Published in final edited form as:
Nat Med. 2007 September ; 13(9): 1032–1034. doi:10.1038/nm1624.

Broad HIV-1 neutralization mediated by CD4-binding site antibodies

Yuxing Li[1], **Stephen A Migueles**[2], **Brent Welcher**[1], **Krisha Svehla**[1], **Adhuna Phogat**[1], **Mark K Louder**[1], **Xueling Wu**[1], **George M Shaw**[3], **Mark Connors**[2], **Richard T Wyatt**[1,4], and **John R Mascola**[1,4]

1 *Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*

2 *Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*

3 *Departments of Medicine and Microbiology, University of Alabama, Birmingham, Alabama 35294, USA*

Abstract

We have identified several patient sera showing potent and broad HIV-1 neutralization. Using antibody adsorption and elution from selected gp120 variants, the neutralizing specificities of the two most broadly reactive sera were mapped to the primary receptor CD4–binding region of HIV-1 gp120. Novel antibodies to the CD4-binding site are elicited in some HIV-1–infected individuals, and new approaches to present this conserved region of gp120 to the immune system may result in improved vaccine immunogens.

The inability to induce antibodies capable of neutralizing diverse HIV-1 isolates has been one of the most important obstacles to the development of an effective AIDS vaccine[1,2]. Whereas current HIV-1 envelope protein (Env)–based vaccines do not elicit potent neutralizing antibody responses[1,3,4], the sera of a subset of HIV-1–infected subjects contain both potent and broadly reactive neutralizing antibodies[1,2]. Little is known about the antibody specificities that mediate this breadth of reactivity[5–7], and an understanding of how broad neutralization is accomplished could provide valuable insights for Env-based immunogen design. Several functionally conserved regions of the Env protein are potential targets of neutralizing antibodies, namely the binding site for the primary receptor CD4 and the chemokine coreceptor–binding site, both on HIV-1 gp120, and regions of HIV-1 gp41 involved in viral fusion to target cells. However, only a few broadly neutralizing monoclonal antibodies (mAb) have been isolated from infected patients over the past 20 years[1]. Two well-characterized mAb, 2F5 and 4E10, are directed against the gp41 membrane-proximal external region (MPER), which consists of 20–30 highly conserved residues that are important in virus fusion. Only two broadly neutralizing mAb are directed against the gp120 region of Env. One is mAb 2G12; it has an unusual domain-swap structure and recognizes a cluster of oligomannose residues on gp120. The second is mAb IgG1 b12; first isolated as an Fab fragment through phage-display technologies, it recognizes the functionally conserved gp120 CD4-binding site[2].

Correspondence should be addressed to R.T.W. (richardwyatt@nih.gov).

[4]These authors contributed equally to this work.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

We tested 32 HIV-1$^+$ sera from a well-characterized patient cohort[8] to identify those that can neutralize a diverse panel of viruses. Several sera neutralized most viruses from clades A, B and C, but two sera stood out as particularly potent and broadly reactive, sera 1 and 45 (Supplementary Fig. 1 online). These two sera were further evaluated to determine the viral epitopes targeted by their neutralizing antibodies. Because two of the most broadly reactive neutralizing mAb are directed against the MPER of gp41, assays were developed to test for neutralizing antibodies directed against this region. Neither neutralization assays based on competition with linear peptides specific for the MPER epitopes nor assays that used a chimeric HIV-2 virus engineered to contain the 25 residues of the HIV-1 MPER detected MPER-directed antibodies (data not shown). We therefore focused further mapping efforts on the gp120 surface. Serum was adsorbed with Env protein covalently linked to magnetic beads (Supplementary Methods online). Initial studies used a conformationally intact gp120 wild-type protein (gp120WT) and a denatured gp120 protein (gp120den). Adsorption with the clade B gp120WT, but not gp120den, removed neutralizing activity against clade B viruses as well as clade A and C viruses (Supplementary Fig. 2 online). These data suggest that the neutralizing activity in both sera is directed against a conformational epitope on gp120 that is conserved across clades.

To evaluate whether antibodies to the CD4-binding site are involved in neutralization, we generated two CD4-binding site–defective mutant Env proteins (Fig. 1a, left) by mutation of residues previously described as crucial for recognition by both CD4 and most CD4-binding site antibodies, including mAb b12 (refs. 9^{-12}). One protein contained a mutation of an aspartic acid residue to an arginine in the CD4-binding region of HIV-1 BaL gp120 (D368R) and is denoted gp120-D368R. The other contained two mutations on the HIV-1 YU2 gp120 backbone (aspartic acid 368 to alanine, D368A, and glutamic acid 370 to alanine, E370A) and is denoted YU2 gp120-D368A/E370A. We confirmed the integrity of the wild-type and mutant proteins, both before and after bead coupling, by testing their reactivity to known panels of HIV-1–specific antibodies (Fig. 1a, center and right). Figure 1b,c shows representative data for sera 1 and 45 adsorbed with both wild-type and mutant gp120s. Each protein adsorbed all measurable binding antibodies, as assessed by ELISA (Fig. 1b). The gp120WT proteins adsorbed a considerable fraction of serum neutralizing activity, whereas the CD4-binding site mutants had either no effect or a much reduced effect (Fig. 1c and Supplementary Table 1 online). These results suggest that both sera contain cross-clade neutralizing antibodies directed against the gp120 CD4-binding site.

To further characterize antibody specificities, gp120-specific IgG was eluted from each protein. We incorporated the YU2 gp120 core protein into the analysis because it contains the CD4-binding site but does not bind major variable-loop antibodies or those directed against the coreceptor-binding site[13]. IgG was eluted from the gp120WT, gp120-D368R and core proteins (Fig. 2a). As the quantity of IgG eluted from each protein was limited, we chose to focus on the neutralization of a non–clade B virus (RW20, clade A) and a clade B virus (PVO) that is highly resistant to mAb b12 (Fig. 2b and Supplementary Methods). For both sera, the IgG eluted from gp120WT and core protein could neutralize both viruses tested (Fig. 2b and Supplementary Table 2 online). For serum 1, the Env-specific IgG eluted from gp120-D368R, which presumably contained no or very few CD4-binding site antibodies, showed only minimal neutralizing activity. This suggests that the predominant neutralizing activity in serum 1 is directed against the CD4-binding site of gp120. For serum 45, the IgG eluted from gp120-D368R was able to modestly neutralize both viruses, suggesting the existence of some neutralizing antibodies directed to a region other than the central region of the CD4-binding site.

To further isolate and characterize the fraction of neutralizing antibodies specific to the CD4-binding site in both sera, we readsorbed the IgG eluted from gp120WT and core protein with

the gp120-D368R protein to remove non–CD4-binding site antibodies. The resulting flow-through fractions would presumably be enriched with CD4-binding site antibodies and were termed gp120WTeluate/368ft and core eluate/368ft, respectively (Fig. 2c–d). Of note, the D368R protein adsorbed only about 40%–50% of the total IgG that had been eluted from gp120WT or core protein (Supplementary Table 2), suggesting that a large proportion of the initial eluted antibodies were directed against the CD4-binding site. To confirm this, we analyzed these IgG fractions in a competition ELISA. Both the CD4-binding site Fab F105 (Fig. 2c) and soluble CD4 (data not shown) completely blocked binding of the core eluate/368ft IgG to gp120 and blocked most binding of the gp120WT eluate/368ft IgG, indicating that these antibody fractions were highly enriched with CD4-binding site antibodies. We then showed that these antibody fractions neutralize several strains of HIV-1, including the mAb b12–resistant virus PVO (Fig. 2d and Supplementary Table 2).

In summary, we tested sera from a well-characterized cohort of HIV-1–infected patients to find those capable of neutralizing diverse strains of HIV-1. Selective antibody adsorption and elution analysis of the two most broadly reactive sera revealed a fraction of neutralizing antibodies directed against the functionally conserved CD4-binding site of gp120. This novel fraction of CD4-binding site antibodies was able to neutralize viruses that were partially or fully resistant to IgG1 b12, the one mAb to the CD4-binding site region that is known to be capable of neutralizing primary patient isolates. Vaccine immunogens capable of generating this type of antibody response are likely to be protective, as mAb b12 can protect monkeys against a viral challenge[14]. These data show that it is possible for B cells in the normal human repertoire to effectively target epitopes located in the gp120 CD4-binding site. Further studies will be needed to determine the prevalence of broadly neutralizing CD4-binding site–directed antibodies elicited in HIV-1–infected individuals. In conjunction with the recent atomic resolution of the mAb b12–binding surface on gp120 (ref. 15), our results suggest that the CD4-binding site of gp120 should be a major target of HIV-1 vaccines designed to better elicit broadly neutralizing antibodies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported in part by the Intramural Research Program of the Vaccine Research Center and Division of Intramural Research, National Institute of Allergy and Infectious Diseases, US National Institutes of Health. G.M.S. and R.T.W. were supported in part by a grant from the Bill & Melinda Gates Foundation Grand Challenges initiative. R.T.W. also receives funding from the International AIDS Vaccine Initiative. We thank P. Kwong and G. Nabel for helpful suggestions; L. Kong for the core molecular surface figure; L. Chen (Vaccine Research Center) for providing the Fab fragment of F105, which was obtained from the laboratory of M. Posner (Dana Farber Cancer Institute); D. Burton (Scripps Research Institute) for mAb IgG1b12; H. Katinger (Polymun Scientific Inc.) for mAb 2G12; S. Zolla-Pazner (New York University School of Medicine) for mAb 447-52D; J. Sodroski and D. Gabuzda (Dana Farber Cancer Institute), J. Overbaugh (Fred Hutchinson Cancer Research Center), L. Morris (National Institute of Communicable Diseases), D. Montefiori (Duke University), J. Binley (Torrey Pines Institute for Molecular Sciences), L. Stamatatos (Seattle Biomedical Research Institute) and V. Polonis and F. McCutchan (U.S. Military Research Program) for providing virus isolates or functional Env plasmids; other members of the Wyatt laboratory who contributed ideas or reagents to the study; and B. Hartman and M. Cichanowski for help in preparation of the figures.

References

1. Haynes BF, Montefiori DC. Expert Rev Vaccines 2006;5:579–595. [PubMed: 16989638]
2. Burton DR, et al. Nat Immunol 2004;5:233–236. [PubMed: 14985706]
3. Mascola JR, et al. J Infect Dis 1996;173:340–348. [PubMed: 8568294]
4. Wyatt R, Sodroski J. Science 1998;280:1884–1888. [PubMed: 9632381]

5. Steimer KS, Scandella CJ, Skiles PV, Haigwood NL. Science 1991;254:105–108. [PubMed: 1718036]
6. Stamatos NM, et al. J Virol 1998;72:9656–9667. [PubMed: 9811699]
7. Dhillon AK, et al. J Virol 2007;81:6548–6552. [PubMed: 17409160]
8. Migueles SA, et al. Nat Immunol 2002;3:1061–1068. [PubMed: 12368910]
9. Thali M, et al. J Virol 1991;65:5007–5012. [PubMed: 1870209]
10. Olshevsky U, et al. J Virol 1990;64:5701–5707. [PubMed: 2243375]
11. Pantophlet R, et al. J Virol 2003;77:642–658. [PubMed: 12477867]
12. Wyatt R, et al. Nature 1998;393:705–711. [PubMed: 9641684]
13. Kwong PD, et al. Structure 2000;8:1329–1339. [PubMed: 11188697]
14. Parren PW, et al. J Virol 2001;75:8340–8347. [PubMed: 11483779]
15. Zhou T, et al. Nature 2007;445:732–737. [PubMed: 17301785]

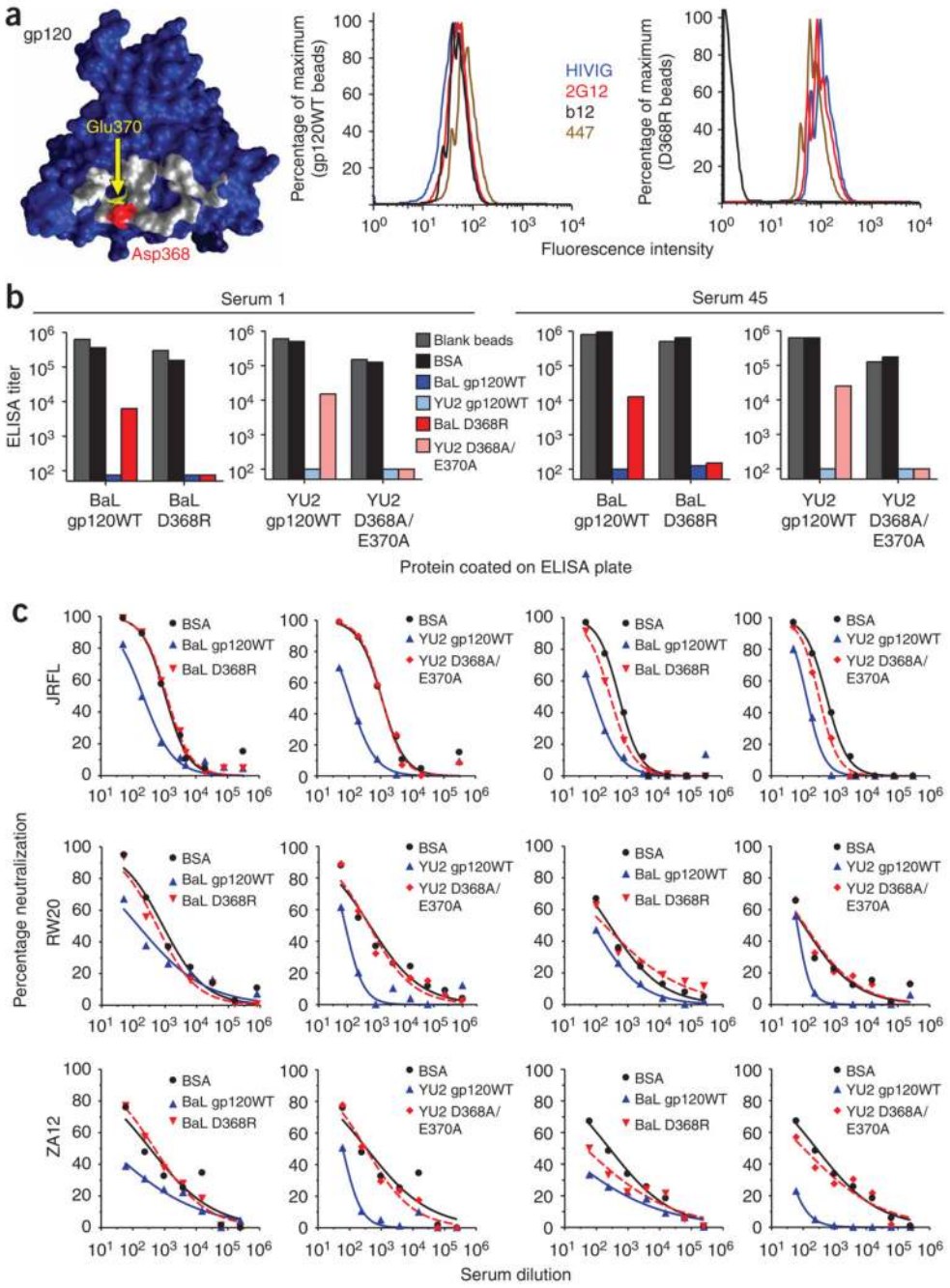

Figure 1.

Adsorption of sera with gp120WT and CD4-binding site mutant proteins. (**a**) Left, molecular surface of the gp120 core. Off-white, CD4-binding site; red, surface-exposed aspartic acid residue Asp368; yellow, glutamic acid residue Glu370. Center and right, flow cytometric analysis of gp120WT (center) and gp120-D368R (right) proteins covalently linked to paramagnetic beads. Protein-linked beads were reacted with the conformational mAbs 2G12 (glycan-dependent), b12 (CD4 binding site) or 447D (V3-directed) at 10 μg/ml, or polyclonal immune globulin from HIV-infected individuals (HIVIG) at 4.0 mg/ml. A PE-conjugated goat antibody to human IgG was used to detect binding. Number of binding events as a percentage of the maximum was plotted against fluorescence intensity. (**b**) End-point ELISA data for sera

1 and 45. Each serum was adsorbed with blank beads or with beads covalently linked to BSA, BaL gp120WT, YU2 gp120WT, BaL gp120-D368R or YU2 gp120-D368A/E370A. The latter two mutant proteins are unable to bind soluble CD4 or most known CD4-binding site antibodies. The protein used to coat ELISA plates is indicated underneath each set of bars. Of note, adsorption with the gp120-D368R or gp120-D368A/E370A mutant proteins left behind a fraction of serum antibodies, presumably to the CD4-binding site, that were detected in the gp120WT protein ELISA. (**c**) Neutralization curves for sera 1 and 45 after adsorption with gp120WT and CD4-binding site–mutant proteins. Control is adsorption with BSA. For serum 1 (two left columns), the CD4-binding site–defective mutants adsorbed little or no neutralizing activity, suggesting that CD4-binding site antibodies were the predominant fraction of neutralizing antibodies in this serum. For serum 45 (two right columns), the CD4-binding site–mutant proteins removed a portion of neutralizing activity against some viruses, suggesting that besides CD4-binding site antibodies, atypical CD4-binding site antibodies or antibodies that bind outside the CD4-binding site were also mediating virus neutralization.

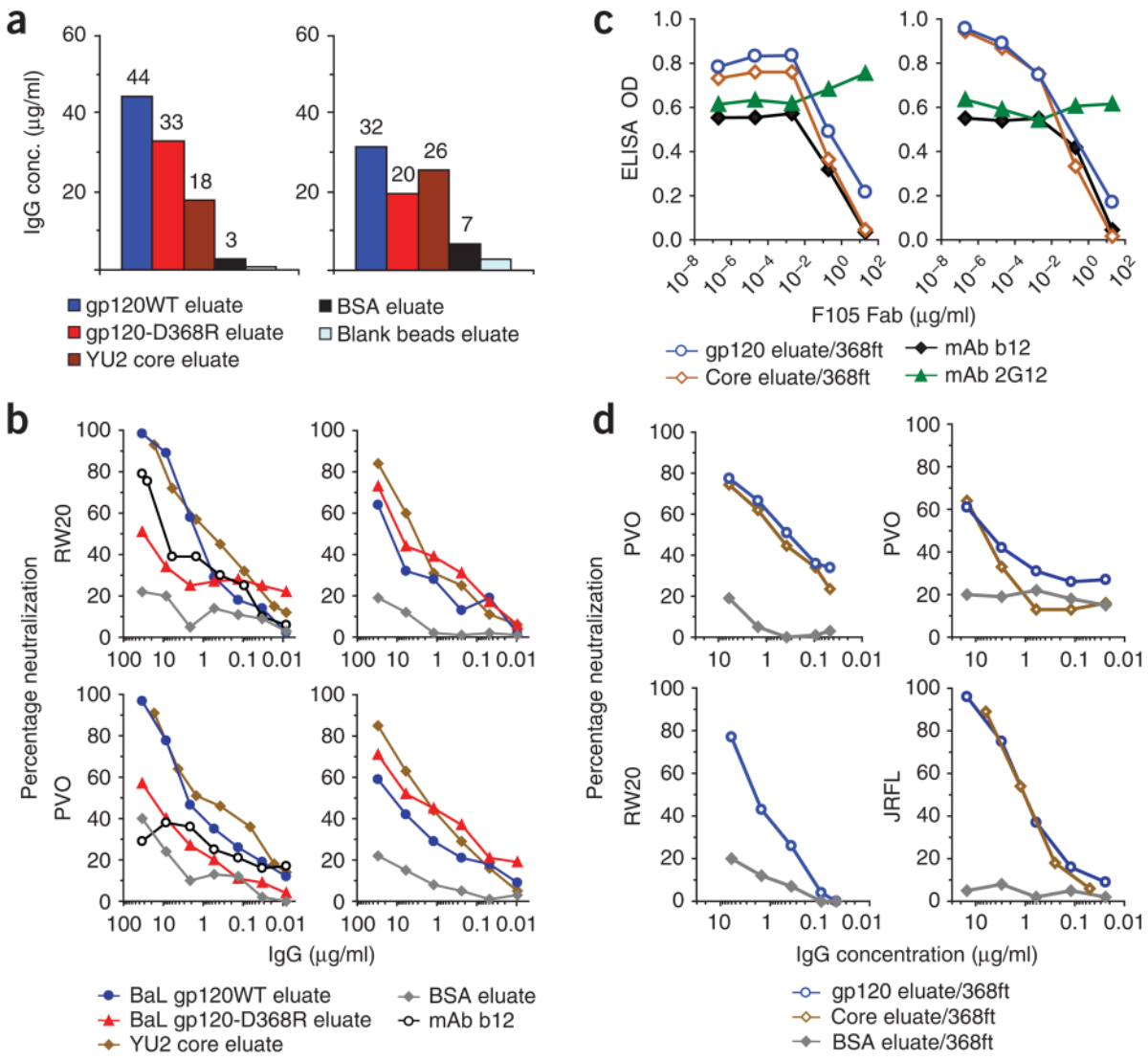

Figure 2.

Analysis of antibodies eluted from Env protein beads and after additional adsorption to enrich for CD4-binding site antibodies. In each panel, serum 1 data are shown on the left and serum 45 data are on the right. (**a**) Concentration of IgG after elution from the indicated proteins. (**b**) Neutralization by IgG eluted from the indicated protein. Top, eluate neutralization of the clade A virus RW20. Bottom, neutralization of the clade B virus PVO. For each virus, left chart shows neutralization by mAb b12 with serum 1 IgG fractions. The BSA eluate contained little IgG; amounts shown here are based on the same physical dilutions as the other IgG fractions. (**c**) Competition ELISA using Fab F105 to block antibody binding to the CD4-binding site of the BaL gp120 used to coat the plate. Monoclonal antibody b12 and 2G12 controls show that increasing concentrations of the Fab F105 have no effect on 2G12 binding but completely block b12 binding to gp120. Open symbols represent the antibody fractions eluted from gp120WT or the core protein and then adsorbed with the gp120-D368R mutant; for example, 'gp120 eluate/368ft' refers to the final flow-though after IgG was eluted from gp120WT and adsorbed with the gp120-D368R protein. (**d**) HIV-1 neutralization of designated isolates by the same IgG fractions described in **c**.