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Target Cell-Specific Determinants of Membrane Fusion within the Human Immunodeficiency Virus Type 1 gp120 Third Variable Region and gp41 Amino Terminus

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The entry of human immunodeficiency virus type 1 (HIV-1) into target cells involves binding to the viral receptor (CD4) and membrane fusion events, the latter influenced by target cell factors other than CD4. The third variable (V3) region of the HIV-1 gp120 exterior envelope glycoprotein and the amino terminus of the HIV-1 gp41 transmembrane envelope glycoprotein have been shown to be important for the membrane fusion process. Here we demonstrate that some HIV-1 envelope glycoproteins containing an altered V3 region or gp41 amino terminus exhibit qualitatively different abilities to mediate syncytium formation and virus entry when different target cells are used. These results demonstrate that the structure of these HIV-1 envelope glycoprotein regions determines the efficiency of membrane fusion in a target cell-specific manner and support a model in which the gp41 amino terminus interacts directly or indirectly with the target cell during virus entry.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS, which is characterized by a depletion of CD4-positive lymphocytes (2, 11, 13, 24, 35). HIV-1 exhibits a tropism for CD4-positive cells owing to a high-affinity interaction between the CD4 glycoprotein, which serves as the viral receptor, and the HIV-1 gp120 exterior envelope glycoprotein (8, 19, 20, 25, 30). Following CD4 binding, the gp120 glycoprotein and gp41, the transmembrane envelope glycoprotein, mediate the fusion of viral and target cell membranes, which is pH independent and necessary for virus entry (14, 22, 46). Similar events mediated by the envelope glycoproteins expressed on the surface of an infected cell result in fusion of infected cells with CD4-positive cells to form syncytia (14, 26, 44). The efficiency of induction of membrane fusion events determines the in vitro cytopathogenicity of HIV-1 (21).

Target cell factors other than CD4 determine the efficiency of the membrane fusion process. Although HIV-1 can bind to many kinds of target cells that express human CD4, syncytium formation and virus entry do not occur in all such cells (1, 5, 6, 27, 48, 49). Some CD4-positive cells resistant to fusion by HIV-1 are susceptible to fusion by the related viruses HIV-2 and simian immunodeficiency virus (SIV_{mac}), suggesting that particular features of the viral envelope glycoproteins can determine fusion-related events in a target cell-specific manner (6, 16). One of the viral determinants of HIV-1 tropism has been mapped to a gp120 region that includes the third variable (V3) loop (4, 17, 18, 32, 41, 47). Whether tropism-associated sequence changes in these regions affect the membrane fusion function or alter other envelope glycoprotein functions in a cell type-specific manner has not been established.

Alterations in at least three regions of the HIV-1 envelope glycoproteins can affect the efficiency of membrane fusion events. Amino acid changes in the gp120 V3 loop, which is the major target for strain-restricted neutralizing antibodies (28, 29, 38), can decrease the efficiency of virus entry and syncytium formation without affecting gp120-CD4 interaction (10, 14, 18, 22). Similar phenotypes have been reported for some amino acid changes in the amino terminus or transmembrane region of the gp41 glycoprotein (3, 9, 14, 15, 21, 22). The gp41 amino terminus is a hydrophobic region that bears distant sequence similarity to the fusion peptides of orthomyxoviruses and paramyxoviruses (31, 33).

To explore the possibility that regions of the HIV-1 envelope glycoproteins previously identified to be important for membrane fusion determine viral tropism, we tested the ability of various mutant glycoproteins to mediate virus entry into different CD4-positive cell lines. By using an envelope complementation assay that measures a single round of virus entry (14), we were able to examine the ability of mutant viruses produced in one cell type to enter a variety of different target cells. In addition, we examined the ability of selected mutants to induce the formation of syncytia in particular target cells.

MATERIALS AND METHODS

Cell lines. The Jurkat, SupT1, H9, and Molt-3 cells are CD4-positive T-lymphocyte lines (36, 43). C8166 cells are CD4-positive lymphocytes that are immortalized by human T-cell leukemia virus type I (HTLV-I), but do not produce the structural proteins of the virus (39). C91/PL lymphocytes are also immortalized by HTLV-I, and they produce infectious HTLV-I particles (35). CEM×174 cells are somatic-cell hybrids of the human T-cell line CEM and the human B-cell line B721.174 (40).

Site-directed mutagenesis. The *Kpn*I-*Bam*HI fragment of the pSVIIIenv plasmid, which expresses the *rev* and *env* genes of the HXBc2 strain of HIV-1, was cloned into the pBluescript vector and used for site-directed mutagenesis by the procedure of Kunkel (23, 34). The presence of the mutation was confirmed by the generation of a novel restriction endonuclease site in some cases and by DNA sequencing. In most cases, two independent clones of each mutated

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envelope fragment were cloned back into the pSVIIIenv plasmid and the phenotypes of the mutant glycoproteins were characterized to ensure that spontaneous mutations distant from the desired mutation were not responsible for the observed phenotype. The nomenclature of the mutants is as follows. The number of the mutant refers to the envelope glycoprotein amino acid of HXBc2, where 1 is the initial methionine. The mutations result in substitution of the amino acid on the right for the amino acid on the left. For example, 313 P/S indicates a substitution of serine for proline at position 313. When a group of residues of the HIV-1 gp41 amino terminus is replaced by the corresponding sequence of another virus, the name of the mutant corresponds to the virus followed by the amino acid number in HIV-1 where the substitution stops. For example, SIV526 is a substitution of the SIV_{mac} sequence from residue 512, which is the first residue of the HIV-1 gp41 glycoprotein, up to and including residue 526.

As a negative control in all experiments, we included a pSVIIIenv plasmid (pSVIIIenvΔKS) containing a deletion extending from the *Kpn*I site (position 5924) to the *Stu*I site (position 6410) in the HXBc2 *env* gene. The *env* reading frame is frameshifted by this deletion.

Envelope glycoprotein expression and CD4-binding assay. COS-1 cells were transfected by the DEAE-dextran method with pSVIIIenv DNA expressing wild-type or mutant envelope glycoproteins as described previously (7, 14). To measure expression, the cells were radiolabeled with [^{35}S]cysteine overnight and precipitated with an excess of a mixture of sera derived from patients with AIDS (14). Expression of mutant glycoproteins, processing of the glycoprotein precursor, and association of gp41 and gp120 glycoproteins were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and densitometric scanning of the autoradiograms. To measure the CD4-binding ability of mutant glycoproteins, we incubated supernatants of radiolabeled transfected cells with an excess of SupT1 lymphocytes at 37°C for 90 min (34). The cells were washed twice with phosphate-buffered saline (PBS), lysed, and immunoprecipitated as described above. The amount of gp120 precipitated from both bound and unbound fractions was measured by densitometric analysis of autoradiograms from SDS-polyacrylamide gels.

Cell surface expression of envelope glycoproteins. COS-1 cells were transfected with 10 μg of pSVIIIenv DNA expressing wild-type or mutant envelope glycoproteins. At 60 h after transfection, the COS-1 cells were washed with PBS containing 2% fetal calf serum and incubated with an ^{35}S-labeled monoclonal antibody against gp120, F105. F105 antibody-producing hybridomas were obtained from Marshall Posner (37), and 1.5×10^7 cells were labeled overnight in 10 ml of RPMI medium containing 1 ml each of [^{35}S]cysteine and [^{35}S]methionine. Following the labeling, a 100-fold excess of unlabeled cysteine and methionine was added to the medium for 3-h chase period. One mililiter of F105 antibody-containing medium was added to each 60-mm dish of transfected COS-1 cells. The envelope-expressing COS-1 cells were incubated with the antibody for 1 h at room temperature, washed three times with PBS containing 2% fetal calf serum, and lysed. The lysates were incubated with protein A-Sepharose beads, which were then washed by the procedure used for immunoprecipitation (14, 34). The labeled antibody was visualized on a reducing SDS-polyacrylamide gel.

Syncytium-forming ability of envelope glycoproteins. To assess the syncytium-forming ability of mutant envelope glycoproteins, 5×10^5 COS-1 cells in 100-mm dishes were transfected with 10 μg of the envelope glycoprotein expressor plasmid pSVIIIenv. At 48 h after transfection, cells were rinsed twice in PBS and incubated with 50 mM EDTA (pH 7.5) at 37°C for approximately 40 min. Cells were removed from the plate by trituration, centrifuged, and washed with 5 ml of PBS, and one-third of the cells were resuspended in 2 ml of medium (Dulbecco modified Eagle medium supplemented with 10% fetal calf serum). To these cells, we added 10^6 SupT1, C91/PL, or C8166 lymphocytes suspended in approximately 1 ml of RPMI 10% fetal calf serum. The cells were transferred to a T25 flask and returned to a 5% CO_2 incubator at 37°C, and syncytia were scored after 8 to 12 h. Cells with a diameter at least five times that of a single cell were counted as syncytia. The C91/PL cells were preincubated with HTLV-I-infected patient serum for 1 h before cocultivation with COS-1 cells; this was done to inhibit any syncytium formation induced by the HTLV-I envelope glycoproteins expressed in the C91/PL cells (35).

Replication complementation assay. Complementation of single-step replication of the *env*-deficient chloramphenicol acetyltransferase (CAT)-expressing provirus, HXBΔenv CAT, with different lymphocyte targets, was performed as described previously (14). COS-1 cells were transfected with 5 μg each of HXBΔenvCAT and envelope glycoprotein expressor pSVIIIenv plasmids. Equivalent amounts of reverse transcriptase activity representing recombinant virions in COS-1 supernatants were added to Jurkat, C91/PL, SupT1, H9, Molt-3, C8166, and CEM×174 lymphocytes. The COS-1 supernatants containing recombinant viruses were all used directly for infection without prior storage, and all supernatants were treated identically. At 72 h following infection, equal numbers of cells were lysed and CAT activity was measured. The background value derived from the negative control, pSVIIIenvΔKS, was subtracted from all the other values.

Determination of envelope glycoprotein concentration on the virion. COS-1 cells cotransfected with the envelope-expressing plasmid and the pHXBΔenvCAT plasmid (14) were labeled with [^{35}S]cysteine (70 μCi/ml) and [^{35}S]methionine (100 μCi/ml) overnight and then subjected to a 3-h chase with medium containing unlabeled methionine and cysteine. Supernatants were centrifuged at $800 \times g$ for 10 min to remove cell debris and then at $12{,}000 \times g$ for 1 h at 4°C to pellet the virions. The supernatants were completely removed, and the viral pellet was lysed and immunoprecipitated as described. The ratio of the gp120 glycoprotein to $p24^{gag}$ protein associated with the pelleted virions was assessed by densitometry of SDS-polyacrylamide gels.

RESULTS

Phenotype of the mutant envelope glycoproteins. HIV-1 envelope glycoprotein mutants containing amino acid alterations in the gp120 V3 region, the gp41 amino terminus, and the gp41 transmembrane region were selected for this study, since changes in each of these regions have been shown to affect the membrane fusion process (3, 9, 10, 14, 15, 18, 21, 22). Mutant 313 P/S has a single amino acid change in the V3 region of the HIV-1 gp120 glycoprotein, which is a target for neutralizing antibodies (28, 29, 38). This amino acid change has been reported to affect the host range of HIV-1 (47).

The amino acid sequence of the mutant envelope glycoproteins containing alterations in the gp41 amino terminus is shown in Fig. 1. Mutant HTLV517 has the first six amino acids of the HTLV-I transmembrane glycoprotein in place of

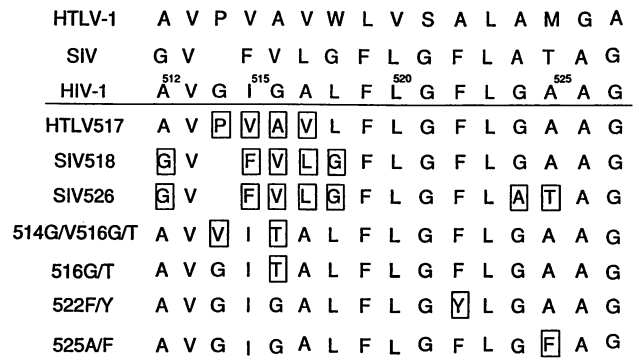

FIG. 1. Amino acid sequence of HIV-1 gp41 mutants. The amino acid sequence of the transmembrane proteins of SIV_{mac}, HTLV-1, HIV-1 (HXBc2), and the mutants are shown in the single-letter code. Numbers represent the amino acid positions in the envelope glycoproteins, with position 1 being the initial methionine of gp120. Mutants are named by the position of the amino acid change, followed by the original amino acid and the substituted residue. Residues in boxes represent amino acids altered in this study.

the six residues found in the HIV-1 gp41 amino terminus. Mutants SIV518 and SIV526 have the first 7 and 15 amino acids, respectively, of the transmembrane glycoprotein of SIV_{mac} in place of the analogous residues in the HIV-1 gp41 amino terminus. Mutant 707 R/I contains a single amino acid change in a charged residue near the membrane-spanning domain of the HIV-1 gp41 glycoprotein and has previously been shown to be partially attenuated in its ability to induce membrane fusion (15).

The mutant glycoproteins were transiently expressed in COS-1 cells, and the phenotypes of the mutants were examined. For all of the mutants, the steady-state level of expression, processing of the gp160 precursor, association of the gp120 and gp41 subunits, cell surface expression, and ability of the gp120 glycoprotein to bind the CD4 receptor were comparable to those of the wild-type glycoproteins (Table 1; Fig. 2 and 3). The ability of the mutant envelope glycoproteins to induce the formation of syncytia was assessed by cocultivating the transfected COS-1 cells expressing the mutant glycoproteins with CD4-positive SupT1 lymphocytes. The results of this assay indicate that most of the mutants were able to induce the formation of syncytia with SupT1 target cells, although at an efficiency lower than that observed for the wild-type HIV-1 glycoproteins (Table 1). The 522 F/Y mutant was significantly attenuated for syncytium-forming ability relative to that of the wild-type glycoproteins.

Target cell preference of the fusion-defective mutant glycoproteins. To test whether alteration of the membrane fusion-associated domains of the HIV-1 envelope glycoproteins might affect the host range of the virus, the ability of the mutant and wild-type glycoproteins to complement the entry of an *env*-defective provirus into different lymphocyte lines was examined. In this assay, recombinant virions produced by COS-1 cell transfection contain the mutant envelope glycoproteins and can encode the bacterial CAT gene if they can gain entry into target cells (14). The ratio of gp120 envelope glycoprotein to p24 core antigen associated with pelleted virions derived from the COS-1 cells was comparable to that of the wild-type glycoprotein for each of the mutant envelope glycoproteins (data not shown). A fixed number of reverse transcriptase units of each recombinant virus preparation was incubated with each of the target

TABLE 1. Phenotype of mutant envelope glycoproteins

Virus	CD4 binding[a]	Processing index[b]	Association index[c]	Fusion (% wt)[d]
SIV 518	ND[e]	0.99	1.00	87
SIV 526	0.98	0.99	1.00	77
HTLV 517	0.98	1.00	1.00	65
514 G/V 516 G/T	0.99	0.99	1.00	62
516 G/T	0.98	0.99	1.01	96
522 F/Y	1.02	1.00	0.98	4
525 A/F	ND	0.99	0.99	68
707 R/I	Normal[f]	1.00	1.01	65
313 P/S	1.00	0.99	0.90	68

[a] The relative CD4-binding ability was calculated by using a binding assay described in Materials and Methods and the following formula: relative binding = (bound mutant gp120 × free wild-type gp120)/(free mutant gp120 × bound wild-type gp120). The relative CD4-binding ability did not vary among different experiments by more than 2% of the reported value.

[b] The amount of gp160 and gp120 glycoproteins was determined by densitometric scanning of autoradiograms of SDS-polyacrylamide gels. The processing index was calculated from the following formula: processing index = (total mutant gp120 × wild-type gp160)/(mutant gp160 × total wild-type gp120). The processing index did not vary among different experiments by more than 2% of the reported value.

[c] The association index indicates the degree of association between the mutant gp120 and gp41 glycoproteins, relative to that of wild-type glycoproteins. Cell lysates and supernatants were treated as described in Materials and Methods, and the association index calculated from the following formula: association index = (mutant cell gp120 × wild-type supernatant gp120)/(mutant supernatant gp120 × wild-type cell gp120). The association index did not vary among different experiments by more than 2% of the reported value.

[d] Syncytium formation with SupT1 target cells was measured as described in Materials and Methods and expressed as percentage of the value observed for the wild-type (wt) glycoproteins. The values shown represent the average of three experiments, with the relative syncytium-forming ability varying by no more than 20% of the reported value.

[e] ND, not done.

[f] The relative CD4-binding ability of the 707 R/I mutant was previously reported and is similar to that of the wild-type gp120 glycoprotein (15).

lymphocyte lines, which were subsequently assayed for CAT expression.

The transfer of CAT activity by the recombinant virions containing the wild-type HIV-1 envelope glycoproteins was most efficient for the C8166 target cells (Fig. 4a). This activity was 5- to 10-fold higher than that observed in the other target cells, consistent with previous reports (44a). The transfer of CAT activity was similar for the Jurkat, SupT1, H9, CEM×174, and C91/PL lymphocytes, which were more efficient target cells than were the Molt-3 lymphocytes (Fig. 4a). The C8166 lymphocytes were also the most efficient target cells for syncytium formation by the wild-type HIV-1 envelope glycoproteins (see below and Table 2). Fluorescence-activated cell sorting with the OKT4 monoclonal antibody indicated that the amount of CD4 present on the surface of these lymphocyte lines is not the sole determinant of the ability to allow virus entry or to support syncytium formation (Fig. 5). For example, the C8166 lymphocytes are more efficient targets than SupT1 cells but express fewer surface CD4 glycoproteins. Apparently, the pattern of target cell preference observed reflects quantitative differences in the ability of the various lymphocyte lines to support HIV-1 entry, a parameter influenced by the level of CD4 expression and other, as yet undefined, factors.

The ability of the mutant envelope glycoproteins to complement virus entry, relative to that of the wild-type glycoproteins, for the various target cells is shown in Fig. 4b. The relative ability of most of the mutant glycoproteins to transfer CAT activity was generally highest for the C8166 target cells and lower for the other target cell lines. CEM

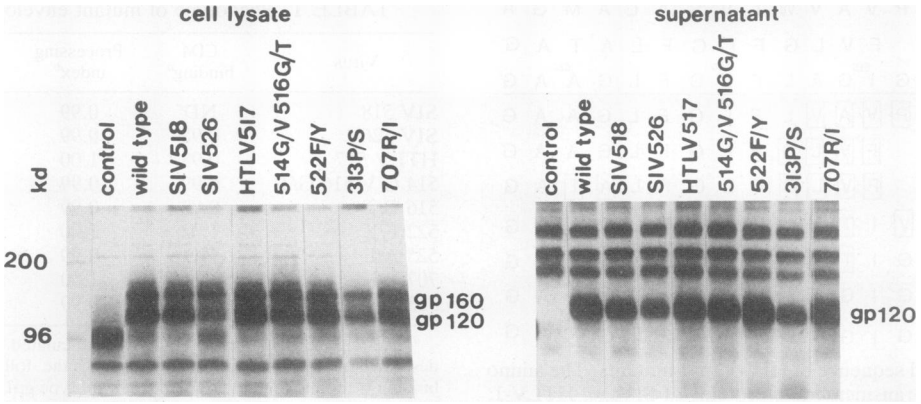

FIG. 2. Expression of envelope glycoprotein mutants in COS-1 cells. Radioimmunoprecipitation of envelope glycoproteins from COS-1 cell lysates and supernatants by using patient serum was performed as described in Materials and Methods. The names of the mutant glycoproteins appear above the lanes.

lymphocytes, one of the fusion partners used to produce the CEM×174 cell line, behaved like H9 cells in terms of allowing entry of virions harboring wild-type or mutant envelope glycoproteins (data not shown). The pattern of target cell preference for two of the mutants, 313 P/S and HTLV 517, revealed an unusual restriction for particular cell lines. While the 313 P/S mutant was able to complement virus entry into most of the cell lines at an efficiency comparable to that of several other mutants (e.g., SIV 526, 525 A/F, 707 R/I), entry into C91/PL cells was selectively attenuated by the amino acid change. Although the HTLV 517 mutant glycoproteins complemented virus entry into SupT1, Jurkat, and Molt-3 lymphocytes as efficiently as did many of the other mutants, entry into C8166 and C91/PL cells was specifically abrogated.

Syncytium induction by envelope glycoprotein mutants. To demonstrate that the 313 P/S and HTLV 517 envelope glycoproteins are specifically deficient for fusion of particular target cells, the ability of wild-type and HTLV 517 glycoproteins to form syncytia with different lymphocyte targets was examined. COS-1 cells expressing the wild-type HIV-1 envelope glycoproteins efficiently formed syncytia with C8166, SupT1, and C91/PL cells (Table 2). The efficiency of syncytium induction by the wild-type HIV-1 glycoproteins exhibited the order C8166 > SupT1 > CP1/PL. COS-1 cells expressing the 313 P/S or HTLV 517 mutants formed syncytia with SupT1 cells at efficiencies of 77 and 56%, respectively, of the wild-type glycoproteins. The efficiencies of syncytium formation by the 313 P/S and HTLV 517 mutants with SupT1 target cells were comparable to those of the SIV 518, SIV 526, and 514 G/V 516 G/T mutants. By contrast, syncytium formation with C8166 and C91/PL target cells was very inefficient for the HTLV 517 mutant compared with that seen for the SIV 518, SIV 526, and 514 G/V 516 G/T mutants. The 313 P/S mutant did not induce cell-cell fusion with C91/PL target cells, even though this mutant exhibited efficient syncytium-forming ability with SupT1 and C8166 target cells. By contrast, several of the mutant glycoproteins (SIV 518, SIV 526, 514 G/V 516 G/T, and 516 G/T) that exhibited syncytium-forming ability in the latter cell lines comparable to that of the 313 P/S mutant were also able to efficiently form syncytia with C91/PL target cells.

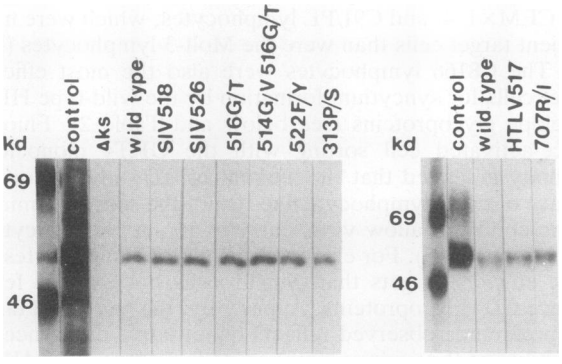

FIG. 3. Cell surface expression of envelope glycoprotein mutants. COS-1 cells expressing envelope glycoproteins were incubated with radiolabeled F105 antibody, which recognizes the gp120 glycoprotein, lysed, and precipitated with protein A-Sepharose beads as described in Materials and Methods. The control lane is a precipitation of the labeled F105 antibody preparation without preincubation with the cells. The names of the mutant glycoproteins appear above the lanes. The first lane indicates the positions of the 69- and 46-kDa markers. kd, kilodaltons.

DISCUSSION

Retrovirus tropism can potentially be mediated at several different stages in the viral life cycle. Several studies have implicated the HIV-1 envelope glycoproteins in both quantitatively and qualitatively distinct patterns of target cell preference (6, 17, 18, 32, 41, 44a, 49, 51). HIV-1 envelope glycoprotein variation could affect viral tropism by directly altering interaction with the target cell or by indirectly affecting cell type-specific differences in precursor processing, glycosylation, subunit assembly, or virion incorporation. In our envelope complementation assay, observed differences in tropism must arise secondary to cell type-specific differences in envelope glycoprotein functions important for the early phase of replication, since all recombinant virions were produced in the same cell type and differ only in the envelope glycoprotein component. The amount of the mutant envelope glycoproteins incorporated into the recombinant virions was demonstrated to be similar for all of the mutants, ruling out the possibility that observed tropic differences merely reflect quantitative differences in envelope glycoprotein density on the virion surface. The patterns

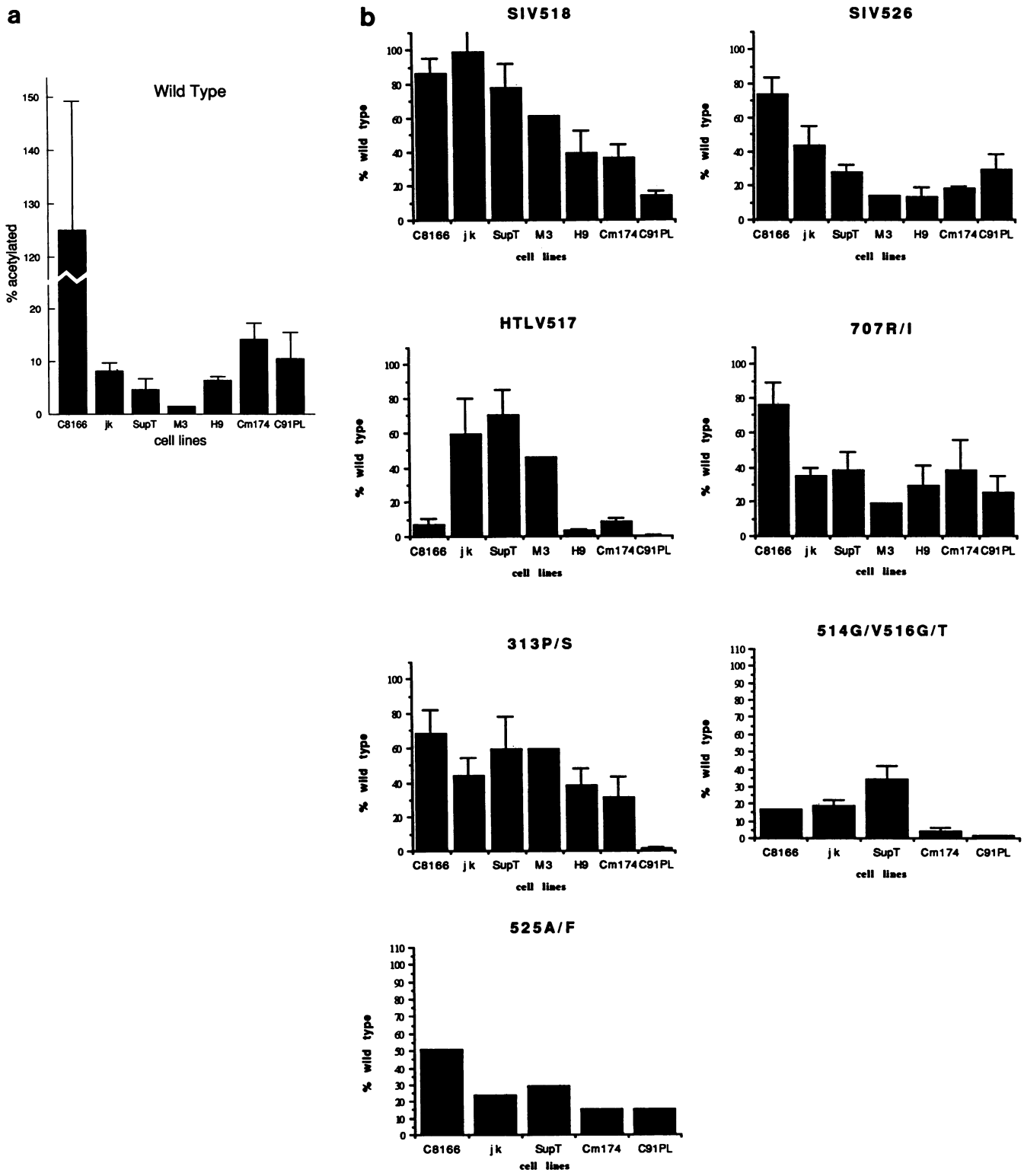

FIG. 4. Replication complementation by HIV-1 envelope glycoproteins, using different cell lines. (a) The percent acetylation of chloramphenicol observed by using the wild-type HIV-1 envelope glycoproteins in the *env* complementation assay is shown for different target cells. For each assay, 5,000 cpm of reverse transcriptase equivalents of recombinant virus was incubated with 10^6 target cells, and 7% (5 μl) of the target cell lysates were used for the CAT assay. The incubation time for the CAT assay was 10 min. For the C8166 cell line only, 2% of the lysate was assayed for only 8 min, but the data were extrapolated to the value shown in the figure, representing the percent acetylation equivalent to 7% of the lysate and a 10-min CAT assay, to allow comparison with the other cell lines. The standard error of the mean is indicated. Abbreviations: jk, Jurkat; SupT, SupT1; cm174, CEM×174; M3, Molt-3. (b) The ability of the mutant envelope glycoproteins to complement a deficient CAT-expressing provirus relative to the wild-type envelope glycoproteins is shown. The standard error of the mean is indicated.

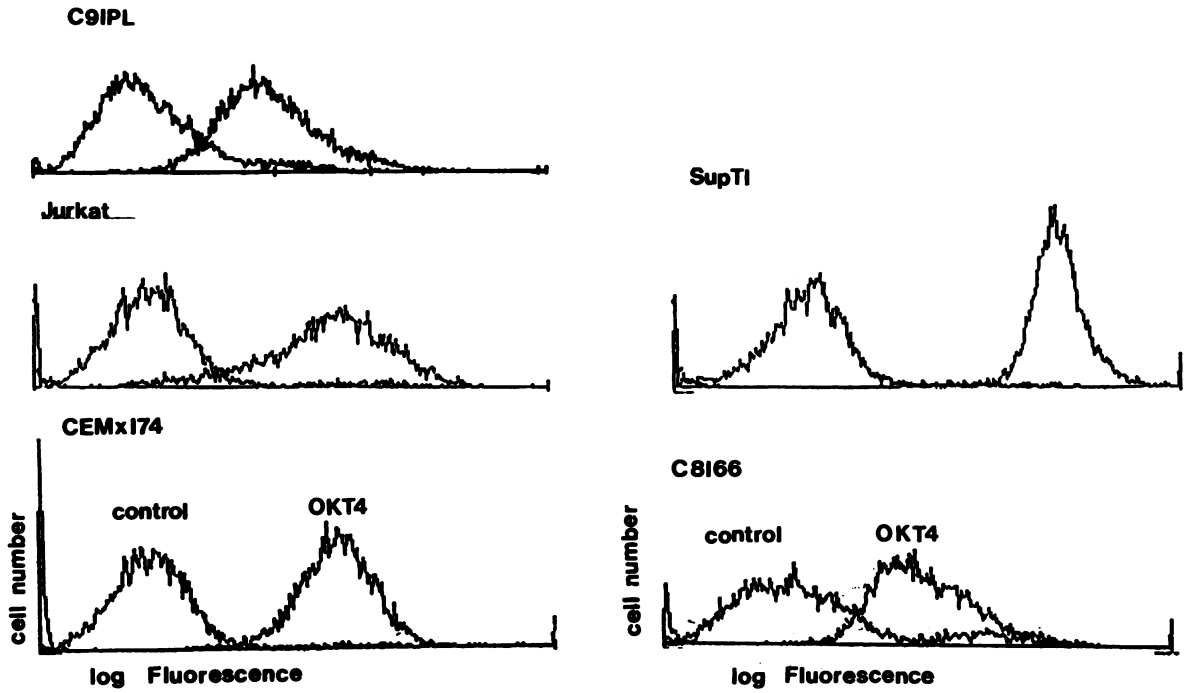

FIG. 5. Cell surface CD4 levels. Fluorescence-activated cell surface analysis was performed on the cell lines by using a monoclonal antibody against the CD4 molecule (OKT4; Ortho Diagnostics) followed by a fluorescein isothiocyanate-conjugated goat anti-mouse monoclonal antibody. The control represents incubation of the cells with the fluorescein isothiocyanate-conjugated antibody without prior addition of OKT4 antibody.

of target cell preference observed for the replication complementation assay were consistent with those seen in the syncytium formation assay.

Since some of the observed differences in tropism among the primate immunodeficiency viruses appear to involve post-CD4-binding events involved in membrane fusion, mutant HIV-1 envelope glycoproteins containing changes in three regions implicated in the fusion function were used in this study. These regions include the gp120 V3 loop (10, 14, 18, 22), which is a target for neutralizing antibodies (28, 29, 38), the hydrophobic domain at the gp41 amino terminus (3, 9, 14, 22), and the gp41 transmembrane region (15). For the purposes of this study, mutant envelope glycoproteins were selected for levels of COS-1 cell expression, precursor processing, gp120-gp41 association, CD4 binding, and virion association equivalent to those of the wild-type envelope glycoproteins. Therefore, observed decreases in replicative or syncytium-forming abilities of the mutants reflect a disruption of some aspect of the membrane fusion process.

TABLE 2. Syncytium formation by HIV-1 envelope glycoprotein mutants[a]

Mutant	No. (%) of syncytia in cell line[b]:		
	SupT1	C8166	C91/PL
mockΔKS	40 (0%)	9 (0%)	6 (0%)
Wild type	950 (100%)	1,250 (100%)	428 (100%)
SIV 518	710 (74%)	625 (50%)	230 (53%)
SIV 526	755 (79%)	1,250 (100%)	366 (85%)
HTLV 517	535 (54%)	25 (1.3%)	30 (5.7%)
514 G/V 516 G/T	658 (68%)	750 (60%)	200 (46%)
516 G/T	913 (96%)	1,250 (100%)	520 (122%)
522 F/Y	37 (0%)	113 (8.4%)	ND[c]
313 P/S	731 (76%)	1,250 (100%)	22 (3.8%)

[a] Syncytium formation was measured as described in Materials and Methods.

[b] Values represent the actual number of syncytia counted in a volume of 300 μl and represent approximately 1/10 of the total number of syncytia formed in the assay. Numbers in parentheses represent the values expressed as a percentage of the values observed for the wild-type glycoproteins. The negative control cells were transfected with the pSVIIIenvΔKS plasmid.

[c] ND, not done.

Human lymphocyte lines could be ranked for their ability to support virus entry and syncytium formation mediated by the wild-type HIV-1 envelope glycoproteins according to the following order: C8166 > CEM×174, C91/PL, Jurkat, H9, SupT1 > Molt-3. In this and another study examining the HIV-1 envelope glycoprotein mutants with decreased CD4-binding affinities (50), the relative replicative abilities of most mutants appear to be higher in cell lines that are more efficient targets for the wild-type glycoproteins. This pattern is probably determined by quantitative factors including, but clearly not limited to, the level of CD4 expression (44a). The level of CD4 expression by the target cell and CD4-binding affinity of the envelope glycoproteins exhibit effects on target cell preference only after significant changes in these parameters (18a, 50).

The 313 P/S mutant exhibited a qualitatively different pattern of target cell preference. Virions harboring the 313 P/S glycoproteins were able to infect C8166, Jurkat, SupT1, Molt-3, H9, and CEM×174 cells at efficiencies comparable to, or better than, those observed for several other mutants, yet were almost completely defective in mediating entry into C91/PL cells. The 313 P/S mutant also demonstrated a selective inability to mediate cell-cell fusion with C91/PL target cells. The 313 P/S change has previously been reported to affect the host range of a different HIV-1 isolate

(47), but the step in the virus replication cycle at which this change exerted its effect was not determined. Similarly, gp120 regions including the V3 loop have been shown to be important for primary macrophage tropism (17, 32, 41). Our study indicates that V3 loop alterations can affect the membrane fusion process of virus entry in a manner dependent upon the target cell and raises the possibility that the other tropic properties mapped to gp120 regions proximal to the V3 loop are also mediated at this level. The results to date could be explained either by a direct interaction of the V3 loop with the target cell or by a more fastidious requirement of particular target cells for specific V3 loop structures and/or functions. Further experiments are required to distinguish these possibilities.

The HTLV 517 mutant also exhibited a qualitatively different pattern of target cell preference. Virions harboring the HTLV 517 glycoproteins were able to infect Jurkat, SupT1, and Molt-3 cells with reasonable efficiency, comparable to or better than that of the SIV 526 mutant, but were unable to mediate entry into C8166, H9, or C91/PL cells. Likewise, although the ability of the HTLV 517 mutant to induce the formation of syncytia with SupT1 cells is comparable to that of other mutants with alterations of the gp41 amino terminus, syncytium formation with C8166 or C91/PL target cells is 7- to 50-fold less efficient for the HTLV 517 mutant. This selective defect in the ability of the HTLV 517 mutant to fuse C8166 membranes is particularly striking, since C8166 lymphocytes are among the most efficient target cells for both HTLV-I- and HIV-1-induced syncytium formation and infection. This observation also indicates that the functional properties of the gp41 amino terminus of the HTLV 517 mutant are distinct from those of the amino termini of either HTLV-I or HIV-1 transmembrane glycoproteins.

Many enveloped viruses possess a hydrophobic region, referred to as the fusion peptide, located at the amino terminus of a transmembrane envelope glycoprotein (31, 33). The fusion peptides of some orthomyxoviruses and paramyxoviruses have been implicated in the membrane fusion process by the observations that mutations that affect these regions alter virus entry (12), that a pH decrease important for influenza virus entry results in a conformational change that exposes the fusion peptide (42, 45), and that fusion peptides can interact with cell membranes (52). The fusion peptide has been proposed to interact with either the viral or target cell membrane to mediate the fusion event (31, 33). The HIV-1 gp41 amino terminus resembles orthomyxovirus and paramyxovirus fusion peptides by virtue of analogous position, distant sequence similarity, and similar phenotypes associated with amino acid alterations (3, 9, 14, 21, 22). The results described herein indicate that the structure of the HIV-1 gp41 amino terminus can qualitatively determine viral tropism at the stage of virus entry. Since several envelope glycoprotein mutants with altered gp41 amino termini efficiently induce fusion with C8166 target cells, the results cannot be explained by a more fastidious requirement of these cells for particular gp41 amino-terminal structures. Instead, the results most probably indicate a direct interaction of this region with components of the target cell, although more complicated models in which the gp41 amino terminus modifies an envelope glycoprotein structure that directly relates to the target cell are still formally possible. Identification of the target cell factors that modulate the membrane fusion process should be facilitated by a characterization of the corresponding envelope glycoprotein determinants.

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