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Crystal structure of a non-neutralizing antibody to the HIV-1 gp41 membrane-proximal external region

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Abstract

The monoclonal antibody 13H11 shares part of its epitope in the HIV-1 gp41 membrane-proximal external region (MPER) with the rare, broadly neutralizing human antibody 2F5. Although 13H11 partially cross-blocked 2F5 binding, 13H11 is non-neutralizing and does not block 2F5 neutralization. We show that unlike 2F5, 13H11 binds to a well-defined helical MPER structure that is consistent with the structure of gp41 in a post-fusion six-helix bundle conformation.

Two rare human monoclonal antibodies, 2F5 and 4E10, broadly neutralize HIV-1 and recognize epitopes on the gp41 membrane-proximal external region (MPER)^{1,2}. The epitope of the non-neutralizing mouse monoclonal antibody (mAb) 13H11 includes the 2F5 core epitope motif gp41_{664–666} DKW^{1–5} and additionally requires Leu669 and Asn671 (ref. 6). Both 2F5 (refs. 7–9) and 4E10 (refs. 8–10) mAbs are polyreactive with lipids and have been proposed to initially bind virion lipids in order to facilitate a second step of high-affinity binding to the MPER when gp41 is likely in the pre-hairpin intermediate conformation during Env-mediated host cell–virion fusion^{11,12}. With overlapping epitopes, 13H11 cross-blocks 2F5 mAb binding to gp41 (ref. 13); however, although the lipid-reactive 2F5 and 4E10 mAbs bind to lipid-gp41 MPER peptide conjugates^{14,15}, 13H11 only interacts with the

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AUTHOR CONTRIBUTIONS

N.I.N. purified proteins and carried out all crystallographic processes and analyses. H.C. and H.-X.L. designed the expression system and produced proteins. S.M.D. and S.M.A. did the binding kinetics measurements and analyses. L.S. assisted in review of the data. B.F.H. and R.M.S. made the mAb 13H11. Y.U. and G.K. sequenced mAb 13H11 VH and VL genes. B.F.H. was responsible for project design and leadership. N.I.N., S.M.A. and B.F.H. wrote the manuscript. N.I.N., S.M.D. and S.M.A. generated the figures.

Accession codes. Coordinates and structure factors for the 13H11–gp41_{652–671} complex have been deposited in the Protein Data Bank with the accession code 3MNW.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

MPER as a free peptide and neither binds peptide-lipid conjugates nor exhibits association with lipids^{11,13}. We set out to address the question of how antigen recognition of a non-neutralizing antibody differs from recognition of broadly neutralizing antibodies that target the HIV-1 gp41 MPER using a combined structural and biochemical approach.

Crystals were obtained of 13H11 Fab complexed with a 20-mer gp41_{652–671} MPER peptide (Ac-QQEKNEQELLELDKWASLWN-NH₂) that includes the 2F5 nominal epitope (Supplementary Tables 1 and 2). The primary structural difference between the non-neutralizing 13H11 mAb and the neutralizing 2F5 antibody structures is a large groove on the 13H11 idiotope between complementarity-determining regions (CDRs) L1 and L2 on the light chain and H1 and H2 on the heavy chain (Fig. 1 and Supplementary Figs. 1 and 2). This cleft is due in large part to the absence of an appreciable CDR-H3 that has a length of four residues (GlyTrpLeuHis)—just enough to form a β -turn between strands F and G of the core IgG fold. In contrast to the long, relatively hydrophobic CDR-H3 of 2F5, the apex of 13H11's CDR-L1 features several amino acid residues with charged and mobile side chains: the sequence from residues 30A to 31 is NSRNERN. As the CDR-H3 on 2F5 (ref. 2) plays a role in interacting with virion lipid^{8,12}, the highly charged CDR-L1 of 13H11 is likely the structural feature of the Fab that precludes lipid-binding activity by 13H11.

The gp41_{652–671} peptide bound in an α -helical conformation to 13H11 Fab (Fig. 2). There was good shape complementarity between 13H11 and the helical MPER peptide with a high shape complementarity statistic (*Sc*) of 0.79 (ref. 16) and an approximately 1,400 Å² surface area buried in the interface. The groove itself shows a mixed charge character, with a hydrophobic pocket within the CDR-H3 region. Specific polar contacts between CDR-L1 and the peptide included H-bonds between the side chains of Arg^{L30F} (where L in the superscripts denotes the light chain and 30F denotes the residue number; residues on the gp41_{652–671} peptide will continue to appear in normal text) on the mAb and Asp664 on the peptide (refer to Supplementary Results for additional detail). The β -hairpin of CDR-L1's canonical class 3 fold was oriented so as to protrude outward from the core IgG fold, creating one wall of the groove.

In previous kinetic studies⁶, three alanine mutations eliminated binding of 13H11 to the gp41_{652–671} peptide: K665A, L669A and N671A (refer to Supplementary Methods). From our crystal structures, several polar contacts were evident between the side chain of Asn671 and the Fab. The role of Leu669 appeared to be the energetically favorable burial of its hydrophobic side chain into a pocket within the CDR-H3 region created by Trp666, Leu^{H101} and Leu^{H94} (Fig. 2 and Supplementary Fig. 3). The backbone carbonyl of Leu669 also formed H-bonds with a backbone amide on the Fab, stabilizing the complex. The role of Lys665 is less clear, but it appears to result from a favorable entropic effect of associating the aliphatic part of the side chain with hydrophobic contacts, facilitated by mitigation of the side-chain charge through a salt bridge with the side chain of Glu662. The alanine scanning results showed E662A reduced 13H11 binding to the MPER by ~66% (ref. 6).

Although 13H11 mAb binding epitope includes the 2F5 core tripeptide (AspLysTrp), it binds to MPER peptides with lower affinities than 2F5 (ref. 13). There is a roughly tenfold difference in the *K_d* of the two mAbs for binding to the linear gp41_{652–671} peptide

(Supplementary Fig. 4b). 13H11 also binds more strongly and with a slower dissociation rate to the full-length heptad repeat II (HR2) peptide DP178 (gp41643–678 with 35 residues) than to shorter versions of the MPER¹³. Because linear peptides in solution often adopt multiple conformations (Supplementary Fig. 4a)³, 13H11 may selectively bind to the preferred α -helical conformation, which may not be equally represented in each of the gp41 MPER peptides studied. The neutralizing determinant of 2F5 has been proposed to be presented on the transient pre-hairpin intermediate conformation of gp41 because 2F5 binds gp41-inter strongly but does not bind to pre-fusion Env on intact virions^{8,12}. However, no binding of 13H11 was observed with the trimeric gp41-inter construct or to the gp41-inter-liposome complex, which 2F5 bound with high affinity (Supplementary Fig. 4c,d). These results suggest either that the 13H11 epitope is not presented in an α -helical conformation on gp41-inter or that access to the helical epitope is obscured by the trimer interface. Because 13H11 also does not bind to intact virions^{8,12} or block 2F5 neutralization (Supplementary Table 3), 13H11 epitope is not exposed on pre-fusion Env and is likely precluded in the intermediate state of gp41. The lack of 13H11 ability to bind to lipids will also deter 13H11 from interacting with MPER residues that are buried in the viral lipid membrane.

Several human gp41 antibodies that bind to the HR2 of gp41 can partially cross-block 2F5, but unlike 2F5, bind strongly to oligomeric gp140 or to complexes of N- and C-terminal gp41 peptides^{12,13,17,18}. It has been suggested that post-fusion forms of gp41 recognized by non-neutralizing gp41 mAbs are present on intact HIV-1 virions as nonfunctional Env spikes, exposed following gp120 shedding^{19,20}. It has therefore been suggested that some non-neutralizing gp41 antibodies bind to the post-fusion complexes of HIV-1 envelope^{11,18}. The structural data appear to indicate this is the case with 13H11. Three residues in the gp41_{652–671} peptide—Asn656, Glu659, and Leu663—are pocket-binding residues involved in the folding and stabilization of the post-fusion six-helix bundle^{13–15}. These residues lie on the exposed face of the MPER α -helix, oriented away from the Fab in our structure. Moreover, in a structural superposition of the helical gp41_{652–671} peptide from the 13H11 complex structure with a recent six-helix bundle construct (Fig. 3), no major clashes are observed between 13H11 and the bundle except for the C-terminal part of the MPER after Leu669, where the helix unwinds in the 13H11 complex structure²¹.

The combined structural and kinetic studies suggest that before membrane fusion, 13H11's epitope on gp41 MPER is inaccessible because it is buried in lipid, is in a nonantigenic conformation or both^{6,11,14}. Even if the epitope is a transiently exposed helix in the pre-fusion conformational ensemble, the lack of a long 2F5-like hydro-phobic CDR-H3 would prevent 13H11 from interacting with the viral membrane. Thus, 13H11 cannot preconcentrate on the membrane as the required initial step in the proposed sequential two-step binding of the neutralizing 2F5 and 4E10 mAbs to gp41 MPER on virions⁸. Neutralizing antibodies to MPER may inhibit HIV-1 by binding and stabilizing the MPER in conformations that preclude folding of the six-helix bundle and subsequent membrane fusion (Supplementary Fig. 3). After fusion, the MPER presents as a stable α -helix in the six-helix bundle—a structure consistent with that of the gp41_{652–671} peptide as it appears in the 13H11 complex structure; therefore, recognition by 13H11 of MPER as an α -helix may fail to prevent the formation of the six-helix bundle, or 13H11 could miss the early pre-

fusion event entirely and bind the post-fusion six-helix bundle directly. Ultimately, 13H11 fails to neutralize HIV-1 because unlike 2F5, it binds only gp41 MPER α -helices reflective of the post-fusion six-helix bundle conformation and fails to prevent membrane fusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Stiegler G et al. *AIDS Res. Hum. Retroviruses* 17, 1757–1765 (2001).11788027
2. Ofek G et al. *J. Virol* 78, 10724–10737 (2004).15367639
3. Barbato G et al. *J. Mol. Biol* 330, 1101–1115 (2003).12860131
4. Cardoso RM et al. *Immunity* 22, 163–173 (2005).15723805
5. Zwick MB et al. *J. Virol* 75, 12198–12208 (2001).11711611
6. Shen X et al. *Proc. Natl. Acad. Sci. USA* 107, 5972–5977 (2010).20231447
7. Ofek G et al. *J. Virol* 84, 2955–2962 (2010).20042512
8. Alam SM et al. *Proc. Natl. Acad. Sci. USA* 106, 20234–20239 (2009).19906992
9. Scherer EM, Leaman DP, Zwick MB, McMichael AJ & Burton DR *Proc. Natl. Acad. Sci. USA* 107, 1529–1534 (2010).20080706
10. Haynes BF et al. *Science* 308, 1906–1908 (2005).15860590
11. Alam SM et al. *J. Immunol* 178, 4424–4435 (2007).17372000
12. Frey G et al. *Proc. Natl. Acad. Sci. USA* 105, 3739–3744 (2008).18322015
13. Alam SM et al. *J. Virol* 82, 115–125 (2008).17942537
14. Song L et al. *Proc. Natl. Acad. Sci. USA* 106, 9057–9062 (2009).19458040
15. Haynes BF & Alam SM *Immunity* 28, 10–12 (2008).18199412
16. Lawrence MC & Colman PM *J. Mol. Biol* 234, 946–950 (1993).8263940
17. Gorny MK & Zolla-Pazner S *J. Virol* 74, 6186–6192 (2000).10846104
18. Yuan W et al. *AIDS Res. Hum. Retroviruses* 25, 319–328 (2009).19292593
19. Poignard P et al. *J. Virol* 77, 353–365 (2003).12477840
20. Moore PL et al. *J. Virol* 80, 2515–2528 (2006).16474158
21. Buzon V et al. *PLoS Pathog* 6, e1000880 (2010).20463810

Figure 1.

Structure of 13H11 Fab. **(a)** 13H11 Fab structure (white) rendered to show surface features contributed to its idiotope by its CDRs. In this view, the presence of a large groove on the idiotope is apparent. **(b)** In a view head-on with respect to the idiotope (rotated 90° from **a**), it is seen that the groove is engendered by the short CDR-H3 and the long CDR-L1.

Figure 2.

Structure of 13H11 bound to MPER. **(a)** 13H11 (white surface) binds the gp41₆₅₂₋₆₇₁ peptide (gray ribbon) in an α -helical conformation. 13H11 epitope residues are highlighted (yellow). Residues in parentheses are behind the helix in this view. Sequences of the peptides gp41₆₅₂₋₆₇₁ in the 13H11 complex structure and gp41₆₅₄₋₆₇₀ crystallized with 2F5 (ref. 2) are indicated with respective epitope residues boxed. **(b)** To show shape and charge complementarity between Fab and peptide, the same view is shown as in **a** but with the Fab and peptide exploded, the peptide rotated by 180° in the plane and surfaces colored by electrostatic potential.

Figure 3.

Relevance to the post-fusion six-helix bundle. **(a)** Pocket binding residues (yellow highlights, labeled) on the gp41₆₅₂₋₆₇₁ peptide (gray ribbon) orient away from 13H11 (white surface) in the complex structure. **(b)** The orientation of the pocket binding residue side chains on the face of the helix directed away from the Fab is more evident in this view looking down the helical axis of the peptide. **(c)** A recent six-helix bundle structure (orange, light orange) superimposes the helical gp41₆₅₂₋₆₇₁ peptide with an r.m.s. deviation of 0.44 Å (ref. 21). Equivalent pocket binding residues from **b** on the bundle structure are shown in green.