

Here we report isolation of a broad and potent gp41 MPER-specific human monoclonal antibody, 10E8, from an HIV-1-infected individual with high neutralization titres. 10E8 is among the most broad and potent antibodies thus far described, and lacks many of the characteristics previously thought to limit the usefulness of MPER-specific antibodies in vaccines or passive therapies, including lipid binding and autoreactivity. In addition, the crystal structure of 10E8, along with biochemical binding studies, demonstrate that the breadth of 10E8 is mediated by its unique mode of recognition of a structurally conserved site of vulnerability within the gp41 MPER.

10E8 isolation and neutralizing properties

To understand the specificities and binding characteristics that underlie a broadly neutralizing antibody response we developed techniques that permitted isolation of human monoclonal antibodies without previous knowledge of specificity21. Serum from one donor, N152, exhibited neutralizing breadth and potency in the top 1% of our cohort against a 20 cross-clade pseudovirus panel (Supplementary Table 1)1. Peripheral blood CD19+IgM+IgD–IgA– memory B cells from this patient were sorted and expanded for 13 days with interleukin (IL)-2, IL-21 and CD40-ligand expressing feeder cells. The supernatants of ~16,500 B-cell cultures were screened and IgG genes from wells with neutralization activity were cloned and re-expressed21 and two novel antibodies (10E8 and 7H6) were isolated.

Nucleotide sequence analysis of DNA encoding 10E8 and 7H6 revealed that both were IgG3 antibodies and were somatic variants of the same IgG clone. These antibodies were derived from IGHV3-15*05 and IGLV3-19*01 germline genes, and were highly somatically mutated in variable genes of both heavy chain (21%) and light chain (14%) compared to germ line. These antibodies also possessed a long heavy-chain complementarity-determining region (CDR H3) loop composed of 22 amino acids (Fig. 1a). The heavy chains of 10E8 and

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of viruses and the potency is comparable to some of the best available monoclonal antibodies.

**10E8 epitope specificity and binding**

To map the epitope of the 10E8 antibody, we tested binding to different subregions of Env by enzyme-linked immunosorbent assay (ELISA). 10E8 bound strongly to gp140, gp41 and the 4E10-specific MPER peptide, but not to gp120 (Fig. 2a). To map further the 10E8 epitope within the MPER, we examined binding of 10E8 to overlapping peptides corresponding to the 2F5 (656–671), Z13e1 (666–677) and 4E10 (671–683) specificities. 10E8 bound to the full MPER and the 4E10-specific peptides, but not 2F5- or Z13e1-specific peptides. Within the 4E10 epitope, when a peptide with a truncated carboxy terminal was tested, 4E10.19 (671–680), 10E8 binding was weakened considerably, indicating that the three terminal amino acids of the MPER—Try681, Ile682 and Arg683—were crucial for 10E8 binding (Supplementary Fig. 3a). Consistent with these results, only the full MPER and 4E10-specific peptides blocked 10E8-mediated neutralization of the chimaeric C1 virus, which contains the HIV-2 Env with the HIV-1 MPER (Supplementary Fig. 3b). Taken together, these data indicate that the minimal 10E8 epitope is located within residues 671–683 of the MPER, although additional contacts towards the amino terminus of the MPER could not be excluded.

To map more precisely the epitope of 10E8, a panel of alanine mutant peptides scanning MPER residues 671–683 was used to block 10E8 neutralization of the chimaeric C1 virus (Fig. 2b)22. 4E10 peptides with alanine substitutions at Thr672, Phe673 or Thr676 failed to block 4E10 or 10E8 neutralization, indicating that these residues are critical for both 4E10 and 10E8 binding. Residues Asn671 and Arg683, both of which are not required for 4E10 binding, were found to be critical for 10E8 binding and neutralization (Supplementary Table 2 and Fig. 2b). We also tested the ability of 10E8 to neutralize HIV-1 JR2 pseudoviruses with alanine substitutions in MPER residues 660–683 (Supplementary Table 3). Consistent with the effects of alanine substitutions on peptide binding, residues Asn671 and Arg683 were critical for 10E8, but not 4E10, neutralization. Individual alanine substitutions at residues 671–673, 680 and 683 resulted in reduced neutralization sensitivity to 10E8 most apparent at the IC₅₀ level rather...
than the IC_{50} level. Although the mechanism for this phenomenon is unclear, a similar effect has been observed previously when MPER mutations cause partial resistance to 4E10^{24}. Taken together, these results indicated that 10E8 recognized a novel epitope that overlaps the known 4E10 and Z13e1 epitopes but differs in a critical dependence on binding to Asn 671 and Arg 683, the last residue of the MPER.

We next investigated whether the greater neutralization potency of 10E8 compared to other MPER antibodies was a result of higher binding affinity to the MPER. Capture of a biotinylated peptide encompassing the full MPER (656–683) to a surface-plasmon resonance (SPR) chip allowed the binding kinetics of antigen-binding fragments (Fabs) 10E8, 2F5 and 4E10 to be examined. In contrast to its higher neutralization potency, the dissociation constant (K_d) of 10E8 to this MPER peptide was weaker than that of 2F5 and 4E10: 17 nM for 10E8 versus 3.8 nM for 2F5 and 0.74 nM for 4E10 (Supplementary Fig. 4). Therefore, the affinity of 10E8 for the MPER in a soluble peptide format did not explain its greater neutralization potency compared to other MPER-specific antibodies.

**Prevalence of 10E8-like antibodies**

We next investigated the prevalence of MPER-specific and 10E8-like neutralizing antibodies in our cohort of HIV-1-infected donors. We selected 78 sera from our cohort with a neutralization ID_{50} > 100 against at least one pseudovirus in a five-virus mini-panel. The median time since diagnosis of these donors was 13.5 years, median CD4 count was 557 cells μl^{-1}, median plasma HIV RNA 5,573 copies ml^{-1}, and they were not receiving antiretrovirals. We tested neutralization against the HIV-2/HIV-1 chimaera C1 (Supplementary Table 4)\(^{29}\). Of 78 sera, 21 exhibited neutralization activity against the HIV-2/HIV-1 C1 virus (Supplementary Table 5). To map the region that was targeted by these sera, neutralization was measured using seven HIV-2/HIV-1 chimaeras containing subdomains of the MPER (Supplementary Table 4)\(^{30}\). Of the 21 sera with neutralization activity against the entire MPER, 8 exhibited a neutralization pattern similar to that observed for 10E8, which entailed neutralization of only those HIV-2/HIV-1 chimaeric viruses that contained the terminal residue of the MPER Arg 683 (C4, C4GW and C8; Supplementary Table 5). For further confirmation of these results, we used peptides corresponding to different portions of the MPER to block sera neutralization of the HIV-2/HIV-1 chimaera C1 (Supplementary Table 6). Of the eight sera found to have a 10E8-like pattern based on neutralization of the chimaeras, three were blocked by peptides consistent with 10E8-like activity. An additional three of the eight 10E8-like sera were blocked by peptides in a pattern consistent with a combination of 10E8- and Z13e1-like antibodies. The six patients whose sera had 10E8-like activity did not differ from the remaining 72 patients with regard to clinical course or HIV neutralization (Supplementary Fig. 5, legend). Overall, 27% of the tested patient sera exhibited anti-MPER neutralizing activity. This prevalence is considerably higher than observed in previous work, possibly related to the known 4E10 and Z13e1 epitopes but differs in a critical dependence on binding to Asn 671 and Arg 683, the last residue of the MPER.

**Analysis of 10E8 autoreactivity**

A property common to the previously characterized MPER monoclonal antibodies 2F5 and 4E10 is that they crossreact with self-antigens\(^{29}\). In addition, binding to both the cell membrane and the Env trimer is thought to be important for optimal neutralization by these antibodies and this autoreactivity may be an obstacle to the elicitation of similar antibodies by a vaccine\(^{29,30}\). Surface plasmon resonance analysis showed that 10E8 did not bind to anionic phospholipids, such as phosphatidyl choline-cardiolipin (PC-CLP) and phosphatidyl choline-phosphatidyl serine (PC-PS) liposomes (Fig. 3a). 10E8 also did not bind HEP-2 epithelial cells, in contrast to 2F5 and 4E10 that bound in a cytoplasmic and nuclear pattern (Fig. 3b). Additionally, 10E8 did not bind autoantigens, such as Sjogren’s syndrome antigens A and B, Smith antigen, ribonucleoprotein, scleroderma 70 antigen, Jo1 antigen, centromere B and histone (Supplementary Table 7). Taken together, these results suggest that 10E8, in contrast to other MPER antibodies, is not autoreactive.

**Virion accessibility of 10E8**

The 2F5 and 4E10 antibodies have been shown to bind relatively poorly to the HIV-1 envelope spike on the surface of infected cells or to cell-free virions, and react more efficiently after Env engagement of the CD4 receptor\(^{31}\). We measured binding to cleaved, full-length envelope spikes on HIVJRFL transfected cells (Supplementary Fig. 6a). Although 10E8 bound less efficiently than other antibodies such as VRC01 or F105, where accessibility is not an issue, it bound more efficiently than either 2F5 or 4E10. In contrast to results of alanine substitution, a mutation in the 4E10 (F673S) region in full-length HIVJRFL envelope spikes enhanced 10E8 binding although the mechanism remains unclear. A mutation in the 2F5 (K665E) region had no influence on 10E8 binding. These data indicate that 10E8 has modestly greater access to the MPER epitope on the cell surface than either 2F5 or 4E10.

To assess binding to cell-free virus, we incubated virions with antibody, washed out unbound antibody, and tested neutralization\(^{31-33}\). During washing, antibodies that cannot access their Env target on free virions will be largely removed and therefore neutralization will be diminished. As a control, neutralization of the HXBc2 isolate was not diminished by washing, because the MPER region is accessible on this laboratory-adapted isolate\(^{33}\). Washing also had little impact on neutralization of JRFL by VRC01. Consistent with previous work, 2F5 and 4E10 neutralization of most virus isolates tested was substantially diminished after washing (Supplementary Fig. 6b)\(^{31,33}\). In contrast to 2F5 and
4E10, washing had a smaller effect on 10E8 neutralization of most viruses tested, as measured by the area under the curve or analysis of the fold-change in neutralization at a fixed inhibitory concentration (Supplementary Fig. 6c). Although 10E8 is not fully able to access its epitope on the native viral spike similarly to VRC01, under most experimental conditions tested it was better able to access its epitope than either 2F5 or 4E10.

**Structure of the 10E8–gp41 complex**

To provide an atomic level understanding of the interaction of 10E8 with HIV-1, we crystallized the Fab of 10E8 in complex with a peptide encompassing the entire 28-residue gp41 MPER (residues 656–683). Monoclinic crystals diffracted to 2.1 Å resolution, and structure solution and refinement to \( R_{\text{cryst}} = 18.01\% \) (\( R_{\text{free}} = 21.76\% \)) revealed two complexes in the asymmetric unit (heretofore referred to as complexes 1 and 2) (Supplementary Table 8). Overall, 10E8 bound to one face of the MPER peptide, which formed two helices, each 15–20 Å in length and oriented 100° relative to each other (Fig. 4a).

Electron density was observed for the entire MPER, ranging from Trp 666 within the N-terminal helix through to Arg 683 of the C-terminal helix (Supplementary Fig. 7). Analysis of main-chain dihedral angles (Supplementary Table 9) indicated that the N-terminal \( \alpha \)-helix extends from residue Asn 657 to Ala 667, tightens into a 3\( \alpha \)-helix from residues Ser 668 and Leu 669, before turning at residues Trp 670 and Asn 671. The C-terminal \( \alpha \)-helix, capped by Asn 671, starts at residue Trp 672 and extends to residue Arg 683, the final residue of the MPER (Fig. 4a, b).

The 10E8 antibody contacts the gp41 MPER primarily through its heavy chain, although crucial contacts are also mediated by the light chain CDR L3 (Fig. 4a–c and Supplementary Tables 10–12). Three predominant loci of interaction are observed between the antibody and gp41 (Supplementary Tables 13 and 14): one between residues of the tip of the CDR H3 loop and the tip of the C-terminal helix of the peptide, a second between residues of the CDR H2 loop and residues of the hinge region of the peptide, and a third at the juncture of the three heavy-chain CDR loops and the light chain CDR L3, which form a hydrophobic cleft that holds residues of the beginning of the MPER C-terminal helix (Fig. 4b).

10E8–gp41 interface

To complement the results observed for the mutagenesis of the highly conserved 10E8 epitope (Fig. 4d, f, h and Supplementary Tables 2 and 3), each residue of the 10E8 paratope, as determined from the crystal structure, was individually mutated to alanine and the resulting 25 10E8 variants assessed for affinity to a soluble MPER peptide. Overall, the most pronounced effects of the alanine mutations on the binding affinity of 10E8 to a soluble MPER peptide occurred within residues of the CDR H3 loop, although mutations between residues Ser 668 and Leu 669, before turning at residues Trp 670 and Asn 671. The C-terminal \( \alpha \)-helix, capped by Asn 671, starts at residue Trp 672 and extends to residue Arg 683, the final residue of the MPER (Fig. 4a, b).

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![Figure 4](https://i.imgur.com/4E10.png)
within the hydrophobic cleft also showed substantial effects (Fig. 4e, Supplementary Table 15 and Supplementary Fig. 8), 10E8 residues identified by an alanine scan as critical for the interaction with gp41 stretched from the cleft all the way to the tip of the CDR H3 (Fig. 4e) and were mirrored by a corresponding stretch of gp41 residues that substantially affected 10E8 binding when mutated to alanine (Fig. 4f).

The same panel of 10E8 alanine mutations was tested for neutralization potency against a panel of five Env pseudoviruses that included both tier 1 and tier 2 viruses (Supplementary Table 16). Similar to the binding data, residues of the 10E8 CDR H3 had marked effects on neutralization, as did residues of the hydrophobic cleft (Fig. 4g). Generally, $K_m$ values of paratope mutants correlated with neutralization (Supplementary Fig. 9). Backbone interactions (on both 10E8 and gp41) also contribute to the interface, especially between the CDR H2 of 10E8 and the hinge region of the MPER, although these are silent in alanine scan analyses. Overall, 10E8 uses a narrow band of residues (~20 X 5 Å) that stretches from the CDR H1 and H2 and extends along most of the CDR H3 to recognize a string of highly conserved hydrophobic gp41 residues, along with a critical charged residue (Arg/Lys 683), that occurs just before the transmembrane region (Fig. 4f, h).

**A conserved gp41–neutralization determinant**

Several structures of neutralizing antibodies in complex with the MPER of gp41 have been reported previously, including those for antibodies 2F5, Z13e1 and 4E10 (Supplementary Fig. 10a). The MPER adopts divergent loop conformations when bound by 2F5 and Z13e1 and an α-helix when bound by 4E10. Comparison of 2F5, Z13e1 and 4E10 epitopes with 10E8-bound gp41 revealed that only the 4E10 epitope has similar secondary structure, with superposition yielding a root mean squared deviation (r.m.s.d.) of 2.49 Å for all atoms of residues 671–683 and 0.98 Å for main-chain atoms (Supplementary Fig. 10b and Supplementary Table 17).

To further compare the recognition of gp41 by 10E8 and 4E10, we examined their angles of epitope approach. As shown in Supplementary Fig. 10c–f, alignment of the recognized MPER helix places 10E8 and 4E10 into similar overall spatial positions. The relative orientations of the recognized helix and the heavy and light chains of the two antibodies, however, differ markedly. With 10E8, the C-terminal helix is perpendicular to the plane bisecting the heavy and light chains (Supplementary Fig. 10c, e); with 4E10, the recognized helix is at the interface between the heavy and light chains (Supplementary Fig. 10d, f). Perhaps relevant to this, 10E8 uses CDR loops almost exclusively in its recognition of gp41, whereas 4E10 incorporates substantial β-strand interactions with gp41.

The differing modes of 10E8 and 4E10 recognition of the conserved C-terminal MPER helix result in a substantial difference in the proportion of the recognized helical face: 10E8 contacts roughly one-third of the helical face, whereas 4E10 contacts over half (Supplementary Fig. 10d, f). Perhaps relevant to this, 10E8 uses CDR loops almost exclusively in its recognition of gp41, whereas 4E10 incorporates substantial β-strand interactions with gp41.

**Sequence variation and 10E8 neutralization**

To place the specificity and structural data into the context of known variation of the MPER, we analysed viral sequences with resistance to 10E8 (Fig. 5a). Of the 181 tested viruses, only 3 were highly resistant to 10E8 with $IC_{50} > 50$ μg ml$^{-1}$. Each of these viruses had substitutions at positions found to affect neutralization by alanine scanning (Asn 671, Trp 672, Phe 673 and Trp 680). Plasma virus from patient N152, from whom 10E8 was cloned, is also probably resistant to 10E8-mediated neutralization$^{40}$. Sequence analysis of plasma viral RNA revealed rare substitutions at positions Trp 680 and Lys/Arg 683 (Fig. 5a). These residues are typically highly conserved with variation only occurring in 1.17% of 3,730 HIV Env sequences in the Los Alamos Database (http://www.hiv.lanl.gov). When the substitutions for the three resistant viruses and the patient virus were placed on the background of the sensitive JR2 virus, substitutions at Asn 671Thr, Trp 672Leu and Phe 673Leu had a modest effect on the IC$_{50}$ values that are ~20-fold compared with JR2 wild-type pseudovirus are highlighted in yellow. Error bars denote one s.e.m.

**A site of gp41 vulnerability.** a. Impact of sequence variation on 10E8 neutralization. Predicted amino acid sequences within the binding epitope of 10E8 for three 10E8-resistant viruses and the patient virus are shown. The 10E8 epitope and differences in sequence compared to the JR2 virus are labelled in red. IC$_{50}$ and IC$_{80}$ values that are ~20-fold compared with JR2 wild-type pseudovirus are highlighted in yellow. Error bars denote one s.e.m.

b. Structural definition of a highly conserved region of gp41 recognized by neutralizing antibodies. Atoms of highly conserved residues that make direct contacts with 10E8 (crystal complex 1) are coloured red and shown in stick representation. Remaining atoms buried by 10E8 are coloured purple, and those making main-chain contacts are coloured cyan. Semi-transparent surfaces of the gp41 MPER are coloured according to the underlying atoms. 90° views are shown, with bond antibody 10E8 in the right panel. The 10E8 CDR H3 interacts with highly conserved hydrophobic residues, whereas the CDR H2 contacts main-chain atoms at the juncture between the N- and C-terminal helices. Many of the unbound residues of the MPER (grey) are hydrophobic, especially those within the C-terminal helix. In the structure of the late fusion intermediate (Supplementary Fig. 11), gp41 residues that contact 10E8 largely face the outside of the helical coiled-coil.
found in the 4E10 epitope, the additional 10E8-bound residue Lys/Arg683 is critical to neutralization. In addition to other differences in binding based on structural analyses noted above, it is possible that the additional potency of 10E8 compared to 4E10 against naturally occurring viral variants may be mediated through binding of highly conserved residues Trp 680 and Lys/Arg 683 that directly interact with the 10E8 CDRH3.

Discussion

10E8 is a broad and potent neutralizing antibody with important implications for efforts to induce such antibodies with vaccines. Previous MPER antibodies were limited in potency, and had a more limited ability to access MPER on Env of primary isolates. In addition, lipid binding and autoreactivity were thought to be characteristics of MPER antibodies and important obstacles to their elicitation by vaccines. However, 10E8 lacks each of these characteristics. In addition, antibodies with a similar specificity were not reported in our chronically infected cohort. This suggests that 10E8-like antibodies were not deleted from the repertoire because of autoreactivity. These results further suggest that 10E8-like antibodies might be raised in a larger fraction of HIV-infected persons receiving a vaccine designed to elicit these antibodies without the B-cell defects of chronic HIV infection. Design of such a vaccine will probably require not only presentation of an intact 10E8 epitope but also use of a platform sufficiently immunogenic to drive the evolution of 10E8-like antibodies.

The extraordinary breadth and potency of 10E8 seems to be mediated by its ability to bind highly conserved residues within MPER. Although the epitope of 10E8 overlaps those of known monoclonal antibodies such as 4E10, it differs in recognition surface, angle of approach, lipid binding and self-reactivity. Alanine scanning, structural analysis and paratope analysis each indicate that 10E8 makes crucial contacts with highly conserved residues Trp 672, Phe 673, Trp 676 and Lys/Arg 683. The extraordinary breadth of some potent monoclonal antibodies, for example that bind the CD4 binding site, is thought to be conferred by blocking a functionally important site that is critical for viral entry. Whether 10E8 impairs Env function or simply acts by binding highly conserved residues remains to be determined. Nonetheless, the breadth and potency of 10E8 demonstrates a conserved site of gp41 vulnerability (Fig. 5b) that is an important target antigen for HIV neutralization and that will probably reinvestigate interest in MPER-based HIV vaccine design.

METHODS SUMMARY

Peripheral blood CD19+ IgM- IgD- IgA- B cells were sorted by flow cytometry, plated at 4 cells per well, and expanded with cytokines and feeder cells. B-cell culture supernatants were screened by microneutralization against HIV MN.03 and HIV 1B 2.2S pseudoviruses. IgG genes from wells with neutralization activity were cloned and re-expressed in 293T cells. Breadth of neutralizing activity was confirmed against a 181-isolate Env pseudovirus panel. Specificity was determined by alanine scanning peptides and mutant Env pseudoviruses. Lipid binding and autoreactivity of 10E8 were measured by SPR plasmon resonance, indirect immunofluorescence on HeP-2 cells and bead arrays. Binding of HIV envelopes on transfected 293T cells was detected by flow cytometry. After pre-incubation with antibody, the impact of washing virions before infecting TZM-bl cells was used to measure access to viral MPER. The frequency of HIV-1 sera with a given specificity was measured by the ability to neutralize HIV-2/HIV-1 chimaeras containing portions of the MPER. 10E8 was co-crystallized with a peptide encompassing the entire 28-residue gp41 MPER (residues 656–683). Structure determination revealed two complexes in the crystal asymmetric unit. Analysis of differences between the two complexes enabled essential interactions to be discerned. The paratope, as defined by residues in the antibody that showed reduced solvent accessibility when in complex with gp41, was subjected to comprehensive alanine scan, with each of the 25 10E8 alanine mutants assessed by SPR for recognition of gp41 and for neutralization of a panel of 5 pseudotyped viruses. The sequence of the patient plasma viral RNA was derived using limiting dilution RT–PCR.

Full Methods and any associated references are available in the online version of the paper.

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1. Doria-Rose, N. A. et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J. Virol. 84, 1631–1636 (2010).
2. Stamatatos, L., Morris, L., Burton, D. R. & Mascola, J. R. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nature Med. 15, 866–870 (2009).
3. Sather, D. N. et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J. Virol. 83, 757–769 (2009).
4. Walker, L. M. et al. A limited number of antibody specificities mediate broad and potent neutralization in selected HIV-1-infected individuals. PLoS Pathog. 6, 1001028 (2010).
5. Simek, M. D. et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J. Virol. 83, 7337–7348 (2009).
6. Binley, J. Specificity of broadly neutralizing anti-HIV-1 sera. Curr. Opin. HIV AIDS 4, 364–372 (2009).
7. Moore, P. L. et al. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. J. Virol. 85, 3128–3141 (2011).
8. Gray, E. S. et al. Antibody specificities associated with neutralization breadth in plasma from human immunodeficiency virus type 1 subtype C-infected blood donors. J. Virol. 83, 8925–8937 (2009).
9. Haynes, B. F., Kelsoe, G., Harrison, S. C. & Kepler, T. B. Cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nature Biotechnol. 30, 423–433 (2012).
10. Walker, L. M. & Burton, D. R. Rational antibody-based HIV-1 vaccine design: current approaches and future directions. Curr. Opin. Immunol. 22, 358–366 (2010).
11. Zwick, M. B. et al. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J. Virol. 75, 10982–10905 (2001).
12. Burton, D. R. et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 266, 1024–1027 (1994).
13. Mustier, T. et al. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. 67, 6642–6647 (1993).
14. Bonsignori, M. et al. Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. J. Virol. 86, 4688–4692 (2012).
15. Wu, X. et al. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 333, 1593–1602 (2011).
16. Scheid, J. F. et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science 333, 1633–1637 (2011).
17. Wu, X. et al. Rational design of envelope identify broadly neutralizing human monoclonal antibodies to HIV-1. J. Virol. 85, 856–861 (2010).
18. Walker, L. M. et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477, 466–470 (2011).
19. Bonsignori, M. et al. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J. Virol. 85, 10098–10109 (2011).
20. Walker, L. M. et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326, 285–289 (2009).
21. Miller, T. et al. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J. Immunol. Methods 329, 112–124 (2008).
22. Kabat, E. A., Foeller, C., Gottesman, K. S., Pery, H. M. & Wu, T. T. Sequences of Proteins of Immunological Interest: Tabulation and Analysis of Amino Acid and Nucleic Acid Sequences of Precursors, V-regions, C-regions, J-chain, T-cell Receptors for Antigen T-cell Surface Antigens, β2-microglobulins, Major Histocompatibility Antigens, Thy-1, Complement, C-reactive Protein, Thymopoietin, Integins, Post-α globulin, α2-macroglobulins, and Other Related Proteins 5th edn (US Dept. Health and Human Services, Public Health Service, National Institutes of Health, 1991).
23. Brune, F. M. et al. Structure-function analysis of the epitope for 4E10, a broadly neutralizing human immunodeficiency virus type 1 antibody. J. Virol. 80, 1680–1687 (2006).
24. Zwick, M. B. et al. Anti–human immunodeficiency virus type 1 (HIV-1) antibodies 2F5 and 4E10 require surprisingly few crucial residues in the membrane-proximal external region of glycoprotein gp41 to neutralize HIV-1. J. Virol. 79, 1252–1261 (2005).
25. Gray, E. S. et al. Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. J. Virol. 81, 6187–6196 (2007).
26. Tonnaras, G. D. et al. Polyclonal B cell responses to conserved neutralization epitopes of individuals of HIV-1-infected donors. J. Virol. 85, 1593–1602 (2011).
27. Morris, L. et al. Isolation of a human anti-HIV gp41 membrane proximal region neutralizing antibody by antigen-specific single B cell sorting. PLoS ONE 6, e23532 (2011).
28. Gray, E. S. et al. Broad neutralization of human immunodeficiency virus type 1 mediated by plasma antibodies against the gp41 membrane proximal external region. J. Virol. 83, 11265–11274 (2009).
HIV-1 antibodies. 

29. Haynes, B. F. et al. Cardiolipin polyclonal autoantibodies in two broadly neutralizing HIV-1 antibodies. *Science* **308**, 1906–1908 (2005).

30. Alam, S. M. et al. Role of HIV membrane in neutralization by two broadly neutralizing antibodies. *Proc. Natl Acad. Sci. USA* **106**, 20234–20239 (2009).

31. Chakrabarti, B. K. et al. Direct antibody access to the HIV-1 membrane-proximal external region positively correlates with neutralization sensitivity. *J. Virol.* **85**, 8217–8226 (2011).

32. Frey, G. et al. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. *Proc. Natl Acad. Sci. USA* **105**, 3739–3744 (2008).

33. Rathinakumar, R., Dutta, M., Zhu, P., Johnson, W. E. & Roux, K. H. Binding of anti-human immunodeficiency virus type 1 antibodies to CD4-ligated and -unliganded human immunodeficiency virus type 1 and simian immunodeficiency virus virions. *J. Virol.* **86**, 1820–1831 (2012).

34. Rupecht, C. R. et al. MPER-specific antibodies induce gp120 shedding and irreversibly neutralize HIV-1. *J. Exp. Med.* **208**, 439–454 (2011).

35. Julien, J. P., Bryson, S., Nieva, J. L. & Pai, E. F. Structural details of HIV-1 recognition by the broadly neutralizing monoclonal antibody 2F5: epitope conformation, antigen-recognition loop mobility, and anion-binding site. *J. Mol. Biol.* **384**, 377–392 (2008).

36. Cardoso, R. M. et al. Structural basis of enhanced binding of extended and helically constrained peptide epitopes of the broadly neutralizing HIV-1 antibody 4E10. *J. Mol. Biol.* **365**, 1533–1544 (2007).

37. Cardoso, R. M. F. et al. Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly conserved fusion-associated motif in gp41. *Immunity* **22**, 163–173 (2005).

38. Olek, G. et al. Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. *J. Virol.* **78**, 10724–10737 (2004).

39. Pejchal, R. et al. A conformational switch in human immunodeficiency virus gp41 revealed by the structures of overlapping epitopes recognized by neutralizing antibodies. *J. Virol.* **83**, 8451–8462 (2009).

40. Wu, X. et al. Selection pressure on HIV-1 envelope by broadly neutralizing antibodies to the conserved CD4-binding site. *J. Virol.* **86**, 5844–5856 (2012).

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Author Contributions M.C., J.H., L.L., G.O., J.R.M. and P.D.K. designed the study, analysed the data, and prepared this manuscript. J.H. and L.L. performed B-cell sorting, antibody cloning, epitope mapping assay, MPER-specific neutralizing sera screening and assessed the impact of sequence variation on 10E8 neutralization. M.K.L. and J.R.M. tested the breadth and potency of 10E8. B.C. performed the autoreactivity assays. G.O., Y.Y. and P.D.K. performed 10E8 structural analysis, with T.W. and B.Z. assisting with paratope alanine scanning. R.T.B. screened the B-cell culture supernatants for neutralization activity. H.I. sequenced the patient nucleotide sequence of 10E8 heavy and light chains have been submitted to GenBank under accession numbers JX645769 and JX645770. Coordinates and structure factors for 10E8 Fab in complex with the gp41 MPER have been deposited with the Protein Data Bank under accession code 4G6F. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.C. (mconnors@nih.gov).
METHODS

Study patients. We selected the plasma and peripheral blood mononuclear cells (PBMCs) from the HIV-1-infected patients enrolled in the National Institute of Health under a clinical protocol approved by the Investigational Review Board in the National Institute of Allergy and Infectious Diseases (NIAID-IRB). All participants signed informed consent approved by the NIAID-IRB. The criteria for enrolment were as follows: having a detectable viral load, a stable CD4 T-cell count above 300 cells μl⁻¹, being diagnosed with HIV infection for at least 4 years, and off antiretroviral treatment for at least 5 years. On the basis of the locations of current and former residences, all patients were presumed to be infected with clade B virus. Donor N152 was selected for B-cell sorting and antibody generation because his serum neutralizing activity is among the most potent and broad in our cohort. He is a slow progressor based on criteria described previously. At the time of leukapheresis, he had been infected with HIV-1 for 20 years, with CD4 T-cell counts of 325 cells μl⁻¹, plasma HIV-1 RNA values of 3,811 copies ml⁻¹ and was not on antiretroviral treatment.

Viruses and plasmids. HIV-1 JR2 MPER alamine mutant pseudovirus plasmids were obtained from M. Zwick (The Scripps Research Institute). HIV-2/HIV-1 chimaeras were provided by G. Shaw and L. Morris.

Memory B-cell staining, sorting and antibody cloning. Staining and single-cell sorting of memory B cells were performed as follows. PBMCs from HIV-1-infected donors N152 and N151 were stained with antibody cocktail consisting of anti-CD19-PE–Cy7 (BD Bioscience), IgG–APC (Jackson ImmunoResearch Laboratories Inc.), IgD–FITC (BD Pharmingen) and IgM–PE (Jackson ImmunoResearch Laboratories Inc.) at 4 °C in dark for 30 min. The cells were then washed with 10 ml PBS-BSA buffer and re-suspended in 500 μl PBS-BSA. A total of 66,000 CD19+ IgG1+ IgD+ IgM+ memory B cells were sorted using a FACSaria III cell sorter (Becton Dickinson) and re-suspended in IMDM medium with 10% FBS containing 100 μM IL-2, 20 ng ml⁻¹ IL-21 and 1 × 10⁻³ ml⁻¹ irradiated 3T3-mscDL40 feeder cells. B cells were seeded into 384-well microtitre plates at a density of 4 cells per well in a final volume of 50 μl. After 13 days of incubation, 40 μl of culture supernatants from each well were collected and screened for neutralization activity using a high-throughput micro-neutralization assay against HIV-1 MN, and HIV-1 YU2. B cells in each well were lysed with 20 μl lysis buffer containing 0.25% of RNase inhibitor (New England Biolabs Inc.), 0.3% of 1 μM Tris pH 8 (Quality Biological Inc.) and 19.45 μl DEPC-treated H₂O. The plates with B cells were stored at −80 °C.

The variable region of the heavy chain and the light chain of the immunoglobulin gene were amplified by RT–PCR from the wells that scored positive in both the HIV-1MN, and HIV-1 YU2 Neutralization assay. The cDNA product was used as template in the PCR reaction. To amplify the highly somatically mutated immunoglobulin gene, two sets of primers as described previously were used in two independent PCRs. One set of primers consisted of the forward primers and the reverse primers specific for the leader region and constant region of IgL, IgX or IgG, respectively. The other set of primers consisted of the forward primer mixes specific for FRW1 and respective reverse primers specific for the IgH, IgX and IgG set of primers. All PCRs were performed in 96-well PCR plates in a total volume of 50 μl containing 20 nM each primer or primer mix, 10 nM each dNTP (Invitrogen), 10 μl 5× Q-solution (Qiagen) and 1.2 μl HotStar Taq DNA polymerase (Qiagen). From the positive PCR reactions, pools of the VH or VL–region DNA were ligated to a pcR2.1-Topo–TA vector (Invitrogen) for sequencing before cloning into the corresponding IgL1, IgX1 and IgG1 expression vector. 10 μg of heavy and light chain plasmids, cloned from the same well and combined in all possible heavy and light chain pairs, were mixed with 40 μl FuGENE 6 (Roche) in 1,500 μl DMEM (Gibco) and co-transfected into 293T cells. The full-length IgG was purified using a recombinant protein-A column (GE Healthcare).

Neutralization assays. Neutralization of the monoclonal antibodies was measured by using single-round HIV-1 Env-pseudovirus infection of TZM-bl cells. HIV-1 Env pseudoviruses were generated by co-transfection of 293T cells with pSG3 delta Env backbone and a second plasmid that expressed HIV-1 Env. At 72 h after transfection, supernatants containing pseudovirus were harvested and frozen at −80 °C until further use. In the neutralization assay, 10 μl of fivefold serially diluted patient serum or monoclonal antibody was incubated with 40 μl of pseudovirus in a 96-well plate at 37 °C for 30 min before addition of TZM-bl cells. After 2 days of incubation, cells were lysed and the viral infectivity was quantified by measuring luciferase activity with a Victor Light luminometer (Perkin Elmer). The 50% inhibitory concentration (IC₅₀) was calculated as the antibody concentration that reduced infectivity by 50%. Supernatants containing virus were captured using immobilized HIV-1 gp120, and purified using HIV-2/HIV-1 chimaera containing different portions of HIV-1 MPER, such as C1 (HIV-2 Env with HIV-1 MPER), C1C (HIV-2 Env with clade C MPER), C3 (HIV-2 Env with HIV-2 Env with short 2F5 epitope), C4 (HIV-2 Env with HIV-1 gp120 epitope), C6 (HIV-2 Env with short 2F5 epitope NWFDDIT), C7 (HIV-2 Env with short 2F5 epitope ALDKWA) and C8 (HIV-2 Env with both Z13 and 4E10 epitope). Fivefold diluted patient serum or monoclonal antibody was incubated with chimaera in a 96-well plate at 37 °C for 30 min before addition of TZM-bl cells. The specificities within patient sera were confirmed by blocking neutralization of the C1 chimaera with 25 μg ml⁻¹ of 2F5, 4E10, MPER, Bal.V3, control peptide, or 50 μg ml⁻¹ of Z13 peptide.

ELISA assays. Each antigen at 2 μg ml⁻¹ was coated on 96-well plates overnight at 4 °C. Plates were blocked with BLOTTO buffer (PBS, 1% FBS, 5% non-fat milk) for 1 h at room temperature, followed by incubation with antibody serially diluted in disruption buffer (PBS, 5% FBS, 2% BSA, 1% Tween-20) for 1 h at room temperature. 1:10,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody was added for 1 h at room temperature. The plates were washed between each step with 0.2% Tween 20 in PBS. Plates were developed using 3,3′,5,5′-tetramethylbenzidine (TMB) (Sigma) and read at 450 nm.

Autoreactivity assays. Binding of 10E8 to phospholipid was measured by SPR conducted on a BIAcore 3000 instrument and data analyses were performed using the BIAevaluation 4.1 software (BIAcore) as described previously. Phospholipid-containing liposomes were captured on a BIAcore L1 sensor chip, which uses an alkyl linker for anchoring lipids. Before capturing lipids, the surface of the L1 chip was cleaned with a 60-s injection of 40 mM octyl-β-D-glucopyranoside, at 100 mM, and the chip and fluids were washed with excess buffer to remove any traces of detergent. Monoclonal antibodies were then injected at 100 μg ml⁻¹ at a flow rate of 30 μl min⁻¹. After each antibody injection, the surface was again cleaned with octyl-β-D-glucopyranoside, and 5 s injections of each 5 mM HCl, then 5 mM NaOH, to clean any adherent protein from the chip.

Reactivity to HIV-1 negative human epithelial (HEp-2) cells was determined by indirect immunofluorescence on slides using Evans Blue as a counterstain and FITC-conjugated goat anti-human IgG (Zeus Scientific) Slides were photographed on a Nikon Optiphot fluorescence microscope. Regarding Fig. 3b, kochromes slides were taken of each monoclonal antibody binding to HEp-2 cells at a 10-s exposure, and the slides scanned into digital format. The Luminex AlexaNeta Multi-Lyte ANA test (Wampole Laboratories) was used to test for monoclonal antibody reactivity to SSA/Ro, SS-B/La, Sm, ribonucleoprotein (RNP), Jo-1, double-stranded DNA, centromere B, and histone and was performed as per the manufacturer’s specifications and as previously described. Monoclonal antibody concentrations assayed were 50, 25, 12.5 and 6.25 μg ml⁻¹. 10 μl of each concentration was incubated with 10 μl of the respective fluorescent beads and the test performed per the manufacturer’s specifications.

Fluorescence-activated cell sorting (FACS) staining of cell-surface HIV-1 Env. FACS staining was performed as previously described. Forty-eight hours after transfection, cells were collected and washed in FACS buffer (PBS, 5% HIFBS, 0.02% azide) and stained with monoclonal antibodies. The transfected cells were suspended in FACS buffer and were incubated with the antibodies for 1 h at room temperature. The monoclonal antibody-cell mixture was washed extensively in FACS buffer and phycocerythrin (PE)-conjugated goat anti-human secondary antibody (Sigma) was added for 1 h at a 1:200 dilution, followed by extensive washing to remove unbound secondary antibody. The PE-stained cells were acquired on a BD LSRII instrument and analysed by Flowjo.

Antibody-virus washout experiments. From a starting concentration of 2 mg ml⁻¹, 12.5 μl of fivefold serially diluted antibodies in PBS were added to 487.5 μl of DMEM containing 10% heat-inactivated FBS and 15 μl of pseudovirus such that the final concentrations of antibodies were 50 μg ml⁻¹ to 0.08 μg ml⁻¹ in a total volume of 500 μl. In the ‘no inhibitor’ control, the same volume of PBS was added instead of antibody. The reaction mixture was incubated for 30 min at 37 °C. The 250 μl reaction mixture was diluted to 10 ml with complete DMEM, centrifuged at 25,000 r.p.m. in a SW41 rotor, for 2 h at 4 °C. The virus pellet was washed twice with 10 ml of PBS. During the washing steps, the virus–antibody complex was centrifuged at 40,000 r.p.m. for 20 min at 4 °C. After the final wash, 250 μl of DMEM was added to the washed virus pellet and it was re-suspended by gentle shaking at 4 °C for 30 min. A total of 100 μl of the suspended virus was used to infect 100 μl of TZM-bl cells (0.2 million per ml), in

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duplicate. From the remaining 250 µl of reaction mixture, an equal volume of the antibody virus mixture was used as a ‘no washout’ control. Plates were incubated at 37 °C in a CO₂ incubator for 2 days. After 2 days, the luciferase assay was done as described previously⁴⁷. The data were then plotted to determine the neutraliza-

Structure determination and analysis. The antigen binding fragment of 10E8 (Fab) was prepared using LysC digestion, as previously described⁴⁷. The IgG was first reduced with 100 µM dithiothreitol (DTT) for 1 h at 37 °C, followed by 1 h of dialysis in HEPES, pH 7.6, to reduce the DTT concentration to 1 mM. Antibodies were then dialysed against 2 mM iodoacetamide for 48 h at 4 °C, and subjected to a final dialysis against HEPES, pH 7.6, for 2 h. After reduction and alkylation, anti-

Binding affinities of 10E8 and 10E8 variants to the gp1 MPER. A biotinylated Surface-Plasmon Resonance (SPR) (Biacore T200, GE Healthcare) was used to assess binding affinity of wild-type 10E8 to a gp41 MPER peptide. A biotinylated peptide composed of residues 656–683 of the gp41 MPER (RRR-NEQELLELDKWASLWNWFDITNWLWYIR-RRK-biotin; American Peptide) was coupled to a biacore SA chip to a surface density of 20–50 response units (RU). Fab was purified by ion exchange (Mono S) and size-exclusion chromatography (S200). Purified 10E8 Fab was incubated with tenfold excess peptide RRR-NEQELLELDKWASLWNWFDITNWLWYIR-RRR (American Peptide) and the complex then set up for 20 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, with the final extension at 72 °C for 7 min. The PCR products were purified with the QIA quick PCR purification kit (Qiagen), and then cloned into pCR2.1-TOPO vector (TOPO TA Cloning it, Invitrogen) for sequence analysis of individual molecular clones. The DNAs from 18 independent clones were sequenced with the ABI BigDye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems) and analysed with the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Statistical analysis. The relationship between the potency of N152 patient serum and 10E8, and the relationship between 10E8 variant binding and neutralization were evaluated by the Spearman rank method.

41. Migueles, S. A. et al. Lytic granule loading of CD8⁺ T cells is required for HIV-infected cell elimination associated with immune control. Immunity 29, 1009–1021 (2008).

42. Kershaw, M. H. et al. Immunization against endogenous retroviral tumor-associated antigens. Cancer Res. 61, 7920–7924 (2001).

43. Li, M. et al. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. J. Virol. 79, 10108–10125 (2005).

44. Koch, M. et al. Structure-based, targeted diglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. Virology 313, 387–400 (2003).

45. Mascola, J. R. et al. Human immunodeficiency virus type 1 neutralization measured by flow cytometric quantification of single-round infection of primary human T cells. J. Virol. 76, 4810–4821 (2002).

46. Ofek, G. et al. Elicitation of structure-specific antibodies by epitope scaffolds. Proc. Natl Acad. Sci. USA 107, 17880–17887 (2010).

47. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. Acta Crystallogr. D 54, 22–30 (1998).