

## Comparative Analyses of Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2 Vif Mutants

THIPPARTHI RAGHAVENDAR REDDY,<sup>1</sup> GÜNTER KRAUS,<sup>1</sup> OSAMU YAMADA,<sup>1</sup>  
DAVID J. LOONEY,<sup>2</sup> MODEM SUHASINI,<sup>1</sup> AND FLOSSIE WONG-STAAAL<sup>1,3\*</sup>

Departments of Medicine<sup>1</sup> and Biology,<sup>3</sup> University of California at San Diego, La Jolla, California 92093-0665,  
and San Diego Veterans Administration Medical Center, San Diego, California 92161<sup>2</sup>

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**Virion infectivity factor (*vif*), a gene found in all lentiviruses, plays an essential role in virus replication in certain target cells. We examined the replication competence of the human immunodeficiency virus type 2 (HIV-2) *vif* mutant in different T-cell lines and primary cells in comparison with that of the HIV-1 *vif* mutant. Both mutant viruses were unable to replicate in peripheral blood-derived mononuclear cells but replicated with wild-type efficiency in certain T-cell lines, such as SupT1 and MOLT-4/8. These results confirm the importance of *vif* in the infection of relevant target cells and imply that some cellular factor(s) could compensate for *vif* function. However, HIV-1 and HIV-2 *vif* mutant viruses also show differential replications in other cell lines, suggesting either different threshold requirements for the same cellular factor(s) or the involvement of different factors to compensate for *vif-1* and *vif-2* functions. By cross complementation experiments, we showed that *vif-1* and *vif-2* have similar functions. Our studies further indicate the existence of two kinds of nonpermissive cells: H9 is unable to complement HIV-1 $_{\Delta vif}$  but is susceptible to a one-round infection with HIV-1 $_{\Delta vif}$  produced from permissive cells. In contrast, U937 is nonpermissive for HIV-2 $_{\Delta vif}$  produced from permissive cells but, once infected, is able to complement the  $\Delta vif$  function. In both types of nonpermissive cells, a step prior to proviral DNA synthesis is affected.**

Lentiviruses, exemplified by human immunodeficiency virus (HIV) and other primate immunodeficiency viruses, have complex genomes that encode a number of regulatory and accessory gene products (4). Extensive efforts have been directed at the characterization of *tat* and *rev*, two regulatory genes that provide critical positive controls for virus replication (5, 28), while the other genes (*vif*, *vpr*, *nef*, *vpu*, and *vpx*) have until recently been deemed nonessential since their deletion is not lethal for virus replication in defined cell culture systems. However, the conservation of these genes among clinical isolates (16) and their expression in patients (8) argue for their important role in vivo. In particular, the *vif* and *nef* genes of simian immunodeficiency virus (SIV) and HIV type 2 (HIV-2) have been shown to be essential for establishing in vivo infection and/or pathogenicity in monkeys (15, 18).

The *vif* open reading frame, encoding a protein of approximately 23 kDa, is conserved among all primate lentiviruses, including HIV-1, HIV-2 (14, 18, 29), and SIV (3), as well as nonprimate lentiviruses, such as visna virus (22) and bovine (11) and feline immunodeficiency viruses (26). Abolition of *vif* gene expression in HIV-1 resulted in the production of a mutant virus reported to be 1,000 times less infectious than the wild-type (WT) virus (7, 24), a defect that can be complemented in *trans* by the *vif* gene (2, 7). The mechanism of *vif* function is not well understood. *Vif* is expressed as a cytoplasmic protein in infected cells from a singly spliced mRNA (20). As such, it is classified as a late gene product since its expression is Rev dependent (10). Furthermore, *Vif* is not known to be incorporated into mature virions (1, 7, 14, 16). Thus, it is paradoxical that the effect of *Vif* seems to be at an early step after virus infection, prior to provirus integration (21, 23). One model is that *Vif* may modify a viral structural protein which is

important for early events in virus replication. However, the proposed activity of *Vif* as a protease which cleaves the cytoplasmic tail of the transmembrane protein (12) or its effect on incorporation of gp120 into virions (19) is controversial (21). An alternative possibility is that *Vif* is virion associated but present at a level difficult to detect. One strong consensus among different investigators is that the requirement of *Vif* is cell specific (7–9, 18, 19, 21).

The focus of the present study was to characterize and compare the roles of *vif* in the replication of HIV-2 (strain KR [25]) and HIV-1 in different cell lines. We report here that cell types supporting the replication of *vif* mutant viruses of HIV-1 and HIV-2 (permissive cells) only partially overlap. However, HIV-2 *Vif* is able to complement an HIV-1 $_{\Delta vif}$  function. Furthermore, the infectivity of *vif* mutant viruses of HIV-1 and HIV-2 can be either producer cell or target cell dependent. However, in both cases, *Vif* appears to act before the integration of proviral DNA into the host genome.

### MATERIALS AND METHODS

**Construction of *Vif* mutants.** The *vif* mutant of HIV-2 $_{KR}$  was constructed by deleting the nucleotides (4956 to 5279) between the two *Bgl*II sites to generate the pHIV-2 $_{KR}$   $\Delta B$  plasmid. Briefly, the pSVL plasmid containing the 5' portion of the HIV-2 genome was digested with the *Bgl*II enzyme, the plasmid ends were ligated, and the DNA was used to transform competent cells. This deletion removes the middle piece of the *vif* gene without interrupting other genes. The *vif* mutant of HIV-1 ( $\Delta S$ ) was constructed as described previously (7). All mutations were confirmed by sequencing prior to the production of virus from these constructs.

**Cells and medium.** U937 (a monocytic cell line) cells were a gift of Robert C. Gallo, and CEM cells were obtained from D. Trono. MOLT-4 clone 8 (MOLT-4/8), SupT1, and H9 cells were obtained from the American Type Culture Collection and maintained in RPMI medium supplemented with 10% fetal calf serum. Fresh peripheral blood lymphocytes were isolated from donors by venipuncture, and this was followed by Ficoll-Hypaque density gradient centrifugation of the cells. Peripheral blood lymphocytes were activated with 4  $\mu$ g of phytohemagglutinin per ml and maintained in RPMI medium containing 10% fetal calf serum and 20 U of interleukin-2 per ml.

**Construction of *Vif* (HIV-2) expression vector.** *Vif-2* expression vector ( $\Delta LCL$ )

\* Corresponding author. Phone: (619) 534-7957. Fax: (619) 534-7743.

was constructed by deleting the *gag*, *pol*, and *env* sequences from a full-length proviral HIV-2 DNA and inserting the *neo* resistance gene, with the resultant construct expressing all the accessory genes, including *vif*, under the control of the HIV-2 long terminal repeat promoter. This construct was transfected into H9 cells by the CaPO<sub>4</sub> method, and G418-resistant H9 cells were selected.

**Preparation of viral stocks.** Two plasmids (2.5 µg each) containing the 5' and 3' halves of the genome of HIV-2<sub>KR</sub> were linearized with the *Sac*I enzyme. The digested DNA was ligated with T4 DNA ligase and transfected into 2 × 10<sup>6</sup> MOLT-4/8 cells in 2 ml by the DOTAP method. After a 1-h transfection, the final volume was brought to 10 ml with RPMI medium. At 8 days posttransfection, when syncytia were detected, the supernatant was harvested and filtered through a 0.45-µm-pore-size filter, and aliquots were made and stored at -70°C until use. The titers of the HIV-2<sub>KR</sub> virus were determined with MOLT-4/8 and peripheral blood lymphocytes as described previously (30). HIV-1 *vif* mutant virus was produced in MOLT-3 by transfection of 5 µg of proviral DNA by DOTAP, and the virus from MOLT-3 cells was concentrated 200-fold by ultracentrifugation.

**Infection of cells.** Cell-free HIV-2<sub>KR</sub> was recovered from supernatant of MOLT-4/8 cells (permissive) and U937 cells (nonpermissive) transfected with WT and *vif* mutant proviral DNAs and used to infect different cell types. The cell-free supernatants collected at different time intervals were assayed for p26 antigen production with the Coulter SIV antigen assay kit. Similarly, HIV-1 virus supernatant derived from MOLT-3 cells (permissive) and H9 cells (nonpermissive) after transfection with WT or *vif* mutant proviral DNA was used as a source of infection. The cell-free supernatants collected at different time intervals were assayed for p24 antigen production with the Coulter HIV-1 antigen capture assay kit.

**PCR.** For the detection of single-round replication, the cell-free virus supernatants were treated with 30 µg of DNase I per ml in the presence of 10 mM MgCl<sub>2</sub> for 30 min at 37°C. For HIV-1 proviral DNA detection experiments, 10<sup>5</sup> H9 cells were infected and lysed in a buffer (40 mM KCl, 50 mM Tris-HCl [pH 8.3], 3 mM MgCl<sub>2</sub>, 0.45% Nonidet P-40) containing 200 µg of proteinase K per ml. The total cell lysate was incubated at 55°C for 1 h, and the proteinase K was inactivated by boiling the lysate for 10 min. The cell lysate was subjected to PCR for *gag* with forward 5' GTACCCCTTCAGGAACAAA 3' and reverse 5' ATA GAACCGGTCTACATA 3' primers. U937 cells were infected with approximately 100 50% tissue culture infective doses (TCID<sub>50</sub>) of HIV-2 WT and *vif* mutant viruses. Aliquots corresponding to 10<sup>6</sup> cells were taken at different time points, and the DNA was isolated according to Invitrogen protocols. The DNA from U937 was subjected to PCR with the *vif* forward 5' CGGCGGATCCGC TATGGAGGAAGGCGAG 3' and reverse 5' CCCGGCACGCGTTCATGCC AGTATCTCCAGC 3' primers. Unless otherwise stated, amplification reactions were performed throughout by first denaturing at 94°C for 7 min, and then each cycle was performed with a 1-min 94°C denaturation, a 30-s annealing at 50°C, and a 45-s elongation at 72°C, with a final elongation of 7 min for 35 cycles.

**Southern blotting.** The PCR DNA products were analyzed with a 1.2% agarose gel and transferred to a nylon membrane (GeneScreen plus) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The filters were prehybridized for 6 h at 55°C in a solution containing 10% Denhardt solution, 1% sodium dodecyl sulfate (SDS), and 1.5 M NaCl; they were then hybridized at 55°C overnight in the same solution containing 100 mg of salmon sperm DNA and 10<sup>6</sup> cpm of the probe per ml. The oligonucleotides used for the detection of proviral DNA were 5' GGGGTCTGCCATTTTGTGTC 3' for HIV-2<sub>KR</sub> from U937 and 5' ATCCTGGGATTAATAAAAATAGTAAGAATGTATAGCCCT AC 3' for HIV-1 from H9, and the oligonucleotides were end labeled with <sup>32</sup>P with the T4 polynucleotide kinase. The filters were washed in 2× SSC for 5 min at room temperature, and then they were washed in 2× SSC-1% SDS at 55°C for 30 min with two changes. Finally, the filters were washed with 0.1× SSC at room temperature for 30 min with two changes, dried, and exposed to X-ray film.

## RESULTS

**Cell-specific replication of HIV-1 and HIV-2 *vif* mutant viruses.** *vif* has been shown to be dispensable for the replication of HIV-1 and HIV-2 in some cell lines (6, 9, 18, 19, 21). To further determine the role of *vif* in HIV-2 virus replication, a *vif* mutant was constructed by deleting 332 bp in the *vif* gene between two *Bgl*II sites of the HIV-2<sub>KR</sub> genome (25). This mutation should result in the synthesis of a truncated *Vif* protein consisting of the N-terminal 35 amino acids. The effect of this *Vif* truncation on the replication kinetics of HIV-2<sub>KR</sub> virus in different human CD4 cell lines was examined. The stocks of HIV-2<sub>KR</sub> *vif* mutant and WT viruses were prepared by transfection of the proviral DNA into MOLT-4/8 cells. Equivalent amounts of WT and *vif* mutant virus supernatant were used to infect the T-cell lines MOLT-4/8, SupT1, CEM, and H9, the monocytic line U937, and peripheral blood mononuclear cells (PBMC). Virus replication was monitored by

TABLE 1. Replication in permissive and nonpermissive cells of HIV-1 and HIV-2 *vif* mutants<sup>a</sup>

Cell type	Antigen production <sup>b</sup>			
	p24		p26	
	HIV-1	HIV-1 <sub>Δvif</sub>	HIV-2 <sub>KR</sub>	HIV-2 <sub>Δvif</sub>
MOLT-4/8	++	++	++	++
H9	++	-	++	++
CEM	++	+	++	++
SupT1	++	++	++	++
U937	-	-	++	-
PBMC	++	-	++	-

<sup>a</sup> Relative replications of HIV-1 and HIV-2 in different cell lines. HIV-1 virus stocks were produced in MOLT-3 cells, and HIV-2<sub>KR</sub> virus stocks were produced in MOLT-4/8 cells as described in Materials and Methods. Cells (10<sup>6</sup>) were infected with approximately 100 TCID<sub>50</sub> of virus, and cell-free supernatants were collected and assayed for p24 and p26 antigen production.

<sup>b</sup> Replication efficiencies at day 6 were graded as follows: ++, 2 × 10<sup>2</sup> to 1 × 10<sup>4</sup> pg of antigen produced per ml and no discernible difference with WT levels or kinetics (permissive); +, 50 to 2 × 10<sup>2</sup> pg of antigen per ml and delayed or reduced levels of viral replication relative to that of WT (semipermissive); and -, 0 to <50 pg of antigen per ml and no detectable viral replication (nonpermissive).

measuring the p26 antigen in cell-free supernatants over time. All cell lines and PBMC supported the growth of WT virus. The HIV-2<sub>KR</sub> *vif* mutant virus replicated with WT virus kinetics in MOLT-4/8, SupT1, CEM, and H9 cells; no replication was detectable in U937 cells or PBMC (Table 1). Similar results were obtained with virus with larger deletions that encompassed the *vif*, *vif-vpr*, and *vif-vpr-vpx* genes (data not shown).

In order to compare the cell-specific replication of *vif* mutant HIV-2<sub>KR</sub> with that of *vif* mutant HIV-1, we constructed the ΔS mutant of HXB-2 by deleting the region between the *Nde*I and *Nco*I restriction sites (7). The replication efficiency of *vif* mutant HIV-1 produced in MOLT-3 cells, a known permissive cell line, was compared with that of *vif* mutant HIV-2<sub>KR</sub> produced in MOLT-4/8 cells in the same target cells. The HIV-1 *vif* mutant also replicated efficiently in MOLT-4/8 and SupT1 cells and poorly, if at all, in PBMC. However, in contrast to the HIV-2<sub>KR</sub> *vif* mutant, the HIV-1 mutant showed delayed virus replication in CEM cells and no replication in H9 cells (Table 1). The replication efficiencies of the two strains in U937 cells cannot be compared since the parental HIV-1 virus (HXB-2) was not infectious for this cell line. The requirement of *vif* for replication of both viruses in primary cells confirms the fundamental role of *vif* in vivo as proposed previously (6, 9, 18, 19, 21). The ability of some cell types to support the replication of *vif* mutant viruses indicates that a certain cellular factor(s) can compensate for *vif* function. However, the differential replications of HIV-1 and HIV-2 mutant viruses in CEM and H9 cells would suggest either that the threshold requirements of these factors are different for the two viruses (i.e., the requirement for HIV-1 is more stringent) or that different or additional factors may be involved for HIV-1.

**Two types of nonpermissive cells for *vif* mutant viruses.** As shown above, U937 and H9 cells are nonpermissive for the replication of *vif* mutant HIV-2<sub>KR</sub> and HIV-1<sub>HXB-2</sub> viruses, respectively (Table 1). However, transfection of these nonpermissive cells with WT and *vif* mutant proviral DNA resulted in the production of extracellular virus. The infectivity of these viruses on permissive cells was then examined. Specifically, 5 µg of WT and *vif* mutant proviral DNAs of HIV-2<sub>KR</sub> were transfected separately into U937, and similarly, WT and mutant HIV-1<sub>HXB-2</sub> DNAs were transfected into H9 cells. At 48

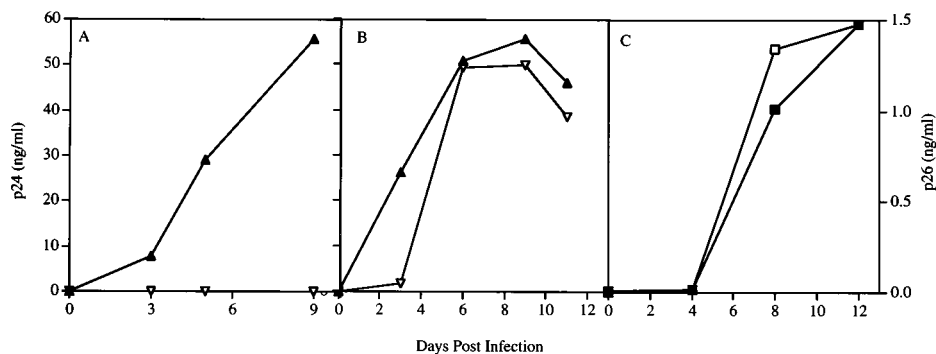


FIG. 1. Transmission of HIV-1 and HIV-2 WT and *vif* mutant viruses from nonpermissive to permissive cell lines. HIV-1 virus stocks were made from H9 (A) and U937 (B) cells. MOLT-3 cells ( $10^6$ ) were infected with approximately 100 TCID<sub>50</sub> of WT (▲) and ΔS (▽) HIV-1 viruses. HIV-2 virus stocks were produced from U937 (C) cells. MOLT-4/8 cells ( $10^6$ ) were infected with approximately 100 TCID<sub>50</sub> of WT (■) and ΔB (□) HIV-2 viruses. Cell-free supernatants collected at different time points were subjected to p24 and p26 antigen assays.

to 72 h posttransfection, the supernatants were filtered through 0.45- $\mu$ m-pore-size filters and the amounts of virus were measured by the p26 and p24 antigen capture assays. Supernatant containing comparable amounts of viral proteins was used to infect MOLT-4/8 cells (HIV-2) and MOLT-3 cells (HIV-1), and virus production was monitored by antigen capture assays. HIV-1 *vif* mutant virus produced from transfected H9 cells failed to replicate in MOLT-3 cells (Fig. 1A), consistent with the previous observation that *vif* or a compensatory cell factor(s) is required at the stage of production of HIV-1 particles (2, 9, 21, 23, 24). In contrast, both WT and *vif* mutant HIV-2<sub>KR</sub> viruses produced from U937 cells were infectious. In both cases (WT and mutant), p26 antigen production increased sharply after 4 days and reached peak levels at days 12 and 9, respectively (Fig. 1C). Furthermore, the same extents of syncytium formation were observed for cells infected with the WT and *vif* mutant viruses (data not shown). In order to rule out contamination of the U937-produced *vif* mutant virus with WT HIV-2<sub>KR</sub> virus, DNA from MOLT-4/8 cells infected with the U937-derived WT and *vif* mutant viruses was subjected to PCR. Bands corresponding to 620 bp for WT *vif* DNA and 320 bp for *vif* mutant DNA were observed (data not shown). We have also examined the replication of HIV-1 *vif* mutant virus in permissive cells, with the virus being produced from U937 by the transfection of proviral DNA. In a manner similar to that for HIV-2, both WT and *vif* mutant HIV-1 viruses produced from U937 cells were infectious, and no difference in the replication of WT and *vif* mutant viruses was observed on day 9 (Fig. 1B). These results suggest that unlike H9, U937 produces infectious *vif* mutant virus particles that can be transmitted to permissive cells, even though it is not susceptible to infection by the *vif* mutant virus itself.

**The role of *vif* in early phase of virus replication in nonpermissive cells.** We also examined the transmission of *vif* mutant viruses from permissive cells to nonpermissive cells. Schwedler et al. (21) reported that HIV-1 *vif* mutant virus produced in permissive cells was infectious for nonpermissive cells and would undergo one round of replication. As Fig. 2A shows, HIV-1 *vif* mutant virus produced from transfected MOLT-3 cells did yield low but detectable virus levels transiently in H9 cells. However, HIV-2 *vif* mutant virus produced from transfected MOLT-4/8 cells did not replicate at all in U937 cells (Fig. 2B). In contrast, HIV-2 *vif* mutant virus produced from permissive cells (MOLT-4/8) replicated with WT virus kinetics in H9 cells, which are nonpermissive for HIV-1 *vif* mutant virus (Fig. 2C). To further dissect this apparent discordance, we examined whether a first-round infection of nonpermissive

cells by HIV-1 and HIV-2 *vif* mutant viruses produced from permissive cells resulted in proviral DNA synthesis. DNA was isolated from H9 and U937 cells at different time intervals within the first 24 h of infection with WT and *vif* mutant viruses of HIV-1 and HIV-2, respectively, and subjected to PCR analysis (see Materials and Methods). The primer pairs were derived from *gag* (HIV-1) and *vif* (HIV-2) and should detect extended, though not necessarily complete, proviral DNA synthesis. A weak signal was detected by Southern blotting immediately after infection ( $T = 0$ ) in some cases, probably repre-

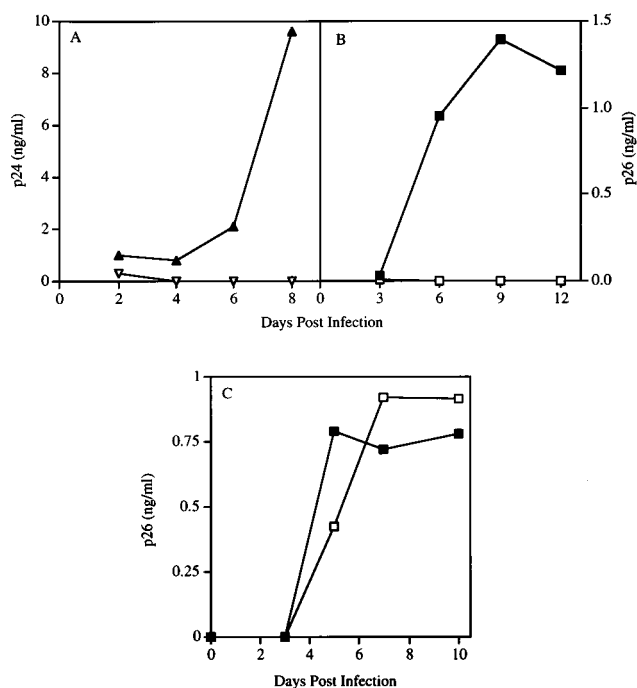


FIG. 2. Transmission of HIV-1 and HIV-2 WT and *vif* mutant viruses from permissive to nonpermissive cell lines. HIV-1 virus stocks were made from MOLT-3 cells. HIV-2 virus stocks were made from MOLT-4/8 cells. (A) H9 cells ( $10^6$ ) were infected with approximately 100 TCID<sub>50</sub> of WT (▲) and ΔS (▽) HIV-1 viruses. The supernatants collected over time were subjected to a p24 antigen assay. (B) U937 cells ( $10^6$ ) were infected with approximately 100 TCID<sub>50</sub> of WT (■) and ΔB (□) HIV-2 viruses. (C) H9 cells ( $10^6$ ) were infected with approximately 100 TCID<sub>50</sub> of WT (■) and ΔB (□) HIV-2 viruses. The cell-free supernatants collected at different time points were subjected to a p26 antigen assay.

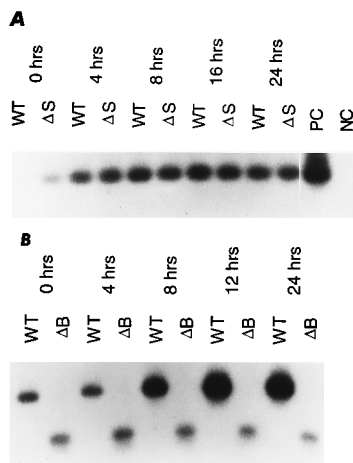


FIG. 3. Effect of *vif* on proviral DNA synthesis in single-round replication. (A) H9 cells were infected with approximately 100 TCID<sub>50</sub> of WT and ΔS HIV-1 viruses produced from MOLT-3 cells. At 0, 4, 8, 16, and 24 h after infection, 10<sup>5</sup> cells were collected and lysed. Cell lysate (25 μl) was subjected to PCR with the *gag* primers as described in Materials and Methods. PC, positive control; NC, negative control. (B) U937 cells were infected with approximately 100 TCID<sub>50</sub> of WT and ΔB HIV-2 viruses produced from MOLT-4/8 cells. At 0, 4, 8, 12, 16, and 24 h after infection, 10<sup>6</sup> cells were collected and DNA was isolated according to Invitrogen protocols. The DNA was subjected to PCR with the *vif* primers as described in Materials and Methods.

senting virion-associated DNA (17, 27). The same signal was also observed with heat-inactivated virus (data not shown). Equal signals of proviral DNA were observed from 4 h onwards in H9 cells infected with WT and *vif* mutant HIV-1 viruses (Fig. 3A), confirming that *vif* mutant HIV-1 produced from permissive cells is competent in undergoing reverse transcription in nonpermissive cells. Again, in contrast, proviral DNA synthesis in U937 cells was only observed in cells infected with WT HIV-2 but not in *vif* mutant-infected cells (Fig. 3B), indicating that HIV-2<sub>KR</sub> *vif* mutant viruses obtained from permissive cells (MOLT-4/8) are still defective in their ability to complete reverse transcription in U937 cells.

**Cross complementation of HIV-1 *vif* mutant virus with HIV-2 *vif*.** The *vif* defect has been shown to be *trans* completable for homologous viruses (7, 21) as well as for genetically heterologous strains of HIV-1 (2). In order to explore whether *vif* function is interchangeable for HIV-1 and HIV-2, we examined the ability of an HIV-1 *vif* mutant virus to replicate in H9 cells stably transfected with an HIV-2 *vif* expression vector (ΔLCL<sub>neo</sub>) in which the *gag*, *pol*, and *env* sequences of an infectious HIV-2 clone had been deleted. *Vif* expression was verified by RNA PCR in this cell line (data not shown). H9 ΔLCLN and H9 cells were infected with WT and *vif* mutant HIV-1 produced from MOLT-3 cells, and virus replication was monitored over time by measuring the p24 antigen levels in cell-free supernatants (Fig. 4). Although HIV-1 *vif* mutant virus showed delayed replication in H9 ΔLCLN, p24 antigen production at day 9 was equal to that of the WT virus. We were not able to carry out the reciprocal complementation since U937 cells were not infectible even by the WT parental HIV-1. These results suggest that although HIV-1 and HIV-2 have different target cell tropisms, the ultimate functions attributed to *vif* for both viruses are similar.

**DISCUSSION**

There is increasing appreciation recently for the role of the nonessential, or accessory, genes of HIV in infection and

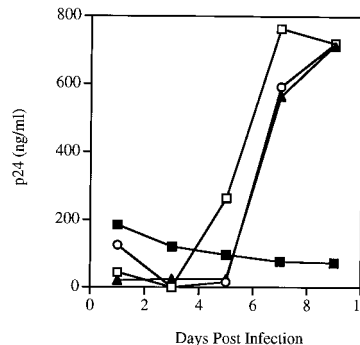


FIG. 4. Cross complementation of HIV-1 *vif* mutant virus with HIV-2 *vif*-expressing cell lines. The H9 cell line (ΔLCLN) stably expressing *Vif*-2 was made as described in Materials and Methods. HIV-1 stocks were made from MOLT-3 cells. H9 (ΔLCLN) cells (10<sup>6</sup>) were infected with approximately 100 TCID<sub>50</sub> of WT (○) and Δ*vif* (▲) HIV-1 viruses. Similarly, H9 cells (control) were infected with approximately 100 TCID<sub>50</sub> of WT (□) and Δ*vif* (■) HIV-1 viruses. Cell-free supernatants collected at different time points were subjected to p24 antigen assays.

pathogenicity and as potential targets for therapy and vaccine development. Although most of the biochemical and molecular analyses of these genes have been carried out with HIV-1, studies pertaining to their relevant *in vivo* functions were by necessity carried out with SIV or HIV-2. The assumption was that the function and mechanism of these gene products would be conserved among primate lentiviruses. The aim of the present study is to directly compare the functional roles of *Vif* in the life cycles of HIV-2 (strain KR) and HIV-1 (HXB-2). The open reading frames of HIV-1 and HIV-2 *vif* are only poorly conserved (Fig. 5), with an amino acid sequence homology of 38%. However, there are short motifs that are highly conserved in HIV-1 and HIV-2 *Vif* proteins, e.g., HIV-2 *Vif* residues 22 to 27, 104 to 115, and 148 to 153. A central sequence bounded by two cysteine residues that was speculated to be an important functional domain (12) for HIV-1 *vif* also showed the same low degree of homology with that of HIV-2:



Therefore, it is of interest to determine if these two divergent protein products may mediate the same function.

Our findings confirmed some of the features previously established for *vif*. First, the requirement of *vif* for both HIV-1 and HIV-2 is cell specific (6, 9, 18, 19, 21). In particular, *vif* mutant viruses could not replicate in primary PBMC, consis-

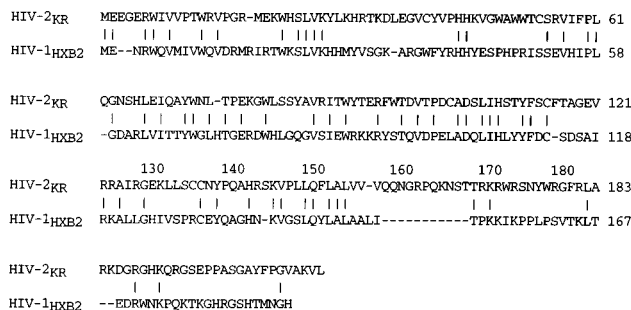


FIG. 5. Alignment of the predicted amino acid sequences of the HIV-1 and HIV-2 *Vif* proteins.

tent with the findings that *vif* mutant SIV and HIV-2 were unable to establish infection in macaque monkeys and with the renewed concept that *vif* is an essential gene for in vivo infection. On the basis of the differential replications of WT and *vif* mutant viruses, cell lines could be grouped into permissive, semipermissive, and nonpermissive phenotypes. Permissive and semipermissive cells apparently can compensate for a defective *vif* function by exploiting either the presence of a positive regulator that substitutes for Vif or the absence of an inhibitory factor that Vif counteracts. Second, *vif* mutant viruses are blocked in an early step in virus replication, most likely at the reverse transcription step. However, in spite of these similarities, there are also distinct differences between HIV-1 and HIV-2<sub>KR</sub>. Whether such differences hold true for all HIV-1 and HIV-2 strains is not known, but for convenience of discussion, we will refer to HIV-2 generically. First, the permissive cell lines for *vif* mutant viruses only partially overlap for HIV-1 and HIV-2. For example, CEM is fully permissive for HIV-2 and semipermissive for HIV-1; H9 cells were permissive for HIV-2 and nonpermissive for HIV-1 (Table 1). The only nonpermissive cell line we could identify for *vif* mutant HIV-2<sub>KR</sub> is the monocytic cell line U937. U937 was also recently reported to be nonpermissive for HIV-2<sub>SBL/ISY</sub> (18). One potential explanation of the differential replications of HIV-1 and HIV-2 *vif* mutant viruses in the same cell lines is that the threshold level of the same cellular factor(s) required to make infectious particles is higher for HIV-1 than for HIV-2. Alternatively, HIV-1 may require additional cellular factors or factors different from those for HIV-2. Our observation that HIV-2 Vif could rescue the replication of HIV-1<sub>Δvif</sub> in H9 cells supports the former hypothesis.

We also demonstrated that there are two kinds of nonpermissive cells. One restricts the production of infectious virions for permissive cells (e.g., H9), and the other restricts the first-round replication of *vif* mutant viruses produced from permissive cells (e.g., U937). We confirmed that HIV-1<sub>Δvif</sub> produced from H9 was not infectious for permissive cell lines like MOLT-3 but that HIV-1<sub>Δvif</sub> produced from MOLT-3 undergoes a one-round infection in H9. In contrast, HIV-2<sub>Δvif</sub> produced from U937 was infectious for permissive cell lines, but HIV-2<sub>Δvif</sub> produced from permissive cells failed to complete the reverse transcription step when transmitted to U937. Further studies would be required to explain this distinct target cell versus producer cell dependence for the two cell types.

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