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A mutation in the human immunodeficiency virus type 1 transmembrane glycoprotein gp41 dominantly interferes with fusion and infectivity

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ABSTRACT **Several domains of the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein have been identified that are involved in HIV-1-mediated membrane fusion. One domain that is involved in membrane fusion is the hydrophobic amino terminus of the HIV-1 transmembrane glycoprotein gp41. Here we show that a polar substitution at gp41 amino acid 2 (the 41.2 mutation) results in an envelope glycoprotein that dominantly interferes with both syncytium formation and infection mediated by the wild-type HIV-1 envelope glycoprotein. The interference by the 41.2 mutant is not a result of aberrant envelope glycoprotein synthesis, processing, or transport. The 41.2 mutant elicits a dominant interfering effect even in the presence of excess wild-type glycoprotein, suggesting that a higher-order envelope glycoprotein complex is involved in membrane fusion. These results shed light on the process by which the HIV-1 envelope glycoproteins induce membrane fusion reactions and present a possible approach to anti-HIV therapy.**

Mutations in several proteins of human immunodeficiency virus type 1 (HIV-1) have been described that dominantly interfere with HIV-1 replication; these include mutations in the regulatory proteins Tat and Rev (1, 2) and the structural protein Gag (3). Mutations that dominantly interfere with viral replication provide valuable insight into the normal functions of viral proteins and may prove useful in antiviral therapy.

The envelope glycoprotein of HIV-1 is synthesized as a polyprotein precursor, gp160, which is proteolytically processed to the mature surface glycoprotein gp120 and the transmembrane glycoprotein gp41. gp120 and gp41 are responsible for the membrane fusion function of HIV-1, which plays an essential role early in the infection process (4). The envelope fusion function also leads to the fusion of permissive cells expressing the HIV receptor molecule CD4, a process known as syncytium formation (5, 6). Syncytium formation contributes to the cytopathic effect of HIV-1 infection in culture and may also play a role in the depletion of $CD4^+$ cells *in vivo* (for review, see ref. 7).

Studies conducted in several laboratories indicate that the envelope glycoproteins of HIV-1 exist as multimers. Evidence supports the formation of HIV-1 envelope glycoprotein dimers, trimers, and tetramers (8–10).

During previous studies, mutations were introduced into the cleavage sequence of the HIV-1 envelope precursor gp160 (11), the hydrophobic amino terminus of the HIV-1 transmembrane glycoprotein gp41 (12), and the principal neutralizing determinant (the V3 loop) of the HIV-1 surface glycoprotein gp120 (13). Characterization of these mutant envelope glycoproteins revealed that a number were defective in syncytium formation.

In this study, we tested the ability of these mutations to dominantly interfere with syncytium formation and virus infection mediated by wild-type HIV-1 envelope glycoproteins. Our results indicate that a mutation at the second amino acid position of gp41 (the 41.2 mutation) dominantly interferes with both processes. The interference phenotype is exhibited even in the presence of a great excess of wild-type to mutant envelope glycoproteins. This result indicates that multiple envelope glycoprotein–CD4 interactions are required for syncytium formation and virus infection. The results are also consistent with the hypothesis that interactions between multiple fusion domains are necessary for membrane fusion.

MATERIALS AND METHODS

HIV-1 Envelope Glycoprotein Mutants. The HIV-1 envelope glycoprotein mutants used in this study have been described (11–13). The 120.518A mutant contains an Arg → Thr substitution at gp120 residue 518 (the final residue of gp120). This mutation abolishes gp160 cleavage and envelope-induced syncytium formation (11). The 41.2 mutant contains a Val → Glu substitution at gp41 amino acid 2; the 41.9 mutant contains a Leu → Arg substitution at gp41 amino acid 9; the 41.15 mutant contains an Ala → Glu substitution at gp41 amino acid 15; the 41.26 mutant contains a Leu → Arg substitution at gp41 amino acid 26; and the 41.29 mutant contains a Gln → Leu substitution at gp41 amino acid 29 (12). gp41 residues 1–28 comprise the highly hydrophobic amino terminus of gp41. gp41 residue 29 lies within the highly conserved polar border of the hydrophobic region. Mutations at all of these positions completely abolish syncytium formation. The 318PR mutant contains a Pro → Arg substitution at gp120 amino acid 318; the 319GA mutant contains a Gly → Ala substitution at gp120 amino acid 319; and the 320RG mutant contains an Arg → Gly mutation at gp120 amino acid 320. These three gp120 mutations lie within the principal neutralizing determinant (the V3 loop) of gp120 (13). The 320RG mutation completely abolishes syncytium formation, whereas the 318PR and 319GA mutations reduce syncytium formation to ≈10% of wild-type levels.

Expression of HIV-1 and HIV-2 Envelope Glycoproteins. The HIV-1 envelope expression plasmids pNL4-3dPst and pHenv have been described (11). pNL4-3dPst is a *gag/pol*-deletion mutant of a full-length proviral clone. pHenv contains the *env* region immediately downstream from the 5′ HIV-1 long terminal repeat. Plasmid pHenvWMJ-2 was constructed by replacing the *env* region from pHenv with the *env* region from the WMJ-2 strain of HIV-1 (14). This replacement was done by cloning the *Kpn* I–*Xho* I fragment from

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Abbreviations: HIV-1 and -2, human immunodeficiency virus type 1 and type 2, respectively.

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plasmid pWMJ II-12 *Sal* I 5′/*Sst* I 3′ (obtained from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD) in place of the *Kpn* I–*Xho* I fragment of pHenv. pHenvWMJ-2 41.2 (a derivative of pHenvWMJ-2 containing the 41.2 mutation) was constructed by introducing the region from the *Bsu*361 site in the gp120 coding region to the *Bam*HI site in the gp41 coding region from pHenv41.2 into pHenvWMJ-2. The HIV-2 envelope expression construct pCMVHIV-2env was constructed by introducing the *env* region from HIV-2_{ROD} into the construct p763 (provided by B. Sugden, University of Wisconsin, Madison, WI). In pCMVHIV-2env, HIV-2 envelope expression is driven by the human cytomegalovirus immediate early enhancer/promoter. The control plasmid pHenvKFS was constructed by introducing a *Bgl* II linker (New England Biolabs linker 1066) into the *Kpn* I site located at the amino terminus of *env*. The insertion of a *Bgl* II linker at the *Kpn* I site results in a frameshift in the *env* open reading frame without affecting any other known HIV-1 open reading frame. The plasmid pCMVHIV-1env41.2 was constructed by cloning the *Sal* I–*Xho* I fragment from pHenv41.2 (12) into p763. Additional details about plasmid constructions will be made available upon request.

HIV-1 Vector Construction. HIV-1 vector pGB108 was made in two steps as follows: The infectious HIV-1 proviral clone pNL4-3 (15) was digested with *Stu* I and *Nhe* I, the digested DNA was treated with Klenow fragment, and the ends were ligated (regenerating the *Nhe* I site) to produce clone pGB107. pGB107 thus contains a 426-base-pair inframe deletion in the *env* gene. The hygromycin B phosphotransferase gene under control of the simian virus 40 early promoter/enhancer was isolated by digesting plasmid pED84 (E.L.D. and A.T.P., unpublished work) with *Nhe* I. After digestion of pGB107 with *Nhe* I, the simian virus 40 hygro^r cassette was ligated into the *Nhe* I site, resulting in plasmid pGB108.

Cell Culture and Fusion Assays. The $CD4^+$ HeLa cell line derivative HeLa T4 (16) was cultured at 37°C in Dulbecco's modified Eagle's medium/5% calf serum (HyClone)/G418 at 300 μg/ml. The HeLa T4 cell line was provided by R. Axel (Columbia University). HeLa T4 transfections were done by the calcium phosphate procedure as described (11) using 15 μg of plasmid DNA per 8×10^5 cells. For the assays that examined the effect of various ratios of pHenv DNA/pHenv41.2 DNA, total amount of transfected DNA per dish was kept constant with pHenvKFS DNA. Wild-type control plates were cotransfected with 7.5 μg of pHenv and 7.5 μg of pHenvKFS. Two days posttransfection, cells were stained, and syncytia (defined as giant cells containing more than four nuclei) were scored microscopically.

Virus Production and Infections. COS-1 cells were maintained at 37°C in Dulbecco's modified Eagle's medium/10% fetal bovine serum. For analysis of the effect of the 41.2 envelope mutation on viral infectivity, 5 μg of pGB108, 5 μg of pHenv, and 5 μg total of various combinations of pHenv41.2 and pHenvKFS DNA were transfected into COS-1 cells by using the DEAE-dextran procedure (17). The pHenv DNA/pHenv41.2 DNA ratios tested were 1:1, 2:1, 5:1, and 10:1; total amount of transfected DNA was kept constant with pHenvKFS. On the second day posttransfection, COS-1 cell medium was changed, and 4×10^5 HeLa T4 cells were plated onto 60-mm-diameter tissue culture dishes for infection the following day. For virus infections, medium was collected from the transfected COS-1 cells and centrifuged at low speed to remove cell debris. The supernatant was removed, and DEAE-dextran was added to a final concentration of 8 μg/ml. One-half milliliter of this supernatant was added to the HeLa T4 cells. After a 3-hr incubation, fresh medium was added. Two days postinfection, selection was initiated at a hygromycin B concentration of 200 μg/ml. Cells were cultured 7–10 days, and virus titer was determined by counting colonies fixed and stained with 0.5% crystal violet/50% (vol/vol) methanol.

Cell Labeling and Radioimmunoprecipitation. Two days posttransfection, 2×10^6 HeLa T4 cells were metabolically labeled with 250 μCi of [^{35}S]methionine (1 Ci = 37 GBq) for 13 hr. Lysates or extracellular media were prepared and immunoprecipitated with AIDS patient serum as described (11, 18). CD4 binding assays were done as described (12). AIDS patient serum was provided by R. Tomar (University of Wisconsin).

RESULTS

A Mutant-Containing a Polar Substitution in the gp41 Fusion Domain of HIV-1 Dominantly Interferes with Syncytium Formation. To determine whether mutant HIV-1 envelope glycoproteins could dominantly interfere with syncytium formation induced by wild-type HIV-1 envelope glycoproteins, we cotransfected the $CD4^+$ HeLa cell line HeLa T4 (16) with equivalent amounts of wild-type and mutant envelope expression constructs. The results (Table 1) indicated that mutations at gp120 amino acids 318, 319, and 320 (within the V3 loop), gp120 amino acid 518 (the site of gp160 proteolytic cleavage), and gp41 amino acids 9, 15, 26, and 29 (within the amino terminus of gp41) had less than a 2-fold effect on syncytium formation induced by wild-type envelope. Mutations at these positions were shown previously to greatly reduce or abolish syncytium formation (11–13). However, coexpression of wild-type envelope glycoprotein with an envelope glycoprotein containing a polar substitution (Val → Glu) at gp41 amino acid 2 resulted in a 25-fold reduction in syncytium formation induced by the wild-type envelope glycoprotein. To insure that the mutant plasmids were not affecting DNA uptake during transfection, an assay was used in which plasmid pCH110 (19), which expresses *Escherichia coli* β-galactosidase, was cotransfected with the wild-type and mutant DNAs. Uptake of pCH110 was quantified by scoring the number of cells that turned blue after treatment with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal). Results of this analysis indicated that none of the mutant envelope expression plasmids inhibited DNA uptake during transfection (data not shown).

To investigate the amount of 41.2 mutant needed to dominantly interfere with syncytium formation, we cotransfected at various pHenv DNA/pHenv41.2 DNA ratios; the ratios were 2:1, 5:1, and 10:1 pHenv/pHenv41.2. Total amount of DNA was kept constant with pHenvKFS DNA. The results (Table 2) indicated that 1:1, 2:1, and 5:1 ratios of pHenv

Table 1. Effect of envelope mutations on syncytium formation

Transfected DNA*	Relative syncytium formation† ± SD
pHenv	
+ pHenvKFS	100
+ pHenv120.518A	57 ± 11
+ pHenv41.2	4 ± 3
+ pHenv41.9	52 ± 24
+ pHenv41.15	68 ± 47
+ pHenv41.26	122 ± 34
+ pHenv41.29	134 ± 32
+ pHenv318PR	123 ± 25
+ pHenv319GA	160 ± 48
+ pHenv320RG	44 ± 18

All data represent the average of five assays.
*HeLa T4 cells (8×10^5) were transfected with 7.5 μg of pHenv DNA plus 7.5 μg of mutant pHenv DNA.
†Syncytium formation resulting from transfection with pHenv plus pHenvKFS was ≈240 syncytia per μg of pHenv.

Table 2. Effect of varying the ratio of wild-type DNA/41.2 DNA on syncytium formation

Transfected DNA*	WT/mutant DNA ratio	Relative syncytium formation† ± SD
pHenv		
+ pHenvKFS	1:1	100
+ pHenv41.2	1:1	4 ± 3
+ pHenv41.2	2:1	6 ± 1
+ pHenv41.2	5:1	18 ± 8
+ pHenv41.2	10:1	39 ± 20

All data represent the average of at least four assays. WT, wild type.

*HeLa T4 cells (8×10^5) were transfected with 7.5 μg of pHenv DNA and various amounts of pHenv41.2 DNA. Total amount of transfected DNA per dish (15 μg) was held constant with pHenvKFS DNA as described.

†Syncytium formation resulting from transfection with pHenv plus pHenvKFS was ≈240 syncytia per μg of pHenv.

DNA/pHenv41.2 DNA dramatically decreased syncytium formation. A 10:1 ratio reduced syncytium formation ≈40%. These data indicate that interference occurs even in the presence of excess wild-type glycoprotein.

The 41.2 Mutant Interferes with Syncytium Formation Induced by a Heterologous Strain of HIV-1 But Not by HIV-2. The *env* gene from the HIV-1 proviral clone pNL4-3 (15) was used to construct pHenv (11). To examine specificity of the 41.2 interference effect, we cotransfected pHenv41.2 with a pHenv derivative, pHenvWMJ-2, which expresses the wild-type envelope glycoprotein of the WMJ-2 strain of HIV-1. We constructed pHenvWMJ-2 by substituting the *env* gene from the WMJ-2 strain of HIV-1 (14) for the *env* gene in pHenv. These two envelope glycoproteins share ≈88% amino acid sequence identity. Table 3 indicates that the 41.2 mutant effectively interfered with syncytium formation induced by the WMJ-2 envelope glycoproteins.

To test the effect of the 41.2 mutant on syncytium formation induced by the HIV-2 envelope glycoprotein, we constructed plasmid pCMVHIV-2env, which efficiently expresses the HIV-2 envelope gene. In contrast to its effect on syncytium formation induced by the HIV-1 envelope glycoprotein, the 41.2 mutant was unable to interfere with syncytium formation induced by the HIV-2 envelope glycoprotein (Table 3). Because the possibility existed that pHenv41.2 was unable to express a sufficient amount of envelope to elicit its effect on the HIV-2 envelope expressed from the cytomegalovirus promoter, we constructed plasmid pCMVHIV-1env41.2, in which expression of the 41.2 mutant envelope is driven by the cytomegalovirus promoter. Cotransfection of pCMVHIV-2env and pCMVHIV-1env41.2 had no effect on syncytium formation induced by pCMVHIV-2env, whereas cotransfection of pCMVHIV-1env41.2 with pHenv blocked pHenv-induced syncytium formation (data not shown). Furthermore, fusion induced by the HIV-2 envelope expressed from the HIV-2 long terminal repeat (using the construct pSP275 provided by M. Emerman, Hutchinson Cancer Research Center, Seattle) was not inhibited by cotransfection with pHenv41.2 (data not shown). These results again indicated that the 41.2 mutant envelope was unable to interfere with syncytium formation induced by the HIV-2 envelope glycoprotein.

Table 3. Effect of 41.2 mutant on syncytium formation induced by the WMJ-2 HIV-1 envelope and the HIV-2 envelope

Transfected DNA*	Relative syncytium formation† ± SD
pHenvWMJ-2	
+ pHenvKFS	100
+ pHenv41.2	13 ± 14
pCMVHIV-2env	
+ pHenvKFS	100
+ pHenv41.2	96 ± 43

All data represent the average of at least four assays.

*HeLa T4 cells (8×10^5) were cotransfected with 7.5 μg of wild-type DNA plus 7.5 μg of mutant DNA.

†Transfection with pHenvWMJ-2 plus pHenvKFS resulted in the formation of ≈400 syncytia per μg of pHenvWMJ-2. Transfection with pCMVHIV-2env plus pHenvKFS resulted in the formation of ≈620 syncytia per μg of pCMVHIV-2env.

Interference Is Not Due to Aberrant Processing, Transport, or CD4 Binding Properties of Coexpressed Envelope Glycoproteins. Previous analysis of the 41.2 mutant indicated that processing and transport were not affected by the Val → Glu substitution (12). However, we investigated the possibility that coexpression of wild-type envelope with the 41.2 mutant might interfere with the transport or processing of coexpressed envelope glycoproteins. Thus, immunoprecipitation analysis was performed on cells cotransfected with pHenv and pHenv41.2. The envelope glycoproteins from cells transfected with pHenv or pHenv41.2 alone were immunoprecipitated in parallel; any significant alteration in transport or processing would thus be readily detectable. Fig. 1*A* indicates that coexpression of wild-type and 41.2 mutant envelope glycoproteins had no detectable effect on envelope processing.

After transport of the gp120–gp41 complex to the cell surface, a significant amount of gp120 is shed into the extracellular medium (20). The amount of gp120 in the medium, therefore, provides a measure of the efficiency with which HIV-1 envelope glycoproteins are transported to the cell surface. To determine whether the 41.2 mutant affected envelope glycoprotein transport to the cell surface in cells cotransfected with pHenv and pHenv41.2, immunoprecipitations were done by using the extracellular medium from labeled cells transfected with pHenv, pHenv41.2, or pHenv and pHenv41.2. Fig. 1*B* indicates that coexpression of wild-

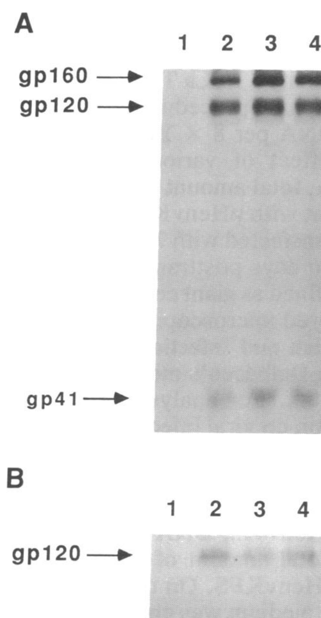

FIG. 1. Immunoprecipitation of wild-type and 41.2 mutant envelope glycoproteins. HeLa T4 cells (8×10^5) were transfected with 15 μg of pHenvKFS (lane 1), 15 μg of pHenv (lane 2), 15 μg of pHenv41.2 (lane 3), or 7.5 μg of pHenv plus 7.5 μg of pHenv41.2 (lane 4). Two days posttransfection, cells were labeled for 13 hr with [^{35}S]methionine, and the envelope glycoproteins were immunoprecipitated as described. (*A*) Immunoprecipitation of cell-associated envelope glycoproteins from cell lysates. (*B*) Immunoprecipitation of secreted gp120 from the extracellular medium.

type and 41.2 mutant envelope glycoproteins had no effect on the amount of gp120 shed into the extracellular medium, strongly suggesting that the 41.2 mutant was not blocking envelope transport to the cell surface.

To determine whether coexpressed envelope glycoproteins could bind the CD4 receptor molecule, CD4 binding assays were done on cells transfected with pHenv, pHenv41.2, or pHenv and pHenv41.2. Our method for analyzing CD4 binding has been described (12). Results of this analysis indicated that coexpression of wild-type and 41.2 mutant envelope glycoproteins does not block gp160 or gp120 binding to CD4 (data not shown).

To rule out the possibility that the 41.2 mutant was in some manner outcompeting wild-type envelope for processing we took advantage of the fact that the envelope glycoprotein of the WMJ-2 strain of HIV-1, although as fusogenic as the envelope glycoprotein expressed by pHenv, is partially defective in processing (E.O.F., unpublished observations). Furthermore, the WMJ-2 gp120, due to differential glycosylation, is somewhat smaller than the pHenv gp120. The 41.2 mutant was introduced into the WMJ-2 envelope expression construct pHenvWMJ-2 to generate pHenvWMJ-2 41.2. Coexpression of pHenv and pHenvWMJ-2 41.2 dominantly interferes with syncytium formation and allows us, as shown in Fig. 2, to distinguish between wild-type and mutant envelope glycoproteins. In lane 1 of Fig. 2 the pHenv gp160 and gp120 are present in approximately equivalent amounts. In lane 2, the WMJ-2 41.2 envelope species are shown; gp120 is present at a reduced level and migrates faster. Lane 3 of Fig. 2 shows the coexpressed pHenv and pHenvWMJ-2 41.2 envelope glycoprotein species. The gp160 band is a composite of pHenv and pHenvWMJ-2 41.2 gp160 and is, therefore, broader and darker than in lanes 1 or 2. The gp120 in lane 3 is derived almost entirely from pHenv. A faint WMJ-2 41.2 gp120 band is evident just below the pHenv gp120 band. That the amount of pHenv-derived gp120 is equivalent in lanes 1 and 3 indicates that the 41.2 mutant does not affect gp160 processing in the cells cotransfected with wild-type and 41.2 mutant expression constructs.

The 41.2 Mutant Dominantly Interferes with HIV-1 Infectivity. To determine whether the 41.2 mutant dominantly interferes with HIV-1 infectivity, an HIV-1 vector system was used that quantitatively analyzes infectivity in a single round of replication. This system, which depends upon envelope trans-complementation for production of infectious vector virus (E.L.D. and A.T.P., unpublished work), makes use of a replication-defective proviral clone (pGB108) containing the hygromycin B resistance gene under control of the simian virus 40 early promoter in place of a 426-base-pair region deleted from the *env* open reading frame. This provirus is unable to encode a functional envelope glycoprotein. When envelope glycoproteins are provided in trans, infectious virions capable of undergoing one round of replication are generated. Infection of permissive $CD4^+$ cell types and selection in medium containing hygromycin result in the generation of hygromycin-resistant colonies. The number of hygromycin-resistant colonies obtained provides a measure of the efficiency with which the envelope provided in trans can rescue infectious virus.

To analyze effects of the 41.2 mutant on the ability of the wild-type HIV-1 envelope glycoprotein to complement the GB108 vector, virus was produced by using pGB108 and various ratios of pHenv DNA/pHenv41.2 DNA as described. Results indicate that even at an excess of pHenv DNA/pHenv41.2 DNA, viral infectivity was substantially diminished (Table 4). Even at a 10:1 ratio of wild-type DNA/41.2 mutant DNA, infectivity was reduced. A similar decrease in titer was seen when wild-type and 41.2 mutant envelopes were expressed by the HIV-1 envelope expression vector pNL4-3dPst (11) (data not shown). These results indicate that the 41.2 mutant, when expressed in cells producing virus, can dominantly interfere with the production of infectious virus.

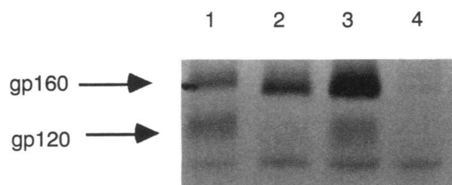

FIG. 2. Immunoprecipitation of pHenv and pHenvWMJ-2 41.2 envelope glycoproteins. HeLa T4 cells (8×10^5) were transfected with 7.5 μg of pHenv (lane 1), 7.5 μg of pHenvWMJ-2 41.2 (lane 2), 7.5 μg of pHenv plus 7.5 μg of pHenvWMJ-2 41.2 (lane 3), or no DNA (lane 4). Two days posttransfection, cells were labeled for 16 hr with [^{35}S]methionine, and the envelope glycoproteins were immunoprecipitated as described.

DISCUSSION

In this paper, we show that a mutant containing a polar substitution at amino acid 2 of the HIV-1 transmembrane glycoprotein gp41 dominantly interferes with both syncytium formation and virus infection mediated by the wild-type HIV-1 envelope glycoprotein. In contrast, mutations elsewhere in the hydrophobic amino terminus of gp41, in the gp160 cleavage region, and in the V3 loop of gp120 do not exhibit a dramatic interference phenotype.

The dominant interference phenotype does not appear to be mediated by an effect on envelope processing, transport, or CD4 binding because coexpression of wild-type and 41.2 mutant envelope glycoproteins has no effect on the amount, size, or ratios of gp160, gp120, or gp41, the amount of gp120 in the medium of coexpressing cells, or the CD4 binding properties of coexpressed envelope glycoproteins. The 41.2 mutant envelope glycoprotein interferes with fusion mediated by the envelope glycoproteins from both the LAV and WMJ-2 strains of HIV-1 but not with fusion mediated by HIV-2 envelope glycoproteins. The effect of the 41.2 mutant on both syncytium formation and infection is apparent even in the presence of a significant excess of wild-type envelope glycoproteins.

Several groups have suggested that membrane fusion mediated by the influenza hemagglutinin protein requires the association of multiple hemagglutinin trimers at the plasma membrane (21–26). In this model, the hemagglutinin proteins form a hydrophobic pore into which flow the lipid bilayers of the plasma membranes. The hydrophobicity of this pore is due largely to the highly hydrophobic amino terminus of the transmembrane protein HA_2. The process of membrane

Table 4. Effect of the 41.2 mutant on infectivity

Transfected DNA*	WT/mutant DNA ratio	Relative Hyg^r transforming units/ml† ± SD
pGB108 + pHenv		
+ pHenvKFS	1:1	100
+ pHenv41.2	1:1	5 ± 2
+ pHenv41.2	2:1	16 ± 4
+ pHenv41.2	5:1	33 ± 10
+ pHenv41.2	10:1	44 ± 4

All data represent the average of four assays. WT, wild type.
*COS-1 cells were transfected with 5 μg of pGB108 plus 5 μg of pHenv plus various amounts of pHenv41.2 DNA. Total amount of DNA was held constant with pHenvKFS DNA as described in text.
†Transfection with pGB108 plus pHenv plus pHenvKFS resulted in a titer of ≈200 hygromycin-resistance (Hyg^r) transforming units per ml.

fusion thus requires cooperation between a number of HA_2 amino termini. A prediction of such a model is that one mutant HA_2 monomer could dominantly interfere with the function of many wild-type subunits. Our results are consistent with such a model for fusion induced by the HIV-1 envelope glycoprotein. Even at a 10:1 ratio of wild-type DNA/41.2 mutant DNA an inhibition of syncytium formation and the production of infectious virus are elicited. Under such conditions the formation of a high percentage of multimeric envelope glycoproteins comprised solely of wild-type subunits would be expected. Because interference is still observed, interference appears to be manifested as a result of a higher-order interaction between envelope glycoprotein molecules or envelope glycoprotein–receptor complexes. Thus, these results suggest that more than one envelope glycoprotein multimer–receptor interaction is required for HIV-1-mediated membrane fusion.

It is interesting that only the 41.2 mutant elicits a dramatic interference phenotype on syncytium formation and the formation of infectious virus. Other fusion-blocking mutations elsewhere in the hydrophobic amino terminus of gp41 or in the V3 loop of gp120 have little or no dominant interfering effect. This result suggests that, at least for HIV-1, only mutations near the amino terminus of the transmembrane glycoprotein fusion domain dominantly interfere with fusion. Mutations further into the gp41 fusion domain or in the V3 region evidently do not alter function of the HIV-1 fusion complex so as to dominantly interfere with fusion.

A recent report describes a mutation, QN-1, in the vesicular stomatitis virus glycoprotein that forms multimers with wild-type glycoprotein and dominantly interferes with virus infectivity (27). As with the 41.2 mutant, the QN-1 mutant glycoprotein cannot induce syncytium formation, and virions containing QN-1 are noninfectious. Although it is not yet known whether the region in which the QN-1 mutation is located is analogous to the amino terminus of gp41, we speculate that the ability of fusion-defective viral envelope glycoproteins to dominantly interfere with syncytium formation and the production of infectious virus may be a general phenomenon among enveloped viruses.

The results of this paper contribute to our understanding of HIV-1 envelope glycoprotein function and the process of membrane fusion. These results suggest the possibility that fusion mediated by the HIV-1 envelope glycoproteins requires the formation of a higher-order fusion complex that can be functionally disrupted by monomers containing a mutation in the gp41 fusion domain. The general model proposed for the dominant interfering phenotype of the 41.2 mutant suggests that similar mutations may be defined in other retroviral systems. Because of its ability to dominantly interfere with both cell fusion and the production of infectious virus, the mutation described here may ultimately prove useful in the development of antiviral therapies.

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