JOURNAL OF VIROLOGY, Mar. 1994, p. 1714–1720
0022-538X/94/$04.00+0

Vol. 68, No. 3

# Cysteine Residues in the Vif Protein of Human Immunodeficiency Virus Type 1 Are Essential for Viral Infectivity

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Received 12 July 1993/Accepted 29 November 1993

**The infectivity factor of human immunodeficiency virus type 1 (HIV-1), Vif, contains two cysteine residues which are highly conserved among animal lentiviruses. We introduced substitutions of leucine for cysteine residues in the *vif* gene of a full-length HIV-1 clone to analyze their roles in viral infection. Mutant viruses containing substitutions in either Cys-114, Cys-133, or both displayed a *vif*-negative infection phenotype similar to that of an isogeneic *vif* deletion mutant, namely, a cell-dependent complete to partial loss of infectivity. The *vif* defect could be complemented by cotransfection of mutant viral DNA with a Vif expression vector, and there was no evidence that recombination contributed to the repair of the *vif* deficiency. The viral protein profile, as determined by immunoblotting, in cells infected with cysteine substitution mutants and that in wild-type virus were similar, including the presence of the 23-kDa Vif polypeptide. In addition, immunoblotting with an antiserum directed against the carboxyl terminus of gp41 revealed that gp41 was intact in cells infected with either wild-type or *vif* mutant HIV-1, excluding that Vif cleaves the C terminus of gp41. Our results indicate that the cysteines in HIV-1 Vif are critical for Vif function in viral infectivity.**

The human immunodeficiency virus type 1 (HIV-1) *vif* gene encodes a 23-kDa nonstructural protein (9, 16) which regulates viral infectivity (9, 19, 30). The requirement for Vif varies from stringent to minimal, depending upon the particular experimental system used (11, 19, 25, 28–30, 34). It has been suggested that cells which do not require Vif for infection contain an endogenous Vif-complementing activity (9, 11, 34). However, recent reports have shown that Vif is essential for HIV-1 replication in primary T lymphocytes (1, 8, 11), indicating that Vif plays an important role in natural HIV-1 infections. Studies using *vif* mutants clarified to some extent the role of this gene product in the HIV-1 life cycle: like viral protease, Vif is synthesized and acts late during virus production, and it functions to increase the infectivity of progeny virions (4, 9, 19, 25, 27, 30), by some estimates up to 1,000-fold (30). The consequences of Vif activity on the virions are critical at the early stages of infection, prior to the establishment of the integrated proviral state (19, 29, 34). Studies with transformed T-cell lines have shown, for example, that *vif* mutants can bind and enter target cells (19, 34) but have a significantly diminished ability to synthesize viral DNA (29, 34). Vif activity is also required for viral spread by cell-to-cell contact (9, 11, 19). In contrast, Vif is dispensable for the expression of transfected or integrated viral DNA (9, 19).

The mechanism by which Vif acts to increase progeny virion infectivity remains obscure. One recent study suggested that Vif modulates the gp120 content in the envelopes of newly synthesized virions and thereby influences infectivity (25). Another study proposed that the Vif protein functions as a thiol protease and implicated a region adjoining cysteine 114 in Vif as an active site of the purported enzyme (12). Although the conclusions of this study have been challenged (11, 34), the cysteine residues may, nonetheless, be important for Vif protein activity. The HIV-1 Vif protein contains two cysteines in positions 114 and 133, which are conserved among HIV-1, HIV-2, and some simian immunodeficiency virus isolates (6, 13, 21, 22). Cysteine residues are critical for the function of HIV-1 Env and Nef proteins (5, 7, 35). The aim of the present study was to determine the functional significance of Cys-114 and Cys-133 residues in Vif during infection by intact virus. We used site-directed mutagenesis to replace either one or both cysteines with leucine in Vif of the infectious molecular clone HIV-1/KS242 (24). The recombinant viruses were tested for infectivity in different CD4-positive T-cell lines, and viral proteins were analyzed by gel electrophoresis. Our data suggest that Cys-114 and Cys-133 are critical for the function of Vif in HIV-1 infection in vitro.

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## MATERIALS AND METHODS

**Cell lines, HIV-1 clones, and vectors.** The cells used included two clones of T-cell leukemia-derived CEM cells (10), CEM-SS (AIDS Reagents Repository, Rockville, Md.) and CEM-CDC (P. Lai, Tampa Bay Research Institute, Fla.), as well as the human T-cell leukemia virus type I-infected T-lymphoblastoid MT-2 cell line (20). The CEM clones and MT-2 cells are susceptible to efficient infection by wild type HIV-1 (14, 19) but differ with respect to their abilities to replicate *vif*-negative HIV-1 (19, 29; also this study). The cells were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum and antibiotics. The infectious wild-type molecular clone of HIV-1 used for the construction of *vif* mutants in this study was pKS242, which contains the genome of the previously described HIV-1/N1T-A virus (23) with shorter cellular flanking sequences (24). The previously described *vif* deletion mutant used as a reference *vif*-negative HIV-1 was KS282 (24). The viral genomes in N1T-A, KS242, and KS282 are identical except for the presence of a 35-bp deletion in KS282 *vif* (24). pKS242 and pKS282 are referred to as the WT and ΔVif viruses, respectively. The eucaryotic Vif expression vector used in Vif complementation experiments, pNLA3 (30), was obtained from M. Martin.

**Construction of HIV-1 clones expressing mutated Vif.** A

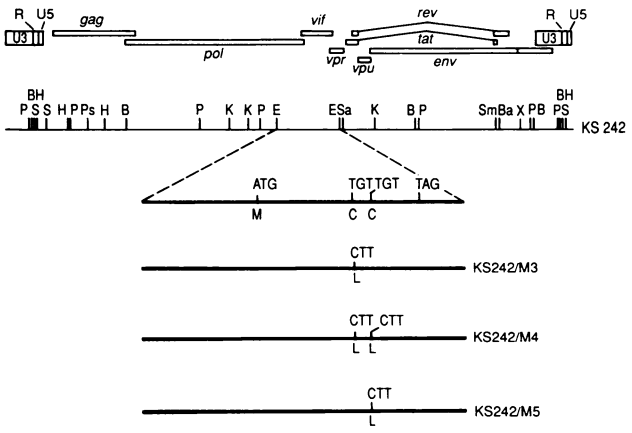


FIG. 1. Structures of the pKS242 HIV-1 proviral DNA clone and Vif cysteine mutants. Restriction sites: P, *Pvu*II; B, *Bgl*II; H, *Hin*dIII; S, *Sac*I; Ps, *Pst*I; K, *Kpn*I; E, *Eco*RI; Sa, *Sal*I; Sm, *Sma*I; X, *Xho*I.

1.1-kb *Eco*RI fragment containing the HIV-1 *vif* gene was excised from pKS242 and cloned into the M13mp18 phage vector. Oligonucleotide-directed mutagenesis of the M13 clone (17) was then used to substitute either one or both of Vif cysteine residues with leucine. Mutagenic oligonucleotides were VF114 (5′-TGTATTACTTTGACCTTTTTTCAG-3′), for replacing Cys-114 with Leu-114, and VF133 (5′-GCCCTA GGCTTGAATATCAAG-3′), for replacing Cys-133 with Leu-133 (altered nucleotides are underlined). The mutated *Eco*RI-*Eco*RI 1.1-kb fragments were reinserted into the proviral clone pKS242. The presence of the desired point mutations in recombinant M13 vectors and in proviral DNA was validated by sequencing the relevant *vif* regions. The sequencing primer was VF161 (5′-CACTAGGCAAAGGTGGC-3′). DNA was sequenced by the chain termination method (26) in an Applied Biosystems automatic sequenator at Columbia Comprehensive Cancer Center. Schematic maps of the KS242 *vif* mutant clones are shown in Fig. 1.

**Production and titration of HIV-1 mutants and viral infectivity studies.** Mutant clones were tested initially in transient expression assays in CEM-SS cells. Cells ($5 \times 10^6$) were transfected with 10 μg of each plasmid DNA by the DEAE-dextran method (3) and cultured under standard conditions; after 24 to 48 h, culture supernatants were tested for HIV-1 p24 core antigen by using the HIV Ag kit (Coulter, Hialeah, Fla.), and the presence of infectious virus was evaluated by cocultivation with CEM-SS cells. For large-scale production of confirmed functional recombinants, mutated and control viral DNAs were electroporated into CEM-SS cells (which in our experience are permissive to replication of *vif*-negative HIV-1 [29]) at 10 μg of DNA per $5 \times 10^6$ cells, by using the Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.) at 230 V, 960-μF capacitance, and a 50- to 70-ms time constant. Ten to 14 days posttransfection, when more than 70% of the transfectants expressed HIV-1-specific antigens, progeny virus present in culture supernatants was collected, filtered through 0.45-μm-pore-size filters, concentrated 50- to 100-fold, and tested for the content of HIV-1 p24 core antigen and for infectivity (by infection of CEM-SS cells with serially diluted virus) as previously described (19, 33). For the majority of viral preparations, a multiplicity of infection of 1 in CEM-SS cells was equivalent to 1 pg of p24 antigen per cell (33). For infectivity studies in Fig. 2, target cells were incubated with the indicated cell-free virus preparation at a multiplicity of infection of 2 for 1 h; the excess virus was washed off, and the infected cells were cultured and tested at the indicated time points for viability, by trypan blue exclusion, and for virus replication, by HIV-1-specific immunofluorescence (IF) on acetone-fixed cells and by measuring the levels of HIV-1 p24 antigens in cell-free culture supernatants.

**Complementation of the mutant viral phenotype by a *vif* expression vector and testing for viral recombinants in cotransfected cultures.** CEM-CDC cells ($5 \times 10^6$) were transfected by electroporation with 5 μg of wild-type or mutant viral DNA with or without 5 μg of pNLA3 vector DNA as described above. The transfectants were washed, cultured under standard conditions, and tested for expression of HIV-1-specific antigens by IF and enzyme-linked immunosorbent assay as described above. To determine whether viral recombination occurred in cotransfected cultures, culture supernatants were collected 6 to 9 days after DNA transfection, filtered through 0.45-μm-pore-size Millipore filters, and treated by DNase to remove any contaminating DNA. Virions in the supernatant were then pelleted by centrifugation at 100,000 × *g*, and viral RNA was extracted by the guanidinium-acid phenol extraction method with RNAzol (CINNA/BIOTECX, Friendswood, Tex.) and used as a substrate for reverse transcription using the Superscript preamplification system (GIBCO BRL, Gaithersburg, Md.). The viral cDNA thus obtained was used as a substrate in a PCR (15) using primers VF52 (5′-GAGAAGCT TTAATACAAGATAATAGTGACAT-3′) and VF32 (5′-CT CGGATCCCATAAGTTTCATAGATATGTTG-3′) to amplify a 750-bp fragment of the HIV-1 *vif* gene or primers VF5071 and VF5411 to amplify a 341-bp fragment. The amplified DNA was electrophoresed through 1.5% agarose gel and analyzed by Southern blot hybridization using $^{32}$P-labeled oligonucleotide VF5282 as a probe. The PCR conditions and oligonucleotides VF5071, VF5411, and VF5282 were described previously (29). To verify the *vif* nucleotide sequence in progeny virions produced by transfected cells, the PCR-amplified *vif* region cDNA obtained from supernatants of cotransfected cells was sequenced in bulk as described above. To determine the infection phenotype of progeny viruses produced by cotransfected cells, culture supernatants obtained 6 days after transfection were used to infect MT-2 cells; HIV-1 infection was evaluated as described above.

**Immunoblot analysis of viral proteins.** For intracellular protein analysis, lysates from HIV-1 DNA-transfected CEM-SS cells were prepared at the time of maximum positivity for HIV-1-specific IF. Cells were washed twice with phosphate-buffered saline, resuspended at $2.5 \times 10^6$/ml in 0.01 M Tris-HCl (pH 8.0)–0.14 M NaCl–0.025% $NaN_3$–1% Triton X-100–0.1% sodium dodecyl sulfate (SDS)–1% Na-deoxycholate–1 mM iodoacetamide–0.1% aprotinin–1 mM phenylmethylsulfonyl fluoride, incubated for 60 min on ice, and centrifuged to remove cell debris. Prior to electrophoresis, cell extracts were diluted 1:1 with a gel loading buffer (24), supplemented with β-mercaptoethanol, and heated to 100°C to denature proteins. Comparable amounts of all lysates were loaded on an SDS-polyacrylamide gel, electrophoresed, and blotted onto a Transblot nitrocellulose filter. For analysis of gp41 in viral particles, virus present in culture supernatants was concentrated by centrifugation, filtered through a 0.45-μm-pore-size Millipore filter, and processed for electrophoresis as described above. Gel concentrations were 10% for total viral protein or gp41 analysis and 15% for Vif analysis. The filters were incubated with pooled AIDS patients' sera for total viral protein detection, with rabbit antiserum R-774 (2) raised against a synthetic peptide representing the C-terminal 15 amino acids of gp41 from BH10 (22) for specific detection of

gp41, and with rabbit antiserum against the synthetic peptide TEDRWNKPQKTKGHR (amino acid positions 170 to 184 in Vif) for Vif detection. The R-774 and Vif antibodies were kindly provided by R. Kennedy and A. Adachi, respectively. The filters were washed, incubated with $^{125}$I-protein A, and autoradiographed.

**Other analytical procedures and reagents.** Unless specified otherwise, plasmid propagation and DNA isolation, DNA recombination, and other molecular biological procedures were performed according to standard methods (3). Fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G antibody F(ab')$_2$ used in the IF assay was purchased from Tago, Inc. (Burlingame, Calif.). Enzymes for recombinant DNA experiments were purchased from New England BioLabs, Inc. (Beverly, Mass.), Rainbow protein molecular weight markers were purchased from Amersham Co. (Arlington Heights, Ill.), and $^{125}$I-protein A was obtained from New England Nuclear Co. (Boston, Mass.). DNase (bovine type IV) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

## RESULTS AND DISCUSSION

**Construction and identification of *vif* mutant viruses.** The HIV-1 clone KS242 contains two cysteines in Vif in positions 114 and 133 (24). As depicted schematically in Fig. 1, we substituted either one or both of the cysteines by leucine, generating three mutant viral clones designated M3 (Cys-114→Leu-114), M4 (both cysteines changed to leucines) and M5 (Cys-133→Leu-133). To determine whether recombinant viruses were viable, replication of mutant clones was tested initially in transient expression assays. All the clones tested, including parental WT virus (55 ng/ml) and the previously described *vif* deletion mutant KS282 (24) (49 ng/ml), yielded similar levels of supernatant p24 antigens 24 h after transfection (38, 59, and 47 ng/ml for clones M3 to -5, respectively). These results indicate that the replacement of either one or both cysteines in Vif does not impair virus activity in this assay and are consistent with previous reports which showed that Vif is not required for viral protein production from proviral DNA (9, 19, 28).

**Cys-114 and Cys-133 are required for Vif function during HIV-1 infection.** To determine whether the cysteine residues in Vif have functional significance in infection with intact virus, cell-free titered preparations of the cysteine mutants, the ΔVif virus, and WT HIV-1 were used to infect CEM-SS, CEM-CDC, and MT-2 T-lymphoid cells at a multiplicity of infection of 2. Cell viability, expression of cell-associated HIV-1 antigens, and levels of HIV-1 p24 core antigens in culture supernatants were determined (Fig. 2). The three T-cell lines were chosen because they allow the determination of a spectrum of host cell responses to *vif*-negative infection ranging from fully permissive to nonpermissive (11, 25, 29). As shown in Fig. 2, with the well-characterized ΔVif virus as a reference (24), CEM-SS cells were highly permissive to *vif*-negative infection, CEM-CDC cells were partially permissive, and MT-2 cells were nonpermissive, allowing only minimal replication of KS282. All three cell lines permitted rapid and efficient replication of the parental WT viral clone, KS242.

The results displayed in Fig. 2 show that the profiles of infection of the three viral mutants containing cysteine substitutions in Vif are indistinguishable from that of the ΔVif mutant: all four mutants replicated well in CEM-SS cells, showed a slow, noncytopathic phenotype of infection in CEM-CDC cells, and replicated poorly in MT-2 cells, which require HIV-1 Vif for infection (29). No differences were detected among the individual Cys mutant viruses in their respective patterns of infection in any of the cell lines tested. All the mutants were able to establish stable infections in CEM cells (Fig. 2), confirming that the replacement of Vif cysteines was not lethal to the replicative machinery of the mutant viruses. Similar results were obtained in three independent experiments using different preparations of mutant and control viruses. These data suggest that both cysteine residues in Vif are important for HIV-1 infection; replacement of either one of the cysteines imposed a typical *vif*-negative phenotype of infection on the mutants, most clearly seen in the block to viral replication in nonpermissive MT-2 cells. To our knowledge, this is the first report of a substitution point mutation abrogating Vif protein function, emphasizing that the cysteines must be intimately involved in the activity of the protein.

**Complementation of Vif cysteine mutants by coexpression of Vif protein.** To confirm that the observed attenuation of HIV-1 infectivity in CEM-CDC and MT-2 cells is caused specifically by Vif-inactivating mutations, we tested whether infection with Vif cysteine mutants can be modified in *trans* by coexpression of intact Vif in the same cells (Fig. 3). CEM-CDC cells were cotransfected with the eucaryotic *vif* expression vector pNLA3 DNA (30) and with either M3, M4, or WT viral DNA, and transfectants were monitored for HIV-1 expression by testing the levels of p24 core antigen in culture supernatants. This experimental system was optimized to distinguish the phenotypes of *vif*-positive and -negative viruses as the progeny of the original transfectants spread infection throughout the culture. In agreement with the infection results shown in Fig. 2, the Vif cysteine mutants M3 and M4 replicated slowly and to low levels after transfection into CEM-CDC cells, while the wild type replicated rapidly and to high levels, reaching a peak of infection of about 900 ng of p24 per ml of supernatant within 10 days. Cotransfection of M3 or M4 with pNLA3 resulted in increased production of p24 approaching the levels measured after the WT viral DNA transfection (about 500 ng of p24 per ml of supernatant on day 10 after cotransfection). These results suggested that the defects caused by mutations in M3 and M4 viral clones were complemented by independent expression of intact Vif in *trans* and that the mutant virus produced in the presence of functional Vif could spread infection.

The cotransfection results shown in Fig. 3 could also be explained by homologous recombination between cotransfected DNAs regenerating WT *vif*. To determine whether this was the case, CEM-CDC cells were cotransfected as described above and progeny virions present in culture supernatants 6 days after cotransfection, when functional complementation of the *vif* defect was clearly detectable (Fig. 3), were analyzed for evidence of recombination. Virion RNA was extracted and subjected to reverse transcription to generate cDNA, which was subjected to PCR amplification using *vif*-specific primers and was sequenced. The results confirmed that the virions carried RNA containing the *vif* mutations; no recombinants between the plasmids within the *vif* gene were evident from the virion RNA (Fig. 4). Specifically, progeny virions produced by transfectants of ΔVif and pNLA3 DNA exclusively carried the ΔVif viral genotype as evidenced by the presence of the shorter *vif* species and by the absence of WT *vif* DNA species of 750 or 341 bp in PCR-amplified cDNA (Fig. 4A); in addition a 35-bp deletion in *vif* cDNA was found in the same location as in the ΔVif virus (Fig. 4B). The progeny viruses produced by M4-pNLA3 cotransfectants uniformly carried the cysteine mutations present in M4 (Fig. 4B). Progeny viruses harvested on day 6 after transfection were tested for infectivity in MT-2 cells and were found to maintain the infection phenotype of transfected

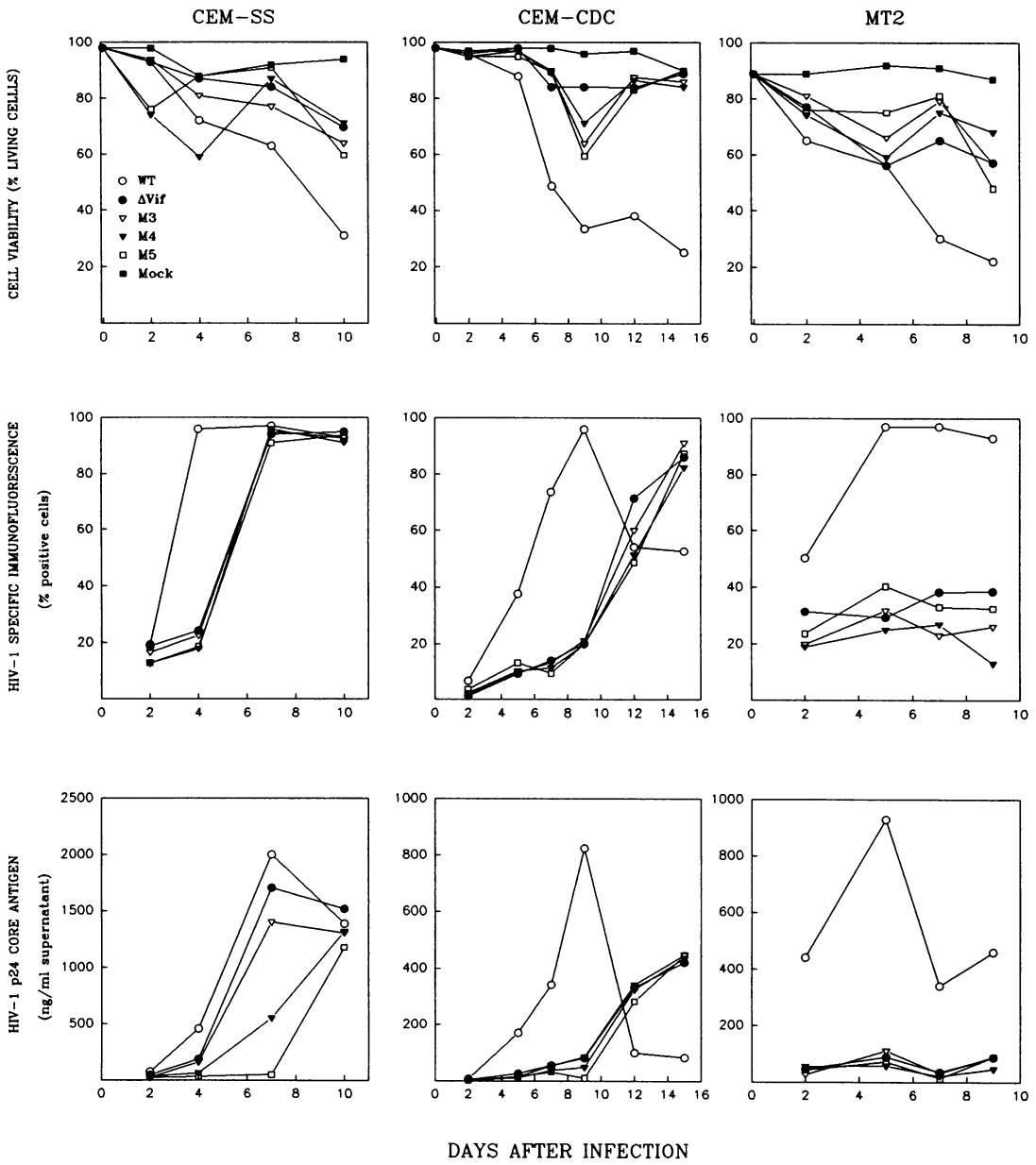


FIG. 2. Biological activities of *vif* mutant HIV-1 clones in permissive and nonpermissive target cells.

ΔVif and M4 DNAs (not shown). Taken together, these results strongly suggest that the biological activity of *vif* mutants cotransfected with pNLA3 is due to functional complementation by Vif protein expressed in *trans* rather than the appearance of recombinant viruses which regenerate an intact *vif* gene. This complementation is likely to be responsible also for the apparent spreading of HIV-1 infection in the cotransfection experiments described here (Fig. 3) and in previous reports (24, 30, 34), because *vif* mutants produced in the presence of Vif protein behave like wild-type viruses in the first cycle of infection (11, 34). These results demonstrate directly that the changes in viral infection phenotype caused by Vif cysteine mutations described here are due to Vif protein inactivation.

**Effect of cysteine substitutions in Vif on viral protein expression and cleavage of gp41.** To determine whether the substitution of the Vif cysteines and the consequential Vif inactivation (Fig. 2) have any effect on total viral protein expression, we compared viral proteins present in CEM-SS cells productively infected with WT and mutant viruses by immunoblotting using different sera (Fig. 5). In agreement with previous reports (9, 24, 28, 30), all the systems tested displayed similar patterns and amounts of major HIV-1 proteins detected by AIDS patient serum, regardless of whether the virus in the infected cells expressed wild-type Vif, mutated Vif, or no Vif (Fig. 5A). Immunoblot analysis using rabbit antiserum to Vif showed a specific band corresponding to a 23-kDa Vif protein in samples of either the WT clone or mutant clones M3 to -5 (Fig. 5B). No equivalent band was seen in uninfected CEM-SS cells or in cells transfected with ΔVif

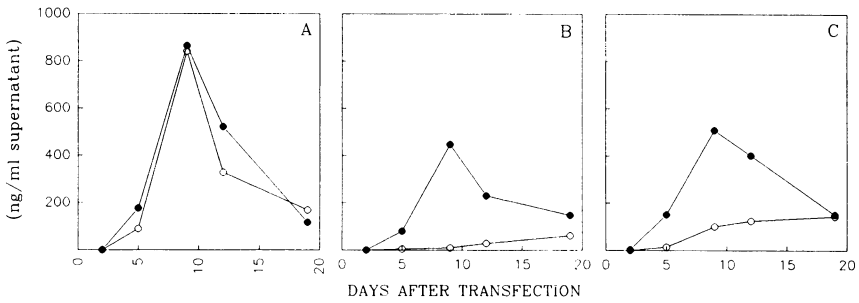


FIG. 3. Complementation of Vif cysteine mutant viruses with a Vif expression vector. Wild-type pN1T-A plasmid (A) and Vif cysteine mutant plasmids M3 (B) and M4 (C) were either transfected alone (open circles) or cotransfected (closed circles) with the Vif expression vector pNLA3; the transfectants were evaluated for HIV-1 expression as described in Materials and Methods.

virus which encodes a truncated Vif protein (not shown). The presence of the full-length 23-kDa Vif protein demonstrates that the cysteine-to-leucine substitutions in Vif did not prevent the synthesis of Vif protein and that mutated Vif proteins were as stable as the wild-type protein. This finding confirms that the cysteine→leucine replacement in Vif alters the function of Vif protein, rather than reduces the amount of the protein below that required for function.

Guy et al. (12) suggested that Vif has a proteolytic activity directed against the cytoplasmic domain of gp41 and that this cleavage of gp41 is required for HIV-1 infection. Substitution of leucine for Cys-114 in Vif abrogated the proteolytic activity shown in their study (12). We thus tested whether virions produced by the Vif Cys-114 mutant M3, which show greatly attenuated infectivity (Fig. 2), or by WT virus contain full-length gp41, including the carboxyl terminus (Fig. 6). Virions were purified from CEM-SS cells productively infected with M3, ΔVif, and WT viruses and were subjected to immunoblotting using rabbit serum R-774 (2) raised against a synthetic peptide consisting of the C-terminal 15 amino acids of BH10 gp41 (22). There were no qualitative or quantitative differences in the detection or the apparent molecular mass of the gp41 with this antiserum (Fig. 6), indicating that the amounts of gp41 with uncleaved cytoplasmic tails in all the viruses tested were similar, regardless of whether they were produced in the presence of intact or Cys-114-mutated Vif or produced in the absence of Vif. Since the WT virus and Vif cysteine mutants in our experiments carried equivalent amounts of full-length gp41

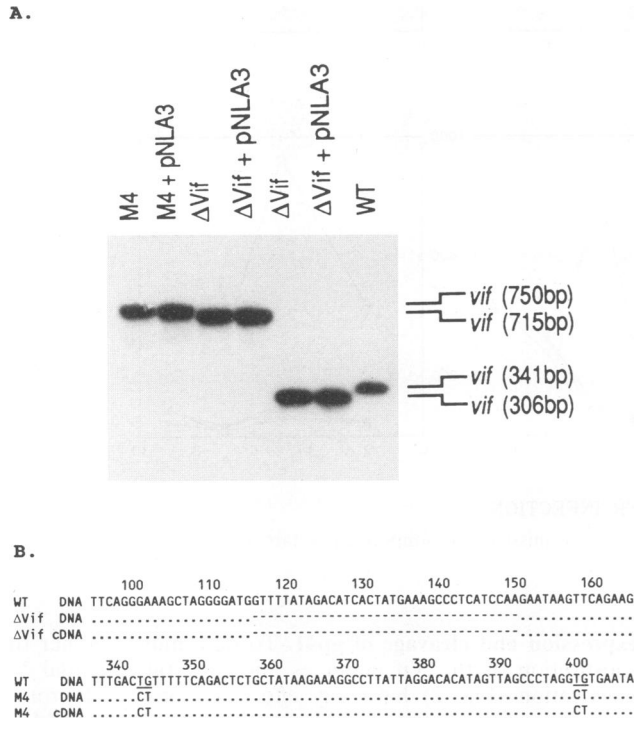


FIG. 4. Genotypic analysis of progeny virions produced by cells cotransfected with *vif* mutant proviral DNA-mutated and Vif expression vectors. CEM cells were cotransfected with either the ΔVif provirus and pNLA3 plasmid DNA or the M4 provirus and pNLA3 plasmid DNA, and progeny virions in culture supernatants were analyzed for *vif* sequences as described in the text. (A) Analysis of PCR-amplified *vif* region cDNA by Southern blot hybridization; (B) comparison of the *vif* region nucleotide sequence of progeny virus genomes and mutant proviral DNAs used for transfection. Dots, identical bases; dashes, gaps in the sequence. The nucleotide pairs in KS242 (WT) *vif* altered to generate the M4 mutant are underlined. The numbering is from the first base of the *vif* gene.

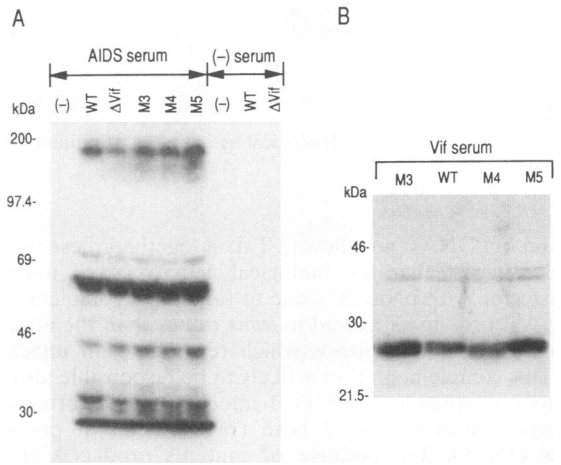


FIG. 5. Immunoblot analysis of HIV-1-specific proteins produced by wild-type- and mutant-HIV-1-infected CEM-SS cells. Cell lysates were prepared from virus-producing cells at the time of the maximum positivity for HIV-1-specific IF and analyzed by immunoblotting using either AIDS patient serum and control negative (−) serum (A) or Vif-specific serum (B).

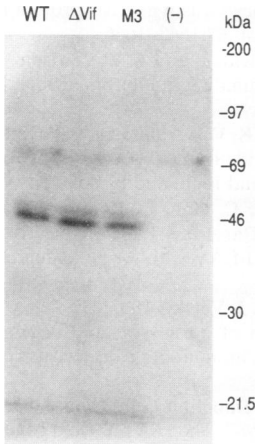


FIG. 6. Immunoblot analysis of gp41 present in viral particles produced in CEM cells by wild-type and *vif* mutant HIV-1. Virus was collected, and virion gp41 was detected with the rabbit monospecific antiserum R-774, directed against the C terminus of gp41 (2), as described in Materials and Methods.

on virions, notwithstanding their major differences in infectivity, we conclude that the postulated Vif-mediated cleavage of the C terminus (the last 15 amino acids) of gp41 plays a limited role in HIV-1 infection. These results differ from those presented by Guy et al. (12), who showed that rabbit antiserum raised against a similar region of gp41 failed to immunoprecipitate gp41 synthesized in the presence of intact Vif but did precipitate gp41 synthesized in the presence of Vif containing a leucine-for-Cys-114 substitution. This discrepancy cannot at present be reconciled; however, there are significant differences in the experimental systems used: full-length infectious HIV-1 clones and CD4-positive T cells described here versus vaccinia virus-based Vif and Env expression vectors tested in CD4-negative BHK-1 cells in the study of Guy et al.

**Conclusions.** The results presented here demonstrate that the two cysteine residues in the Vif protein are critical for HIV-1 infectivity, because substitution of either one or both cysteines by leucine confers an infection phenotype indistinguishable from that of the *vif* deletion mutant KS282, most apparent in the impaired replication pattern in MT-2 or CEM cells. That this phenotype derives solely from the *vif* mutations is confirmed by the phenotypic complementation observed when any of the cysteine mutants or the ΔVif mutant was cotransfected with a Vif expression vector. Although previous *vif* mutagenesis experiments indicated that the C-terminal 90 amino acids of Vif are required for infectivity (9), the study reported here is the first time to our knowledge that specific amino acids in this region can be credited with a role in virus infectivity.

The mechanism by which Vif affects HIV-1 infectivity is unknown. Although we find that both cysteines are required for Vif function, and so virus infectivity, like other studies (11, 25, 34) our data provide little support for the hypothesis that Vif acts as a cysteine protease that cleaves the C terminus of gp41 (12). The requirement for both cysteines could be due to their role in the formation of inter- or intramolecular disulfide bonds or their direct role in the functional domain of the protein. The results presented here do not distinguish between these possibilities. Disulfide bonds are critical for the function of HIV-1 Env and Nef proteins (7, 31, 35), and the existence of such bonds in Vif is under intensive investigation in our laboratory.

## ACKNOWLEDGMENTS

We thank R. Kennedy and A. Adachi for the gifts of antisera to gp41 and Vif, respectively, P. Lai for CEM-CDC cells, M. Martin and K. Strebel for the pNLA3 vector, G. Bentsman for skillful technical help, M. J. Potash for fruitful discussions and interest, and L. Peters for typing the manuscript.

This work was supported by PHS grant CA 37465 and by the Aaron Diamond Foundation, New York, N.Y.

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