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Complementation of *vif*-Defective Human Immunodeficiency Virus Type 1 by Primate, but Not Nonprimate, Lentivirus *vif* Genes

JAMES H. M. SIMON,[1] TAMARA E. SOUTHERLING,[1] JEFFREY C. PETERSON,[2,3] BARBARA E. MEYER,[1] AND MICHAEL H. MALIM[1,3]*

Howard Hughes Medical Institute,[1] Graduate Group in Molecular Biology,[2] and Departments of Microbiology and Medicine,[3] University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6148

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The productive infection of many susceptible human cells, including lymphocytes and macrophages derived from peripheral blood, by the pathogenic lentivirus human immunodeficiency virus type 1 requires expression of the virally encoded *vif* (for virion infectivity factor) gene. Interestingly, this gene appears to have been conserved among all of the lentiviruses of primates and almost all of the lentiviruses of nonprimates. Using T cells constitutively expressing *vif* genes derived from diverse sources and virus replication assays, we show that the *vif* gene of a second primate lentivirus, simian immunodeficiency virus from macaques, complements *vif*-defective human immunodeficiency virus type 1 but that those of three distinct nonprimate lentiviruses do not. Although the molecular basis for Vif function has yet to be defined, the potential implications of this noted restriction of *vif* complementarity are discussed.

One of the features that distinguishes members of the lentivirus subfamily of retroviruses from the majority of replication-competent oncogenic retroviruses is their possession of genes in addition to *gag*, *pol*, and *env*. Although the prototypic lentivirus of primates, human immunodeficiency virus type 1 (HIV-1), carries six such genes (namely, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*), equivalents of only three of them have been noted among the lentiviruses of nonprimates (for reviews, see references 7, 17, 23, 40, 41, and 45). Each lentivirus encodes, first, a transcriptional *trans* activator, Tat, that acts on the viral long terminal repeat (LTR) promoter element to stimulate expression of all viral genes and, second, a posttranscriptional *trans* activator, Rev, that is required for the cytoplasmic expression of unspliced viral mRNA. The third conserved gene, *vif*, appears to play a role in modulating viral infectivity (10, 13, 15, 16, 20, 27, 33, 38, 49, 50, 52, 54, 55, 57, 60, 62) and is found in all known lentiviruses except equine infectious anemia virus (EIAV).

In contrast to Tat and Rev, both of which are essential for efficient HIV-1 replication in all cultured cell systems, Vif influences replication only in certain cell types (10, 13, 15, 16, 49, 55, 62). For example, *vif*-defective HIV-1 is unable to replicate in primary human lymphocytes or macrophages (nonpermissive cells) but spreads efficiently through cultures of T-cell lines such as C8166 or Sup-T1 (permissive cells). Nevertheless, the observation that intact *vif* genes are maintained by the viral populations of HIV-1-infected individuals (63), taken together with the demonstration that development of an AIDS-like disease in rhesus macaque monkeys infected with simian immunodeficiency virus from macaques (SIV_{mac}) requires a functional *vif* gene (20), does provide compelling evidence that Vif plays an essential role in the life cycles of primate lentiviruses in vivo. However, the mechanism by which Vif, a protein that in the case of HIV-1 comprises ~192 amino acids, has a mass of ~23 kDa, and is primarily cytoplasmic (22), induces replication in nonpermissive cells remains poorly understood. For instance, data have been presented suggesting that Vif may stimulate efficient reverse transcription following infection (55, 62), modulate viral particle maturation (25), increase $gp160/120^{Env}$ incorporation into virions (49), catalyze a truncation within the carboxy-terminal 15 amino acids of $gp41^{Env}$ (24), or even increase the release of virions from infected cells (3).

Experiments performed in many laboratories have addressed the issue of cross-complementarity between the Tat or Rev *trans* activators of various lentiviruses. Although some degree of reciprocity is frequently found among the Tat or Rev proteins of primate lentiviruses (2, 8, 9, 11, 18, 32, 35, 48, 53, 61), there are as yet no instances of complementation between primate- and non-primate-derived *tat* or *rev* genes. Importantly, this absence of cross-complementation has made it possible to design and construct chimeric genes with a view to identifying potential "virus specificity" and "effector" domains within a number of these proteins. For example, a number of experiments of this type have been described for both the Tat and the Rev proteins of HIV-1 and EIAV (6, 14, 37). In each case, the viral specificity was shown to be determined by the interaction of the chimeric *trans* activator's RNA binding domain with its cognate RNA target sequence. It is thought that once this has occurred, effector function and ensuing *trans* activation, which presumably lacks virus specificity, can then take place. Thus, a chimeric HIV-1:EIAV Tat protein in which the basic (RNA binding) and carboxy-terminal domains of HIV-1 Tat were replaced by the corresponding domains of the EIAV protein stimulated transcription from the EIAV LTR almost as well as wild-type EIAV Tat but had little effect on the noncognate HIV-1 LTR (6).

As a first step towards performing similar domain swap experiments with lentivirus Vif proteins and potentially defining specificity and effector domains, it was important for us to establish patterns of cross-complementation between the *vif* genes themselves. Here, we report that the *vif* gene of SIV_{mac} can act in *trans* to restore replication to *vif*-defective HIV-1 but that the analogous genes from three distinct lentiviruses of nonprimates, namely, visna virus, bovine immunodeficiency virus (BIV), and feline immunodeficiency virus (FIV), cannot.

* Corresponding author. Phone: (215) 573-3493. Fax: (215) 573-2172. Electronic mail address: malim@hmivax.humgen.upenn.edu.

MATERIALS AND METHODS

Molecular clones. The wild-type HIV-1 proviral expression plasmid, pIIIB, has been described previously (19). This vector contains a proviral clone of the HIV-1_{IIIB} isolate HXB-3 in which the internal *Sph*I-*Sal*I fragment has been replaced with the analogous fragment from the NL4-3 recombinant provirus (1); all six additional genes of this provirus are therefore intact. pIIIB/Δ*vif*, a *vif*-defective derivative of pIIIB in which there are nonsense mutations at codon positions 26 and 27 of *vif* (AAA.CAC→TAA.TAG [the periods indicate the reading frame]), was created by PCR-mediated site-directed mutagenesis. Importantly, these mutations are 3′ to the region of overlap between the *vif* and *pol* open reading frames.

The 5′ and 3′ hemiviral clones, p239SpSp5′ and p239SpE3′, used for generating the infectious, pathogenic 239 isolate of SIV_{mac} have been described previously (28). The *vif* gene of p239SpSp5′ was disrupted with nonsense mutations at codon positions 33 and 34 (ACT.AAA→TAA.TAG) by PCR-mediated mutagenesis to generate p2395′/Δ*vif*. p2393′/TM^{BK} was derived from p239SpE3′ by the introduction of a premature stop codon (CAG→TAG) 146 codon positions upstream from the natural stop codon. An analogous foreshortening of the cytoplasmic domain of the transmembrane $gp41^{Env}$ segment of Env has previously been shown to correlate with efficient replication of the BK28 isolate of SIV_{mac} in HUT 78 cells (29).

All of the retrovirus vectors are derivatives of the G418 resistance-conferring murine leukemia virus-based vector LXSN (39). To obtain the vector LN-M, a 238-bp fragment was amplified by PCR as a *Bam*HI-*Bgl*II fragment from the 3′ LTR (proviral coordinates 8007 to 8245) of Mason-Pfizer monkey virus (M-PMV) and inserted into the unique *Bam*HI site of LXSN. Importantly, this *cis*-acting element of M-PMV has been shown to afford constitutive expression of viral sequences that are otherwise dependent on the interaction of Rev with its target sequence, the Rev response element (5). The *vif* (or putative *vif*) genes of HIV-1 (hVif), SIV_{mac} (sVif), BIV (bVif), FIV (fVif), and visna virus (vVif) were amplified by PCR from pIIIB, p239SpSp5′, $BIV127^{inf}$ (4), 34TF10 (46), and LV1-1KS1 (56), respectively; inserted into LN-M at the *Bam*HI site that is immediately 5′ to the M-PMV sequences; and assessed for integrity by DNA sequencing. The mutated *vif* gene of pIIIB/Δ*vif* (ΔhVif) was also inserted into LN-M; this served as a negative control for these studies. The amphotropic murine leukemia virus Env and Moloney murine leukemia virus Gag/Pol vectors SV-A-MLV-*env* and SV-Ψ$^-$-*env*$^-$-MLV have been described previously (31).

Monoclonal antibodies. The immunogen used for the generation of a Vif-specific monoclonal antibody was a His_6:Vif fusion that comprised the 192-amino-acid Vif protein encoded by pIIIB with an N-terminal extension of Met-Arg-Gly-Ser-His_6-Ser. This protein was overexpressed in *Escherichia coli* BL21(DE3)pLysS by using one of the pET vectors (Novagen Inc., Madison, Wis.) and purified by nickel (Ni^{2+}) chelate affinity chromatography under denaturing conditions (6 M guanidine hydrochloride) according to the manufacturer's instructions (QIAGEN Inc., Chatsworth, Calif.). The column eluate was dialyzed against 10 mM Na_2PO_4 (pH 5)–150 mM NaCl, and the >95% pure His_6:Vif protein was used to immunize BALB/c mice by standard procedures. Following three subsequent booster injections, the spleen of one mouse was recovered, and hybridomas with the mouse plasmacytoma cell line SP2/0Ag were generated. Culture supernatants derived from these hybridomas were screened by enzyme-linked immunosorbent assay (ELISA) for reactivity against plastic-adhered antigen. The antibody secreted by one of the positive hybridomas was characterized further and was shown to be highly reactive in Western blot (immunoblot) analyses; this antibody is termed MAb319. The monoclonal antibody 4F4 has been described previously; it is specific for the heterogeneous ribonucleoprotein particle C1/C2 proteins (47).

Construction of *vif*-expressing cell lines. The recombinant retrovirus stocks used for transducing lentivirus *vif* genes into the human T-cell lines HUT 78 and Jurkat were obtained by transient transfection of the African green monkey kidney cell line COS. Thirty-five-millimeter-diameter cultures were transfected with 2.5 μg each of SV-A-MLV-*env*, SV-Ψ$^-$-*env*$^-$-MLV, and the relevant LN-M-derived vector by using DEAE dextran and chloroquine. At 48 h, 10^6 T cells were infected by cocultivation for 24 h, harvested, and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 μg of gentamicin sulfate per ml, and 1 mg of G418 (GIBCO BRL Inc., Gaithersburg, Md.) per ml. Resistant cultures emerged in 2 to 3 weeks, and these were named according to the parental T-cell line and the particular *vif* gene they expressed.

Northern (RNA) blot analyses. Total RNAs were isolated from ~5×10^6 T cells, resolved on formaldehyde-containing 1.5% agarose gels, and transferred to nitrocellulose. The filters were then hybridized to ^{32}P-labeled DNA probes generated by random priming (Pharmacia Biotech Inc., Piscataway, N.J.) and subjected to autoradiography. The *vif*-specific mRNAs were detected with a probe specific for the M-PMV sequence common to all LN-M derived vectors, and equal loading between lanes was subsequently determined by rehybridization with a γ-actin-specific probe.

Western analyses. Total lysates from ~5×10^5 T cells were resolved in sodium dodecyl sulfate–14% polyacrylamide gels and electroeluted onto nitrocellulose filters. The HIV-1 Vif and host cell C1/C2 heterogeneous ribonucleoprotein particle (hnRNP) proteins were detected by initial hybridization with MAb319 or 4F4, respectively. Bound antibody was visualized by secondary hybridization with a horseradish peroxidase-conjugated anti-mouse antibody raised in goats (Fisher Biotech, Pittsburgh, Pa.), enhanced chemiluminescence (Amersham Corp., Arlington Heights, Ill.), and autoradiography.

Analysis of HIV-1 and SIV_{mac} replication. Initial viral inocula of HIV-1 and HIV-1/Δ*vif* were obtained by transient transfection of pIIIB and pIIIB/Δ*vif*, respectively, into COS cells; importantly, viral particles produced from COS cells do not require Vif expression to be infectious (62). The ensuing virus-containing supernatants were used to establish acute infections in the permissive cell line C8166 (see Fig. 1C) (15). At a time when virus expression was close to its maximum, the supernatants were clarified by passage through 0.45-μm-pore-size nitrocellulose filters and stored in aliquots at −70°C, and their viral contents were assessed by ELISA for soluble $p24^{Gag}$ (Dupont, NEN, Inc., Billerica, Mass.). All determinations of replicative capacity were performed by challenging 2×10^6 T cells with wild-type or Δ*vif* viral stocks containing equivalent levels of $p24^{Gag}$ in a final volume of 1 ml. At 24 h, the cells were washed twice, and they were maintained thereafter in RPMI 1640 medium containing 10% fetal bovine serum and gentamicin sulfate (HUT 78, Jurkat, and C8166 parental lines) or in medium additionally supplemented with 1 mg of G418 per ml (transduced HUT 78 and Jurkat lines) at 0.5×10^6 to 2.5×10^6 cells per ml. Virus production, and hence replication, was monitored over time by measuring $p24^{Gag}$ expression in the culture medium by ELISA.

Full-length SIV_{mac} proviruses were generated by the ligation of *Sph*I-digested hemiviral clones. Specifically, p2393′/TM^{BK} was used in combination with either p239SpSp5′ or p2395′/Δ*vif* to create wild-type or Δ*vif* SIV_{mac}, respectively. These ligation mixtures were transfected into the human embryonal kidney cell line 293T by using calcium phosphate to generate stocks that were assessed for viral content by assaying reverse transcriptase activity (21), filtered, and stored in aliquots at −70°C; as with COS cells, Δ*vif* and wild-type viruses from 293T cells are equally infectious. Equivalent inocula of wild-type and mutant viruses were used to challenge various HUT 78 cell lines as described above, and the ensuing virus replication was determined by measuring supernatant reverse transcriptase activity.

RESULTS

The contribution of *vif* to HIV-1 replication differs among T-cell lines. The major goal of these studies was to determine which lentivirus *vif* genes could functionally complement the *vif* gene of HIV-1. However, before these experiments could be initiated, it was important to identify T-cell lines in which the efficient replication of HIV-1 is dependent on Vif expression. In particular, it has been demonstrated by several groups that although Vif is essential for HIV-1 replication in certain cell lines, it is largely dispensable in others (10, 13, 15, 16, 49, 55, 62); these cell types have been termed nonpermissive and permissive, respectively. From a historical standpoint, it was this enigmatic cell-type-specific dispensability of Vif that led to the previously held notion that *vif* was a nonessential gene.

Stocks of wild-type and Δ*vif* HIV-1 were obtained by infecting the permissive T-cell line C8166 (15) with supernatants derived from COS cells transiently transfected with pIIIB or pIIIB/Δ*vif*. These were adjusted for viral content and used to challenge the T-cell lines HUT 78 and Jurkat as well as C8166 itself. Virus production, and therefore replication, was monitored over time by measuring the levels of soluble $p24^{Gag}$ in the culture medium (Fig. 1). As would be expected, HIV-1 carrying an intact and functional *vif* gene replicated efficiently in all three cell lines and attained maximum levels of virus production within 1 to 3 weeks. In contrast, there were dramatic differences among these cell lines in the replication of HIV-1/Δ*vif*. Specifically, virus growth in the absence of Vif was undetectable in HUT 78 cells (Fig. 1A), severely reduced and delayed in Jurkat cells (Fig. 1B), and unaltered in C8166 cells (Fig. 1C). These observations are mostly consistent with those of others and serve to illustrate that cells can be either nonpermissive (HUT 78 and Jurkat) or permissive (C8166) to the rapid and efficient replication of *vif*-defective HIV-1.

Complementation of HIV-1 *vif*. The most straightforward approach to examine complementation of HIV-1 *vif* by the *vif* genes of other lentiviruses is to analyze HIV-1/Δ*vif* replication in cells constitutively expressing those genes. The *vif* genes (confirmed and/or putative) of HIV-1, SIV_{mac}, visna virus, BIV, and FIV, in addition to the disrupted version from HIV-

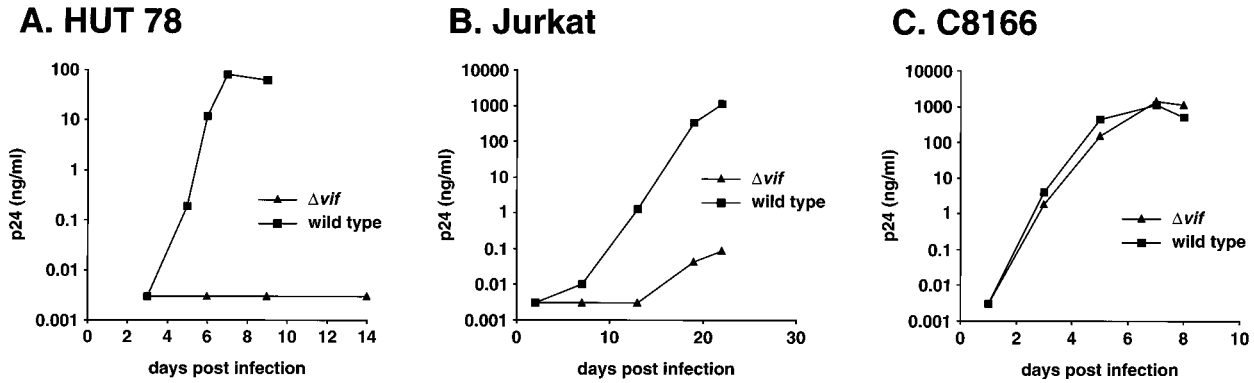

FIG. 1. Effect of *vif* on HIV-1 replication. HUT 78 (A), Jurkat (B), or C8166 (C) cells (2×10^6) were challenged with wild-type or Δ*vif* stocks of HIV-1 corresponding to 10, 10, or 1 ng of $p24^{Gag}$, respectively. The cultures were maintained in complete medium, and the extents of virus replication was assessed by ELISA for soluble $p24^{Gag}$ expression.

1/Δ*vif*, were therefore amplified by PCR and inserted into the retrovirus vector LN-M. This particular vector was chosen because it contains, immediately 3′ to the site used for the introduction of heterologous sequences, a *cis*-acting sequence from the 3′ LTR of M-PMV known to activate the expression of HIV-1 genes that are otherwise dependent on Rev and the Rev response element (5). It was considered advantageous to include this sequence element in the parental vector used here, since it has previously been suggested that Rev is required for the expression of HIV-1 Vif (19, 51). Although the presence of this M-PMV sequence did not prove to be essential for the expression of Vif in the context of a retrovirus vector, we have found that this element can often act as a moderate enhancer of gene expression in stable lines (data not shown). In any event, we have yet to encounter an instance in which this element reduces gene expression, and we therefore favor the idea that it may be useful to include it in general-purpose retrovirus vectors.

Infectious recombinant retroviruses were generated by cotransfection of COS cell monolayers with each of the *vif*-carrying LN-M vectors in combination with SV-A-MLV-*env* and SV-Ψ^--*env*$^-$-MLV. These stocks were used to infect Jurkat cells, and polyclonal transduced populations were then derived by selection in G418-containing medium. To confirm that the expected transcripts corresponding to the various *vif* genes were expressed in these cell lines, total cellular RNA was isolated from each culture and subjected to Northern analysis (Fig. 2A). By utilizing the M-PMV sequences common to all LN-M-derived vectors as the probe, we were able to ensure that hybridizations to the different *vif* mRNAs were equivalent. Although the lengths of the transcripts varied in accordance with the length of each *vif* coding region, significant expression of each gene was readily detectable (Fig. 2A, lanes 2 to 7). In the case of the LN-M/hVif-transduced culture, functional expression of the *vif*:M-PMV mRNA in terms of protein synthesis was verified by Western analysis of whole-cell lysates with a monoclonal antibody specific for HIV-1 Vif (Fig. 2B, lane 2).

To evaluate the replication of HIV-1/Δ*vif* in the transduced Jurkat lines, cultures were challenged with virus stocks produced in C8166 cells, and the expression of soluble $p24^{Gag}$ was measured over time (Fig. 3A). As expected, the growth of the Δ*vif* virus in Jurkat/ΔhVif cells was severely retarded and was therefore reminiscent of that noted in untransduced Jurkat cultures (compare Fig. 3A with Fig. 1B). The rapid replication of the Δ*vif* virus in the hVif-expressing culture (Fig. 3A) was also anticipated, since the HIV-1 *vif* gene harbored by these cells was derived from the wild-type parental expression vector pIIIB. The noncognate Vif proteins segregated into two categories with respect to their capacities to cross-complement HIV-1/Δ*vif*. Specifically, whereas the other primate lentivirus Vif, that encoded by SIV_{mac}, induced replication almost as efficiently as the Vif protein of HIV-1 itself, the *vif* genes from

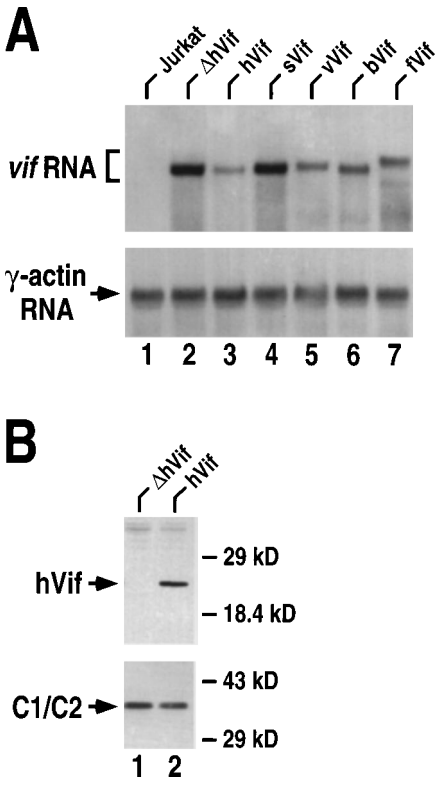

FIG. 2. Stable expression of primate and nonprimate lentivirus *vif* genes in Jurkat cells. (A) Northern analysis of total cellular RNAs isolated from parental Jurkat cells (lane 1) and cultures transduced with LN-M-based vectors carrying a disrupted HIV-1 *vif* gene (lane 2) or the intact *vif* gene of HIV-1 (lane 3), SIV_{mac} (lane 4), visna virus (lane 5), BIV (lane 6), or FIV (lane 7). The filter was hybridized initially with the M-PMV-derived sequences (upper panel) and subsequently with a γ-actin-specific probe to confirm that equal quantities of RNA had been applied to each lane (lower panel). (B) Western analysis of total cell lysates derived from cultures transduced with LN-M/ΔhVif (lane 1) or LN-M/hVif (lane 2). The HIV-1 Vif protein and cellular C1/C2 proteins (the latter served as a loading control) were visualized following initial hybridization with the antibodies MAb319 and 4F4, respectively. The positions of prestained protein molecular mass standards (GIBCO BRL Inc.) are indicated at the right.

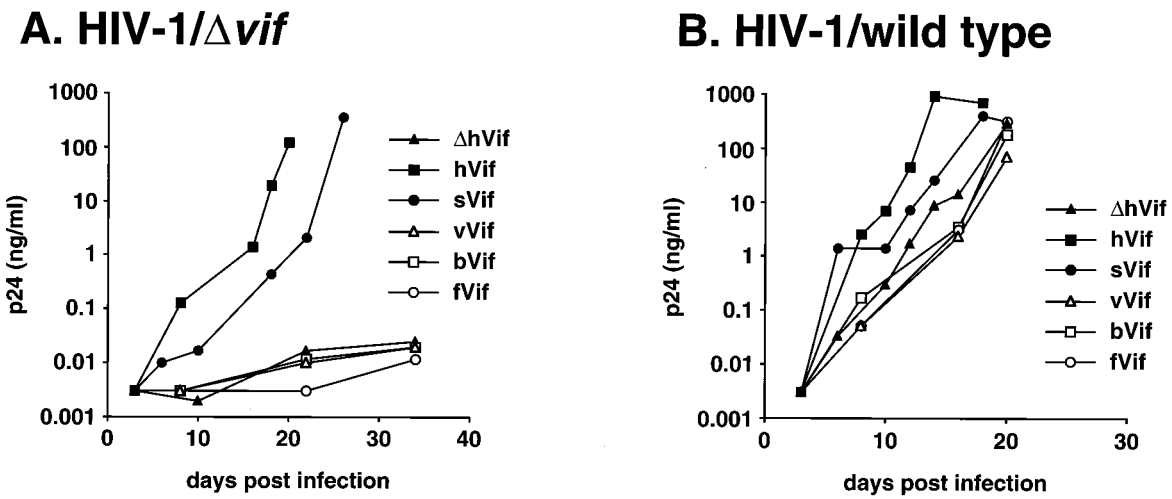

FIG. 3. Replication of Δ*vif* (A) and wild-type HIV-1 (B) in Jurkat cell lines expressing lentivirus *vif* genes. HIV-1 carrying either a disrupted or a wild-type *vif* gene and corresponding to 5 ng of $p24^{Gag}$ was used to challenge 2×10^6 cells expressing a mutated HIV-1 *vif* gene or the intact genes of HIV-1, SIV_{mac}, visna virus, BIV, or FIV. Subsequent virus replication was monitored by determining soluble $p24^{Gag}$ expression by ELISA.

visna virus, BIV, and FIV all failed to stimulate replication to levels higher than that observed in Jurkat/ΔhVif cells (Fig. 3A). Because we demonstrated earlier that the *vif* mRNA levels in the vVif-, bVif-, and fVif-expressing cells are at least as high as that in the Jurkat/hVif culture (Fig. 2A; compare lanes 5, 6, and 7 with lane 3), we have concluded that the inability of each of these cell lines to support efficient HIV-1/Δ*vif* replication is unlikely to be caused by insufficient levels of Vif expression. Rather, we consider it probable that the nonprimate lentivirus Vif proteins are unable to influence the nature of HIV-1 virion expression in nonpermissive human T cells in a manner that is concordant with robust viral replication.

Parallel infections of the transduced Jurkat cultures with wild-type HIV-1 derived from C8166 cells were also performed. Consistent with the data described above, replication in Jurkat/ΔhVif cells was similar to that observed in the parental line, but replication was noticeably accelerated in cultures expressing the Vif protein of either HIV-1 or SIV_{mac} (Fig. 3B). Although not conclusive, this suggests that the levels of Vif that are normally expressed in HIV-1-infected cells may limit the extent to which the virus can replicate. Moreover, we also noted that the growth of wild-type HIV-1 was neither induced nor suppressed in the vVif-, bVif-, or fVif-expressing cells relative to that seen in the cells transduced with LN-M/ΔhVif. It was useful to establish this second point, since there are reports in the literature of lentivirus gene function being suppressed in a *trans*-dominant manner by the wild-type version of the corresponding gene of another lentivirus. For example, the HIV-1 Tat and Rev *trans* activators are inhibited by overexpression of the EIAV Tat and HIV-2 Rev proteins, respectively (18, 34).

The *vif* gene of SIV_{mac} is complemented by HIV-1 *vif*. Having established that the Vif protein of SIV_{mac} efficiently complements HIV-1 Vif, we wanted to determine whether the converse is also true. In other words, can HIV-1 Vif restore replicative capacity to SIV_{mac} that lacks a functional *vif* gene? Although it is not possible to address this question with Jurkat cells since SIV_{mac} is unable to productively infect this cell line (data not shown), replication in a number of other human T lymphoid lines, for example, HUT 78, has been reported (29, 30). Since we had already established that HUT 78 cells are nonpermissive for the replication of viruses that lack *vif* (Fig. 1A; see below), this cell line appeared to be ideally suited to these experiments. Accordingly, cultures of HUT 78 were stably transduced with the LN-M-based retrovirus vectors that

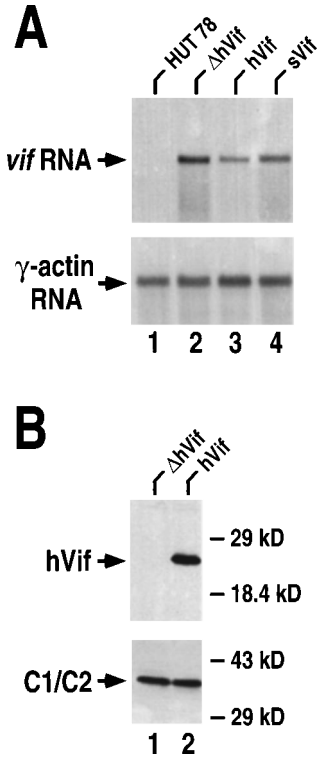

FIG. 4. Stable expression of primate lentivirus *vif* genes in HUT 78 cells. (A) Northern analysis of total cellular RNAs isolated from HUT 78 cells (lane 1) and cultures transduced with LN-M based vectors carrying a nonfunctional HIV-1 *vif* gene (lane 2) or the intact *vif* gene of HIV-1 (lane 3) or SIV_{mac} (lane 4). (B) Western analysis of whole-cell lysates derived from HUT 78 cultures transduced with LN-M/ΔhVif (lane 1) or LN-M/hVif (lane 2). The *vif*:M-PMV and γ-actin transcripts as well as the HIV-1 Vif and C1/C2 proteins were visualized as described for Fig. 2.

TABLE 1. Replication of wild-type and Δ*vif* SIV_{mac} in HUT 78 cell lines expressing HIV-1 and SIV_{mac} *vif*

Cell line	Reverse transcriptase activity[a] (cpm/ml)	
	SIV_{mac}/Δ*vif*	SIV_{mac} (wild type)
HUT 78/ΔhVif	<200	330,000
HUT 78/hVif	400,000	130,000
HUT 78/sVif	410,000	310,000

[a] Levels of reverse transcriptase activity in filtered culture supernatants were determined 12 days after challenge with SIV_{mac} stocks derived from transfected 293T cells.

bear either a nonfunctional *vif* gene or the *vif* gene of HIV-1 or SIV_{mac}. As with the Jurkat cell lines discussed above, it was directly demonstrated that each of the HUT 78-derived cultures expressed the predicted *vif*:M-PMV transcript (Fig. 4A, lanes 2 to 4) and, at least in the case of the HUT 78/hVif cells, the expected Vif protein (Fig. 4B, lane 2).

It has previously been determined by others that potent replication of SIV_{mac} in HUT 78 cells (as well as a number of other human T-cell lines) can be induced by removing ~146 amino acids from the carboxy terminus of the cytoplasmic tail of the transmembrane glycoprotein $gp41^{Env}$ (29). The *env* gene of the 3′ hemiviral clone of SIV_{mac}, p239SpE3′, was therefore foreshortened in an analogous fashion by the introduction of a nonsense mutation. Stocks of Δ*vif* and wild-type SIV_{mac} that each carried a truncated transmembrane glycoprotein were then obtained by transiently transfecting 293T cells with appropriately in vitro-reconstructed proviruses and used to challenge the three transduced HUT 78 cell lines. Subsequent virus replication was measured by assaying for reverse transcriptase activity that had been released into the culture medium (Table 1). As predicted, SIV_{mac}/Δ*vif* replicated well in cells harboring the cognate SIV_{mac}-derived gene but failed to productively infect the culture that lacked an intact *vif* gene. Importantly, however, this *vif*-defective virus also grew efficiently in the culture expressing the HIV-1 Vif protein. In addition, and as expected, SIV_{mac} carrying a functional *vif* gene was able to replicate in all three HUT 78 cell lines tested (Table 1). In conclusion, we have therefore demonstrated that the Vif proteins of HIV-1 and SIV_{mac}, which have only ~27% amino acid sequence identity, are able to cross-complement each other in the context of virus replication assays.

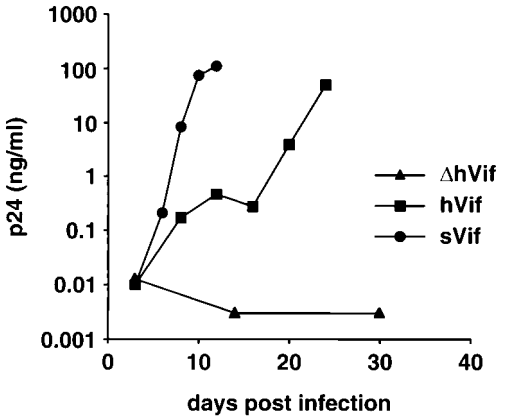

FIG. 5. Replication of *vif*-defective HIV-1 in HUT 78 cells expressing HIV-1 or SIV_{mac} *vif*. Cells (2×10^6) expressing the disrupted HIV-1 *vif* gene or the intact gene of either HIV-1 (closed squares) or SIV_{mac} (closed circles) were challenged with HIV-1/Δ*vif* equivalent to 5 ng of $p24^{Gag}$. Ensuing virus replication was measured as soluble $p24^{Gag}$ expression.

As a confirmation of the complementation of HIV-1 *vif* by the Vif protein of SIV_{mac} in Jurkat cells (Fig. 3A), we also infected the three transduced HUT 78 cell lines with HIV-1/Δ*vif* (Fig. 5). Consistent with our earlier findings, this virus replicated in cultures that expressed the *vif* gene of either HIV-1 or SIV_{mac} but not in the negative control cells containing the disrupted *vif* gene.

DISCUSSION

In this report, we have described experiments in which the abilities of a number of proven and predicted lentivirus *vif* genes to functionally substitute for HIV-1 *vif* were determined. Importantly, we found that SIV_{mac} Vif can restore replication to *vif*-defective HIV-1 (Fig. 3A and 5) but that the counterpart genes of three distinct lentiviruses of nonprimates, namely, visna virus, BIV, and FIV, cannot (Fig. 3A). In fact, the complementation of HIV-1 gene activity by the corresponding gene of SIV_{mac} appears to be more complete in the case of *vif* than has been noted for either *tat* or *rev*. Specifically, SIV_{mac} Tat activates transcription from the HIV-1 LTR at ~20% efficiency compared with HIV-1 Tat (61), whereas SIV_{mac} Rev is unable to induce the cytoplasmic expression of Rev-responsive unspliced HIV-1 mRNAs (35). Moreover, and in keeping with the full activity displayed by the HIV-1 Tat and Rev proteins on SIV_{mac} or SIV_{agm} (a related virus of African green monkeys) substrates (48, 61), we also found that the Vif protein of HIV-1 appeared to be fully functional in terms of complementing the replication of *vif*-defective SIV_{mac} (Table 1).

A major question that arises from these studies is why the *vif* genes of nonprimate lentiviruses are incapable of complementing HIV-1 *vif*. Here, we offer three potential hypotheses, the last of which we view as the more likely explanation. First, it is conceivable that Vif serves different roles in the life cycles of nonprimate and primate lentiviruses. If this was the case, it would be anticipated that vVif, bVif, or fVif would each fail to cross-complement HIV-1/Δ*vif*. However, this notion seems highly improbable given (i) the invariant positioning of these genes at (and overlapping with) the 3′ terminus of *pol*, (ii) the similar lengths of the various Vif proteins, ranging from ~192 amino acids in HIV-1 to ~251 amino acids in FIV; (iii) the strong conservation of a Ser/Thr-Leu-Gln-X-Leu-Ala motif in each protein (43, 63); and (iv) at least with HIV-1, SIV_{mac}, and FIV, the dramatic loss of (or reduction in) replicative capacity that is caused by disruption of the gene (3, 10, 13, 15, 16, 20, 27, 33, 38, 49, 50, 52, 54, 55, 57, 60, 62). Thus, in the light of these shared attributes, it seems likely that the function of Vif has been conserved among the lentiviruses.

Second, it is possible that human T cells fail to provide the cellular milieu necessary for the activity of nonprimate lentivirus Vif proteins. Although this hypothesis cannot be tested at this time, since a cell type that is nonpermissive to the replication of Δ*vif* lentiviruses and yet is susceptible to infection by HIV-1 as well as one of the nonprimate viruses has not been identified, this possibility again seems implausible. Indeed, it appears to be especially unlikely given that the other two conserved nonstructural proteins of lentiviruses, Tat and Rev, are active, albeit only partially in some instances, in cells derived from a wide variety of different species (6, 12, 26, 34, 36, 42, 44, 58, 59). Thus, it would be surprising if Vif had been tailored to function in cells of the host species, or closely related species, but the Tat and Rev *trans* activators had not.

Third, and this is the explanation that we currently favor, it is possible that the Vif proteins of nonprimate lentiviruses are unable to interact with the gene products of HIV-1 in such a way as to render assembled HIV-1 virions fully infectious. The molecular basis for this lack of function can only be speculated upon at this time, as it is not yet known how HIV-1 Vif itself functions. In this regard, it has recently been demonstrated that HIV-1/Δ*vif* viral particles are severely debilitated in their capacity to complete reverse transcription following virus challenge compared with their wild-type counterparts (55, 62). These findings, in combination with the observation that the nucleocapsids of Δ*vif* virions appear to be nonhomogeneously packed and less electron dense than those of wild-type particles (25), are suggestive of a model for Vif function in which Vif modulates an aspect of particle maturation that then influences viral infectivity by stimulating efficient reverse transcription. Accordingly, an explanation for the noted inability of the Vif proteins of visna virus, BIV, and FIV to substitute for HIV-1 Vif would be that they are unable to recognize and interact with HIV-1-encoded gene products (presumably proteins) in a way that results in the required virion maturation events taking place.

If it is indeed true that each Vif protein functions by the same mechanism, then the use of variants with different virus substrate specificities to create chimeric proteins will, potentially, allow both the virus specificity and the effector domains of Vif proteins to be defined. Such information will enhance our understanding of this critical nonstructural protein of lentiviruses.

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