Part IV-D: Antibody References
Antibody References

[Abacioglu (1994)] Y. H. Abacioglu, T. R. Fouts, J. D. Laman, E. Claassen, S. H. Pincus, J. P. Moore, C. A. Roby, R. Kamin-Lewis, & G. K. Lewis. Epitope Mapping and Topology of Baculovirus-Expressed HIV-1 gp160 Determined with a Panel of Murine Monoclonal Antibodies. *AIDS Res Hum Retroviruses* **10**:371–381, 1994. Thirty MAbs were obtained from BALB/c mice immunized with rgp160 LAI expressed in baculovirus. These antibodies map to 4 domains: gp120 C1, C2, C3/V4, and the cytoplasmic tail of gp41. All epitopes were exposed on rgp160 without denaturing the protein, but 6/8 epitopes mapped in gp120 are not exposed unless the protein is denatured, showing rgp160 and gp120 fold differently.


[Arendrup (1995)] M. Arendrup, L. Akerblom, P. M. Heegaard, J. O. Nielsen, & J. E. Hansen. The HIV-1 V3 domain on field isolates: participation in generation of escape virus in vivo and accessibility to neutralizing antibodies. *Arch Virol* **140**:655–670, 1995. The anti-V3 Ab titre in patient serum was generally low against autologous virus isolated later than the serum sample, in contrast to a higher titre against peptides corresponding to virus isolated earlier than the serum sample. The authors conclude that the V3 domain is subject to immunoselection in vivo, and that V3 on early field virus is less accessible to NAbs than the V3 loop on laboratory strains.


[Barsov (1996)] E. V. Barsov, W. E. Huber, J. Marcotrigiano, P. K. Clark, A. D. Clark, E. Arnold, & S. H. Hughes. Inhibition of Human Immunodeficiency Virus Type 1 Integrase by the Fab Fragment of a Specific Monoclonal Antibody Suggests that Different Multimerization States Are Required for Dif-
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Different Enzymatic Functions. *J Virol* **70**:4484–4494, 1996. MAb 35 does not inhibit HIV-1 IN, but Fab 35 inhibits 3’-end processing, strand transfer and disintegration. This appears to be through interfering with multimerization, and suggests that the C-terminal region is important for IN function.


[Binley (1996)] J. M. Binley, H. J. Ditzel, C. F. Barbas III, N. Sullivan, J. Sodroski, P. W. H. I. Parren, & D. R. Burton. Human antibody responses to HIV type 1 glycoprotein 41 cloned in phage display libraries suggest three major epitopes are recognized and give evidence for conserved antibody motifs in antigen binding. *AIDS Res Hum Retroviruses* **12**:911–924, 1996. A panel of anti-gp41 human Fab fragments were generated by panning phage display antibody libraries prepared from HIV-1 positive donors with gp41. Fabs tended to be directed against three epitopes, designated clusters I-III. None were neutralizing. A common CDR3 motif was found in several of the heavy chain sequences.

[Binley (1997b)] J. M. Binley, P. J. Klasse, Y. Cao, I. Jones, M. Markowitz, D. D. Ho, & J. P. Moore. Differential regulation of the antibody responses to Gag and Env proteins of human immunodeficiency virus type 1. *J Virol* **71**:2799–809, 1997b. Retention of anti-Env antibodies and loss of anti-Gag antibodies during progression was studied, and suggested to be the result of the loss of T-cell help and the ability of Env to stimulate B cells even with declining CD4 cells, because of Env’s unique ability to bind to the CD4 molecule.


[Bolmstedt (1996)] A. Bolmstedt, S. Sjolander, J. E. Hansen, L. Akerblom, A. Hemming, S. L. Hu, B. Morein, & S. Olofsson. Influence of N-linked glycans in V4-V5 region of human immunodeficiency virus type 1 glycoprotein gp160 on induction of a virus-neutralizing humoral response. *J AIDS Hum Retrovirol* **12**:213–220, 1996. Because N-linked glycans on viral glycoproteins can protect otherwise accessible neutralization epitopes of the viral envelope from neutralizing antibodies, the aim of this study was to explore the possibility of achieving a more broadly neutralizing immune response with a gp160 depleted of three N-linked glycans in the CD4-binding domain. Mutant and wild type gp160 were formulated into immunostimulating complexes (iscoms), and guinea pigs were vaccinated. Both preparations induced high serum antibody response to native gp120 and V3 peptides. The sera from animals immunized with the mutated glycoprotein lacking CD4 glycosylation sites did not neutralize nonrelated HIV strains better than did sera from animals immunized with wild type glycoprotein, but animals immunized with mutant gp160 neutralized mutant virus better than wild type virus, and vice versa.


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[Boudet (1995)] F. Boudet, H. Keller, M. P. Kiely, & J. Theze. Single peptide and anti-idiotype based immunizations can broaden the antibody response against the variable V3 domain of HIV-1 in mice. *Mol Immunol* **32**:449–457, 1995. Given the high degree of sequence variability of the V3 loop, the humoral response to this region tends to be type specific. An anti-idiotypic antibody could broaden the anti-V3 antibody polyclonal response in BALB/c mice, relative to the original Ab used to generate the anti-idiotype response. A synthetic peptide derived from the V3 determinant of HIV-1 MN induced an antibody response to multiple HIV-1 strains, but the extent of this cross-reactivity was inversely correlated with the binding affinity to V3 MN peptide.

[Boudet (1995)] F. Boudet, H. Keller, M. P. Kiely, & J. Theze. Single peptide and anti-idiotype based immunizations can broaden the antibody response against the variable V3 domain of HIV-1 in mice. *Mol Immunol* **32**:449–457, 1995. Given the high degree of sequence variability of the V3 loop, the humoral response to this region tends to be type specific. An anti-idiotypic antibody could broaden the anti-V3 antibody polyclonal response in BALB/c mice, relative to the original Ab used to generate the anti-idiotype response. A synthetic peptide derived from the V3 determinant of HIV-1 MN induced an antibody response to multiple HIV-1 strains, but the extent of this cross-reactivity was inversely correlated with the binding affinity to V3 MN peptide.

[Broder (1994)] C. Broder, P. Earl, D. Long, S. Abedon, B. Moss, & R. Doms. Antigenic implications of human immunodeficiency virus type 1 envelope quaternary structure: Oligomer-specific and -sensitive monoclonal antibodies. *Proc Natl Acad Sci USA* **91**:11699–11703, 1994. 35 anti-gp41 and 27 anti-gp120 murine MAbs generated by immunization with oligomeric HIV-1 IIIB envelope were studied. These MAbs tended to react with conformational epitopes. 21 of the anti-gp41 MAbs reacted preferentially with oligomeric env, while only 1 of the anti-gp120 MAb reacted more strongly with the oligomer, and 14 of the anti-gp120 preferentially recognized monomeric env.


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proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. *AIDS Res Hum Retroviruses* **10**:359–369, 1994. A panel of 33 human monoclonal antibodies were produced. Linear epitopes for some of this set of MAbs were mapped using peptide ELISA. Linear epitopes were mapped in gp41, and a single epitope was mapped in p24. While multiple gp120 specific MAbs were generated, all seemed to be conformational or carbohydrate dependent, or both.


**[Bukawa (1995)]** H. Bukawa, K.-I. Sekigawa, K. Hamajima, J. Fukushima, Y. Yamada, H. Kiyono, & K. Okuda. Neutralization of HIV-1 by secretory IgA induced by oral immunization with a new macromolecular multicomponent peptide vaccine candidate. *Nature Med* **1**:681–685, 1995. This paper studies the anti-HIV-1 antibodies raised in response to a multicomponent peptide vaccine given orally. It consisted of: V3 loop peptides based on sequences from cyclized B consensus sequence; a PND common in Japan; IIIB PND; Thai B strains PND; a CD4 binding site peptide; and a Gag peptide, HPG30. BALB/c mice were immunized. Serum IgA and IgG and fecal IgA were detected. IgA from fecal samples was capable of neutralizing lab strains.


**[Burton (1991)]** D. R. Burton, C. F. Barbas III, M. A. Persson, S. Koenig, R. M. Chanock, & R. A. Lerner. A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. *Proc Natl Acad Sci USA* **88**:10134–10137, 1991. A panel of human monoclonal antibody Fab fragments was generated against the surface of the gp120 glycoprotein of HIV-1 by antigen selection from a random combinatorial library prepared from 5 ml of bone marrow from an asymptomatic individual who had been HIV-positive for 6 years. These Fab variable regions were sequenced and were found to be diverse. Binding constants were measured and the Fabs generally bound gp120 with high affinity. The methods used to obtain this panel could be used to obtain antibodies to test passive immunization as a therapy for AIDS.


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[Cao (1997)] J. Cao, N. Sullivan, E. Desjardin, C. Parolin, J. Robinson, R. Wyatt, & J. Sodroski. Replication and neutralization of human immunodeficiency virus type 1 lacking the V1 and V2 variable loops of the gp120 Envelope glycoprotein. *J Virol* pages 9808–12, 1997. An HIV-1 mutant lacking the V1-V2 loops can replicate in Jurkat cells and revertants that replicate with wild-type efficiency rapidly evolve in culture. These viruses exhibited increased neutralization susceptibility to V3 loop or CD4i MAbs, but not to sCD4 or anti-CD4BS MAbs. Thus the gp120 V1 and V2 loops protect HIV-1 from some subsets of neutralizing antibodies.


[Cavacini (1994b)] L. A. Cavacini, J. Power, C. L. Emes, K. Mace, G. Treacy, & M. R. Posner. Plasma pharmacokinetics and biological activity of a human immunodeficiency virus type 1 neutralizing human monoclonal antibody, F105, in cynomolgus monkeys. *Tumor Immunol* **15**:251–256, 1994b. MAB F105 was administered intravenously to four cynomolgus monkeys. At 15 days post-dose, total serum F105 was 230 +/- 79 µg/ml and F105 was immunoreactive with cells infected with the MN and IIIB strains of HIV-1 as determined by flow cytometry.

[Cavacini (1998b)] L. A. Cavacini, M. H. Samore, J. Gambertoglio, B. Jackson, M. Duval, A. Wisnewski, S. Hammer, C. Koziel, C. Trapnell, & M. R. Posner. Phase I study of a human monoclonal antibody directed against the CD4-. *AIDS Res Hum Retroviruses* **14**:545–50, 1998b. In an immunotherapeutic study, administration of a single dose of F105 was non-toxic and the Ab persisted, yet no benefit was observed in 4 individuals. The authors suggest it may be more helpful in other settings, for example, patients with no pre-existing anti-CD4 BS Abs, or in combination with other MAbs.


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[Conley (1996)] A. J. Conley, J. A. Kessler II, L. J. Boots, P. M. McKenna, W. A. Schleif, E. A. Emini, G. E. Mark III, H. Kattinger, E. K. Cobb, S. M. Luceford, S. R. Rouse, & K. K. Murthy. The consequence of passive administration of an anti-human immunodeficiency virus type 1 neutralizing monoclonal antibody before challenge of chimpanzees with a primary virus isolate. *J Virol* 70:6751–6758, 1996. The MAb 2F5 was infused into two chimpanzees which were then given an intravenous challenge with a primary HIV-1 isolate – both became infected, but with delayed detection and prolonged decrease in viral load relative to controls, indicating that preexisting, neutralizing antibodies (passively administered or actively elicited) affect the course of acute-phase virus replication and can be influential after the Ab can no longer be detected in the peripheral circulation.

[Conley (1994b)] A. J. Conley, J. A. Kessler II, L. J. Boots, J.-S. Tung, B. A. Arnold, P. M. Keller, A. R. Shaw, & E. A. Emini. Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, and anti-gp41 human monoclonal antibody. *Proc Natl Acad Sci USA* 91:3348–3352, 1994b. 2F5 is capable of neutralizing a broad range of primary isolates and lab strains. Susceptibility to neutralization was dependent on presence of a conserved antibody binding site. Kinetic studies were done, and 2F5 has a very long t1/2 of dissociation, 156 minutes for gp41. The authors point out that LDKW core is present in highly diverged international isolates.


[Cook (1994)] D. G. Cook, J. Fantini, S. L. Spitalnik, & F. Gonzalez-Scarano. Binding of human immunodeficiency virus type 1 HIV-1 gp120 to Galactosylceramide (GalCer): relationship to the V3 loop. *Virology* 201:206–214, 1994. Antibodies against GalCer can block infection of CD4-negative cells from the brain and colon that are susceptible to HIV infection. This paper explores the ability of a panel of MAbs to inhibit binding of gp120 to GalCer, and also of the binding of GalCer to inhibit MAb-gp120 interaction. MAbs to the V3 loop and GalCer showed mutual inhibition of binding to gp120, and anti-CD4 binding site MAbs showed reduced inhibition. N- and C-terminal MAbs didn’t influence GalCer binding.


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[Denisova (1996)] G. Denisova, B. Stern, D. Raviv, J. Zwickel, N. I. Smorodinsky, & J. M. Gershoni. Humoral immune response to immunocomplexed HIV envelope glycoprotein 120. *AIDS Res Hum Retroviruses* **12:**901–909, 1996. Mice were injected with the gp120 in different configurations: free, complexed with CD4, and as an immunocomplex bound to a V3 loop MAb (M77) of the protein. Polyclonal sera, as well as monoclonal antibodies produced in each case, were analyzed. The free gp120 and gp120-CD4 complex immunogens stimulated responses were directed mainly toward conformational epitopes, but gp120 immunocomplexed with MAb M77 also produced numerous and varied MAbs directed toward linear epitopes that were presumably inaccessible on the gp120, gp120-CD4 proteins.

[Denisova (1995)] G. Denisova, J. Zwickel, & J. M. Gershoni. Binding of HIV-1 gp120 to an anti-V3 loop antibody reveals novel antigen-induced epitopes. *FASEB J* **9:**127–132, 1995. This paper describes the characterization of five antibodies that bind M77-epitopes that are only revealed upon M77-gp120 interaction.


[DeVico (1995)] A. L. DeVico, R. Rahman, J. Welch, R. Crowley, P. Lusso, M. G. Sarnigadharan, & R. Pal. Monoclonal antibodies raised against covalently crosslinked complexes of human immunodeficiency virus type 1 gp120 and CD4 receptor identify a novel complex-dependent epitope on gp120. *Virology* **211:**583–588, 1995. To explore the immunogenicity of regions of gp120 that are exposed due to conformational changes in gp120 upon CD4 binding, CD4 was covalently linked to gp120 and this complex was used as an immunogen for BALB/c mice. Two MAbs were produced, both of which bind preferentially to the gp120-CD4 complex, and are conformational. Competition assays indicate these MAbs bind to epitopes that are recognized by sera from HIV-1 infected humans.

[di Marzo Veronese (1986)] F. di Marzo Veronese, T. D. Copeland, A. L. DeVico, R. Rahman, S. Oroszlan, R. C. Gallo, & M. G. Sarnigadharan. Characterization of highly immunogenic p66/p51 as the reverse transcriptase of HTLV-III/LAV. *Science* **231:**1289–1291, 1986. This study identified the 66 and 51 kilodaltons bands in western blots as RT. Enzymatic activity was shown, and the protein was defined by Edmund ... and comparison to HIV-1 pol nucleotide translation. A mouse hybridoma was generated that inhibited enzyme activity.


[Ditzel (1995)] H. J. Ditzel, J. M. Binley, J. P. Moore, J. Sodroski, N. Sullivan, L. S. W. Sawyer, R. M. Hendry, W.-P. Yang, C. F. Barbas III, & D. R. Burton. Neutralizing recombinant human antibodies to a conformational V2- and CD4-binding site-sensitive epitope of HIV-1 gp120 isolated by using an epitope-masking procedure. *J Immunol* **154**:893–906, 1995. A panel of Fabs was obtained from a library prepared from the bone marrow of a long-term asymptomatic HIV-1 seropositive male donor. Four Fabs recognize the CD4BS. An additional four Fabs were retrieved after epitope masking gp120 with the CD4BS Fabs at the screening stage. 3/4 of these Fabs bind to a V2 dependent conformational epitope.

[Ditzel (1997)] H. J. Ditzel, P. W. Parren, J. M. Binley, J. Sodroski, J. P. Moore, C. F. B. 3rd, & D. R. Burton. Mapping the protein surface of human immunodeficiency virus type 1 gp120 using human monoclonal antibodies from phage display libraries. *J Mol Biol* **267**:684–95, 1997. (Genbank: U82767 U82768 U82769 U82770 U82771 U82772 U82942 U82943 U82944 U82945 U82946 U82947 U82948 U82949 U82950 U82951 U82952 U82961 U82962) Recombinant monoclonal antibodies from phage display libraries provide a method for Env surface epitope mapping. Diverse epitopes are accessed by presenting gp120 to the library in different forms, such as sequential masking of epitopes with existing MAbs or scCD4 prior to selection or by selection on peptides. Fabs identified by these methods have specificities associated with epitopes presented poorly on native multimeric envelope.


tested the 6 human MAbs 1125H, TH9, 4.8D, 257-D-IV, TH1, 2F5, and also HIVIG for neutralization of MN, JRCSF, the two B clade primary isolates 301657 and THA/92/026, and the D clade isolate UG/92/21. 2F5 was the most broadly neutralizing, better than HIVIG. The other MAbs showed limited neutralization of only MN (anti-CD4BS MAbs 1125H, TH9, and 4.8D), or MN and JRCSF (anti-V3 MAbs 257-D-IV and TH1).


[Earl (1994)] P. L. Earl, C. C. Broder, D. Long, S. A. Lee, J. Peterson, S. Chakrabarti, R. W. Doms, & B. Moss. Native oligomeric human immunodeficiency virus type 1 Envelope glycoprotein elicits diverse monoclonal antibody reactivities. *J Virol* **68**:3015–3026, 1994. In a study of the repertoire of response to oligomeric versus monomeric Env protein, 138 murine MAbs were generated in response to an immunogen that was a gp120/bp41 oligomeric molecule that was not cleaved due to a mutation in the cleavage site. The oligomeric molecule was found to elicit a response that was very different than the monomer. Most MAbs were conformational, many were to gp41 or if in gp120, to the CD4 BS. Few MAbs to linear V3 epitopes were produced in response to oligomeric protein, though this was a common specificity in response to immunization with gp120 monomeric protein.


[Eddleston (1993)] M. Eddleston, J. C. de la Torre, J.-Y. Xu, N. Dorfman, A. Notkins, S. Zolla-Pazner, & M. B. A. Oldstone. Molecular Mimicry Accompanying HIV-1 Infection: Human Monoclonal Antibodies That Bind to gp41 and to Astrocytes. *AIDS Res Hum Retroviruses* **10**:939–944, 1993. In this paper, three anti-HIV-1 gp41 specific MAbs were found to react with astrocytes: 98-6, 167-7 and 15G1. Reactive astrocytes in the hippocampus were most prominently involved, and the antibodies stained no other cell type in the brain, kidney or liver. All three mapped to a conformationally dependent epitope between aa 644-663.


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[Fouts (1997)] T. R. Fouts, J. M. Binley, A. Trkola, J. E. Robinson, & J. P. Moore. Neutralization of the human immunodeficiency virus type 1 primary isolate JR-FL by human monoclonal antibodies correlates with antibody binding to the oligomeric form of the envelope glycoprotein complex. *J Virol* 71:2779–2785, 1997. To test whether antibody neutralization of HIV-1 primary isolates is correlated with the affinities for the oligomeric envelope glycoproteins, JRFL was used as a model primary virus and a panel of 13 human MAbs were evaluated for: half-maximal binding to rec monomeric JRFL gp120; half-maximal binding to oligomeric - JRFL Env expressed on the surface of transfected 293 cells; and neutralization of JRFL in a PBMC-based neutralization assay. Antibody affinity for oligomeric JRFL Env but not monomeric JRFL gp120 correlated with JRFL neutralization.

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[Franke (1998)] S. S. Frankel, R. M. Steinman, N. L. Michael, S. R. Kim,


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with LAI strain, but not other virus strains, when BAT123 was given 1 hour before virus inoculation, or up to 4 hours post-exposure. No therapeutic effect was observed when BAT123 was administered after infection had been established.

[Gauduin (1998)] M. C. Gauduin, R. Weir, M. S. Fung, & R. A. Koup. Involvement of the complement system in antibody-mediated post-exposure protection against human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* **14**:205–11, 1998. Post-exposure passive transfer of murine BAT123 can confer protection to hu-PBL-SCID mice challenged with HIV-1 LAI, and the mechanism is by complement-mediated cytolysis or virolysis. This protection was not conferred by CGP 47 439, a BAT123 chimera that has a human IgG1 Fc domain, substituted in place of a murine IgG1 Fc domain, suggesting that the protection is mediated by complement. Further evidence was that the protective ability of BAT123 is lost when mice were treated with cobra venom factor, which inactivates serum complement. IgG1 does not fix complement efficiently, so an IgG2 MAb might perform better. Therefore, in spite of the potential for enhancement in some circumstances, in this circumstance complement activation provided a protective advantage.


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[Ho (1991a)] D. D. Ho, M. S. C. Fung, Y. Cao, X. L. Li, C. Sun, T. W. Chang, & N.-C. Sun. Another discontinuous epitope on glycoprotein gp120 that is important in human immunodeficiency virus type 1 neutralization is identified by a monoclonal antibody. *Proc Natl Acad Sci USA* **88:**8949–8952, 1991a. A description of the neutralizing murine MAb G3-4. Evidence suggested that the G3-4 epitope was discontinuous, but later studies showed marginal peptide binding in the V2 region.

[Ho (1992)] D. D. Ho, M. S. C. Fung, H. Yoshiyama, Y. Cao, & J. E. Robinson. Discontinuous epitopes on gp120 important in HIV-1 neutralization. *AIDS Res Hum Retroviruses* **8:**1337–1339, 1992. Further description of the human MAb 15e and the murine MAb G3-4, gp120 mutants that affect 15e epitope binding: 113, 257, 368, 370, 421, 427, 475; four of these coincide with amino acids important for the CD4 binding domain. G3-4 is neutralizing and behaves like a discontinuous epitope, and partially blocks sCD4 binding.


[Huang (1997)] X. Huang, J. J. Barchi, Jr., F. D. Lung, P. P. Roller, P. L. Nara, J. Muschik, & R. R. Garrity. Glycosylation affects both the three-dimensional structure and antibody binding properties of the HIV-1IIIB GP120 peptide. *Biochemistry* **36:**10846–56, 1997. Glycosylated analogues of the V3 loop of gp120 were studied using NMR and circular dichroism spectroscopies, and by AB binding properties to MAb 0.5 β. A 24-residue peptide from the HIV-1 IIIB isolate (residues 308-331) designated RP135, was glycosylated with both N- and O- linked sugars.


[Inouye (1998)] P. Inouye, E. Cherry, M. Hsu, S. Zolla-Pazner, & M. A. Wainberg. Neutralizing antibodies directed against the V3 loop select for different escape variants in a virus with mutated reverse transcriptase (M184V) than in wild-type human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* **14:**735–40, 1998. The M184V substitution in RT yields high level resistance to 3TC and low level resistance to ddl and ddC, and alters the properties of RT. Virus containing the wt form of RT grown in the presence of the MAb 447-D develops 447-D resistance in 36 days, with the GPGR to GPGK substitutions (AGA(R) to AAA(K)). 447-D resistance took longer to acquire in virus with the M184V substituted RT, and had the form CTRPN to CTRPY (AAC(N) to TAC(Y)) at position 5 of the V3 loop.

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[Janvier (1996)] B. Janvier, J. Lasarte, P. Sarobe, J. Hoebeke, A. B.-B. F. Borras-Cuesta, & F. Barin. B-cell epitopes of HIV type 1 p24 capsid protein: a reassessment. AIDS Res Hum Retroviruses 12:519–525, 1996. The reactivity pattern of 45 overlapping synthetic pentadecapeptides, spanning amino acids 133 to 363 (p24) of HIV-1 p55 gag precursor, using sera from 20 HIV-1 positive and 8 HIV negative individuals was determined by ELISA. A peptide covering aa 178-192 was recognized by 40 of 45 covering 288-302 of p55 by 45aa 272-322 of p55 was recognized by most human sera. A conformational epitope involving sequences from aa 183-186 and 289-292 was proposed, based by analogy to the structure of the Mengovirus VP2 protein.


[Jensen (1997)] T. H. Jensen, A. Jensen, A. M. Szilvay, & J. Kjems. Probing the structure of HIV-1 Rev by protein footprinting of multiple monoclonal antibody-binding sites. FEBS Lett 414:50–4, 1997. Rev was mapped using MAb protein footprinting, which gave results that agreed well with peptide mapping, but was useful for identifying a discontinuous interaction between two regions. Footprints supported a previously proposed structure (Auer et al., Biochemistry, 33 (1994) 2988-2996) predicting that a helix-loop-helix motif in Rev brings the termini of the protein into proximity.


[Kalland (1994a)] K. H. Kalland, A. M. Szilvay, K. A. Brokstad, W. Saetrevik, & G. Haukenes. The human immunodeficiency virus type 1 Rev protein shuttles between the cytoplasm and nuclear compartments. Mol Cell Biol 14:7436–7444, 1994a. Ten anti-Nef MAbs were generated and mapped. Nef is expressed in two isomorphic forms, and was shown to be expressed mainly in the Golgi complex and at the nuclear membrane, but occasionally in the nucleus, particularly in MT-4 cells.

[Kalland (1994b)] K. H. Kalland, A. M. Szilvay, E. Langhoff, & G. Haukenes. Subcellular distribution of human immunodeficiency virus type 1 Rev and


[Kang (1994)] C.-Y. Kang, K. Hariharan, P. L. Nara, J. Sodroski, & J. P. Moore. Immunization with a soluble CD4-gp120 complex preferentially induces neutralizing anti-human immunodeficiency virus type 1 antibodies directed to conformation-dependent epitopes of gp120. *J Virol* **68:**5854–5862, 1994. Most of the MAbs generated in this study were conformational, but there were four that bound a V3 loop peptide. These four could neutralize lab strains with different efficiencies. These MAbs were very sensitive to substitutions in the V3 loop, but also to substitutions in the base of the V1/V2 loop structure (120/121 VK/LE), indicating an underlying conformational character. Additionally, many anti-CD4 binding site MAbs were described, that shared a sensitivity to substitutions at residues 368 and 370. Another class of MAbs was found that appeared to be conformationally sensitive, and shared a reduction in binding with the amino acid substitution 88 N/P in the C1 domain.

[Karwowska (1992a)] S. Karwowska, M. K. Gorny, A. Buchbinder, V. Gianakakos, C. Williams, T. Fuerst, & S. Zolla-Pazner. Production of human monoclonal antibodies specific for conformational and linear non-V3 epitopes of gp120. *AIDS Res Hum Retroviruses* **8:**1099–1106, 1992a. A single linear MAAB was generated, to the immunodominant domain in the C-terminal portion of gp120. This antibody did not inhibit rCD4-rgp120 binding or neutralize IIIIB or MN. Three conformational epitope binding MAbs were also described in this paper that could neutralize IIIIB and MN.


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[Lescar (1997)] J. Lescar, R. Stouracova, M. M. Riottot, V. Chitarra, J. Brynda, M. Fabry, M. Horejsi, J. Sedlacek, & G. A. Bentley. Three-dimensional structure of an Fab-peptide complex: structural basis of HIV-1 protease inhibition by a monoclonal antibody. *J Mol Biol* **267**:1207–22, 1997. (Genbank: U62632 U62633) F11.2.32 is a MA b raised against HIV-1 protease which inhibits proteolytic activity. The structure of the complex of the Fab fragment and the synthetic peptide that it binds to, residues 36 to 46 of protease, have been determined at 2.2 AA resolution, and that of the Fab in the free state has been determined at 2.6 AA resolution. The conformation of the bound peptide shows no overall structural similarity to the corresponding segment in HIV-1 protease.

[Lescar (1997)] J. Lescar, R. Stouracova, M. M. Riottot, V. Chitarra, J. Brynda, M. Fabry, M. Horejsi, J. Sedlacek, & G. A. Bentley. Three-dimensional structure of an Fab-peptide complex: structural basis of HIV-1 protease inhibition by a monoclonal antibody. *J Mol Biol* **267**:1207–22, 1997. (Genbank: U62632 U62633) F11.2.32 is a MA b raised against HIV-1 protease which inhibits proteolytic activity. The structure of the complex of the Fab fragment and the synthetic peptide that it binds to, residues 36 to 46 of protease, have been determined at 2.2 AA resolution, and that of the Fab in the free state has been determined at 2.6 AA resolution. The conformation of the bound peptide shows no overall structural similarity to the corresponding segment in HIV-1 protease.


or immunoprophylaxis. Because HIV can replicate in rhesus macaques, such approaches can potentially be studied in an in vivo monkey model.


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[Moore & Ho (1995)] J. P. Moore & D. D. Ho. HIV-1 neutralization: the consequences of adaptation to growth on transformed T-cells. *AIDS* **9 suppl A**:S117–S136, 1995. This review considers the relative importance of a neutralizing antibody response for the development of a vaccine, and for disease progression during the chronic phase of HIV-1 infection. It suggests that T-cell immunity may be more important. The distinction between MAbs that can neutralize primary isolates, and those that are effective at neutralizing only laboratory adapted strains is discussed in detail. Alternative conformations of envelope and non-contiguous interacting domains in gp120 are discussed. The suggestion that soluble monomeric gp120 may serve as a viral decoy that diverts the humoral immune response in vivo is put forth.

[Moore (1994a)] J. P. Moore, F. E. McCutchan, S.-W. Poon, J. Mascola, J. Liu, Y. Cao, & D. D. Ho. Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. *J Virol* **68**:8350–8364, 1994b. Four of five anti-V3 MAbs were slightly cross-reactive within clade B, but not very reactive outside clade B. Two discontinuous CD4 binding site MAbs appear to be pan-reactive. Anti-V2 MAbs were only sporadically reactive inside and outside of clade B.


[Moore (1994c)] J. P. Moore, Q. J. Sattentau, R. Wyatt, & J. Sodroski. Probing the Structure of the Human Immunodeficiency Virus Surface Glycoprotein gp120 with a Panel of monoclonal antibodies. *J Virol* **68**:469–484, 1994c. This study compared a large number of MAbs that bind to linear epitopes of gp120, and compared binding affinities for: i) native and SDS-DDT denatured gp120, (clone BH10 of the LAI isolate expressed in CHO cells);
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ii) recombinant gp120 lacking the V1, V2, V3 loops; iii) a panel of 20 mer peptides; iv) a panel of gp120 mutants; and v) oligomeric versus monomeric gp120. The binding ratio of native versus denatured monomeric gp120 is included in the table in this database. These numbers should be considered with the following points in mind: a continuous epitope may be partially exposed on the surface; and a preparation of gp120 is not homogeneous and contains fully folded, partly denatured, and some completely unfolded species, so the conformation of what is considered to be a native protein will not only reflect fully folded gp120. The authors suggest that a fivefold increase in the affinity for a MAb binding to denatured versus native gp120 indicates that the epitope is inaccessible in the native form. We also have included here information extracted from Moore et al’s list of the gp120 mutations that reduced the binding of a particular MAb. In mapping of exposed regions of gp120, C2, C3, and C5 domain epitopes were found to bind preferentially to denatured gp120. V1, V2 and V3, part of C4, and the extreme carboxy terminus of C5 were exposed on the native monomer. In the oligomeric form of the molecule, only V2, V3 and part of C4 are well exposed as continuous epitopes.


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[Moore (1993c)] J. P. Moore, H. Yoshiyama, D. D. Ho, J. E. Robinson, & J. Sodroski. Antigenic variation in gp120s from molecular clones of HIV-1 LAI. *AIDS Res Hum Retroviruses* 9:1185–1193, 1993c. The binding of MAbs to four molecular clones of HIV-1 LAI: HxB2, HxB3, Hx10, and NL4-3, was measured. Despite the close relationship between these clones, there is considerable variation in their antigenic structure, judged by MAb reactivities to the V2, V3, and C4 domains and to discontinuous epitopes. Small variations in sequence can profoundly affect recognition of gp120 by all five groups of defined anti-gp120 neutralizing antibodies.


[Moore (1995b)] J. P. Moore, A. Trkola, B. Korber, L. J. Boots, J. A. Kessler II, F. E. McCutchan, J. Mascola, D. D. Ho, J. Robinson, & A. J. Conley. A human monoclonal antibody to a complex epitope in the V3 region of gp120 of human immunodeficiency virus type 1 has broad reactivity within and outside clade B. *J Virol* 69:122–130, 1995b. The epitope was defined as including amino acids on both sides of the loop of the V3 loop: -I—G–FY-T, where the G is the second G of the GPGR tip of the loop. This antibody bound well to gp120 molecules from clades A,B,C,E, and F, when the critical amino acids were present. Binding did not parallel neutralization however; 19b could produce a 50-fold reduction of infectivity in some primary B isolates, and in C clade isolates at low virus input concentrations, but not in isolates from all clades where binding could occur (A,E, and F).


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[Nyambi (1998)] P. N. Nyambi, M. K. Gorny, L. Bastiani, G. van der Groen, C. Williams, & S. Zolla-Pazner. Mapping of epitopes exposed on intact human immunodeficiency virus type 1 (HIV-1) virions: a new strategy for studying the immunologic relatedness of HIV-1. *J Virol* **72**:9384–91, 1998. 18 human MAbs binding to gp120 and gp41 were tested using a novel assay to test binding to intact HIV-1 virions. The new method involves using MAbs to the host proteins incorporated into virions to bind them to ELIZA plates. Antigenic conservation in epitopes of HIV-1 in clades A, B, D, F, G, and H was studied. MAbs that were selected were directed against V2, V3, CD4bd, C5 or gp41 regions. Antibodies against V2, the CD4BS, and sp41 showed weak and sporadic reactivities, while binding strongly to gp120, suggesting these epitopes are hidden when gp120 is in its native, quaternary structure.


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hu-PBL-SCID mice by passive immunization with a neutralizing human monoclonal antibody against the gp120 CD4-binding site. AIDS 9:F1–F6, 1995. The Fab b12, at 1.9 mg/kg, was able to protect 25 mice from HIV-1 infection showing that complete protection against HIV-1 infection can be achieved in the hu-PBL-SCID model by passive immunization with physiologically relevant doses of antibody.


[Parren (1998a)] P. W. Parren, I. Mondor, D. Naniche, H. J. Ditzel, P. J. Klasse, D. R. Burton, & Q. J. Sattentau. Neutralization of human immunodeficiency virus type 1 by antibody to gp120 is determined primarily by occupancy of sites on the virion irrespective of epitope specificity. J Virol 72:3512–9, 1998a. The authors propose that the occupancy of binding sites on HIV-1 virions is the major factor in determining neutralization, irrespective of epitope specificity. Neutralization was assayed T-cell-line-adapted HIV-1 isolates. Binding of Fabs to monomeric gp120 was not correlated with binding to functional oligomeric gp120 or neutralization, while binding to functional oligomeric gp120 was highly correlated with neutralization. The ratios of oligomer binding/neutralization were similar for antibodies to different neutralization epitopes, with a few exceptions.


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[Poignard (1996a)] P. Poignard, T. Fouts, D. Naniche, J. P. Moore, & Q. J. Sattentau. Neutralizing antibodies to human immunodeficiency virus type-1 gp120 induce envelope glycoprotein subunit dissociation. *J Exp Med* **183**:473–484, 1996a. Binding of Anti-V3 and the CD4 neutralizing MAbs induces shedding of gp120 on cells infected with the T-cell line-adapted HIV-1 molecular clone Hx10. This was shown by significant increases of gp120 in the supernatant, and exposure of a gp41 epitope that is masked in the oligomer. MAbs binding either to the V2 loop or to CD4BS discontinuous epitopes do not induce gp120 dissociation. This suggests HIV neutralization probably is caused by several mechanisms, and one of the mechanisms may involve gp120 dissociation.

[Poignard (1996b)] P. Poignard, P. J. Klasse, & Q. J. Sattentau. Antibody neutralization of HIV-1. *Immunology Today* **17**:239–246, 1996b. Comprehensive review of HIV envelope gp120 and gp41 antibody binding domains, and different cross-reactivity groups of MAbs ability to neutralize primary isolates. The distinction between neutralization of laboratory strains and primary isolates is discussed. The only three epitopes that have confirmed broad neutralization against a spectrum of isolates are gp120 epitopes for IgG1b12 and 2G12, and the gp41 epitope of 2F5.


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assay using a soluble, oligomeric form of HIV-1IIIB Env (gp140) that contains gp120 and the gp41 ectodomain was developed. The gp140, captured by various monoclonal antibodies (MAbs), retained its native oligomeric structure: it bound CD4 and was recognized by MAbs to conformational epitopes in gp120 and gp41, including oligomer-specific epitopes in gp41.


[Rizzuto (1998)] C. D. Rizzuto, R. Wyatt, N. Hernandez-Ramos, Y. Sun, P. D. Kwong, W. A. Hendrickson, & J. Sodroski. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* **280:**1949–53, 1998. This paper compares the epitope for CD4 inducible MAbs with the chemokine co-receptor binding site on the gp120 molecule. Site-directed mutagenesis of YU2 Env was guided by information obtained from the crystallized CD4-17b-gp120 core structure, Kwong et al., 1998. YU2 is a primary macrophage tropic R5 isolate with high affinity for both CD4 and CCR5. A protein with the V1-V2 loops deleted, called wtΔ was the basis for the assay which detected binding of virus to cells expressing CCR5 in the presence of sCD4. Preincubation with MAb 17b blocks binding, as did the natural lig- and for CCR5, MIP-1β and anti-CCR5 MAb 2D7. Mutations 437 P/A and 442 Q/L increased CCR5 binding affinity. The region of gp120 CCR5 binding is shown to be the highly conserved β-sheet bridging structure, located proximal to the V3 loop.


[Robert-Guroff (1994)] M. Robert-Guroff, A. Louie, M. Myagkikh, F. Michaels, M. P. Kiemy, M. E. White-Scharf, B. Potts, D. Grogg, & M. S. Reitz, Jr. Alteration of V3 loop context within the envelope of human immunodeficiency virus type 1 enhances neutralization. *J Virol* **68:**3459–3466, 1994. MN-V3 loop inserted into a HBX2 background results in enhanced neutralization of anti-MN V3 MAb 50.1 and human HIV+ sera when the chimeric virus was compared to MN. Enhanced affinity, and greater proportions of labeled infected H9 cells by FACS analysis, were also observed using two anti-MN V3 MAbs, 50.1 and 83.1.


[Robinson (1990c)] W. E. Robinson, Jr., T. Kawamura, D. Lake, Y. Masuho, W. M. Mitchell, & E. M. Hersh. Antibodies to the Primary Immunodominant


that antibodies specific for one of five different binding regions on gp120 are associated with viral neutralization: V2, V3, C4, the CD4 binding site, and a complex discontinuous epitope that does not interfere with CD4 binding. Kinetic binding properties of a set of MAbs that bind to these regions were studied, analyzing binding to both functional oligomeric LAI gp120 and soluble monomeric LAI BH10 gp120; neutralization ID50s were also evaluated. It was found that the neutralization ID50s was related to the ability to bind oligomeric, not monomeric, gp120, and concluded that with the exception of the V3 loop, regions of gp120 that are immunogenic will be poorly presented on cell-line-adapted virions. Further, the association rate, estimated as the $t_1/2$ to reach equilibrium binding to multimeric, virion associated, gp120, appears to be a major factor relating to affinity and potency of the neutralization response to cell-line-adapted virus.


[Schneider (1991)] T. Schneider, H.-P. Harthus, P. Heldebrandt, M. Niedrig, M. Broker, W. Weigelt, A. Beck, & G. Pauli. Epitopes of the HIV-1-negative factor reactive with murine monoclonal antibodies and human HIV-1-positive sera. *AIDS Res Hum Retroviruses* 7:37–43, 1991. Epitopes for 9 murine MAbs were mapped, and found to be located in 4 immunogenic regions. 7/10 sera from HIV-1 positive individuals reacted to the four nef immunogenic regions.


[Schutten (1997)] M. Schutten, A. C. Andeweg, G. F. Rimmelzwaan, & A. Osterhaus. Modulation of primary human immunodeficiency virus type 1 envelope glycoprotein-mediated entry by human antibodies. *J Gen Virol* 78:999–1006, 1997. A series of HIV-1 envelope glycoproteins from related primary virus isolates of different SI phenotypes, together with chimeras of these proteins, were tested in an envelope trans-complementation assay for their sensitivity to either antibody mediated inhibition or enhancement of HIV-1 entry. In contrast to the inhibition of HIV-1 entry, antibody mediated enhancement was not temperature dependent and could not be mediated by F(ab) fragments, implicating cross-linking as an important step. Enhancement or inhibition seemed to be determined by virus isolate rather than by the specificity of the antiserum used. 2F5 was the only MAb that inhibited the entry of all viruses.

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[Shang (1991)] F. Shang, H. Huang, K. Revesz, H.-C. Chen, R. Herz, & A. Pinter. Characterization of monoclonal antibodies against the human immunodeficiency virus matrix protein, p17 gag: identification of epitopes exposed at the surfaces of infected cells. J Virol 65:4798–4804, 1991. Six MAbs with linear epitopes were mapped. These Abs could only bind to HIV-infected cells that had been permeabilized with acetone. Only G11g1 and G11h3, two antibodies that did not bind to peptides, but only to intact p17, could react with live HIV-1 infected cells. These antibodies were not neutralizing.


[Shotton (1995)] C. Shotton, C. Arnold, Q. Sattentau, J. Sodroski, & J. A. McKeating. Identification and characterization of monoclonal antibodies specific for polymorphic antigenic determinants within the V2 region of the human immunodeficiency virus type 1 envelope glycoprotein. J Virol 69:222–230, 1995. Anti-V2 linear and conformation dependent MAbs were studied. All V2 Abs studied could bind IIIb, but failed to neutralize non-clonal stocks. Epitope exposure is different in rgp120 compared to native gp120. HXB2 V2-MAb neutralization escape mutants were sequenced.


[Skinner (1988a)] M. A. Skinner, A. J. Langlois, C. B. McDanal, J. S. McDougal, D. P. Bolognesi, & T. J. Matthews. Neutralizing Antibodies to an Immunodominant Envelope Sequence Do Not Prevent gp120 Binding to CD4. J Virol 62:4195–4200, 1988a. This report was an early suggestion that there are at least two classes of biologically active antibodies to HIV: one class is isolate restricted, primarily directed to a hypervariable loop structure of gp120 and not involved in CD4 binding; the second class is directed at more conserved structures that may directly block CD4 binding.


were neutralized by anti-V3 loop MAbs. The chimeric viruses elicited potent NAb s against ALA-1 and MN in guinea pigs.


[Spear (1993)] G. T. Spear, D. M. Takefman, B. L. Sullivan, A. L. Landay, & S. Zolla-Pazner. Complement activation by human monoclonal antibodies to human immunodeficiency virus. *J Virol* 67:53–59, 1993. This study looked at the ability of 16 human MAbs to activate complement. MAbs directed against the V3 region could induce C3 deposition on infected cells and virolysis of free virus, but antibodies to the CD4BS and C-terminal region and two regions in gp41 could induce no complement mediated effects. Pretreatment with sCD4 could increase complement-mediated effects of anti-gp41 MAbs, but decreased the complement-mediated effects of V3 MAbs. Anti-gp41 MAbs were able to affect IIIB but not MN virolysis, suggesting spontaneous shedding of gp120 on IIIB virions exposes gp41 epitopes. IgG isotype did not appear to have an effect on virolysis or C3 deposition.


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[Sullivan (1993)] N. Sullivan, M. Thali, C. Furman, D. Ho, & J. Sodroski. Effect of amino acid changes in the V2 region of the human immunodeficiency virus type 1 gp120 glycoprotein on subunit association, syncytium formation, and recognition by a neutralizing antibody. *J Virol* 67:3674–3679, 1993. Recognition of neutralizing MAb G3-4 was altered by substitutions in 176 to 184 in the V2 loop. Some changes in the V2 loop can affect subunit assembly; other changes allow expression and CD4 binding but inhibit syncytium formation and viral entry, suggesting that V1/V2 may be involved in post receptor binding events.


[Szilvay (1992)] A. M. Szilvay, S. Nornes, I. R. Haugan, L. Olsen, V. R. Prasad, C. Endresen, S. P. Goff, & D. E. Helland. Epitope mapping of HIV-1 reverse transcriptase with monoclonal antibodies that inhibit polymerase and RNase H activities. *J AIDS* 5:647–657, 1992. 20 MAbs are described, only five are able to bind to short peptides. These five MAbs are insensitive to mutations throughout the rest of RT.

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[Tani (1994)] Y. Tani, E. Donoghue, S. Sharpe, E. Boone, H. C. Lane, S. Zolla-Pazner, & D. I. Cohen. Enhanced in vitro human immunodeficiency virus type 1 replication in B cells expressing surface antibody to the TM env protein. *J Virol* 68:1942–1950, 1994. The MAb 98-6 was expressed as a surface anti-gp41 monoclonal antibody receptor for gp41 (sIg/gp41) by transfection into a CD4-negative B-cell line. Transfected cells could bind HIV envelope, but could not be infected by HIV-1. When CD4 delivered by retroviral constructs was expressed on these cells, they acquired the ability to replicate HIV-1, and sIg/gp41 specifically enhanced viral replication.


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that contains V1 and V2, and the hydrophobic region in C2 from Arg 252 to Asp 262. Additionally changes in Glu 370, and Met 475 in C5, affected binding and neutralization. The hydrophobic character of these critical regions is consistent with the limited exposure on gp120 prior to CD4 binding.


and outside clade B with a high potency. IgG1b12 and 2G12 could potently neutralize isolates from within clade B, but showed a reduction in efficacy outside of clade B. 2F5 neutralization was dependent on the presence of the sequence: LDKW.


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subunit vaccines (Genentech gp120IIIB, MicroGeneSys gp160IIIB, or ImmunoAG gp160IIIB) preferentially induced Abs reactive only to the denatured form of gp120. This may explain the inability of the vaccinee sera to neutralize primary HIV-1 isolates.

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flow cytometry. *J Virol* **69**:3807–3815, 1995. A set of 13 human MAbs to a variety of epitopes were tested against a panel of primary isolates of HIV-1, representing different genetic clades. The V3 loop tended to be B clade restricted, and a single gp120 C-terminus binding antibody was clade specific. Two other gp120 C-terminus binding antibodies were group specific.


