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Identification of a novel CD4i human monoclonal antibody Fab that neutralizes HIV-1 primary isolates from different clades

Mei-Yun Zhang [a,b], Yuuei Shu [a], Igor Sidorov [a], Dimiter S. Dimitrov [a,*]

[a] *Human Immunovirology and Computational Biology Group, LECB, CCR, NCI-Frederick, NIH, Frederick, MD 21702, USA*
[b] *BRP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA*

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Abstract

A new human monoclonal antibody (hmAb), designated m16, was selected by sequential antigen panning (SAP) of a human phage display library against recombinant soluble HIV-1 envelope glycoproteins (Envs) (gp140s) and their complexes with soluble CD4. It bound with high (nM) affinity to gp120 and gp140; the binding was further enhanced by interactions of the Envs with CD4. m16 inhibited cell fusion mediated by the Envs of 9 HIV-1 isolates from clades A, B, E and G with potency on average comparable to that of the broadly neutralizing human monoclonal antibody Fab X5. The identification of a new hmAb with broad neutralizing activity that exhibits differential inhibitory profile suggests a potential for its use as a component of anti-HIV-1 treatments.
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Major problems in prevention and treatment of HIV-1 infections are the virus' ability to rapidly generate mutants resistant to immune responses and drugs, and the side effects of antiretroviral drugs currently in use. Several human monoclonal antibodies (hmAbs) exhibit potent and broad HIV-1 neutralizing activity in vitro, and can prevent HIV-1 infection in animal models (reviewed in (Burton, 2002; Ferrantelli and Ruprecht, 2002), see also a recent article (Veazey et al., 2003)). A recent clinical trial suggested that two of these broadly HIV-1 neutralizing hmAbs, 2F5 and 2G12, are without side effects in humans (Armbruster et al., 2002; Stiegler et al., 2002). However, the potency of 2F5 and 2G12 used in combination in this clinical trial was significantly lower than currently used HAART regimens and relapses did occur (Stiegler et al., 2002). Further increase in the potency of the currently available broadly HIV-1 neutralizing hmAbs and development of new neutralizing hmAbs might help in the development of better approaches for prevention and treatment of HIV-1 infection.

Here we described the identification of a new hmAb, m16 that was selected by a methodology termed sequential antigen panning (SAP), based on sequentially changing the antigen during the panning of phage display libraries (Zhang et al., 2003a). The materials and the basic methodologies were essentially the same as those we used for identification and characterization of the broadly HIV-1 neutralizing hmAb Fab X5 (Moulard et al., 2002) except for the human antibody phage library that was provided by T. Evans (University of California, Davis).

We hypothesized that by sequentially changing antigens during panning of phage display libraries and screening the panned libraries using different antigens, the selected phage will display Fabs against conserved epitopes shared among all antigens used during the entire selection process. Complexes of two different recombinant soluble Envs ($gp140_{89.6}$ and $gp140_{IIIB}$) with two-domain soluble CD4 (sCD4) were used as antigens for phage library panning. Purified $gp120_{89.6}$, $gp140_{89.6}$ and $gp140_{IIIB}$ were expressed by using a recombinant vaccinia virus (gift of R. Doms, University of Pennsylvania, Philadelphia, PA), and purified by using a combination of lentil lectin affinity chromatography and size exclusion chromatography. Phage (5×10^{12} cfu/ml) were preadsorbed on streptavidin-M280-Dynabeads in PBS for 1 h at room temperature (RT) followed by depletion in an immunotube (Nunc, Denmark) coated with 10 μg/ml sCD4 for 1 h at 37 °C. Depleted phage library was incubated with 50 nM biotinylated $gp140_{89.6}$ complexed with sCD4 in solution ($gp140_{89.6}$:sCD4 = 1:1 on molar level)

* Corresponding author. Tel.: +1-301-846-1352; fax: +1-301-846-6189.
E-mail address: dimitrov@ncifcrf.gov (D.S. Dimitrov).

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for 2 h at RT with gentle agitation. Phage bound to biotinylated $gp140_{89.6}$ were separated from the phage library using streptavidin-M280-Dynabeads and a magnetic separator (Dynal). The beads were washed 20 times with 1 ml of PBS containing 0.1% Tween-20 and another 20 times with 1 ml of PBS. Bound phage were eluted by incubation at RT for 10 min with 1 ml of 100 mM TEA followed by neutralization with 0.5 ml of 1 M, pH 7.5 Tris–HCl. Eluted phage were rescued by infection of *Escherichia coli* TG1 cells and phage library was prepared for the next round of panning. For the second round of panning, the phage library was preadsorbed on streptavidin-M280-Dynabeads and immobilized sCD4 as before and 50 nM of biotinylated $gp140_{IIIB}$ complexed with sCD4 (1:1 on molar level) used as antigen. For the third and fourth rounds of panning, 10 nM (2 nM for fifth and sixth rounds) of biotinylated $gp140_{89.6}$ and $gp140_{IIIB}$ alone were sequentially used as antigens. After four, five and six rounds of panning, screening of individual phage clones was performed in a phage ELISA with $gp140_{89.6}$, $gp120_{JR\text{-}FL}$ and $gp140_{IIIB}$, and their complexes with sCD4. Two clones were selected based on their significant binding to all six antigens used for screening including sCD4-gp120 and gp120 from an HIV-1 isolate (JR-FL) which was not used for panning and has significant sequence differences compared to 89.6 and IIIB. One phage clone, designated m16, showed enhanced binding to Env complexes with sCD4 and was selected for further characterization. Phagemid DNA of m16 was prepared and sequenced. In a control experiment to assess the efficiency of the SAP methodology, the panning was performed only with one antigen (sCD4-$gp140_{89.6}$). In this case none of the clones tested bound to $gp140_{IIIB}$, sCD4-$gp140_{IIIB}$, $gp120_{JR\text{-}FL}$ and sCD4-$gp120_{JR\text{-}FL}$ (data not shown).

m16 bound to $gp120_{JR\text{-}FL}$ and $gp140_{89.6}$ with high (nM and subnanomolar) affinity as measured by an ELISA assay; its binding was significantly enhanced by sCD4 (Fig. 1 and Table 1). These results suggest that m16 can strongly bind to gp120 and gp140 and that its binding is significantly increased by the CD4 interaction with gp120 (i.e. m16 is a CD4i antibody).

To determine the breadth and potency of HIV-1 neutralization by m16 we measured its ability to inhibit cell fusion mediated by Envs of primary isolates from different clades. m16 inhibited various isolates with potency on average comparable to that of Fab X5 (Table 2). Similar results were obtained by counting syncytia (data not shown). One should note that m16 is a Fab fragment. The neutralizing activity of Ig m16 could be lower for some isolates because of restriction access effects due to size as observed for IgG1 X5 (Labrijn et al., 2003) but higher for others in dependence on the interplay between avidity and size effects. Experiments

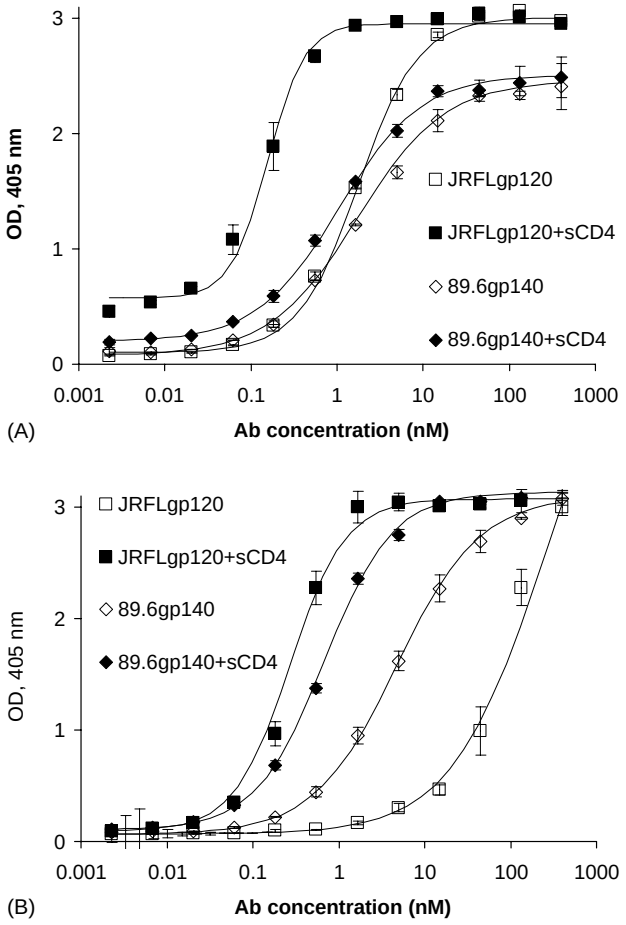

Fig. 1. Binding of m16 to gp120 (gp140) and sCD4-gp120 (gp140). Gp120 or gp140 (1 μg/ml) were captured by the polyclonal sheep anti-gp120 antibody D7324 that was coated (at 5 μg/ml) on microplates (captured ELISA) (Fig. 1A) or directly coated on microplates (coated ELISA) (Fig 1B). Three-fold serially diluted m16 was added to the wells in the presence or absence of sCD4 (2 μg/ml). Bound m16 was detected by anti-human IgG $F(ab')_2$-HRP and measured as optical densities at 405 nm. The background was estimated by the amount of Fabs bound to BSA and subtracted. The data were fitted to the Langmuir adsorption isotherm: $B/B_{max} = F^n/(EC_{50}{}^n + F^n)$, where B is the amount of bound Fab, B_{max} is the maximal amount of bound Fab, F is its bulk concentration and EC_{50} is the concentration of half-maximal binding approximately equal to the equilibrium dissociation constant, n is a constant close to 1. The lines represent the data fitting and the symbols the data.

Table 1
Binding of m16 to gp120 or gp140, and sCD4-gp120 or sCD4-gp140

Env	Captured ELISA		Coated ELISA		
	$gp140_{89.6}$	$gp120_{JR\text{-}FL}$	$gp140_{89.6}$	$gp120_{JR\text{-}FL}$	$gp120_{IIIB}$
Without sCD4	1.7 ± 0.02	1.8 ± 0.02	4.8 ± 0.5	240 ± 90	6.3 ± 0.9
With sCD4	1.0 ± 0.07	0.16 ± 0.01	0.68 ± 0.03	0.27 ± 0.02	5.0 ± 1.1

The data were obtained by captured and coated ELISA as described in the legend of Fig. 1. EC_{50} and standard deviation of EC_{50} in nM are shown.

Table 2
Inhibition of HIV-1 Env-mediated fusion by Fab m16 and Fab X5

Env	Clade	Antibody	
		m16	X5
RW020.5	A	65	83
US715.6	B	88	89
HT593.1	B	34	45
US05.11	B	88	89
89.6	B	63	100
NL43	B	83	63
TH022.4	EA	78	87
UG975.1	G	44	57

$10^5 \times 293$ cells, transfected with plasmids encoding various HIV-1 Envs under the control of T7 promoter and infected with recombinant vaccinia virus encoding T7 polymerase gene, were preincubated with X5 or m16 at 50 μg/ml for 30 min at 37 °C, and then mixed with 10^5 CEM-CCR5 cells infected with recombinant vaccinia virus encoding β-galactosidase gene. The extent of cell fusion was quantified colorimetrically 2 h after mixing the cells. The data are averages of duplicate samples and presented as percentage of fusion inhibition. The standard deviation was on average 9% and less than 15% for each of the experiments. In a control experiment the CEM-CCR5 cells were preincubated with the anti-CD4 antibody Q4120 (Healey et al., 1990) at 100 μg/ml for 30 min at 37 °C that reduced the signal to a background level (on average 10% of signal intensity in the absence of antibody). Similar background level was also observed for CD4-negative cells that do not fuse.

are in progress to evaluate this possibility. These results suggest that m16 can neutralize a broad range of HIV-1 primary isolates, and perhaps its efficacy could be further increased in scFv format (Zhang et al., 2003b) or in combination with sCD4 or as a fusion protein with CD4.

Of the large number of mAbs and Fabs that have been generated against HIV-1 Envs until recently only several human mAbs were identified that exhibit broad and potent HIV-1 neutralizing activity (D'Souza et al., 1997): two against gp120–IgG1 b12 (Burton et al., 1994; Roben et al., 1994) and 2G12 (Sanders et al., 2002; Scanlan et al., 2002; Trkola et al., 1996), and one against gp41, 2F5 (Muster et al., 1993). Two other antibodies against gp120, the Fab X5 (Moulard et al., 2002) and IgG 447-52D (Conley et al., 1994) and two against gp41, 4E10 (Stiegler et al., 2001; Zwick et al., 2001) and Fab Z13 (Zwick et al., 2001) are also known to neutralize a variety of HIV-1 primary isolates from different clades. Of note is that some of these broadly neutralizing antibodies, e.g. 2G12, do not efficiently neutralize important clade C HIV-1 isolates. The CD4i antibody Fab X5 does neutralize at least one clade C isolate (BR025) (Moulard et al., 2002), and the recently developed CD4i scFvs m6 and m9 (Zhang et al., 2003b) neutralize three clade C isolates (ZA003, CN006 and IN017). As a CD4i antibody m16 may also neutralize clade C isolates but that remains to be shown. Recently, a broadly HIV-1 -neutralizing hmAb (IgG1 b12) was shown to protect macaques from vaginal challenge with SHIV-1 (Veazey et al., 2003) supporting the concept that hmAbs can be used for protection of sexually transmitted virus. Further studies are needed to determine whether m16 alone or in combination has protective activity in vivo. Only experiments in animal models and clinical trials in humans will show whether this new antibody has potential as an HIV-1 therapeutic.

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