Neutralizing antibodies to the HIV-1 envelope glycoproteins
Labrijn, A.F.

Citation for published version (APA):
Labrijn, A. F. (2004). Neutralizing antibodies to the HIV-1 envelope glycoproteins
Chapter 5

Novel broadly cross-reactive HIV-1 neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes

Maxime Moulard1*
Sanjay Phogat2*
Yuuei Shu2*
Aran F. Labrijn1
Xiaodong Xiao2
James M. Binley1
Mei-Yun Zhang2
Igor A. Sidorov2
Christopher C. Broder3
James Robinson4
Paul W.H.I. Parren1
Dennis R. Burton1
Dimiter S. Dimitrov2

1Departments of Immunology and Molecular Biology, The Scripps Research Institute, La Jolla, CA
2Laboratory of Experimental and Computational Biology, National Cancer Institute-Frederick, NIH, Frederick, MD
3Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD
4Department of Pediatrics, Tulane University Medical Center, New Orleans, LA
*Authors contributed equally

PNAS (99): 6913-6918 '2002
HIV-1 entry into cells involves formation of a complex between gp120 of the viral envelope glycoprotein (Env), a receptor (CD4) and a coreceptor, typically CCR5. Here we provide evidence that purified gp120, gp120-x5, CD4-CCR5 complexes exhibit an epitope recognized by a novel Fab (X5) obtained by selection of a phage display library from a seropositive donor with a relatively high broadly neutralizing serum antibody titer against an immobilized form of the trimolecular complex. X5 bound with high (nM) affinity to a variety of Envs including primary isolates from different clades and Envs with deleted variable loops (V1, V2, 3). Its binding was significantly increased by CD4 and slightly enhanced by CCR5. X5 inhibited infection of peripheral blood mononuclear cells by a selection of representative HIV-1 primary isolates from clades A, B, C, D and G with an efficiency comparable to that of the broadly neutralizing antibody lgG1 b12. Furthermore X5 inhibited cell fusion mediated by Envs from R5, X4 and R5X4 viruses. Of the five broadly cross-reactive HIV-1 neutralizing human monoclonal antibodies known to date, X5 is the only one which exhibits increased binding to gp120 complexed with receptors. These findings suggest that X5 could be possibly used as entry inhibitor alone or in combination with other antiretroviral drugs for the treatment of HIV-1 infected patients, and have important conceptual and practical implications for development of vaccines and inhibitors.

Introduction
Binding of the HIV-1 envelope glycoprotein (Env, gp120-gp41) to CD4 and coreceptors initiates a series of conformational changes that are the heart of the fusogenic machinery leading to viral entry (13, 15). The elucidation of the nature of the Env conformational changes is not only a clue to the mechanism of HIV-1 entry but may also provide new tools for the development of inhibitors and vaccines (9, 27, 38). It has been proposed that the interaction of coreceptor molecules with the Env-CD4 complex leads to intermediate Env-CD4-CCR5 complexes and used for selection of human monoclonal antibodies which in turn could be helpful for characterization of HIV-1 entry intermediates. These antibodies could be broadly neutralizing if their epitopes include conserved intermediate structures that are exposed during entry. In addition, the use of complexed coreceptor molecules may prevent selection of antibodies against the coreceptor binding site on gp120 that may be minimally accessible following attachment of native Env to cell surface associated CD4; such antibodies as 1b7 and CG10 are only weakly neutralizing against primary isolates (39). Here we report the identification of novel

Materials & Methods

Cells, viruses, plasmids, soluble CD4 (sCD4), gp120, gp140 and monoclonal antibodies (mAbs). 3T3 cells expressing CD4 and CCR5 were a gift from D. Libman (New York University, New York, NY); C127 cells expressing high concentrations of CCR5 were a gift from J. Sodroski (Dana Farber Institute, Boston, MA); the parental cells were purchased from ATCC and used as a negative control. The stable cell line TF228 expressing LAI Env was a gift from J. Zonak (SmithKline Beecham Pharmaceuticals, Philadelphia, PA) through R. Blumenthal (NCI-Frederick, Frederick, MD). The CEM cells expressing CCR5 (CEM-CCR5) were a gift from J. Moore (Cornell University, New York, NY). The T-cell line H9 was obtained from the MRC AIDS Reagent Project. Peripheral blood mononuclear cells (PBMCs) were isolated and pooled from three wild-type CCR5 donors. All HIV isolates were also obtained from the NIH AIDS Research and Reference Program (ARRP). Recombinant vaccinia viruses used for the reporter gene fusion assay were described previously (26). Plasmids used for expression of various Envs were obtained through the ARRP from B. Hahn (University of Alabama). Two-domain soluble CD4 (sCD4) was obtained from the ARRP. Purified gp120-x5 and gp140-x5 were produced by recombinant vaccinia virus (gift of R. Doms, University of Pennsylvania, Philadelphia, PA) with a combination of lentil lectin affinity chromatography and size exclusion chromatography. Recombinant gp140 from the primary isolates 92UG007, 92HT593, 93M190.5, 93RZ001, and gp120 from 93RZ001 were prepared similarly using clones obtained from the ARRP. Recombinant gp120-x5 was a gift from A. Schultz and N. Miller (NAID, Bethesda, MD). The single chain fusion protein gp120-x5-CD4 (17) was a gift from T. Fouts (Institute of Human Virology, Baltimore, MD); gp120-x5 and gp140-x5, with deleted variable loops and the disulfide bond stabilized (140w) were previously described (4, 34). The anti-gp120 mAbs 1b7 and 43b were previously derived by a method described in (32) and characterized in (40); 2b6 and 21c are newly derived and characterized (47). The anti-gp120-CD4 complex specific mAb CG10 was previously described (18). Various other anti-gp120 mAbs were previously described; 1b9 (28); F91, A32, G3-136, G3-S19 (26); b12 (6); G212 (43). The mAb MAG45 was a gift from C-Y Kang (IDEC Pharmaceuticals). The anti-CCR5 mAb 5C7 was a gift from C-Y Kang (IDEC Pharmaceuticals). The anti-CCR5 mAb 5C7 was a gift from C-Y Kang (IDEC Pharmaceuticals). The anti-CCR5 mAb 5C7 was a gift from C-Y Kang (IDEC Pharmaceuticals).
produced by a methodology described previously (48) but modified to allow production of larger amounts. NIH 3T3 transfectants (10^5 in 100 ml) expressing high levels of CD4 and CCR5 were washed twice with cold (4°C) phosphate-buffered saline (PBS) then pelleted by centrifugation and incubated in 10 ml PBS buffer at 4°C for 14 hours. The pellets were washed five times with 100 ml of cold ice lysing buffer and incubated with gp20-CCR5, at 5 μg/ml in 20 ml lysis buffer for 1 hour at 4°C. They were again washed five times with 100 ml cold ice lysis buffer, once with cold PBS, incubated with 0.2% formaldehyde overnight, and finally washed twice for two days in an ice bath. The protein was purified by precipitation with 10 μg CD4, 10 μg CCR5 and 20 μg gp120 as quantified by monitoring optical absorbance of soluble CD4, detergent solubilized CCR5 (dsCCR5) and gp120. For quantification of CD4 and gp120, two duplicate samples each containing 0.1% of the total amount of bead-associated gp120-CD4-CCR5 complexes were used. They were eluted by adding 4X sample buffer for SDS-PAGE gel, kept overnight at 37°C and run on a 10% SDS-PAGE gel simultaneously with calibrating amounts (1, 3, 10, 30, 100 ng) of soluble four-domain CD4 or gp120. Quantification of CCR5 was performed similarly but the calibrating amounts of dsCCR5 were themselves pre-calibrated with a 38 amino acid residue N-terminal CCR5 peptide by using rabbit polyclonal anti-CCR5 antibodies against this peptide sequence. The samples were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 20 ml Tris-HCl (pH 7.6) buffer containing 140 mM NaCl, 0.1% Tween-20 and 5% nonfat powdered milk. For Western blotting these membranes were incubated with anti-CD4, anti-gp120 or anti-CCR5 antibodies, then washed, incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Bethyl, Montgomery, PA). The antibodies were then detected by using the supersignal chemiluminescent substrate from Pierce (Rockford, IL). The images were acquired by a BioRad phosphorimager (BioRad, Hercules, CA). The signal from the calibrating molecules was integrated for each band and plotted on a calibration curve. The amounts of CCR5, CD4 and gp120 were then calculated by interpolating the calibration curves. DsCCR5 was produced by a methodology described previously (23).

Phage display library screening. A phage library (IgGlK) from a seropositive individual with a relatively high cross clade neutralizing titer (FDAZ) (31, 44) was used. Phage (5 μl) were preadsorbed on protein G beads with HEPES buffer at pH 7.5 under gentle agitation. Beads were washed 10 times with PBS containing 0.5% Tween. Phage were eluted from the beads by incubation with 50 μl 0.1M HCl-glycine (pH 2.2) solution containing BSA at 1 mg/ml for 10 min at room temperature. The solution was neutralized with 3 μl of 2M Tris base. X1L-Blue E. coli cells were reinfected and panning repeated for total of 5 rounds.

Preparation of soluble Fab fragments. Phagemid DNA was isolated from the panned library and digested with Spe I and Nhe I to remove the gene III fragment and self-replicated as described elsewhere (2). The gene III-charged library was used to transform X1L-Blue E. coli. 50 individual clones were grown up. The corresponding Fabs were obtained by lysing the cell pellet. Cells were frozen in a dry ice-ethanol bath for 5 min followed by thawing in a 37°C water bath. This process was repeated four times and the cell debris was pelleted by centrifugation at 15,000 rpm for 15 minutes at 4°C. Soluble Fabs were produced as described (2). Protein G columns were used for purification.

ELISA analysis of Fab supernatants. ELISA wells were coated overnight at 4°C with 50 μl of gp120 (10 μg/ml in PBS), blocked in 100 μl of 3% BSA/PBS for 1 hour at 20°C. After 5 washes with 0.05% Tween20/PBS washing buffer (WB), wells were incubated with 50 μl of Fab supernatants for 1 hour at 20°C. After 10 washes with WB, 50 μl of 1:1000 dilution of alkaline phosphatase-conjugated goat-anti-human IgG-F(ab')2 was added and incubated for 1 hour at 20°C. Following 10 washes with WB, the assay was developed at 20°C for 30 minutes with p-nitrophenyl phosphate substrate (Sigma) and monitored at 405 nm. Positive clones showed absorbance values >1.

The CDR3 region of the heavy chains from positive clones were sequenced using the Sequenator (S)-GAAGATTAGCACCTTGACAGC-3).
was selected. The CDR3 sequence of the heavy chain for X5 binding to gp120 (data represented by empty data were fitted to the Langmuir adsorption isotherm by the amount of X5 bound to BSA and subtracted. The optical density at 405 nm. The background was estimated detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')
r
added at the indicated concentrations. Bound X5 was coated directly on 96-well plates, washed and X5 was gp120-sCD4. Gp120 and gp120-sCD4 complexes were detected by anti-human F(ab')

of this Fab was unique - it was not identified previously either in the laboratory (DRB) or reported in the Genbank database. Phage-displayed X5 exhibited binding activity to protein G cross-linked to Sepharose beads with an apparent affinity (equilibrium dissociation constant) of 1.4 nM which was 15-fold lower than the affinity for gp120,FL-sCD4 complexes (0.09 nM).

Binding of X5 to gp120 and gp140 from different isolates that is significantly increased by CD4 and slightly enhanced by dsCCR5

X5 bound gp120 from the primary isolate 89.6 with an affinity (equilibrium dissociation constant) of 9.4 nM which was increased 10-fold to 1 nM after binding of sCD4 to gp120 as measured by an ELISA assay (Fig. 5.1). Similarly, the affinity of X5 binding to gp120,FL was increased from 10 nM to 2 nM after addition of sCD4 (Fig. 5.1). Binding of X5 to gp120 from the dual tropic primary isolate 93Z001.3 (Clade D) and gp120,FL was also increased significantly by CD4 (not shown). X5 also bound a single chain fusion protein, where gp120,FL and CD4 are intramolecularly associated (17), at a level similar to that for the scD4-gp120,FL complex (not shown).

X5 bound a cleaved disulfide bond stabilized gp140,AV3,AV2 (4), and the binding was increased by scD4 (Fig. 5.2). Binding of X5 to gp140,AV3,AV2 and its complex with scD4 was somewhat lower but comparable to the binding to gp120,FL and scD4-gp120,FL. Recombinant gp140 from primary isolates from different clades (92UG037.8, Clade A, R5; 92HT593.1, Clade B, R5X4; 93MW965.26, Clade C, R5; 93Z001.3, Clade D, R5X4) complexed with scD4 bound X5 (not shown). The binding was at levels similar or lower than to 89.6 gp140 complexed with scD4; for two isolates (92UG037.8 and 92HT593.1) binding to gp140 in the absence of scD4 was significantly lower than to gp140,FL. X5 also bound gp120 with deleted V3 loop, and gp140 with deleted V1 and V2 or V1 and V2 and V3 loops (34) in a CD4-dependent manner (Fig. 5.2).

The amount of bound X5 to gp120,FL-sCD4 complexes and to single chain gp120-sCD4 molecules was slightly (on average 1.3-fold) enhanced in presence of dsCCR5 (not shown). Although small this increase was statistically significant (p=0.005 as calculated by a t-test); to ensure that the enhancement was not due to artifacts and was reproducible, this experiment was performed six times during a three month period using different aliquots of dsCCR5 either freshly prepared or stored for up to one week. In addition, the native conformation of dsCCR5 was
tested by using the conformationally dependent anti-CCR5 mAB 2D7 and sCD4-gp120 complexes which bound specifically to dsCCR5 (not shown, and previously reported (23)). Finally, in another set of experiments the enhancing effect of dsCCR5 was partially reversed by adding RANTES or MIP-1β (not shown). The results of these control experiments suggested that the enhancement of the X5 epitope by dsCCR5 was specific and reproducible. X5 did not bind denatured gp120s96 suggesting discontinuity of its epitope. Therefore, X5 binds to a conserved conformational gp120 epitope that is significantly affected by CD4 and slightly by CCR5 but differs from their binding sites.

X5 competition with known antibodies to gp120
To further characterize the X5 epitope we measured X5 competition with anti-gp120 mAbs in the presence or absence of sCD4. The major results are (Table 5.1): i) X5 competed to varying degrees with the antibodies 17b, CG10, 48d, 23e and 21c which bind CD4-inducible epitopes. ii) X5 binding to gp120 was enhanced by the A32 mAb which binds a CD4-inducible epitope; in turn X5 binding enhanced the exposure of the A32 epitope (not shown). iii) X5 competed to some extent with mAbs to the CD4 binding site such as lgG1b12 and F91. iv) X5 did not compete with mAbs against other regions of gp120 including the V3 (19b) and V2 (G3-519) loops, C1 (MAG45, C11), C3/V4 (2G12), C4 (G3-136) and C5 (C11). v) The competition pattern was not significantly dependent of the Env used at least for the Env's from the two isolates investigated in detail (89.6 and JR-FL). These results suggest that the X5 epitope is likely located in close proximity to the coreceptor and CD4 binding sites.

Inhibition of HIV-1 infection and Env-mediated cell fusion
To determine the breadth and potency of HIV-1 neutralization by X5 we measured its ability to inhibit infection of PBMCs by different clades in comparison with the potent broadly neutralizing mAbs with X5 for gp12019b and gp12021c, gp12019b was captured by the anti-gp120 Ab D7324, incubated with X5 (5 μg/ml) and various antibodies at different concentrations in absence or presence of sCD4 (2 μg/ml), and IgG Fc of the bound antibodies was detected with goat anti-mouse or anti-human IgG Fc alkaline phosphatase conjugate. Gp120s96 was directly coated on 96-well plates, incubated with biotinylated X5 (0.5 μg/ml) and various antibodies, and the amount of bound X5 was detected by using streptavidin-HRP. Here + denotes some competition, ++ significant competition and +++ complete displacement; similarly - denotes various degrees of enhancement; 0 denotes lack of measurable effects and na - not applicable.
antibody b12 (Table 5.2). X5 neutralized all tested primary isolates with a potency that was generally comparable to or in some cases better than IgG1 b12. The potency was particularly noteworthy given that X5 was assessed as an Fab fragment; its potency may improve as a whole IgG molecule. This molecule is currently being generated. X5 was also able to neutralize several representative R5 (JR-FL and Bal), X4 (NL4-3) and X4R5 (89.6) viruses at IC50 in the range of 0.1 to 10 ng/ml in another assay based on a luciferase reporter HIV-1 Env pseudotyping system (not shown). X5 inhibited cell-cell fusion mediated by Env of primary isolates from different clades with a potency comparable to that of IgG b12 as measured by a reporter gene assay (Table 5.3). X5 almost completely inhibited sCD4-induced fusion mediated by Env from NL4-3, HXB2, 89.6, JR-FL, ADA, Bal and SF162 at very low (100 ng/ml) concentrations; inhibition of fusion mediated by X4 Env was less efficient (71 to 83 % inhibition) compared to inhibition of R5 Env-mediated fusion (96 to 100%) (not shown). These results suggest that X5 is a potent broadly HIV-1 neutralizing antibody.

Discussion

The major result of this study is the identification of a novel human monoclonal antibody Fab, X5, which binds to a conserved epitope on gp120 induced by CD4 that is different from the coreceptor binding site and is slightly enhanced by CCR5 binding. X5 neutralizes a broad range of HIV-1 isolates. To our knowledge this is the first report of an antibody selected for binding to purified gp120-CD4-coreceptor complexes; compared to the four known potent broadly neutralizing antibodies (b12, 2F5, 2G12 and 4E10/Z13) it is the only one against a receptor inducible epitope. The use of a coreceptor complexed with gp120-C4E10/Z13) it is the only one against a receptor inducible epitope. The use of a coreceptor complexed with gp120-CD4 allowed for selection of an antibody to an epitope that differs from the coreceptor binding site and is somewhat more exposed after CCR5 binding. Interestingly an Fab almost identical to X5 was also selected by panning the FDA2 library against proteoliposomes (24) associated with gp160膜 suggesting that the X5 epitope can become available in differing environments. The characterization of epitopes such as that of X5 may provide valuable information for the nature of the initial conformational changes of the Env required for its fusogenic activity.

It has been previously demonstrated that binding of CD4 to the Env induces conformational changes as measured by the increased binding of antibodies to the V3 loop of gp120 and its enhanced cleavage by an exogenous protease (36). Subsequent studies, including the solution of the crystal structure of a trimolecular CD4-gp120-17b complex, provided a wealth of information about the gp120 conformation in presence of CD4 (20, 30). However, the conformational changes

Table 5.2: Neutralization of HIV infections of PBMCs by X5 and b12.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clade</th>
<th>IC50 μg/ml</th>
<th>IC50 μg/ml</th>
<th>IC50 μg/ml</th>
<th>IC50 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>RW020.5</td>
<td>A</td>
<td>52</td>
<td>28</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>US715.6</td>
<td>B</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>HT593.1</td>
<td>B</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>US005.11</td>
<td>B</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>RU570</td>
<td>G</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>BR029</td>
<td>F</td>
<td>6.5</td>
<td>3.3(69)</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>RU570</td>
<td>G</td>
<td>125</td>
<td>4.4</td>
<td>32</td>
<td>1.9</td>
</tr>
<tr>
<td>IC50 ug/ml</td>
<td>IC50 ug/ml</td>
<td>IC50 ug/ml</td>
<td>IC50 ug/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.05</td>
<td>0.025</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3: Inhibition of cell-cell fusion by X5 and b12.

<table>
<thead>
<tr>
<th>Env</th>
<th>Clade</th>
<th>Fab X5</th>
<th>IgG1 b12 (Fab b12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UG037.8</td>
<td>A</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>RW020.5</td>
<td>A</td>
<td>56</td>
<td>58</td>
</tr>
<tr>
<td>US715.6</td>
<td>B</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>HT593.1</td>
<td>B</td>
<td>20</td>
<td>39</td>
</tr>
<tr>
<td>US005.11</td>
<td>B</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>BR025.6</td>
<td>C</td>
<td>62</td>
<td>22</td>
</tr>
<tr>
<td>ZR001.3</td>
<td>D</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>TH022.4</td>
<td>EA</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>BR029.2</td>
<td>F</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>BR019.4</td>
<td>FB</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>UG975.10</td>
<td>G</td>
<td>28</td>
<td>41</td>
</tr>
</tbody>
</table>

10 293 cells, transfected with plasmids encoding various HIV Env under the control of T7 promoter and infected with recombinant vaccinia virus encoding T7 polymerase gene, were preincubated with X5 or IgG b12 at 100 μg/ml for 30 min at 37°C, and then mixed with 10 4 CEM-CCR5 cells infected with recombinant vaccinia virus encoding p-glucosidase gene. The extent of cell fusion was quantified colorimetrically 2 hours after mixing the cells. The data are averages of duplicate samples and presented as % of fusion inhibition.
induced by coreceptors, which are most likely responsible for driving the fusion process to completion are less well characterized. It was proposed that the interactions of the coreceptors with Env and possibly with CD4 may help in the relocation of the fusion peptide from the interior of gp41 to a position close to the surface of the host membrane (14). The existence of major conformational changes specifically induced by coreceptor interactions with the Env-CD4 complex was demonstrated by using fluorescent dyes as indicators of exposure of hydrophobic regions of the Env or membrane destabilization (12, 19). Models of fusion intermediate states containing coiled coils derived from gp41 sequences have also been proposed and experimental evidence provided for their existence (5, 7-9, 22, 37, 45, 46). However, the nature of the conformational changes leading to these intermediate structures remains unclear. The new antibody identifies an epitope which may play a role in the coupling of the gp120 conformational changes to those in gp41. One can speculate that the X5 binding to the CD4-gp120-CCR5 complex interferes with those conformational changes in gp41 that are required to transduce an activation signal for gp41 to undergo the major conformational changes leading to fusion.

The X5 epitope is conserved and partially overlaps the epitope of the antibody 17b which binds a CD4 inducible epitope that partially overlaps the coreceptor binding site on gp120. 17b neutralizes weakly TCLA HIV-1 and does not neutralize primary isolates (30). The X5 epitope is significantly different in that it is outside the CCR5 binding site. Its precise localization is currently under investigation but it is likely located at close proximity to the coreceptor and CD4 binding sites as also indicated by the X5 competition with b12 which binds to the CD4 binding site on gp120. X5 binds with higher affinity to gp120 than 17b and neutralizes various HIV-1 including primary isolates with potency comparable to that of IgG1 b12. The crystal structure of gp120 complexed with CD4 and the Fab 17b reveals an orientation of the 17b epitope toward the target cell membrane (35). It was also shown recently that access to the CD4-induced coreceptor-binding domain on gp120 is largely blocked at the fusing cell membranes and is unlikely to represent a target for neutralizing antibodies (16). Thus one might speculate that the X5 epitope is oriented differently than the 17b epitope and the coreceptor binding site, and it is easier to access in agreement with its potent HIV neutralizing activity. The precise mechanism of HIV-1 inhibition by X5 is likely to involve blocking of post-CD4-coreceptor binding events and is currently under investigation.

The identification of X5 also shows that antibodies against conserved epitopes whose exposure is enhanced by interaction with receptor and coreceptor molecules may exist in infected individuals. Importantly, the results imply that certain epitopes, distinct from the coreceptor binding site, can be recognized by the immune system and elicit broadly HIV-1 neutralizing antibodies. This result may have important conceptual consequences for development of vaccines able to induce broadly cross-reactive antibodies. In addition, X5 could be possibly used as entry inhibitor alone or in combination with other antiretroviral drugs for the treatment of HIV-1 infected individuals.

Acknowledgment

We wish to thank J. Sodroski, J. Moore, E. Berger, R. Doms, T. Fouts, L. Wu, G. Quinnan and N. Miller for generous gifts of reagents. This study was supported by CPA from the CCR, NCI, and by the NIH Intramural AIDS Targeted Antiviral Program (DSD). DRB and JR acknowledge financial support from the NIH (grants AI32922 and AI24030, respectively). M.M was supported by a grant from the Philipe Foundation (New York, USA, and Paris, France). The gp140 constructs from the primary isolates 92UG037.8, 92TH939.1, 93MW956.26 and 93ZR001.3 were prepared by Agnes Jones-Trower using clones obtained from the NIH AIDS Research and Reference Reagent Program; their development was supported by NIH grant AI42599 to C.C. Broder.

References

epitopes of human immunodeficiency virus type 1 (HIV-1) gp120 by human monoclonal antibodies produced by EBV-transformed cell lines. AIDS Res Hum Retroviruses 6:567-79.


AIDS Res Hum Retroviruses 18:1207-17.
