I-C

Human Monoclonal Antibodies that Neutralize HIV-1

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Abstract

The HIV Immunology Database provides continuously updated information on monoclonal antibodies (mAbs) against HIV-1 produced by various techniques, including cellular methods utilizing Epstein-Barr transformation, phage display technology, antigen stimulation of lymphoid cells *in vitro*, and preparation of hybridomas from cells of transgenic mice. In this review, particular attention is focused on those human mAbs that are able to inhibit the infectivity of HIV virions. These mAbs target several clusters of neutralizing epitopes in the HIV-1 envelope proteins, including V1, V2, V3, CD4bs, CD4i in gp120, and cluster I, cluster II, and a region adjacent to cluster II in gp41. Only five of the 174 mAbs listed here can be classified as broadly and potently neutralizing for HIV-1 primary isolates; these include mAbs 2G12, IgG1b12, and 447-52D (specific for gp120) and 2F5 and 4E10 (specific for gp41). Certain other monoclonal reagents are capable of broad neutralization as Fab fragments but not as IgG molecules. The existence of human mAbs with neutralizing activity against diverse HIV-1 isolates demonstrates the ability of the human immune response to recognize B cell epitopes on the HIV-1 envelope that are shared and that, when complexed with antibody, prevent virus infectivity. The paucity of such broadly neutralizing mAbs highlights the challenge faced by designers of HIV-1 vaccines: to design a vaccine that will induce that small proportion of antibodies with broad neutralizing activity.

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I-C-1 Introduction

The HIV Immunology Database catalogues existing rodent and human monoclonal antibodies (mAbs) that are specific for various proteins of HIV-1. The human mAbs, which are reviewed herein, were produced by a variety of different techniques, although the two techniques that have given rise to the majority of useful mAbs are based on hybridoma and recombinant technologies. Both techniques have resulted in extremely valuable mAbs that have provided important information about the antigenic structure of neutralizing epitopes. Hybridoma techniques are based on the transformation of B cells from the cells of infected individuals [Gorny1989], and the mAbs derived from these cells represent antibodies that are part of the normal human B cell repertoire. In contrast, while the recombinant technology has several advantages over the cellular technology [Barbas1992], given the random recombination of heavy and light chains that takes place in vitro during this process, the resulting Fab fragments and IgG molecules may not actually represent antibodies that exist in human hosts [Huang2004].

The 174 mAbs summarized in this review are grouped on the basis of the epitopes that they recognize in gp120 and gp41. Among these mAbs and Fab fragments, 11 regions are recognized, however, only five human mAbs specific for four regions of gp120 and gp41 have been established as capable of broad and potent neutralization of HIV-1; these include mAbs 2G12, IgG1b12, and 447-52D (specific for various epitopes in gp120) and 2F5 and 4E10 (specific for gp41). Thus, while the existence of these latter mAbs demonstrates that the human immune system has the capacity to recognize shared neutralizing epitopes on the HIV-1 envelope glycoproteins, the paucity of broadly neutralizing mAbs highlights the limited immunogenicity and/or restricted availability of the regions in the native virions that are critical for virus infectivity.

Several phenomena may explain the rarity of functional antibodies against regions of the virus that are indispensable for its infectivity. These include, but are not limited to, the following: (a) the ability of the virus to continually vary its sequence in critical regions while maintaining function [Zolla-Pazner2004a];

(b) carbohydrate masking of critical epitopes [Back1994, Wei2003]; (c) conformational masking of receptor-binding sites [Kwong2002]; (d) poor immunogenicity of some neutralizing epitopes [Trkola1996b]; and (e) inaccessibility or transient exposure of neutralizing epitopes on the intact virus and on the virus as it binds to cell receptors.

Despite these phenomena, which form the basis for virus escape from the neutralizing potential of antibodies, the human humoral immune response is able to produce antibodies that neutralize HIV-1. The characterization of neutralizing epitopes recognized by human mAbs provides the basic data for the rational design of effective vaccine immunogens. In addition, the identification and characterization of neutralizing human mAbs may provide reagents for passive immunization to prevent infection, as in the setting of maternal-fetal transmission. These mAbs should also provide important information about mechanisms of virus neutralization, the antigenic structure of the virus, and the nature of the B-cell repertoire that can be tapped to provide protective humoral immune responses against the virus.

The characteristics of 174 human mAbs that target the envelope glycoproteins of HIV-1 are reviewed below. The epitopes within gp120 and gp41 that are recognized by these mAbs have been mapped and are catalogued in the HIV Immunology Database. For the convenience of the reader, we have linked the mAbs discussed in this review with each antibody's web page in the HIV Immunology Database (`http://www.hiv.lanl.gov/`).

I-C-2 Anti-V1 mAbs

No anti-V1 mAbs have been derived from the cells of infected humans. However, the HIV Immunology Database provides information on ten human anti-V1 mAbs (Table I-C-1) that were generated from transgenic mice carrying the genes coding for fully human IgGκ. These "xeno-mAbs" were derived from transgenic mice that had been immunized with native recombinant gp120 from HIV-1_{SF162}; the Ab-producing cells were selected with recombinant $gp120_{SF162}$ [He2002]. All of these anti-V1 xeno-mAbs display potent type-specific neutralizing activity against the autologous strain SF162 with 50% neutralizing doses (ND50) in the range of 0.3 to 4.5 µg/ml, as determined in a luciferase assay. Ten out of the 35 xeno-mAbs selected with $rgp20_{SF162}$ were specific for V1, suggesting that in this transgenic model, V1 is an immunodominant epitope [He2002]. The dearth of human anti-V1 mAbs derived from the cells of infected humans is probably related to the very high diversity of the V1 domain; this would render the selection of anti-V1 mAbs with heterologous gp120 reagents a difficult task. However, the lower immunogenicity of this region in the setting of natural infection cannot be excluded.

I-C-3 Anti-V2 mAbs

Anti-V2 antibodies have the capacity to neutralize HIV, but their activity is generally weak and their cross-reactivity is usually limited, suggesting that the antibodies with this specificity may have limited utility in mediating vaccine-induced, broad-based protection. The HIV Immunology Database contains a list of five anti-V2 human mAbs, two Fab fragments generated from HIV-infected individuals, and one xeno-mAb (Table I-C-2). The most thoroughly analyzed of the anti-V2 mAbs, mAb 697-D, could only weakly neutralize three of four primary isolates when a relatively high dose of mAb was used against a low virus input [Gorny1994]; this mAb displayed no neutralizing activity against four TCLA strains [Gorny1994, Nyambi1998]. mAb 697-D recognizes a conformational epitope, showing only weak reactivity with a V2 peptide; it binds weakly and only sporadically to intact virions from clades A, B and D [Nyambi2000]; similarly, other human anti-V2 mAbs, such as mAbs 830A, 1357, 1362 and 1393A, could bind to soluble gp120 but showed only weak and sporadic binding to virions of primary isolates, with the most frequent binding to B, C and D clades [Nyambi1998, Nyambi2000]. mAb 697-D could not inhibit the ability of gp120 to block the binding of MIP-1 beta to CCR5, suggesting that the V2 region does not block envelope/coreceptor interaction [Trkola1996a].

Two Fab fragments, L15 and L17, are specific for the V2 domain (Table I-C-2) and four Fab fragments, L25, L39, L40 and L78, are directed to complex epitopes that include the V2 loop and the CD4 binding site (CD4bs) (Table I-C-3) [Ditzel1995, Ditzel1997]. These latter Fab fragments were retrieved after epitope masking of gp120 with CD4bs Fab fragments during the screening stage. They were characterized by their V2 region-dependence, indicated by their sensitivity to amino acid changes in the V2 loop and by competition with murine anti-V2 mAbs. In addition, they are sensitive to amino acid changes usually associated with CD4 binding, and their binding to gp120 can be inhibited by soluble CD4. Among these several Fab molecules, only L25 and L78 mediate weak neutralization of T cell line-adapted (TCLA) viruses; neutralizing activity against primary isolates was not reported. The poor performance of these anti-V2 and anti-V2/CD4bs Fab fragments in neutralization assays does not exclude the possibility that, as whole IgG molecules, they could display neutralizing activity since Fab fragments usually display affinities that are lower by two to three orders of magnitude than intact IgG molecules. However, no studies of these

Table I-C-1: Anti-V1 mAbs

mAbs	Ab type	Neutralization	Reference
35D10/D2, 40H2/C7, 43A3/E4, 43C7/B9, 45D1/B7, 46E3/E6, 58E1/B3, 64B9/A6, 69D2/A1, 82D3/C3	Xeno-mAb	psSF162	He2002

Table I-C-2: Anti-V2 mAbs

mAbs	Ab type	Neutralization	Reference
697-D	mAb	PI (weakly)	Gorny1994
830A	mAb	psSF162	Pinter2004
1357, 1361, 1393A	mAb	non-neutralizing	Nyambi1998
8.22.2	Xeno-mAb	psSF162 (weakly)	He2002
L15, L17	Fab	non-neutralizing	Parren1998

Table I-C-3: Anti-V2-CD4bs mAbs

mAbs	Ab type	Neutralization	Reference
L25, L78	Fab	TCLA (weakly)	Ditzel1995
L39, L40	Fab	non-neutralizing	Ditzel1995

fragments as whole IgG molecules have been published, and the lack of potent neutralizing activity by anti-V2 IgG mAbs (see above) is not an encouraging indicator.

In summary, the information about anti-V1 and anti-V2 mAbs is incomplete because the number of mAbs, Fab fragments and xeno-mAbs is still relatively small. The results utilizing these mAbs suggest that anti-V1 mAbs are rather type-specific but can be quite potent, while anti-V2 are more broadly neutralizing but of low activity. A notable exception is the chimp mAb C108G, which is directed against a glycan-dependent epitope localized in V2 and was shown to neutralize primary HIV-1BaL isolate and TCLA strain IIIB [Vijh-Warrier1996].

I-C-4 Anti-V3 mAbs

Human antibodies directed to the V3 loop of gp120 constitute the major group of human mAbs in the database. There are currently 33 IgG mAbs, two Fab fragments generated from HIV-infected individuals, four xeno-mAbs produced by transgenic mice, and three IgM mAbs produced by in vitro stimulation of peripheral blood mononuclear cells (PBMC) with V3 peptides (Table I-C-4). These numbers reflect both the strong immunogenicity of the V3 region and the early interest in anti-V3 Abs generated when it was shown that they could neutralize TCLA virus strains [Rusche1988]; subsequent studies have documented the ability of anti-V3 antibodies to also neutralize primary isolates [Conley1994, Gorny2004, Gorny2002, Scott1990] and to mediate protection as demonstrated

Table I-C-4: Anti-V3 mAbs

mAbs	Ab type	Neutralization	Reference
N70-1.9b	mAb	TCLA	Robinson1990
19b	mAb	TCLA	Moore1994
257-D, 268-D, 311-11-D, 386-D, 391/95-D, 412-D, 418-D, 419-D, 453-D 504-D, 694/98-D, 782-D, 838-D, 908-D, 1006-15D, 1027-15D, 1108, 1324-E, 1334-D	mAb	TCLA	Karwowska1992, Gorny1993
447-52D, 2182, 2191, 2219, 2412, 2442, 2456	mAb	PI	Gorny1993, Gorny2002
4117C, 41148D	mAb	TCLA	Tilley1992, Pinter1993
M77	mAb	TCLA	diMarzo Veronese1992
MN215	mAb	TCLA	Schutten1995
TH1	mAb	TCLA	D'Souza1995
loop 2, DO142-10	Fab	TCLA	Barbas1993, Seligman1996
8E11/A8, 8.27.3	Xeno-mAb	TCLA	He2002
6.1, 6.7	Xeno-mAb	non-neutralizing	He2002
MO96/V3, MO97/V3, MO99/V3	IgM mAb, in vitro stimulation	non-neutralizing	Ohlin1992

in passive immunization experiments in various animal models [Andrus1998, Emini1992].

Many of the human anti-V3 mAbs were produced from cells of HIV-infected individuals who had been infected for several years; these were shown to neutralize TCLA and/or primary isolates [Gorny2004, Gorny1997, Gorny2002, Gorny1993, Moore1995, Scott1990]. In contrast, the IgG anti-V3 xeno-mAbs displayed neutralizing activity that appeared to be specific for the immunizing SF162 virus strain [He2002], while the IgM mAbs that were induced *in vitro* by antigenic stimulation of cells from HIV-uninfected individuals displayed no neutralizing activity [Ohlin1992]. These results suggest that the induction of broadly reactive and potent anti-V3 antibodies may require prolonged antigenic stimulation, resulting in mature and broadly reactive anti-V3 antibodies. This is consistent with the slow appearance of broadly reactive antibodies in the sera of infected individuals [Pilgrim1997], a factor that could have profound implications for the development of vaccine regimens.

Another important element in the generation of broadly reactive and potent human anti-V3 mAbs is the method used to select for cells producing the appropriate mAbs. Although the most broadly reactive of the neutralizing anti-V3 mAbs, 447-52D, was selected using a V3 peptide, the majority of mAbs selected with V3 peptides do not efficiently neutralize primary isolates [Gorny1993]. This is most probably due to the fact that peptides are highly flexible and may assume a myriad of conformations. Thus, most of the V3 mAbs selected with peptides are directed to structures which are irrelevant in the context of primary isolate infectivity; by chance, the structure of the $V3_{MN}$ peptide that captured mAb 447 bore a relevant conformation. In contrast, the use of a V3 fusion protein for mAb selection in which the V3 region retains a structure which approximates that of the native V3 loop [Kayman1994] resulted in the identification of mAbs that recognize conformation-sensitive V3 epitopes; the majority of these latter anti-V3 mAbs neutralize many primary isolates [Gorny2002]. Thus, within the repertoire of anti-V3 antibodies elicited in HIV-infected subjects, there are broadly reactive neutralizing antibodies, and these can be rescued if appropriate selection methods are employed.

The conformation-sensitive anti-V3 mAbs can neutralize primary isolates, and most can cross-neutralize a variety of isolates from clade B and, to a lesser extent, those from other clades [Gorny2004, Gorny2002]. The mAb 447-52D, though selected with a peptide, recognizes a conformational determinant [Gorny2002, Sharon2003, Stanfield2004], and it is the most broadly neutralizing of all the currently existing anti-V3 mAbs. This mAb interacts with the 14 residues at the crown of the V3 loop; its "core epitope" is defined by the sequence GPxR, a motif that is highly conserved among clade B viruses and which exists in a minority of other HIV subtypes [Gorny1992]. The presence and recognition of the arginine (R) residue in the core epitope is required for mAb 447-52D to exercise its activity [Zolla-Pazner2004b]), a fact that is explained by structural studies of this mAb in complex with a V3 peptide showing salt bridge and cation-pi interactions formed between the R residues in the core epitope and residues in the heavy chain of the mAb [Stanfield2004]. Other anti-V3 mAbs, such as mAbs 2182, 2191, 2219, 2412, 2442 and 2456 (Table I-C-4), do not recognize the same epitope as 447-52D, yet also display the ability to cross-neutralize primary isolates [Gorny2004, Gorny2002]. This cross-reactivity reveals the presence of features within the V3 loop which are conserved despite the sequence variation in this region. This structural conservation is also reflected in the role of the V3 loop in binding to the chemokine receptors which act as coreceptors for the virus [Cormier2002, Hill1997, Suphaphiphat2003, Trkola1996a].

I-C-5 Anti-CD4-binding site (CD4bs) mAbs

The HIV Immunology Database provides information about 30 anti-CD4bs human mAbs and 15 recombinant Fab fragments generated from PBMCs or bone marrow of HIV-1-infected individuals. In addition, there are six human anti-CD4bs mAbs listed which were produced from the cells of transgenic mice (Table I-C-5). Antibodies specific to the CD4bs inhibit the binding of sCD4 to gp120 and, as a consequence, interfere with virus attachment to the target cells. The CD4bs is made up of residues from C2, C3 and C4, conferring the conserved character of this domain and explaining the cross-reactivity of anti-CD4bs mAbs when tested for their ability to bind to gp120 molecules from viruses of diverse subtypes [Jeffs2001]. This immunochemical cross-reactivity is reflected in the ability of these mAbs to neutralize a broad array of TCLA strains; surprisingly, however, most anti-CD4bs mAbs cannot neutralize primary isolates [D'Souza1997, McDougal1996, Sullivan1995]. Since the CD4bs is a key feature of both TCLA and primary isolates, the selective neutralization of TCLA strains as opposed to primary isolates by most anti-CD4bs mAbs is surprising and still not fully explained. MAb IgG1b12 is a striking exception to the generalization that anti-CD4bs do not neutralize primary isolates [Burton1994]. Indeed, this mAb neutralized half of 90 primary isolates from diverse subtypes, and when tested against 30 primary isolates of subtype B, it neutralized 73% [Binley2004, Burton2004].

The differential activity of IgG1b12 vs. other anti-CD4bs mAbs has not been fully explained. It is possible that IgG1b12, which was produced using recombinant technology [Barbas1992], possesses a paratope that does not exist in na-

Table I-C-5: Anti-CD4bs mAbs

mAbs	Ab type	Neutralization	Reference
F105	mAb	TCLA	Posner1991
IgG1b12	mAb	PI	Burton1991
15e, 21h, F91	mAb	TCLA	Ho1991, Moore1993, Thali1992
1125H, 5145A	mAb	TCLA	Tilley1991
448-D, 559/64-D, 588-D, 654-D, 729-D, 830D, 9CL, 1027-30D, 1202-D, 1331E, 1570, 1595, 1599	mAb	TCLA	Karwowska1992
GP13, GP44, GP68	mAb	TCLA	Schutten1993
S1-1	mAb	TCLA	Moran1993
120-1B1	mAb	TCLA	Watkins1993
50-61A	mAb	TCLA	Fevrier1995
48-16	mAb	non-neutralizing	Fevrier1995
TH9	mAb	TCLA	D'Souza1995
205-43-1(HT5), 205-46-9(HT7), 205-42-15(HT6)	mAb	TCLA	Fouts1997
L28, L33, L41, L42, L52	Fab	TCLA	Ditzel1995
DA48, DO8i, b3, b6, b11, b13, b14, 2G6	Fab	TCLA	Parren1998
MTW61D	Fab	TCLA	Fouts1998
28A11/B1, 35F3/E2, 38G3/A9	Xeno-mAb	TCLA	He2002
55D5/F9, 46D2/D5, 67G6/C4	Xeno-mAb	non-neutralizing	He2002

ture. There is, however, nothing notably unusual about the structure of this mAb [Saphire2001]. Other explanations for its broad and potent activity may lie in its unusual dependence on regions within the V2 domain in order for binding to occur. Thus, it was shown that the Fab fragment of b12, as opposed to that of other anti-CD4bs mAbs, has reduced binding activity to V2-deleted gp120, and that deletion of V2 from isolate SF162, but not of V1, diminished the neutralizing activity of IgG1b12 for this virus; this distinguished IgG1b12 from another anti-CD4bs mAb, 654-D, and from IgG-CD4 [Stamatatos1998]. Additional evidence that the IgG1b12 epitope includes a portion of V2 comes from data showing that escape mutants generated when JR-FL was cultured in the presence of IgG1b12 displayed two substitutions in V2 as well as one in C3 [Mo1997]. The specificity of IgG1b12 for the CD4bs and V2 is reminiscent of the "V2/CD4bs" Fab fragments described by Dietzl *et al.* [Ditzel1995, Ditzel1997] (see above); however, no primary isolate neutralizing activity has been described for these latter Fab fragments, nor have they been compared to IgG1b12 in any published experiments.

A notable feature of mAb IgG1b12 is its long CDR H3 region, consisting of 18 amino acids. The CDR H3 projects above the rest of the antigen-binding site of the mAb, fitting into the pocket of the CD4bs of gp120 [Saphire2001]. Interestingly, several other broadly reactive and potent neutralizing human mAbs, such as anti-V3 mAb447-52D (see above), anti-CD4i mAbX5 (see below) and anti-gp41 mAb 2F5 (see below), also have long CDR H3 regions consisting of 20 to 22 residues [Darbha2004, Kunert1998, Stanfield2004], a characteristic that is shared by many human anti-viral Abs [Stanfield2004].

I-C-6 Anti-CD4-induced (CD4i) epitopes

This is a small group of human mAbs (Table I-C-6) which binds to the gp120 bridging sheet, a beta-sheet consisting of four anti-parallel beta-strands contributed by the C4 region and the V1/V2 stem [Kwong1998].

Several anti-CD4i mAbs were derived by Epstein-Barr virus transformation of B cells from the PBMCs of HIV-infected subjects [Thali1991, Xiang2002], while Fab X5 was selected from a phage display library derived from an HIV-1 infected donor whose serum displayed strong neutralizing activity [Moulard2002]. The phage library was screened with gp120-CD4-CCR5 complexes which exposed the CD4i epitope. The X5 epitope is near the CD4bs and CCR5 binding site but does not overlap with them; its specificity is slightly different than the 17b epitope which reacts with the CCR5 binding site only [Darbha2004, Moulard2002]. Immunochemical analyses show that the CD4i epitope only becomes accessible after the binding of gp120 to CD4. For example, anti-CD4i mAbs have little or no ability to bind to gp120 in ELISA until the envelope protein is preincubated with sCD4, which, by definition, induces the conformational changes that expose the CD4-induced epitope.

Since the bridging sheet interacts with the chemokine receptors, the mechanism of neutralization of the anti-CD4i mAbs is thought to be through the inhibition of gp120 binding to CCR5 and CXCR4 [Trkola1996a]. However, the anti-CD4i mAbs display no neutralizing activity against primary isolates. Only the Fab or single chain forms of antibodies with specificity for the CD4i epitope display neutralizing activity [Labrijn2003]. Thus, the scFv and Fab fragments of mAbs 17b and 48d displayed more potent neutralizing activity against JR-CSF, JR-FL and ADA than did the intact IgG forms of these mAbs, and, similarly, the scFv and Fab fragments of X5 potently neutralized these viruses but lost this activity when converted into a whole IgG molecule [Labrijn2003]. These data suggest that the size of the neutralizing molecule is a critical factor, and models juxtaposing the gp120 molecule on the virus particle and the chemokine receptor on the surface of the target cell indicate that the bulk of an intact IgG molecule prevents its insertion between gp120 and the coreceptor [Dey2003, Labrijn2003, Moulard2002]. Thus, steric hindrance precludes the ability of anti-CD4i IgGs from effectively neutralizing virus infectivity. Nevertheless, the X5 Fab neutralizes primary isolates from clades A, B, C, D, E, F and G, and neutralizes R5, X4 and R5X4 isolates, showing the exceptionally conserved character of CD4i epitopes [Moulard2002].

I-C-7 Anti-carbohydrate mAbs

There is only one neutralizing human mAb, 2G12, which recognizes an epitope composed of carbohydrates (Table I-C-7); this mAb is specific for high-mannose and/or hybrid glycans at residues 295, 332 and 392 with peripheral glycans from 386 and 448 on either flank [Sanders2002, Scanlan2002]. These carbohydrate moieties are located on an exposed surface of the gp120 trimer that does not interact with CD4 or the chemokine receptors. Nonetheless, mAb 2G12 inhibits gp120 interaction with CCR5 as shown in MIP-1beta-CCR5 competition studies [Sanders2002, Trkola1996a]. These data led to the hypothesis that the neutralizing activity of mAb 2G12 is an indirect, steric effect manifested by a binding site that is physically close to the receptor-binding sites of gp120 [Scanlan2002]. mAb 2G12 potently neutralizes TCLA and was recently shown to neutralize 41% of primary isolates representing various subtypes [Binley2004, Burton2004, Scanlan2002, Trkola1996b].

Table I-C-6: Anti-CD4i mAbs

mAbs	Ab type	Neutralization	Reference
17b, 21c, 23e, 48d, 49e	mAb	TCLA	Thali1993, Xiang2002
X5	Fab	PI	Moulard2002

Table I-C-7: Anti-Carbohydrate mAbs

mAbs	Ab type	Neutralization	Reference
2G12	mAb	PI	Buchacher1994

Despite the standard cellular technique used in the generation of mAb 2G12 and the commonly employed approach for selection of the mAb by measuring binding to gp160 [Buchacher1994], mAb 2G12 is unique both in terms of structure as well as specificity. Recent crystallographic studies revealed that two Fabs of mAb 2G12 assemble into an interlocked VH domain-exchanged dimer forming an extended binding site which targets the aforementioned conserved cluster of oligomannose moieties on the surface of gp120 [Calarese2003].

The 2G12 epitope is poorly immunogenic as reflected by competitive binding assays that demonstrated that 2G12-like antibodies were absent from all of 16 sera from HIV-infected long-term survivors and AIDS patients [Trkola1996b]. These factors—the unusual structure of mAb 2G12, its unusual epitope, and the poor immunogenicity of this epitope—suggest that, despite the undeniable potency and breadth of activity of mAb 2G12, the probability of inducing similar Abs with a vaccine may be quite low.

I-C-8 Anti-gp41 mAbs

As shown in Table I-C-8, there are 28 human mAbs and 24 Fab fragments directed to the highly immunogenic regions of gp4 [Binley1996, Xu1991]. Twenty-four mAbs and Fab fragments are directed to the most immunodominant region, cluster I (aa 579–604), 19 mAbs and Fab fragments are specific for cluster II (aa 644–663), three are specific for an epitope adjacent to cluster II, and six Fab fragments are specific for cluster III (a conformational epitope involving aa 619–648). Only four out of these 52 mAbs and Fab fragments have documented neutralizing activity: mAbs 2F5, 4E10, clone 3 and Fab fragment Z13.

MAb 2F5 is one of the best studied of the human mAbs. It has broad and potent activity, neutralizing 67% of 90 isolates from various virus subtypes [Binley2004, Burton2004, Trkola1995]. The linear 2F5 epitope is located near the transmembrane domain at aa 662–667, a region which is adjacent to cluster II and is not well exposed on the virus or on virus-infected cells [Nyambi1998, Ofek2004, Sattentau1995]. Immunochemical data show that 2F5 reacts strongly with peptide C43 representing a portion of the C-heptad repeat region of gp41, while there is no reactivity with peptide N51, the N-heptad repeat region of gp41. While mAb 2F5 reacts with the N51:C43 complex, which forms a coiled-coil complex, it reacts with the complex less strongly than it does with C43 alone [deRosny2004, Gorny2000]. While this might suggest that 2F5 interferes with the formation of the gp41 coiled-coil, this was not borne out by recent studies [deRosny2004]. Thus, the mechanism of neutralization by mAb 2F5 is still unknown, although interference with the late fusion process has been suggested [deRosny2004].

MAb 4E10 and Fab Z13 are also specific for a region that is adjacent to cluster II. They recognize the same epitope, and both are specific for a predominantly linear and relatively conserved epitope at aa 671–677 that is proximal to that of 2F5 [Zwick2001]. mAbs 4E10 and Z13 neutralize primary isolates of diverse clades, including A, B, C, D and E [Zwick2001]. While mAb 4E10 is the most broadly neutralizing mAb currently described, it is less potent than the other well-described, broadly neutralizing mAbs [Binley2004, Burton2004, Zwick2001].

MAb clone 3 binds to a linear epitope between aa 597 and 606 at the C-terminal end of cluster I, the most immunodominant region of gp41 [Cotropia1996]. The epitope is quite conserved, a fact reflected by its ability to neutralize three diverse TCLA viruses from clade B and three primary isolates

Table I-C-8: Anti-gp41 mAbs

mAbs	Ab type	Neutralization	Reference
Cluster I*			
1B8	mAb	non-neutralizing	Banapour1987
86	mAb	non-neutralizing	Sugano1988
50-69, 181-D, 240-D, 246-D, 1367	mAb	non-neutralizing	Gorny1989, Nyambi1998
V10-9	mAb	non-neutralizing	Robinson1990
3D6	mAb	non-neutralizing	Felgenhauer1990
2F11	mAb	non-neutralizing	Eaton1994
1F11, 1H5, 3D9, 4B3, 4D4, 4G2	mAb	non-neutralizing	Buchacher1994
clone 3	mAb	TCLA, PI	Cotropia1996
F240	mAb	non-neutralizing	Cavacini1998
A1, A4, M8B, M12B, M26B, T2	Fab	non-neutralizing	Binley1996
Cluster II*			
98-6, 126-6, 167, 1281, 1342, 1379	mAb	non-neutralizing	Gorny1989, Xu1991
ND-15GI	mAb	non-neutralizing	Eddleston1993
Md-1	mAb	non-neutralizing	Chen1995
D5, D11, G1, M10, M12, M15, S6, S8, S9, S10, T3	Fab	non-neutralizing	Binley1996
Adjacent to Cluster II*			
2F5	mAb	PI	Buchacher1994
4E10	mAb	PI	Buchacher1994
Z13	Fab	PI	Zwick2001
Cluster III*			
A9, G5, G15, L1, L2, L11	Fab	non-neutralizing	Binley1996

* Cluster I: aa 596–613; Cluster II: aa 644–663; Adjacent to Cluster II: aa 662–676; Cluster III: conformational epitope involving aa 619–648.

from group O [Cotropia1996, Ferrantelli2004]. Beyond the activity of clone 3 against these viruses, few details are known about its functional breadth. The clone 3 epitope overlaps with the epitope of mAbs 246 and 240 (aa 590–597 and 592–600, respectively). Interestingly, these latter mAbs bind to cluster I but have little or no neutralizing activity [Hioe1997] despite their ability to bind to intact virus particles [Nyambi1998].

As noted above, the mechanism(s) of neutralization of all four neutralizing anti-gp41 mAbs is still unknown. An indication that at least some anti-gp41 mAbs may interfere with virus/cell fusion was provided by studies of the fusion process at a suboptimal temperature (31.5°C), which prolongs the time during which fusion intermediates are exposed to mAbs [Golding2002]. These experiments showed that human mAbs against cluster II (mAbs 98-6, 1281 and 167-D) strongly inhibited fusion between HIV envelope-expressing effector cells and target cells expressing appropriate receptors [Golding2002].

I-C-9 Concluding remarks

Among the 174 human mAbs summarized in Tables I-C-1–I-C-8, only five mAbs (2G12, IgGb12, 447-52D, 2F5 and 4E10) and two Fab fragments (X5 and Z13) can be classified as broadly and potently neutralizing for HIV-1 primary isolates. Presently, the epitopes targeted by some of these mAbs do not appear to be practical targets for vaccine development due to their weak immunogenicity (2G12, 2F5) or their inaccessibility to antibody molecules (17b, X5, etc.). The CD4bs, which would appear to be an ideal target for neutralizing antibodies, induces antibodies which, for the most part, have little or no activity against primary isolates. And, while the epitopes recognized by neutralizing V3 mAbs are accessible and immunogenic, they induce a spectrum of antibodies ranging from narrowly to broadly cross-reactive, but the latter apparently require prolonged antigenic stimulation to emerge.

While the search for and design of effective immunogens continues, the most efficacious of the mAbs described to date may serve as beacons to illuminate the effort. Meanwhile, continuing work is needed, using new screening techniques, to identify neutralizing mAbs to additional classes of epitopes in order to provide the maximum number of viral determinants to target with a vaccine.

I-C-10 Acknowledgments

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