

Determinants of Human Immunodeficiency Virus Type 1 Envelope Glycoprotein Activation by Soluble CD4 and Monoclonal Antibodies

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Infection by some human immunodeficiency virus type 1 (HIV-1) isolates is enhanced by the binding of subneutralizing concentrations of soluble receptor, soluble CD4 (sCD4), or monoclonal antibodies directed against the viral envelope glycoproteins. In this work, we studied the abilities of different antibodies to mediate activation of the envelope glycoproteins of a primary HIV-1 isolate, YU2, and identified the regions of gp120 envelope glycoprotein contributing to activation. Binding of antibodies to a variety of epitopes on gp120, including the CD4 binding site, the third variable (V3) loop, and CD4-induced epitopes, enhanced the entry of viruses containing YU2 envelope glycoproteins. Fab fragments of antibodies directed against either the CD4 binding site or V3 loop also activated YU2 virus infection. The activation phenotype was conferred on the envelope glycoproteins of a laboratory-adapted HIV-1 isolate (HXBc2) by replacing the gp120 V3 loop or V1/V2 and V3 loops with those of the YU2 virus. Infection by the YU2 virus in the presence of activating antibodies remained inhibitable by macrophage inhibitory protein 1β, indicating dependence on the CCR5 coreceptor on the target cells. Thus, antibody enhancement of YU2 entry involves neither Fc receptor binding nor envelope glycoprotein cross-linking, is determined by the same variable loops that dictate enhancement by sCD4, and probably proceeds by a process fundamentally similar to the receptor-activated virus entry pathway.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (5, 29, 56). The envelope glycoproteins of HIV-1, gp120 SU and gp41 TM, are assembled in an oligomeric spike on the virion surface and mediate virus entry into target cells (20, 34, 40, 53, 57, 58, 70, 79). The initial steps in virus entry involve a specific high-affinity binding of gp120 to the cell surface receptor CD4, as well as an interaction with proteins of the chemokine receptor family which serve as co-receptors for HIV-1, HIV-2, and simian immunodeficiency virus (SIV) (1, 12, 16, 19, 22, 23, 30, 33, 69). Primary isolates of HIV-1, HIV-2, and SIV use CCR5 as a coreceptor, while T-cell-tropic and laboratory-adapted variants of these viruses acquire the ability to use additional chemokine receptors. Association of gp120 with target cell receptors triggers conformational changes in gp120 and gp41 that promote fusion of the virus and cell membranes. Some of the CD4-induced changes involve the conformation of variable loops on the gp120 glycoprotein (61, 64, 72, 77). Among other possible functions, alterations in the conformation of the gp120 variable loops, particularly the third variable (V3) loop, may play a role in the exposure and/or formation of the chemokine receptor binding site. Chemokine receptor binding, in turn, is believed to trigger additional changes in the envelope glycoprotein complex, possibly including the exposure of the gp41 ectodomain. Mutagenic, biochemical, and structural studies suggest that gp41 mediates membrane fusion by insertion of its hydrophobic,

amino-terminal fusion peptide into the target cell membrane (6, 27, 28, 35).

While all HIV and SIV strains undergo fundamentally similar steps during virus entry, there are structural differences in the envelope glycoproteins that distinguish virus isolates from one another. One important distinction is the interaction of different viruses with CD4. All HIV and SIV can attach to cells via the CD4 receptor, but some strains of HIV-2 exhibit CD4-independent tropism, utilizing the chemokine receptor CXCR4 as a receptor (22). Virus strains also exhibit functional differences in their response to incubation with a soluble form of the CD4 receptor (sCD4). Early observations from studies of sCD4 as a potential inhibitor of HIV-1 infection demonstrated that the envelope glycoproteins of laboratory-adapted HIV-1 become unstable when incubated with sCD4 and shed gp120, one proposed mechanism for the inhibitory effect of sCD4 on HIV-1 (7, 26, 32, 38, 46–48, 76, 80). Primary HIV-1 isolates require significantly higher concentrations of sCD4 to induce gp120 shedding and neutralize virus infection (46, 47, 73, 80). Likewise, SIVagm and some strains of HIV-2 are relatively resistant to the inhibitory effects of sCD4, and this results, at least in part, from the diminished ability of sCD4 to bind the oligomeric envelope glycoprotein complex (2, 3, 13, 66, 73). Similarly, primary HIV-1 neutralization by antibodies against gp120 is best predicted by the ability of the antibody to bind the oligomeric envelope glycoprotein complex (25, 44, 45, 52, 60, 73).

Viruses, like SIVagm and some HIV-2 strains, that require high concentrations of sCD4 for neutralization often exhibit an enhancement of infection following incubation with subinhibitory concentrations of sCD4 (2, 3, 13, 66, 73). A similar phe-

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nomenon for some primary strains of HIV has been observed (66, 67, 73). Further, it was demonstrated that monoclonal antibodies directed against gp120 were also capable of enhancing entry of these virus strains (66, 67, 73). Similar to activation by sCD4, enhancement by the F105 antibody, which is directed against the CD4 binding site (CD4BS) of gp120, was observed for particular primary isolates but not for laboratory-adapted isolates (73).

Here we report on antibody-mediated activation of entry by viruses containing the envelope glycoproteins of a primary HIV-1 isolate, YU2. The infectious YU2 provirus was molecularly cloned directly from the brain of an HIV-1-infected individual and therefore represents an isolate unaltered by passage in tissue culture (41, 42). We examined whether antibodies directed against gp120 epitopes removed from the CD4BS could enhance YU2 virus entry and whether antibody fragments lacking the ability to cross-link envelope glycoproteins or bind Fc receptors could efficiently activate the YU2 envelope glycoproteins. The gp120 determinants for antibody enhancement were mapped by the study of viruses with chimeric envelope glycoproteins. Finally, we examined whether antibody enhancement bypassed the requirement for the CCR5 coreceptor in virus entry.

MATERIALS AND METHODS

Antibodies, sCD4, and chemokines. The F105, 1.5e, and immunoglobulin G1b12 (IgG1b12) antibodies bind to epitopes overlapping the CD4-binding site on gp120 and have been described elsewhere (9, 50, 60). Fab DO8i, MTW61D, and DA48 were isolated by panning phage display libraries prepared from bone marrow from seropositive donors against recombinant monomeric gp120. The libraries and panning procedures used have been described previously (8, 54). Fab DO8i and MTW61D were obtained by panning libraries, derived from long-term asymptomatic (>6 years) donors, against recombinant BRU gp120 (Intracell, Cambridge, Mass.), and recombinant gp120 from the Dutch primary isolate W61D (obtained from C. Bruck via the MRC AIDS Reagent Project, Potters Bar, England), respectively. Fab DA48 was obtained by panning a library derived from a >15-year long-term nonprogressor against recombinant BRU gp120. All three Fabs were mapped to CD4BS-related epitopes. Fab DO142-10 and IgG1 loop 2 recognize the gp120 V3 loop (4, 17, 55). Rabbit polyclonal antiserum against CD4 and sCD4 were kind gifts from Raymond Sweet (Smith-Kline Beecham) and macrophage inhibitory protein 1 β (MIP-1 β) was purchased from R&D Systems.

Cells and cell lines. Human peripheral blood mononuclear cells (PBMC) were prepared by Ficoll gradient separation, stimulated with phytohemagglutinin for 48 to 72 h, and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 5% interleukin-2.

COS-1 cells were grown in Dulbecco's modified Eagle medium containing 10% FBS. The T-cell line Molt 4 clone 8 and was maintained in RPMI 1640 medium containing 10% FBS.

Envelope glycoprotein and sCD4 expressor plasmids. The pSVIIIenv plasmids expressing the HXBc2 and YU2 envelope glycoproteins have been described previously (73). Lee Ratner provided an HIV-1 proviral clone that encoded an HXBc2 envelope glycoprotein containing the V3 loop of the YU2 virus (10). The *Kpn*I (6347)-*Bam*HI (8475) fragment of the chimeric envelope gene was inserted into the pSVIIIenv plasmid to make the YU2 V3 envelope glycoprotein expressor plasmid. The expression plasmid for the YU2 V1/V2 chimera was constructed by substituting the *Dra*III (6599)-*Stu*I (6836) fragment of the YU2 *env* for the corresponding fragment of the HXBc2 *env*. The plasmid expressing the YU2 V1/V2/V3 chimera was constructed by substituting the *Dra*III (6599)-*Stu*I (6836) fragment of YU2 into the YU2 V3 expressor plasmid. The plasmid expressing the Δ V1/V2 YU2 V3 protein was made by substituting the *Stu*I (6836)-*Bam*HI (8475) fragment of the HXBc2 Δ V1/V2 envelope expressor (82, 83) into the YU2 V3 expressor plasmid. For the sCD4 expressor plasmid, the sCD4 gene was obtained by PCR amplification from a vector containing full-length CD4. The plus-strand primer (5'-TATAAATAACTCGAGGCAAGGCCACAATGAACCGGGG3') spanned the CD4 open reading frame start site and inserted an *Xho*I restriction site. The minus-strand primer (5'-TATAAATAATCTAGATTACACCGGGGTGGACCATGTGGGG3') inserted a premature stop codon 1,176 bp downstream of the start codon flanking the *Xba*I restriction site. The *Xho*I-*Xba*I fragment of the PCR product was cloned into pCDNA3 (Invitrogen).

Envelope glycoprotein expression and sCD4 binding assay. COS-1 cells were transfected by the DEAE-dextran method with pSVIIIenv DNA expressing envelope glycoproteins, as described previously (34).

To measure protein expression, the cells were radiolabeled with [³⁵S]cysteine overnight and precipitated with an excess of a mixture of sera derived from HIV-1-infected individuals.

To measure the CD4 binding ability of envelope glycoproteins, metabolically labeled sCD4 was prepared from COS-1 cells transfected with the sCD4-pCDNA3 plasmid and radiolabeled with [³⁵S]cysteine and [³⁵S]methionine. The supernatant containing radiolabeled sCD4 was incubated for 90 min at room temperature with COS-1 cells expressing HIV-1 envelope glycoproteins. The COS-1 cells were then washed four times with ice-cold phosphate-buffered saline (PBS) containing 2% FBS and lysed in 0.75 ml of Nonidet P-40 buffer (0.5% Nonidet P-40, 0.5 M NaCl, 10 mM Tris HCl [pH 7.5]), and sCD4-gp120 complexes were precipitated on protein A-Sepharose, using rabbit polyclonal antiserum against human CD4. The amount of sCD4 bound was measured by densitometric analysis of autoradiograms of sodium dodecyl sulfate-polyacrylamide gels. To observe the influence of antibodies on sCD4 binding, antibodies were incubated with envelope glycoprotein-expressing COS-1 cells in 1 ml of PBS for 30 min at room temperature prior to addition of 1 ml of supernatant containing radiolabeled sCD4, and incubation was continued for an additional 90 min at room temperature. The cells were washed four times with ice-cold PBS containing 2% FBS, lysed, and immunoprecipitated as described above. The amount of sCD4 bound was measured by densitometric analysis of autoradiograms from sodium dodecyl sulfate-polyacrylamide gels.

Virus neutralization assay. Complementation of a single round of replication of the *env*-deficient chloramphenicol acetyltransferase (CAT)-expressing provirus by the various envelope glycoproteins was performed as described previously (34). Either monoclonal antibody or sCD4 was incubated with recombinant virus for 90 min at 37°C before addition of the virus to target lymphocytes. Three days after infection, the target cells were lysed and CAT activity was measured as described previously (34). The standard deviation in this assay was experimentally determined and was less than 10% of the mean (data not shown).

RESULTS

Activation of YU2 envelope glycoproteins by monoclonal antibodies. In previous studies, sCD4 and monoclonal antibodies directed against the CD4BS enhanced virus entry mediated by macrophagetropic primary envelope glycoproteins (66, 67, 73). These experiments did not address whether enhancement required ligand binding to a particular region of the viral envelope glycoproteins. Therefore, we examined the ability of a panel of antibodies directed against different regions of gp120 to enhance entry of virions bearing the YU2 envelope glycoproteins.

A previously described envelope complementation assay (34) was used to examine the ability of recombinant virions containing different envelope glycoproteins to enter Molt 4 clone 8 lymphocytes in the presence of various concentrations of antibodies. We showed previously that the F105 antibody, which recognizes a gp120 region overlapping the CD4BS, failed to neutralize virions bearing the YU2 envelope glycoproteins even at concentrations exceeding 50 μ g/ml, while virions bearing the HXBc2 envelope were neutralized at much lower concentrations (73). The presence of the F105 antibody increased the efficiency of entry of virus with the YU2 envelope glycoproteins. Here we show (Fig. 1) that another CD4BS antibody, 1.5e, also enhanced YU2 entry and did not neutralize virus, even at the highest concentration tested (50 μ g/ml). We also tested antibodies that recognize other regions of gp120. The loop 2 antibody binds to a linear epitope within the V3 loop (17) which is present in YU2 but not HXBc2 envelope sequences. Virions bearing YU2 envelope glycoproteins exhibited enhanced entry at IgG1 loop 2 antibody concentrations of up to 20 μ g/ml. The 17b antibody recognizes a CD4-induced gp120 epitope, which is a discontinuous structure composed of sequences within each of the conserved gp120 regions (77). Incubation of YU2 virions with the 17b antibody also resulted in a greater than 10-fold enhancement of entry, whereas the viruses containing the HXBc2 envelope glycoproteins were neutralized by the antibody. These experiments indicate that antibody-mediated activation is a property of the particular envelope glycoproteins present on the infecting virion and is not restricted to ligands that bind to a specific gp120 region.

Activation of YU2 envelope glycoproteins by an Fab antibody fragment. Enhancement of HIV-1 entry observed in

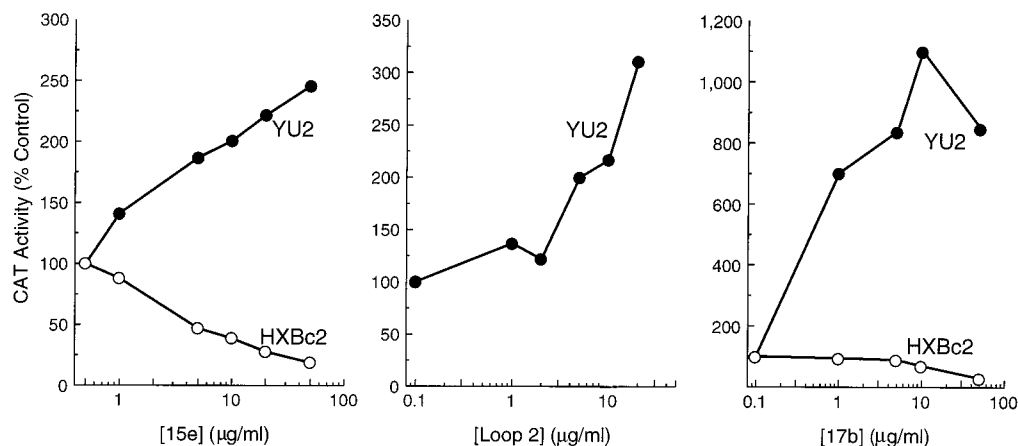


FIG. 1. Effects of monoclonal antibodies on infection by viruses with HXBc2 or YU2 envelope glycoproteins. Recombinant viruses bearing the HXBc2 or YU2 envelope glycoproteins were produced in COS-1 cells and incubated with monoclonal antibodies directed against a CD4-induced epitope (17b), the CD4BS (1.5e), or the V3 loop (loop 2). CAT activity was measured 72 h after the addition of Molt 4 clone 8 target cells and is expressed as a percentage of the CAT activity in samples containing no antibody. The baseline conversions of chloramphenicol to acetylated forms for the HXBc2 and YU2 viruses in the absence of added antibody were 36 and 8%, respectively.

other systems has been reported to be dependent on either Fc-mediated processes or antibody cross-linking of the viral envelope glycoproteins (14, 15, 37, 43, 67, 71, 75). We examined whether the antibody-mediated enhancement observed for the YU2 virus depended on Fc interactions by testing the ability of Fab antibody fragments to enhance virus entry. Because the Fab is monovalent, these experiments also tested the requirement for a bivalent interaction between the ligand and YU2 envelope glycoproteins. DO142-10 is an Fab fragment that binds to the V3 loop of gp120 (68). As shown in Fig. 2, DO142-10 mediated a sixfold enhancement of YU2 entry into PBMC target cells, while HXBc2 was completely neutralized under identical conditions.

We extended this type of analysis further by including Fab fragments that bind to epitopes overlapping the CD4 binding site (Table 1). Fab b12, DO8i, MTW61D, and DA48 are all CD4BS-directed Fab fragments and exhibited from two- to almost sevenfold enhancement of YU2 entry into Molt 4 clone

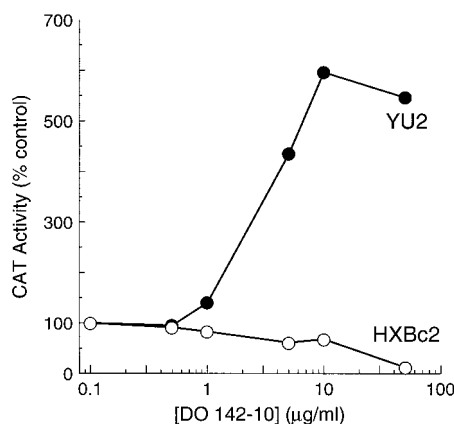


FIG. 2. Effects of an Fab fragment on infection of HIV-1 isolates with different envelope glycoproteins. Recombinant viruses bearing the HXBc2 or YU2 envelope glycoproteins were produced in COS-1 cells and incubated with an Fab directed against the V3 loop (DO142-10). CAT activity, expressed as a percentage of the CAT activity in control samples containing no antibody, was measured 72 h after the addition of PBMC target cells. The baseline CAT conversions for HXBc2 and YU2 were 29 and 8%, respectively.

8 target cells at the single concentration tested (10 μg/ml). All of the Fab fragments examined efficiently neutralized virions bearing the HXBc2 envelope glycoproteins. These data indicate that antibody enhancement of YU2 virus entry is not dependent on the Fc portion of the antibody and is not due to antibody cross-linking of gp120 molecules.

YU2 envelope glycoprotein determinants of enhancement. The variable loops of the HIV-1 gp120 envelope glycoprotein contain important determinants for fusion, cell type specificity, and coreceptor usage (6, 12, 21, 24, 31, 36, 39, 49, 65, 74, 82). The gp120 variable loops have also been suggested to contribute to the resistance of some strains of HIV-1 to neutralization (51). Therefore, we examined whether the variable loops contribute to the antibody-mediated activation of the YU2 envelope glycoproteins. We constructed a series of chimeric glycoproteins, using the envelope glycoproteins of the HXBc2 virus, which is sensitive to neutralization by antibodies, and those of the YU2 virus, which is resistant to and enhanced by antibodies (Fig. 3A). Substitution of the V3 loop of the YU2 gp120 glycoprotein in an HXBc2 envelope resulted in a virus that exhibited enhanced entry following incubation with antibody (Fig. 3B, YU2 V3). At a concentration of 1 μg/ml, the 17b antibody enhanced the entry of the YU2 V3 virus 160% rela-

TABLE 1. Abilities of various Fab fragments to activate YU2 envelope glycoproteins^a

| Fab fragments | CAT activity (% conversion) | | | |
|---------------|-----------------------------|------|------|------|
| | HXBc2 | | YU2 | |
| | -Fab | +Fab | -Fab | +Fab |
| DO142-10 | 43 | 7 | 13 | 28 |
| b12 | 39 | 1 | 14 | 27 |
| DO8i | 42 | 1 | 12 | 50 |
| MTW61D | ND | ND | 13 | 88 |
| DA48 | 60 | 10 | 16 | 82 |

^a Recombinant viruses bearing the HXBc2 or YU2 envelope glycoproteins were produced in COS-1 cells. Virions were incubated alone (-) or with (+) Fab fragments directed against the V3 loop (DO142-10) and CD4BS (b12, DO8i, MTW61D, and DA48) at a concentration of 10 μg/ml. CAT activity was measured 72 h after the addition of Molt 4 clone 8 target cells. The values expressed are the percentage conversion of chloramphenicol to acetylated forms.

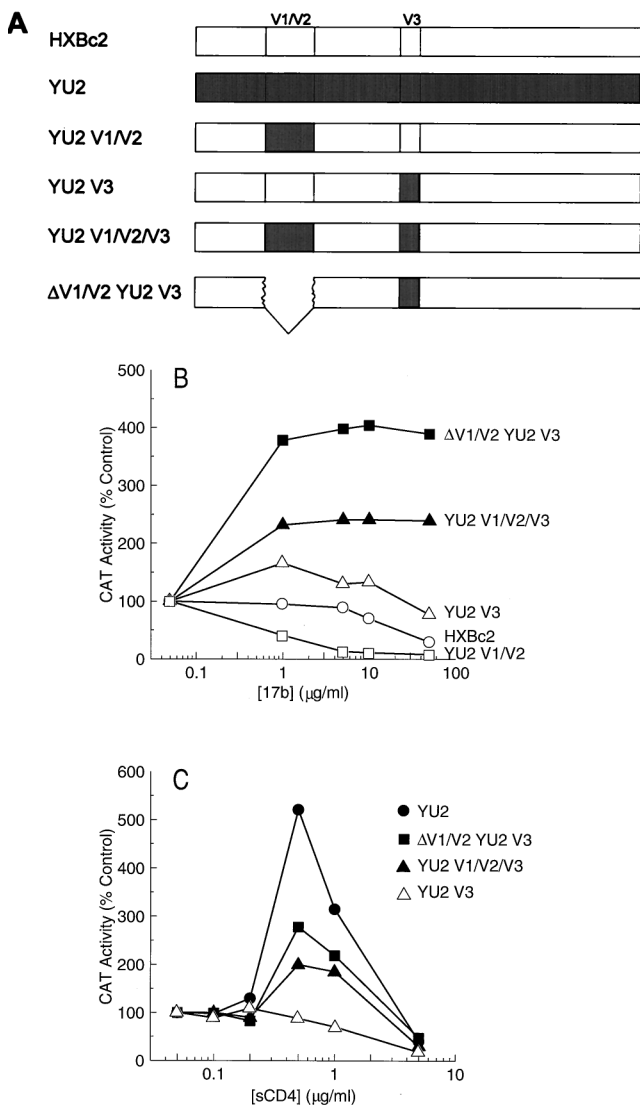


FIG. 3. Effects of the 17b antibody on infection of viruses with chimeric HXBc2-YU2 envelope glycoproteins. Recombinant viruses bearing the HXBc2 or chimeric envelopes containing variable loop changes (A) were produced in COS-1 cells and incubated for 90 min with a monoclonal antibody directed against the CD4-induced epitope 17b (B) or sCD4 (C). PBMC target cells were added, and CAT activity, expressed as a percentage of the CAT activity in samples containing no antibody, was measured 72 h later. The baseline conversions of chloramphenicol to acetylated forms in the absence of 17b for the different viruses were as follows: HXBc2, 56%; YU2 V3, 10%; YU2 V1/V2, 30%; YU2 V1/V2/V3, 24%; and ΔV1/V2 YU2 V3, 40%.

tive to entry in the absence of antibody. The presence of the YU2 V1 and V2 loops in addition to the YU2 V3 loop further increased the 17b-mediated enhancement (Fig. 3B, YU2 V1/V2/V3). While enhancement of YU2 V3 diminished at higher concentrations of 17b antibody, YU2 V1/V2/V3 enhancement remained at 250% of the control level even at 50 μg of the 17b antibody per ml. However, this level of enhancement was still less than that observed for the complete YU2 envelope glycoprotein (1,100% of the untreated control level) (data not shown). These results indicate that the V1/V2 and V3 loops of the YU2 gp120 glycoproteins are the major determinants of the enhancement phenotype but that the efficiency of enhance-

ment may also be influenced by other envelope glycoprotein regions.

Studies performed on the monomeric gp120 glycoprotein indicate that movement of the V1 and V2 loops is responsible for some of the conformational changes in gp120 that are induced by CD4 binding (82). To examine the role of the V1 and V2 loops in antibody-mediated enhancement of YU2 entry, we constructed a gp120 chimera (YU2 V1/V2) bearing only the V1/V2 loops of YU2 substituted for those of the HXBc2 gp120 glycoprotein. The YU2 V1/V2 virus was more sensitive to neutralization by the 17b antibody than were viruses bearing wild-type HXBc2 envelope glycoproteins (Fig. 3B). Comparison of the effects of the 17b antibody on infection by the YU2 V1/V2, YU2 V1/V2/V3, and YU2 V3 viruses suggests that the V1/V2 loops require the presence of the appropriate V3 loop to contribute to the enhancement phenotype. This interpretation is supported by comparing the effects of the 17b antibody on the YU2 V3 virus and on an identical virus from which the HXBc2 V1 and V2 loops have been deleted (Fig. 3B), ΔV1/V2 YU2 V3. The ΔV1/V2 YU2 V3 virus is enhanced more than twice as much as the YU2 V3 virus, which contains the V1 and V2 loops from HXBc2. These results indicate that while the V3 loop alone is capable of conferring some antibody-induced enhancement, the V1 and V2 loops influence the efficiency of this effect.

We have shown previously that while YU2 is enhanced at low concentrations of sCD4, this effect is overcome by the neutralizing ability of sCD4 at higher concentrations. The chimeric envelope glycoproteins showed a pattern of enhancement by sCD4 (Fig. 3C) similar to that seen for the 17b antibody. The YU2 V3 loop alone was unable to confer enhancement properties to the HXBc2 glycoproteins in the context of the HXBc2 V1/V2 loop. However, removal of the V1/V2 loop allowed the YU2 V3 enhancement phenotype to be manifest (Fig. 3C, ΔV1/V2 YU2 V3). The presence of the YU2 V1/V2 and V3 loops together also allowed sCD4-mediated enhancement. By contrast, the wild-type HXBc2 and YU2 V1/V2 viruses were neutralized by sCD4 at concentrations of greater than 0.5 μg/ml (data not shown).

Inhibition of YU2 activation by MIP-1β. Primary macrophagotropic HIV-1 isolates utilize the chemokine receptor CCR5 as a coreceptor, and the CCR5 ligand, MIP-1β, inhibits entry of these virus isolates (1, 11, 12, 18, 33, 59). We examined whether the entry of viruses with the YU2 envelope glycoproteins depends on CCR5 to the same degree in the presence and absence of enhancing antibodies. The effect of MIP-1β on the entry of YU2 viruses was examined in the absence and presence of the F105 antibody, previously shown to enhance YU2 virus entry. Figure 4 shows that MIP-1β inhibited the F105-enhanced entry of YU2 in a dose-dependent fashion. In these experiments, the addition of F105 resulted in a 200% increase in the efficiency of YU2 virus entry (data not shown). The levels of relative inhibition of YU2 virus entry by MIP-1β were similar in both the presence and absence of the F105 antibody. Therefore, antibody-mediated enhancement of YU2 utilizes an entry mechanism that remains dependent on CCR5.

Effect of antibodies on sCD4 binding to the native YU2 envelope glycoproteins. One possible explanation for antibody-mediated enhancement of entry is that binding of an antibody to one subunit of the YU2 envelope glycoproteins results in an increase in the CD4 binding affinity of the other subunits of the oligomer. We tested this hypothesis by examining the effects of enhancing antibodies on the binding of sCD4 to the oligomeric YU2 envelope glycoproteins expressed on a cell surface. As expected, unlabeled sCD4 effectively competed with [³⁵S]sCD4 for binding to the YU2 envelope glycoproteins, with half-max-

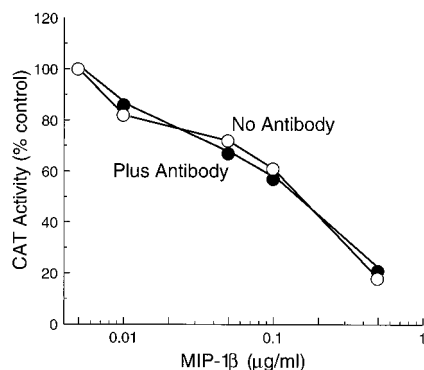


FIG. 4. Effects of MIP-1 β on YU2 virus infection in the absence and presence of 17b antibody. Recombinant virions bearing the YU2 envelope glycoprotein were incubated with either MIP-1 β alone or MIP-1 β plus F105 for 90 min at 37°C. PBMC target cells were added, and CAT activity, expressed as a percentage of CAT conversion relative to samples containing no MIP-1 β , was measured after 72 h.

imal inhibition of binding occurring at less than 5 μ g of sCD4 per ml (data not shown). This value is consistent with prior measurements of sCD4 binding affinity to HIV-1 envelope glycoprotein oligomers (46, 62). The concentration of [35 S] sCD4 in these experiments was significantly below the gp120-sCD4 dissociation constant, and thus any antibody-induced increase in sCD4 binding affinity should be detectable. Neither the gp120 V3 loop-directed monoclonal antibody IgG1 loop 2 nor Fab DO142-10, both of which enhanced YU2 virus entry, affected the binding of [35 S] sCD4 to YU2 envelope glycoproteins expressed on the surface of COS-1 cells (data not shown). The V3-directed 110.4 antibody, which does not recognize the YU2 envelope glycoproteins, also had no effect on sCD4 binding in these experiments, as expected. We did not find evidence to support the hypothesis that antibody-mediated enhancement of YU2 virus entry occurs by increasing the affinity of the viral envelope glycoproteins for the CD4 receptor.

DISCUSSION

While the majority of primary HIV-1 isolates exhibit resistance to neutralization by antibodies compared with laboratory-adapted viruses passaged on T-cell lines (44, 63, 73), only a subset of primary viruses have been shown to exhibit detectable enhancement (66, 67, 71, 73). Primary virus envelope glycoproteins, as assembled oligomers, bind anti-gp120 antibodies less efficiently than do the envelope glycoproteins of laboratory-adapted viruses (44, 63, 73). Our studies demonstrate that neutralization resistance and enhancement are determined primarily by the configuration of the major variable (V1/V2 and V3) loops of the gp120 envelope glycoprotein. Previous studies have suggested the ability of these loops to mask HIV-1 neutralization epitopes and have demonstrated that these effects are most evident in the context of the assembled envelope glycoprotein complex (82, 83). The variable loops on the YU2 envelope glycoprotein may be particularly effective at achieving this masking effect. Thus, at antibody concentrations typically used in neutralization experiments, most of the YU2 oligomeric envelope glycoprotein spikes may be occupied by an insufficient number of antibody molecules to achieve neutralization. The high affinity of sCD4 for gp120 enables it to neutralize YU2, but only at higher concentrations, again supporting the notion that enhancement occurs when there is suboptimal occupation of binding sites on the envelope glycoprotein oligomers.

One consequence of epitope masking by the gp120 variable loops is that elements of the envelope glycoproteins that require exposure during the entry process (e.g., receptor binding regions) may also be occluded. Indeed, the relative resistance of many primary HIV-1 isolates to soluble CD4 has been attributed to a lower binding affinity of the primary virus envelope glycoprotein oligomers for CD4 (44, 63, 73). While the monomeric gp120 glycoproteins of primary viruses bind CCR5 in the presence of CD4 with high affinity (78, 81), the relative ability of envelope glycoprotein oligomers of primary and laboratory-adapted HIV-1 isolates to bind their respective chemokine receptors has not been assessed. The extreme degree of neutralization resistance achieved by viruses like YU2 may necessitate some means of achieving more efficient receptor binding *in vivo*. Activation by neutralizing antibodies, which are abundant in most HIV-1-infected individuals after several months of infection, would provide a means to do so. Indeed, in the absence of antibodies, the entry of viruses with the YU2 envelope glycoproteins into PBMC is less efficient than that of viruses with envelope glycoproteins derived from other HIV-1 isolates (73). In the presence of antibodies, these differences in entry efficiency are nullified.

Antibodies and Fab fragments directed against a number of gp120 epitopes that map to the presumably exposed surface of the assembled oligomeric spike were able to enhance YU2 virus entry. These results indicate that cross-linking of envelope glycoproteins or Fc-mediated uptake does not play a necessary role in enhancement of YU2 virus entry. The observation that binding of antibodies or Fab fragments to a number of nonoverlapping gp120 regions can activate virus entry suggests that enhancement might be triggered by the binding of any ligand that can access the envelope glycoprotein oligomer. If the YU2 envelope glycoproteins need to assume a conformation of high free energy to achieve an antibody-enhanceable state, the binding of any ligand to the glycoproteins could induce a return to a lower-energy conformation that is favorably disposed to progress along the pathway toward membrane fusion. Based on inhibition by MIP-1 β , this pathway appears to require CCR5 interaction, indicating that enhancement does not involve use of alternative or additional coreceptors. The similarity in pattern of enhancement by sCD4 and antibodies observed for the chimeric envelope glycoproteins suggests that antibody-mediated enhancement shares fundamental features with the receptor-activated virus entry pathway.

Enhancement is mediated by antibodies that have been shown to interfere with gp120 binding to either CD4 or chemokine receptors. This observation supports a model in which antibody binding to one subunit of the envelope glycoprotein oligomer induces conformational changes in the unoccupied subunits conducive to entry. Our mapping of the determinants of antibody-mediated enhancement makes it likely that the major variable loops of the gp120 glycoprotein participate in this process. While the precise nature of the conformational changes involved in enhancement requires further investigation, we could not find evidence that they result in an increase in CD4 binding affinity.

The prevalence of natural HIV-1 strains that exhibit enhanced entry into target cells in response to antibodies is unknown. However, it is noteworthy that the YU2 virus demonstrates the most dramatic enhancement among the viruses tested. Since the YU2 sequences were cloned directly from an HIV-1-infected individual into phage, changes in the envelope glycoproteins due to virus passage in culture or due to provirus cloning were minimized. Our observation that some other primary HIV-1 envelope glycoproteins do not exhibit the degree of antibody-mediated enhancement of virus entry seen for the

YU2 envelope glycoproteins (73) raises the question of whether even minimal tissue culture passage in PBMC alters this phenotype. The availability of infectious HIV-1 proviral clones derived by PCR technology should help to address this important issue.

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