JOURNAL OF VIROLOGY, Dec. 1995, p. 7630–7638 Vol. 69, No. 12
0022-538X/95/$04.00+0

The Vif Protein of Human and Simian Immunodeficiency Viruses Is Packaged into Virions and Associates with Viral Core Structures

HONGMEI LIU,[1] XIAOYUN WU,[1] MARGARET NEWMAN,[1,2] GEORGE M. SHAW,[1,2] BEATRICE H. HAHN,[1,2] AND JOHN C. KAPPES[1,2]*

Departments of Medicine[1] and Microbiology,[2] University of Alabama at Birmingham, Birmingham, Alabama 35294

Received 3 April 1995/Accepted 25 August 1995

The *vif* gene of human and simian immunodeficiency viruses (HIV and SIV) encodes a late gene product that is essential for viral infectivity in natural target cells. Virions produced in the absence of Vif are abnormal in their ultrastructural morphology and are severely impaired in the ability to complete proviral DNA synthesis upon entry into new target cells. Because previous studies failed to detect Vif protein in virus particles, Vif is believed to influence virus infectivity indirectly, by affecting virion assembly, release, and/or maturation. In this report, we reexamined the possibility that Vif is a virion-associated protein. Utilizing high-titer Vif-specific antibodies, a sensitive immunoblot technique, and highly concentrated virus preparations, we detected a 23-kDa Vif-reactive protein in wild-type HIV type 1 (HIV-1) and a 27-kDa Vif-reactive protein in wild-type SIV_{SM} virions. Neither protein was present in virions derived from *vif*-deficient HIV-1 and SIV_{SM} proviral constructs. Vif protein content was similar among different strains of HIV-1 and was independent of the cell type (permissive or nonpermissive) used to produce the virus. To determine the subvirion localization of Vif, HIV-1 virions were treated with proteinase K or Triton X-100 to remove virion surface proteins and the viral membrane, respectively, purified through sucrose, and analyzed by immunoblot analysis. Vif protein content was not affected by the removal of external surface proteins or by the removal of the viral membrane and submembrane $p17^{Gag}$ matrix protein. Instead, Vif colocalized with viral core structures which sedimented at a density of 1.25 g/ml on linear sucrose gradients (enveloped HIV-1 particles sediment at a density of 1.17 g/ml). Finally, the amount of Vif protein packaged into virions was estimated to be on the order of 1 molecule of Vif for every 20 to 30 molecules of $p24^{Gag}$, or between 60 and 100 molecules of Vif per particle. These results indicate that Vif represents an integral component of HIV and SIV particles and raise the possibility that it plays a direct role in early replication events.

Primate lentiviruses encode a number of auxiliary proteins which are not absolutely necessary for growth in vitro but are essential for viral replication and pathogenicity in vivo (for reviews, see references 15, 18, 43, and 44). One of these, termed Vif, has long been known to represent an important determinant of viral infectivity, particularly in natural target cells (10, 12, 13, 23, 36, 37, 39, 42). The HIV-1 *vif* gene is expressed in vitro (3) and in vivo (21, 26), and its reading frame has been found intact in the majority of proviruses characterized directly in primary (uncultured) patient tissue (40, 46). This finding, along with its conservation in the genome of most lentiviruses (30), including all known primate lentiviruses (18), strongly suggests an important role for Vif in lentiviral biology. Numerous studies have reported an influence of Vif on the replicative capacity of human and simian immunodeficiency viruses (HIV and SIV) in various target cells. Vif is known to increase viral infectivity, although the magnitude of this effect is dependent on the cell type that is producing the virus (5, 8, 12, 32, 33, 36, 41, 45). For example, Vif is absolutely required for virus growth in peripheral blood lymphocytes and macrophages (1, 6, 8, 12, 13, 45). By contrast, established T-cell lines vary in the ability to support replication of *vif*-deficient mutants (5, 12, 36). Cell lines have been classified as nonpermissive (e.g., H9) if they require Vif to support HIV type 1 (HIV-1) replication, semipermissive (e.g., SupT1 and CEMx174) if they allow replication of *vif*-deficient HIV-1 at intermediate levels, and permissive (e.g., HeLa, COS, and C8166) if they replicate *vif*-deficient HIV-1 to wild-type levels (5). The existence of cell types that support a productive infection of *vif*-deficient viruses suggests that Vif can be complemented by (or eliminates the negative effect of) certain cellular factors.

Although the precise mechanism of Vif action remains unknown, current evidence suggests that Vif acts late in the replication cycle at the time of particle formation. When produced from permissive cells, Vif-minus virions are able to enter new target cells (including nonpermissive ones) efficiently, complete proviral DNA synthesis, integrate into the host cell genome, express wild-type amounts of genomic and subgenomic messages, and produce normal quantities of progeny virions (6). In contrast, when produced from nonpermissive cells, Vif-minus virions have an abnormal protein profile (5, 36) and altered ultrastructural morphology (5, 19) and are severely impaired in the ability to synthesize proviral DNA upon infection of new target cells (5, 41, 45). Vif-dependent loss of infectivity can be rescued by expression of Vif protein in *trans* (4, 45). However, for successful complementation to occur, Vif must be expressed in the producer cell rather than the target cell (45). Finally, Vif protein has been reported to associate with the cytoplasmic side of cellular membranes, suggesting a role at the cell surface during particle formation (16). In the absence of evidence indicating Vif incorporation into virions (37, 45), these data have been interpreted to indicate

* Corresponding author. Mailing address: University of Alabama at Birmingham, Department of Medicine, 701 19th St. South, LHRB 639, Birmingham, AL 35294. Phone: (205) 934-0051. Fax: (205) 975-7300.

that Vif is required for the proper incorporation, processing, and/or assembly of virion proteins and thus influences virus infectivity only indirectly.

The experimental basis for concluding that Vif is not associated with HIV or SIV particles derives from only few studies (37, 45). More recently, two studies have provided suggestive evidence that very small amounts of Vif may be present in virions (5, 50). However, these studies did not distinguish between specific and unspecific particle association (e.g., contamination of virus pellets with Vif-containing vesicles). Because the Vif-minus phenotype (abnormal assembly of the viral nucleoprotein core, reduced proviral DNA synthesis upon entry of new cells, and ability to complement Vif in the producer but not the target cell) could also be explained by a more direct role for Vif in virion assembly, morphogenesis, and early infection events, we elected to reexamine the possibility that Vif is a virion-associated protein. Using high-titer Vif-specific antibodies (16) and sensitive immunodetection techniques, we found that Vif is indeed packaged into HIV-1 and SIV_{SM} particles. Incorporation of Vif was specific, occurred in quantities comparable to the viral *pol* gene products, and was not due to an association with viral surface proteins or the viral membrane. Most importantly, virion subfractionation studies showed that Vif is associated with the purified viral core. These results are in agreement with the phenotypic characteristics of *vif*-minus viruses, in particular with the reported differences in nucleoprotein core morphology (19), and raise the possibility of a direct function for Vif in early replication events.

MATERIALS AND METHODS

Cells and viruses. HeLa-tat (HLtat) cultures were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. These cells express the first exon of HIV-1 *tat* constitutively (9) and were kindly provided by B. Felber and G. Pavlakis. SupT1 and CEMx174 immortalized T-cell lines were maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 μg of gentamicin per ml, and 15% fetal bovine serum (complete medium). Human peripheral blood mononuclear cells (PBMCs) were obtained from healthy adult volunteers by Ficoll-Hypaque density gradient centrifugation as described previously (35). PBMCs were stimulated with phytohemagglutinin (2 μg/ml) for 24 h, washed free of lectin, and maintained in complete medium supplemented with 30 U of interleukin-2 (Boehringer Biologicals) per ml.

Infectious molecular clones of $HIV\text{-}1_{YU\text{-}2}$ (cloned from uncultured human brain; see references 27 and 28 for details), $HIV\text{-}1_{SG3}$ (cloned from productively infected HUT 78 cells [14]), and SIV_{SM}PBj1.9 (cloned from short-term-infected PBMC DNA [7]) have been described elsewhere. Virus derived from $HIV\text{-}1_{SG3}$ replicates to very high titers both in immortalized T-cell lines and in PBMCs (14). Virus derived from SIV_{SM}PBj1.9 replicates well in human and macaque PBMCs and causes an acute illness in experimentally infected pigtailed macaques similar to primary HIV-1 infection (20). Virus derived from $HIV\text{-}1_{YU\text{-}2}$ replicates well in primary lymphocytes and macrophages but not in established T-cell lines (27). Both SG3 and YU-2 genomes lack a functional *vpu* gene (YU-2 lacks the *vpu* initiation codon, and SG3 contains a 23-bp deletion in the *vpu* open reading frame). The PBj1.9 genome contains uninterrupted open reading frames for all proviral genes.

To obtain a genomically intact YU-2 clone, PCR mutagenesis was performed to reinstate the *vpu* initiation codon. A 458-bp fragment including the *vpu* gene was amplified by using a pair of sense (5′-TAGTACATGTAATGCAATCTTTACAAG-3′) and antisense (5′-CCACATGTTAAAATTTTCTGT-3′) primers. The sense primer contained an A at position 5613 (underlined) which restored the *vpu* initiation codon (CTG to ATG). The mutagenized fragment was reintroduced into the YU-2 provirus, resulting in a proviral clone, termed pYU-2C, which differs from pYU-2 by only a single base pair. This was confirmed by sequence analysis of pYU-2C.

Construction of *vif*-deficient HIV-1 and SIV_{SM} proviruses. PCR mutagenesis was used to generate a *vif*-deficient YU-2C provirus. Briefly, a 566-bp (*vif*-containing) DNA fragment was amplified from pYU-2 by using sense (5′-TCACGAGCTCGCACCATATGTAGATTTAAGGGA-3′) and antisense (5′-AAGCCATGTAGCCATGGCCTAGGA-3′) primers. The sense primer introduced a G (instead of a T) at position 4679 and an A (instead of a C) at position 4684 (underlined), generating two in-frame stop codons within the *vif* open reading frame at amino acid positions 30 (TAT to TAG) and 32 (TCA to TAA) (Fig. 1A). The mutagenized fragment was reinserted into the YU-2C genome by using a series of different subcloning steps, generating $pYU\text{-}2C^{vif-}$.

Site-directed mutagenesis of the SIV_{SM}PBj1.9 *vif* gene was accomplished by using a Muta-Gene kit (Bio-Rad). Briefly, an internal 4.5-kb *Eco*RI fragment was excised from the PBj1.9 genome and ligated into pTZ19. An internal *Bam*HI-*Sst*I (*vif*-containing) DNA fragment was then subcloned into M13 and mutagenized to abrogate Vif translation. Two mutagenic primers were used. One (antisense, 5′-CCACTATCTAATCCTTTTCCTCCTCCGTAGTCTTT-3′) introduced a C (instead of a T) at position 5108 and an A (instead of a G) at position 5126; the other (antisense, 5′-TTCAGGTGTTAGATGAGGCTA-3′) changed an A to a T at position 5185 (Fig. 1B). These substitutions changed the Met initiation codon to a Thr and introduced in-frame translational stop codons at positions 5125 and 5185. None of the introduced nucleotide substitutions resulted in amino acid changes in the overlapping *pol* open reading frame (Fig. 1B). The mutagenized DNA fragment was ligated back into the pTZ19 shuttle vector, and the 4.5-kb *Eco*RI fragment was the ligated back into the PBj1.9 provirus, generating $pPBJ1.9^{vif-}$. Both YU-2C and PBj1.9 *vif* mutations were confirmed by DNA sequence analysis.

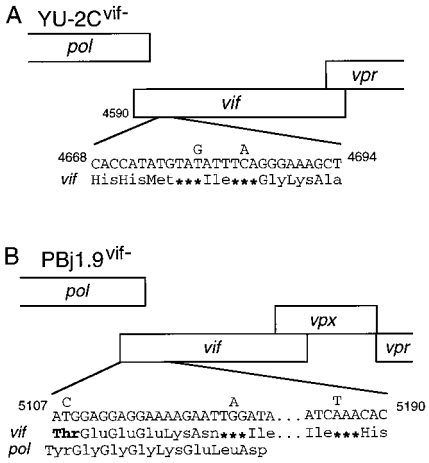

FIG. 1. Construction of *vif*-deficient HIV-1 and SIV_{SM} proviruses. The position of the *vif* open reading frame in the genome of HIV-1 and SIV_{SM} is indicated. Mutagenesis of the YU-2C and PBj1.9 *vif* open reading frames was performed as described in Materials and Methods. Nucleotide substitutions introduced to create translational stop codons (asterisks) are shown above the wild-type sequence. Sequences are numbered according to database entries (7, 14).

Antibodies. Antibodies used in this study included monoclonal antibodies reactive with the $p24^{Gag}$ protein of HIV-1 (ATC1; obtained from a hybridoma cell line purchased from the American Tissue Culture Collection) and the $p27^{Gag}$ protein of HIV-2 (29, 47). Polyclonal and monoclonal antibodies were also obtained from the AIDS Research and Reference Reagent Program. These included anti-HIV-1 (HXB2) Vif antibody 2221 (contributed By D. Gabuzda), anti-HIV-1 reverse transcriptase (RT) antibody 634 (contributed by the Division of AIDS), anti-HIV-1 $p17^{Gag}$ antibody 286 (contributed by M. Phelan), and anti-HIV-1 gp41 antibody 1242 (contributed by S. Zolla-Pazner).

HIV-2 and SIV_{SM} Vif-specific antibodies were raised against a synthetic oligopeptide. A 21-mer (MEEDKRWIVVPTWRVPGRMEK) corresponding to a region of predicted protein antigenicity in the Vif protein of $HIV\text{-}2_{ROD}$ (coordinates 4869 to 4931 [17]) was synthesized on a model 430 peptide synthesizer (Applied Biosystems, Inc.). The oligopeptide was coupled to keyhole limpet hemocyanin in a glutaraldehyde single-step coupling procedure and used to immunize New Zealand White female rabbits subcutaneously with 200 μg of peptide emulsified in an equal volume of Freund's complete adjuvant. Rabbits were boosted at 2-week intervals with 200 μg of coupled peptide mixed with an equal volume of Freund's incomplete adjuvant. Sera were titered by enzyme-linked immunosorbent assay (ELISA), using an uncoupled oligopeptide, and collected at peak titers for analysis.

Transfection and virus purification. Transfections of proviral clones were performed on 40 to 60% confluent HLtat cells by the calcium phosphate DNA precipitation method as recommended by the manufacturer (Stratagene). Sixty hours after transfection, culture supernatants were collected, clarified by low-speed centrifugation (1,000 × *g*, 10 min), and filtered through a 1.2-μm-pore-size filter. Supernatants from productively infected cells were prepared similarly. Virions were concentrated by ultracentrifugation (125,000 × *g*, 2 h) through 20% sucrose. Viral pellets were solubilized for immunoblot analysis or resuspended in TNE buffer (10 mM Tris-HCl [pH 7.2], 100 mM NaCl, 1 mM EDTA) for sucrose gradient analysis. Sucrose-purified particle preparations were quantified by RT analysis or p24 antigen capture.

Proteinase K digestion of viral surface proteins. Supernatants (360 ml) from SupT1 cells productively infected with $HIV\text{-}1_{SG3}$ (2.6×10^7 RT counts/ml) were pelleted through cushions of 20% sucrose and resuspended in proteinase K

digestion buffer (40 mM Tris-HCl [pH 8.0], 2 mM $CaCl_2$). One half of the virus preparation was adjusted with proteinase K (Gibco/BRL) to a final concentration of 1 mg/ml, while the other was diluted with equivalent volumes of digestion buffer. After incubation at 22°C for 18 h, the proteinase inhibitor phenylmethylsulfonyl fluoride was added (5 mg/ml, final concentration), and both aliquots were centrifuged again through cushions of 20% sucrose. Lysates of both proteinase-treated and untreated virion preparations were analyzed by immunoblot analysis.

Preparation of viral core structures. Core structures of HIV-1 were prepared by methods previously described for the purification of SIV_{MAC} cores (49). Briefly, culture supernatants (500 ml) from SupT1 cells productively infected with HIV-1_{SG3} (1.4×10^7 RT counts/ml) were pelleted through 20% sucrose and dissolved in TNE buffer (800 μl) by continuous mixing for 2 h at 4°C. To remove the viral membrane, one aliquot (400 μl) was treated with Triton X-100 at a final concentration of 0.5% at 20°C for 8 min, diluted in TNE, and pelleted through 50% sucrose (to remove any enveloped particles that may have remained). The other aliquot was mock treated in TNE. Both detergent-treated and untreated particle preparations were then diluted with TNE and centrifuged (100,000 × *g*, 18 h, 4°C) over linear gradients of sucrose prepared by mixing equal volumes of 30% (wt/vol) sucrose dissolved in water and 70% (wt/vol) sucrose dissolved in deuterium oxide (Sigma). Fourteen fractions were collected from the bottom of each tube, diluted in phosphate-buffered saline (PBS), pelleted by ultracentrifugation, and examined for protein content by immunoblot analysis. The density of each fraction was determined with a refractometer.

Western blot (immunoblot) analysis. Virions were concentrated from the supernatants of transfected or infected cells by ultracentrifugation through 20% sucrose (125,000 × *g*, 2 h, 4°C). Infected cells and virus pellets were solubilized in loading buffer (62.5 mM Tris-HCl [pH 6.8], 0.2% sodium lauryl sulfate [SDS], 5% 2-mercaptoethanol, 10% glycerol), boiled for 5 min, and separated on 12.5% polyacrylamide gels containing SDS. Following electrophoresis, proteins were transferred to nitrocellulose (0.2-μm pore size; Schleicher & Schuell) by electroblotting, incubated for 1 h at room temperature in blocking buffer (5% nonfat dry milk in PBS), and then incubated for 2 h with the appropriate antibodies diluted in blocking buffer (the anti-HIV-1 Vif antiserum was used at a dilution of 1:1,000; the anti-SIV_{SM} Vif antiserum was used at a dilution of 1:100). Protein bound antibodies were detected with horseradish peroxidase-conjugated specific secondary antibodies, washed extensively, and developed by using the highly sensitive ECL (enhanced chemiluminescence) detection system (Amersham).

Quantitation of virion-associated Vif protein. Dilution panels (external standards) were prepared from two different sources of recombinant Vif protein, including a histidine-tagged, nickel column-purified recombinant Vif protein provided by Dana Gabudzda (11) and a commercially available preparation of *Escherichia coli*-expressed Vif obtained from Intracel (Cambridge, Mass.). Also used as an external standard was a dilution panel of highly purified p51 RT protein (provided by Jacob Lebowitz [25]). Protein concentrations and purity (>95%) were confirmed in two independent laboratories. SG3 virions were produced by SupT1 cells, banded on a 20 to 60% linear gradient of sucrose, and quantitated by p24 antigen capture ELISA (Coulter, Hialeah, Fla.). Western blot band intensities were determined on a Lynx 5000 molecular biology work station with matched custom software (Applied Imaging, Santa Clara, Calif.).

RESULTS

Detection of Vif protein in HIV-1 particles. Concentrated preparations of HIV-1 virions were prepared from SupT1 cells productively infected with HIV-1_{SG3}. We chose this particular strain of HIV-1, since it replicates to high titers in both PBMCs and immortalized T-cell lines (14). HIV-1_{SG3} proviral DNA (pSG3) was transfected into HLtat cells, and infectious virus was transmitted to SupT1 cells by cocultivation. Virus replication was monitored by RT analysis, and at peak titers (2×10^7 cpm of RT activity per ml), culture supernatants were collected and clarified by low-speed centrifugation (1,000 × *g*, 15 min). Virus was then concentrated by ultracentrifugation through 20% sucrose (125,000 × *g*, 2 h, 4°C), resuspended in electrophoresis buffer, and examined for the presence of Vif and Gag proteins by immunoblot analysis. Infected cell lysates were analyzed in parallel. Using an anti-Vif antibody raised against bacterially expressed HXB2 Vif protein (16) and the highly sensitive ECL detection method, we detected a 23-kDa protein in both infected cells and purified virions (Fig. 2A). Gag proteins were detected in cell and viral lysates with an anti-$p24^{Gag}$ monoclonal antibody (Fig. 2B; the lanes probed with the anti-Vif antibody contain five times more lysate than those probed with anti-Gag antibody). These results demonstrated the presence of a 23-kDa Vif-reactive protein in infected cell lysates and sucrose-purified HIV-1 particles and suggested that the relative proportion of cell-associated Vif packaged into virions was less than that of $p24^{Gag}$.

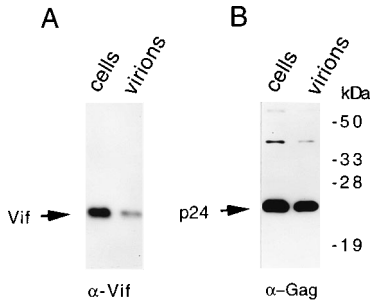

FIG. 2. Vif protein in HIV-1-infected cells and cell-free virions. HIV-1_{SG3} virions from productively infected SupT1 cultures were concentrated by ultracentrifugation through 20% sucrose. Transfected cells and pelleted virions were lysed in electrophoresis buffer and examined by immunoblot analysis. Replica blots were reacted with either an anti-Vif antibody (A) or an anti-Gag antibody (B). Bound antibodies were visualized by the ECL method (Amersham).

To confirm that Vif was indeed packaged into HIV-1 virions and not just simply cosedimenting with the pelleted material, immunoblots were performed on banded virus preparations collected from continuous sucrose density gradients. For these experiments, HIV-1_{SG3} virions were pelleted from supernatants (50 ml) of infected SupT1 cultures (3×10^7 cpm of RT activity per ml), resuspended in 800 μl of PBS, loaded onto continuous gradients of 20 to 60% sucrose, and centrifuged overnight (100,000 × *g*, 18 h, 4°C). Fractions were collected, concentrated by ultracentrifugation (125,000 × *g*; 1 h), and analyzed by immunoblotting (Fig. 3). Replica blots were probed independently with anti-Gag (Fig. 3A) and anti-Vif (Fig. 3B) antibodies. The results demonstrated peak concentrations of both Gag and Vif proteins in the same fractions at a specific gravity of 1.17 g/ml, suggesting a direct association of Vif protein with virus particles.

Detection of Vif protein in HIV-1 virions derived from permissive and nonpermissive producer cells. To examine whether the association of Vif protein with virions was influenced by the cells in which the virus was propagated, three different cell types were used to produce infectious HIV-1_{SG3}. At peak levels of virus replication (as determined by RT assay), virions were concentrated from the culture supernatants of infected phytohemagglutinin-stimulated normal human PBMCs and SupT1 cells, and transfected HLtat cells by ultracentrifugation through 20% sucrose. Pellets were lysed, and equal concentrations of virions (RT equivalents) were examined by immunoblot analysis (Fig. 4). Vif protein was detected in similar quantities in viral pellets derived from permissive HLtat cultures, semipermissive SupT1 cultures, and nonpermissive PBMC cultures (Fig. 4A). Similar amounts of p24 Gag were detected for all virus preparations (Fig. 4B; the lanes probed with the anti-Vif-antibody contain five times more viral lysate than those probed with the anti-Gag antibody). These results indicate that Vif protein is incorporated into virions in similar amounts relative to Gag, independent of the producing cell type.

Incorporation of Vif protein into virions derived from different HIV-1 strains. Our results indicated efficient incorporation of Vif protein into HIV-1_{SG3} virions. Since this provirus was cloned after continuous passage in an established T-cell line and contains a defective *vpu* gene (14), we wanted to confirm virion association of Vif in a second, biologically relevant virus strain. The YU-2C strain was thus selected for comparison, since it was cloned without interim tissue culture

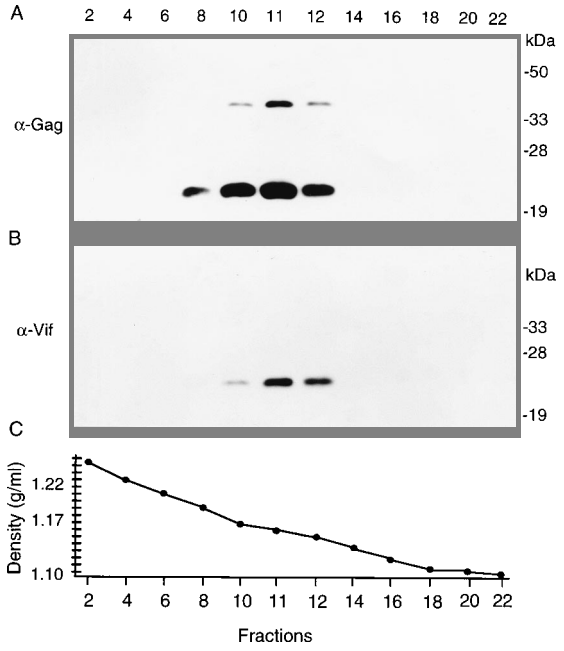

FIG. 3. Vif protein content of particles purified by sedimentation over continuous sucrose gradients. HIV-1_{SG3} virions were concentrated from supernatants (50 ml) of productively infected SupT1 cells (3×10^7 RT counts per ml) by ultracentrifugation through 20% sucrose. Pellets were resuspended in PBS, layered over a 20 to 60% linear gradient of sucrose, and centrifuged for 18 h at 100,000 × *g* in an SW41 Beckman rotor. Fractions (0.5 ml) were collected from the bottom of the tube, diluted, pelleted a second time by ultracentrifugation in a TL45 Beckman rotor (125,000 × *g*, 1 h, at 4°C), and lysed in electrophoresis buffer for immunoblot analysis. Replica blot were probed separately with anti-Gag (A) and anti-Vif (B) antibodies. Fraction 2 represent the second collection from the bottom of the gradient, and fraction 22 represents the last. (C) Fractions were also analyzed for density with a refractometer.

directly from infected human brain (27, 28) and since it contains a complete set of accessory genes after restoration of its *vpu* initiation codon (see Materials and Methods). For control, a *vif*-deficient mutant of YU-2C and wild-type SG3 were analyzed in parallel. Proviruses were transfected into HLtat cells, supernatant was collected 60 h later, and virions were examined by immunoblot analysis following concentration through 20% sucrose cushions (Fig. 5). Replica blots were probed separately with anti-Vif (Fig. 5A) and anti-Gag (Fig. 5B) antibodies. Vif protein was detected in pellets derived from pYU-2C and SG3 but not in similarly prepared pellets of pYU-$2C^{vif-}$. Similar amounts of Gag protein were detected for the three different viruses, confirming equivalent numbers of particles

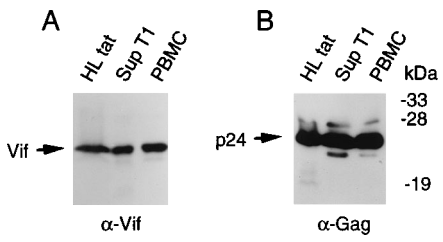

FIG. 4. Vif protein content of HIV-1 produced by different cell types. HIV-1_{SG3} virions were pelleted from the culture supernatants of transfected HLtat cells and productively infected SupT1 cells and phytohemagglutinin-stimulated normal human PBMCs. Equal numbers of virions (RT equivalents) derived from each cell type were examined by immunoblot analysis. Replica blots were probed separately with anti-Vif (A) and anti-Gag (B) antibodies as described in the legend to Fig. 2.

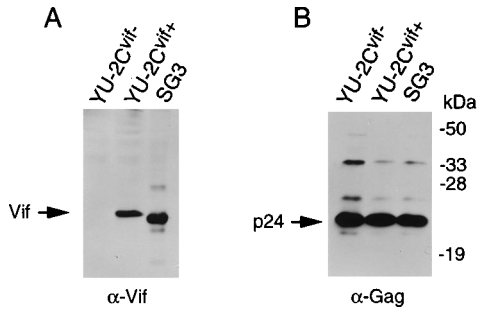

FIG. 5. Detection of Vif protein in HIV-1_{YU-2C} virions. HLtat cells were transfected with the pYU-$2C^{Vif-}$, pYU-2C, and pSG3 proviruses. Sixty hours later, virions were concentrated from culture supernatants by ultracentrifugation through 20% sucrose. Pellets were solubilized in electrophoresis buffer and examined by immunoblot analysis. Replica blots were probed with either an anti-Vif (A) or an anti-Gag (B) antibodies as described in the legend to Fig. 2.

(Fig. 5B). These results indicate that different strains of HIV-1, including viruses present in vivo, package Vif in similar quantities.

Detection of Vif protein in SIV_{SM} virions. The *vif* gene is conserved in all primate lentiviral lineages, and its function is thought to be similar to that of HIV-1 (30, 32, 33, 38). To determine whether the Vif protein of HIV-2 and SIV_{SM} strains is also incorporated into virions, we examined wild-type and *vif*-deficient SIV_{SM}PBj1.9 particles. Proviral DNAs were transfected into HLtat cells, and progeny virions were pelleted through sucrose cushions and examined by immunoblot analysis. Transfection-derived preparations of wild-type and *vif*-minus PBj1.9 were also transmitted to CEMx174 cells, propagated to high titers, and concentrated for immunoblot analysis (Fig. 6). Replica blots were probed with a Vif-specific antibody raised against a synthetic oligopeptide and a monoclonal antibody reactive with HIV-2 Gag (Fig. 6). A 27-kDa protein was detected in viral pellets of wild-type PBj1.9 derived from both HLtat (Fig. 6A) and CEMx174 (Fig. 6B) cells but not in virions produced from *vif*-mutant PBj1.9. Comparable amounts of p27 Gag antigen confirmed equivalent amounts of wild-type and mutant viruses. These results suggest that the ability to package Vif protein is a general property of primate lentiviruses.

Association of HIV-1 Vif protein with viral core structures. To exclude the possibility that Vif might nonspecifically adsorb

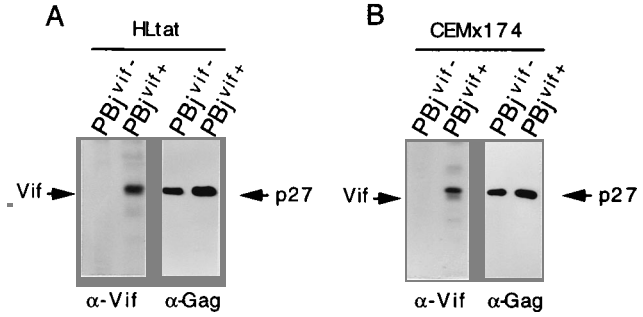

FIG. 6. Detection of Vif protein in SIV_{SM} virions. HLtat cells were transfected with wild-type and *vif*-deficient SIV_{SM}PBj1.9 proviruses. Sixty hours later, progeny virions were concentrated from culture supernatants by ultracentrifugation through 20% sucrose (A). Vif-plus and Vif-minus PBj1.9 virions were also concentrated from the supernatants of productively infected CEMx174 cells (B). Pellets were lysed in electrophoresis buffer and analyzed by immunoblot analysis. Replica blots were probed with anti-Vif and anti-Gag antibodies.

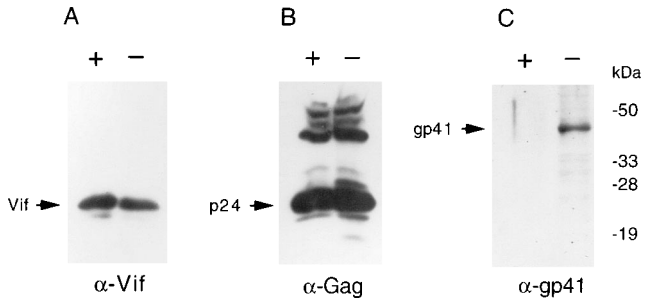

FIG. 7. Detection of Vif protein in proteinase K-treated virions. HIV-1_{SG3} virions were pelleted from the culture supernatants of productively infected SupT1 cells by ultracentrifugation through 20% sucrose. Pellets were resuspended in proteinase K digestion buffer (40 mM Tris-HCl [pH 8.0], 2 mM $CaCl_2$) containing either 1 (+) or 0 (−) mg of proteinase K per ml. After incubation at 22°C for 18 h, the protease inhibitor phenylmethylsulfonyl fluoride (5 mg/ml, final concentration) was added, and both samples were centrifuged through 20% sucrose. Pellets were lysed and examined by immunoblot analysis. Replica blots were probed separately with anti-Vif (A), anti-Gag (B), and anti-gp41 (C) antibodies.

to the outer surface of HIV-1 virions, we determined its subvirion localization. HIV-1_{SG3} virions, derived from productively infected SupT1 cultures, were pelleted through 20% sucrose and resuspended in proteinase digestion buffer, and one half of the preparation was treated with proteinase K (final concentration of 1 mg/ml). Because HIV-1 particles contain a lipid bilayer, structural components inside this membrane are protected from proteolytic digestion, whereas those on the outer surface such as gp120 or the exterior portion of gp41 are not. Following incubation at 22°C for 18 h, virions were treated with a protease inhibitor (phenylmethylsulfonyl fluoride; 5 mg/ml), pelleted through 20% sucrose, and lysed for immunoblot analysis. Non-proteinase K-treated SG3 particles were prepared in parallel. The protein profiles of treated and untreated virions, analyzed on replica blots with anti-Vif, anti-$p24^{Gag}$, and anti-gp41 antibodies, are depicted in Fig. 7.

The viral transmembrane protein, gp41, was detected in untreated but not in proteinase K-treated virions (Fig. 7C). Since the monoclonal antibody used in this experiment reacts with an epitope on the exterior portion of gp41 (34, 48), these results indicated that proteinase K digestion had indeed removed proteins from the surface of virions. As expected, anti-$p24^{Gag}$ antibodies detected Gag proteins in both treated and untreated preparations with comparable intensities (Fig. 7B). This result confirmed the integrity of the proteinase K-treated virions and indicated that blots contained equivalent numbers of particles. Finally, anti-Vif antibodies detected similar quantities of Vif protein in both treated and untreated preparations (Fig. 7A). This result suggested that Vif (like Gag) is protected from proteinase K digestion and must therefore be located inside the viral envelope.

To further explore the localization of Vif within the HIV-1 particle and to exclude the possibility that Vif-containing vesicles could copurify with virions, we examined whether Vif protein would remain particle associated after solubilization of the virion lipid bilayer. HIV-1_{SG3} was propagated in SupT1 cells and concentrated from 500 ml of culture supernatants at peak virus production (1.4×10^7 RT counts/ml). Pelleted virions were resuspended in TNE and treated with Triton X-100 at a final concentration of 0.5% for 8 min at room temperature. Virus was then separated into two equal aliquots. One aliquot was centrifuged (125,000 × *g*, 2 h) through 20% sucrose, while the other was centrifuged similarly through 50% sucrose. For controls, equal amounts (as determined by RT activity) of non-Triton X-100-treated virions were prepared in parallel. Following centrifugation, viral pellets were solubilized in electrophoresis buffer and examined for differences in their protein profiles by using anti-Vif, anti-$p24^{Gag}$, anti-RT, and anti-$p17^{Gag}$ (matrix) antibodies.

Figure 8A depicts replica blots of detergent-treated and untreated virions following centrifugation through 20% sucrose. The results revealed virtually identical Vif, RT, and Gag reactivities, indicating similar quantities of each of these proteins in wild-type and membrane-denuded core structures. By contrast, matrix protein reactivity was significantly reduced in Triton X-100-treated virions, suggesting that removal of the viral membrane had led to the partial removal of the submembrane matrix protein as well. This inference was further confirmed by analysis of immunoblots prepared following centrifugation through cushions of 50% sucrose, which enriches for viral core structures relative to enveloped particles (Fig. 8B). Enveloped HIV-1 virions sediment in sucrose gradients at 1.15 to 1.17 g/ml, while viral cores are much denser and sediment at approximately 1.25 g/ml (49). Accordingly, centrifugation through 50% sucrose resulted in a significant loss of Vif, Gag, and RT reactivities in untreated virions but not in detergent-treated virions, which, because of their higher density, pelleted to the bottom of the tube (Fig. 8B).

As a final confirmation of the association of Vif with viral core structures, Vif protein content of Triton X-100-treated virions banded over continuous sucrose gradients was determined. SG3 virions were concentrated from culture supernatants of infected SupT1 cells as described above, treated with Triton X-100, pelleted through 50% sucrose to enrich for core

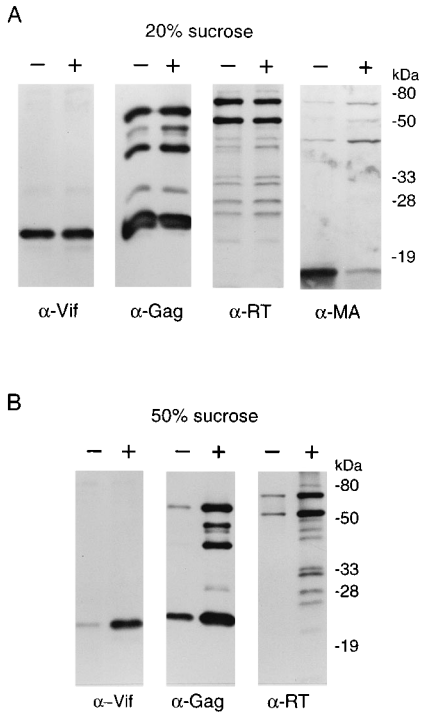

FIG. 8. Detection of Vif protein in HIV-1 virions treated with Triton X-100. HIV-1_{SG3} virions were collected from the culture supernatants of SupT1 cells and pelleted through 20% sucrose. Pellets were resuspended in TNE buffer and aliquoted into two tubes. One aliquot was treated with 0.5% Triton X-100 (+) at 20°C for 8 min; for control, the other was not treated (−). Each aliquot was separated into two tubes and centrifuged over 20% (A) or 50% (B) sucrose. Pellets were lysed and examined by immunoblot analysis. Replica blots were probed with anti-Vif, anti-Gag, anti-RT, and anti-matrix (MA) antibodies.

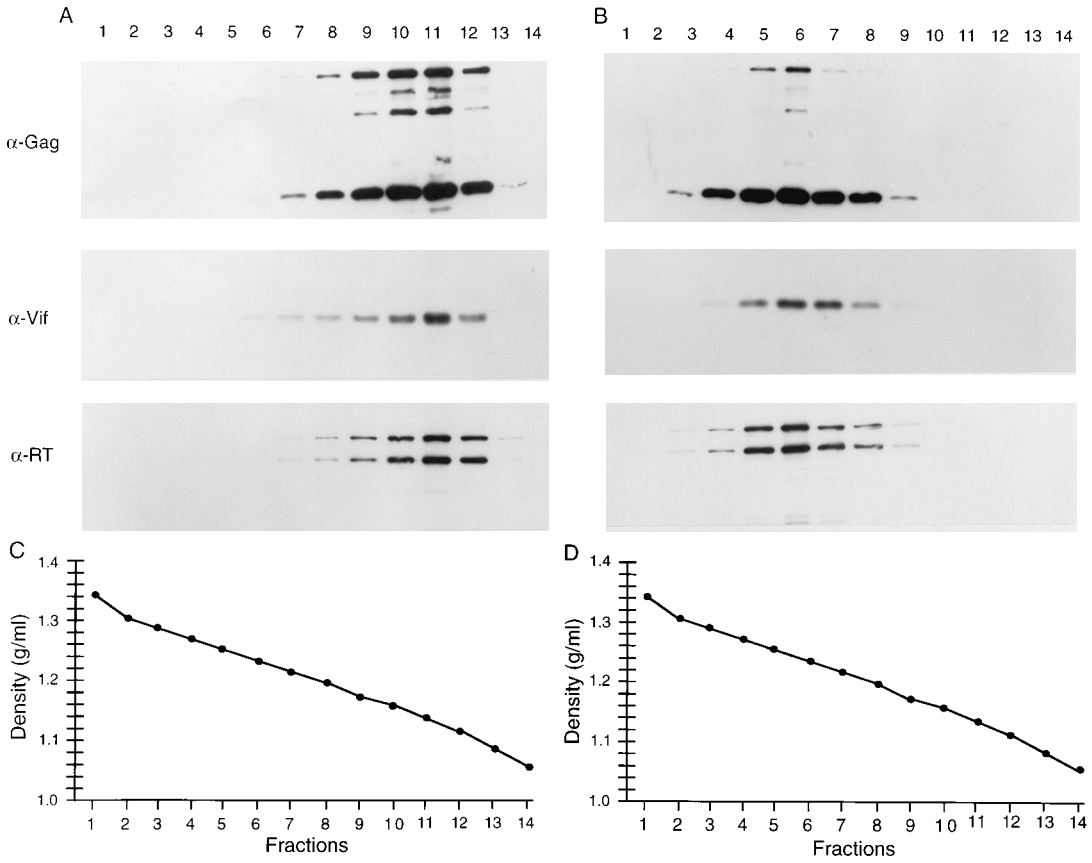

FIG. 9. Sucrose gradient analysis of HIV-1 virions treated with Triton X-100. HIV-1_{SG3} virions were collected from the culture supernatants of SupT1 cells and pelleted through cushions of 20% sucrose. Pellets were resuspended in TNE buffer and aliquoted into two tubes. One aliquot was treated with 0.5% (final concentration) Triton X-100 at 20°C for 8 min and then centrifuged through 50% sucrose to enrich for viral core structures. The other aliquot was mock treated for control. Both untreated (A and C) and treated (B and D) preparations were centrifuged (100,000 × *g*, 18 h, 4°C) over linear gradients of sucrose prepared by mixing 4 ml of 30% (wt/vol) sucrose in water with 4 ml of 70% (wt/vol) sucrose in deuterium oxide (Sigma). Fourteen fractions were collected from the bottom of each tube. Each fraction was diluted with PBS, pelleted again in a Beckman TL45 rotor, and examined by immunoblot analysis (A and B). Replica blots were probed with anti-Vif, anti-Gag, and anti-RT antibodies. Sucrose fractions were also analyzed for density with a refractometer (C and D).

structures, and centrifuged (100,000 × *g*, 18 h, 4°C) through a linear gradient of 30 to 70% sucrose dissolved in deuterium oxide to ensure solubility. Identical quantities of untreated SG3 virions were analyzed in parallel. Fractions were collected, diluted in TNE, and centrifuged again to concentrate virions. Pellets were solubilized and examined by immunoblot analysis (Fig. 9). Replica blots were probed separately with anti-Vif, anti-Gag, and anti-RT antibodies. For untreated virions, peak concentrations of Gag and RT proteins were detected in fractions 10 and 11 (Fig. 9A), corresponding to a density of 1.15 to 1.16 g/ml. For detergent-treated virions, peak concentrations of Gag and RT shifted closer to the bottom of the gradient (fractions 5 and 6), corresponding to a density of 1.24 to 1.25 g/ml. This result confirmed that Triton X-100 treatment had resulted in the removal of the viral envelope and that Vif protein colocalized with Gag and RT proteins in purified HIV-1 core structures.

Quantitation of Vif protein in virions. To determine how much Vif was packaged into HIV-1 particles, we prepared dilution panels of recombinant Vif protein and used them as external standards to quantify Vif in sucrose-banded virion preparations by Western blot analysis (Fig. 10). We used two independent recombinant Vif protein preparations, which gave virtually identical results (Fig. 10B depicts results obtained with prokaryotically expressed Vif kindly provided by Dana Gabuzda). Their concentrations and purity (>95% by Coomassie blue gel staining) were confirmed. HIV-1_{SG3} virions were derived from productively infected SupT1 cultures, banded on a 20 to 60% linear gradient of sucrose, and quantified by p24 antigen capture ELISA (Coulter). Virions from a single gradient fraction (at peak p24 concentration) were then used for all subsequent Western blot analyses (Fig. 10).

Both sucrose-banded SG3 virions and recombinant Vif protein were serially diluted, run on the same SDS-polyacrylamide gel, and electroblotted onto nitrocellulose (Fig. 10A). Following probing with an anti-Vif antibody, immunoblot intensities of the Vif dilution panel (ranging from 0.3 to 10 ng of purified Vif) were quantified on a Lynx densitometer (Applied Imaging) and used to plot a standard curve (Fig. 10B). This graph was then used to determine the amount of Vif protein present in different dilutions of sucrose-banded virions. Using only values within the linear signal range of the ECL detection system, we determined that 4.3, 1.7, and 0.8 ng of Vif correspond to 100, 50, and 25 ng of virion-associated p24 Gag, respectively (Fig. 10B). Since Vif and p24 have similar molecular weights, these values indicate a packaging ratio of 20 to 30 molecules of p24 Gag for each molecule of Vif, or between 60 to 100 molecules of Vif per virus particle (assuming approximately 2,000 molecules of p24 per virion [2, 24]).

Because our estimates of virion-associated Vif protein were higher than a previous suggestion (50), we sought to validate our experimental approach by quantifying an HIV-1 protein of

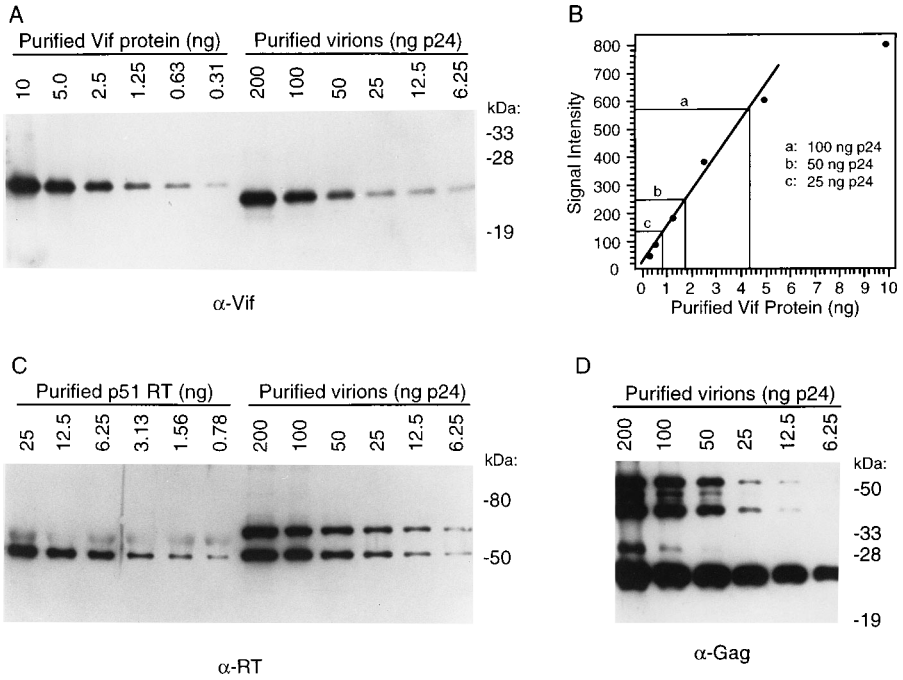

FIG. 10. Quantitation of virion-associated Vif protein. (A) Recombinant Vif protein (obtained from Dana Gabuzda) was serially diluted (10 to 0.31 ng) as an external standard for immunoblots of sucrose-banded HIV-1 virions ranging in concentration between 200 and 6.25 ng of p24 per lane. (B) A standard curve (solid dots and corresponding line) was constructed by plotting known protein concentrations of recombinant Vif standards (*x* axis) against measured immunoblot signal intensities (*y* axis; Vif standards between 0.31 and 5 ng fell within the linear range of the ECL detection system). The Vif contents of virions containing 100, 50, and 25 ng of p24 were then deduced by determining their signal intensities and plotting them against the standard curve. (C) As in panel A, recombinant p51 RT (obtained from Jacob Lebowitz) was serially diluted (25 to 0.78 ng) and used as a standard to quantify the RT content of virions. A standard curve was plotted from p51 RT signal intensities as described for panel B (data not shown). (D) Western blot of the identical preparation of sucrose-banded virions shown in panels A and C probed with an anti-Gag antibody.

known virion content, RT (Fig. 10C). Using a dilution panel of purified recombinant RT (p51 RT [25]), we determined that 4.2, 3.0, and 1.7 ng of p51 RT correspond to 50, 25, and 12.5 ng of particle-associated p24 Gag, respectively (standard curve not shown). Calculating the corresponding numbers of RT and p24 molecules (assuming p51-p66 heterodimers), we determined a ratio of 10 to 20 molecules of p24 Gag for each molecule of RT. These results are in excellent agreement with previously reported estimates of particle-associated RT (24, 31), thereby corroborating our quantitative determinations of Vif. Sucrose-banded SG3 virions were also probed with an anti-Gag antibody (Fig. 10D), which confirmed a normal protein profile with respect to processed and unprocessed Gag proteins. Taken together, these results indicate that Vif is packaged into HIV-1 particles in quantities comparable to (or slightly less than) those of the viral *pol* gene products.

DISCUSSION

Vif is known to influence the replication potential of HIV and SIV by enhancing viral infectivity (10, 12, 13, 23, 32, 33, 36, 37, 39, 42). Since earlier reports failed to demonstrate its presence in the virus particle (37, 45), it has been proposed that Vif affects viral infectivity indirectly through its actions on virion assembly, release, and/or maturation (5, 6). Accordingly, particles assembled in the absence of Vif are believed to be noninfectious because their structural proteins have been either not properly modified before budding from the producer cell or not processed normally subsequent to budding and extracellular release. Because the Vif-minus phenotype could also be due to the absence of Vif from virus particles, we reexamined the possibility that Vif is a virion-associated protein. To increase our chances for Vif detection, we used high-titer Vif-specific antibodies (16), highly concentrated virion preparations (generally on the order of 50 to 200 ng of particle-associated p24 antigen per immunoblot), and the ECL immunoblot detection method, which is at least 10 times more sensitive than most other conventional methods. Using these optimized conditions, we were able to reproducibly detect Vif protein in cell-free virion preparations of two different primate lentiviruses (HIV-1 and SIV_{SM}). The virion-associated HIV-1 and SIV_{SM} Vif proteins were identical in size to their cellular counterparts (23 and 27 kDa, respectively), suggesting that packaging did not result in (or require) protein processing. In addition, the presence of Vif in virions was not the property of any particular virus strain or dependent on the type of producer cell. Comparison of the Vif content in $HIV\text{-}1_{SG3}$ (*vpu*-minus) and $HIV\text{-}1_{YU\text{-}2C}$ (*vpu*-plus) indicated that *vpu* was not required for Vif packaging. Finally, the amount of virion associated Vif protein was estimated to be on the order of 1 Vif molecule per 20 to 30 molecules of p24 Gag, or 60 to 100 molecules per particle.

Because the presence of Vif protein in concentrated virions could have been the result of unspecific adsorption to the viral envelope, we performed virion fractionation studies to exclude this possibility. In a first step, virions were treated with proteinase K to remove surface proteins. To control for this process, we used an antibody specific for an external epitope of gp41 (34, 48). As expected, no gp41 reactivity was detectable in treated virions, while the same protein was readily visible on blots of untreated virus. By contrast, no differences in Gag and Vif reactivities were observed on immunoblots of the same virus preparations. These results suggested that Vif (like Gag) was protected from proteolytic digestion because it is located internal to the viral membrane.

Since Vif has been reported to associate with the inner surface of the lipid bilayer (16), we also considered that Vif may have been detected in association with virions because it was present in the viral membrane. Moreover, the presence of Vif in viral pellets could be explained by cosedimentation of Vif-containing cell-derived vesicles or micelles. We thus treated concentrated virions with a mild detergent (Triton X-100) to disrupt the viral membrane (and membranes of any copurifying vesicles) and isolated viral core structures following centrifugation through sucrose. Using a methodology previously employed to determine the virion sublocalization of Vpx in SIV_{MAC} (49), we were able to titrate the detergent treatment such that the lipid bilayer of most particles could be removed without concomitant destruction of the remainder of the particle. Western blot analysis revealed significantly lower quantities of $p17^{Gag}$ in virions treated with Triton X-100. However, Vif protein content (like that of p24 and RT) was unaffected by the removal of the viral membrane and submembrane matrix protein. This was also the case following centrifugation through 50% sucrose which, due to their greater density, selectively enriched for viral core structures. Particle association of Vif was further confirmed by analysis of detergent-treated and untreated virions on linear sucrose gradients. Enveloped virions sedimented at the expected density of 1.15 to 1.17 g/ml as determined by peak Gag and RT concentrations. By contrast, detergent-treated core structures sedimented at a much higher density of 1.25 g/ml. Importantly, in both instances Vif protein cosedimented with maximal Gag and RT reactivities. Taken together, these results indicate that Vif represents an integral component of the virus core and that within the virus particle, the majority of Vif protein is not associated with the viral membrane or submembrane compartments.

Given the intensities of Vif protein on immunoblots (compare Fig. 2 to 9), it is somewhat surprising that the presence of Vif in virions has not been reported earlier. Two technical factors may explain this circumstance. One is the choice of antibodies. Clearly, not all antibodies that detect cell-associated or bacterially expressed Vif protein can detect Vif in the virus particle (37). For example, we failed to detect virion-associated Vif protein with a different anti-HIV-1 Vif antibody from the NIAID Repository (data not shown). Similarly, our anti-SIV_{SM} Vif antibody was much less active and required up to 100-fold more virion RT equivalents to yield a positive Western blot. The second factor relates to the amount of virus particles analyzed per immunoblot. We used highly concentrated HIV-1 preparations, on the order of 50 to 200 ng of particle-associated p24 per lane. Most previous studies, including those that failed to detect Vif in virions (37, 44), did not specify how many particles were analyzed.

Zombeck et al. were the first to describe an association of Vif with the HIV-1 virion (50). However, these investigators found only very small quantities (i.e., 5 to 15 molecules) of Vif per particle, leading them to conclude that the packaging of Vif was likely unspecific and thus of questionable biological relevance. Our estimate of virion-associated Vif is nearly 1 order of magnitude higher and on the order of quantities described for the viral *pol* gene products. It should be emphasized that we used a number of controls to derive these values. First, all quantitation was performed in the linear range of the ECL detection system (Fig. 10B). Second, the anti-Vif antibody used for quantitation in Fig. 10 was raised against the same Vif protein included as the external standard (11). Third, a second dilution panel prepared from commercially available Vif protein yielded virtually identical results (data not shown). Fourth, simultaneous use of highly purified p51 RT protein as an external standard confirmed the reliability our assay system. Fifth, signal intensities of external standards and virion preparations were always compared on the same immunoblot. Thus, our estimate of virion-associated Vif protein should be accurate, at least within the limitations of the assay system. In addition, it should be noted that our findings have most recently been confirmed by other investigators (22). Coexpressing an epitope-tagged Vif protein with a *vif*-deficient HIV-1 provirus, Karczewski and Strebel identified Vif protein in the viral core in readily detectable quantities (22). Moreover, these investigators also report an association between Vif and p24 Gag, as evidenced by coimmunoprecipitation of both proteins. Although this may suggest how Vif is packaged, additional experiments are necessary to determine whether Vif is incorporated into the HIV-1 particle by a specific mechanism.

Although there is currently no formal proof that the presence of Vif in the particle has functional consequences, evidence is increasing that the virion association of Vif is important for early replication events. Vif-mutant virus produced by nonpermissive cells is characterized by an abnormal ultrastructural morphology and protein composition (5, 19). The structure of the nucleoprotein core in particular seems to be altered in the absence of Vif, exhibiting nonhomogeneous packing or redistribution of electrodense material (19). It is thus possible that a direct interaction of Vif with either Gag or Gag/Pol precursor molecules before, during, and/or after budding is necessary for their proper folding, assembly, and/or processing. As a core protein, Vif may also associate with the preintegration complex and facilitate (either directly or indirectly) its nuclear transport upon internalization into new target cells. The recent finding that Vif interacts with the cellular cytoskeletal network further supports this possibility (22). Finally, as a virion component, Vif may also affect proviral DNA synthesis by interacting with the RT, the NC protein, or the viral RNA. Whatever its exact mechanism of action may be, it will be important to determine whether incorporation of Vif into virions is of functional relevance. Given the absolute requirement of Vif for virus replication in natural target cells, inhibition of its incorporation into virions could represent a new therapeutic strategy against HIV.

ACKNOWLEDGMENTS

We thank Xiaofang Yu (Harvard School of Public Health, Boston, Mass.) for advice concerning the preparation and purification of HIV-1 core structures; Louis Henderson (National Cancer Institute, Frederick, Md.) for helpful suggestions concerning the treatment of virions with proteinase K to remove surface proteins; Dana Gabuzda and Xiaoyu Yang (Dana-Farber Cancer Institute, Boston, Mass.) for bacterially expressed Vif protein and anti-Vif antibodies; and Jacob Lebowitz (University of Alabama at Birmingham) for purified p51 RT protein. We also thank Dana Gabuzda and Klaus Strebel (National Institute for Allergy and Infectious Diseases, Bethesda, Md.) for communication of unpublished data. The following reagents were obtained through the AIDS Research and Reference Reagents Program, Division of AIDS, NIAID: $HIV\text{-}1_{HXB2}$ Vif antiserum from D. Gabuzda, HIV-1 RT antiserum from the Division of AIDS, and $HIV\text{-}1\ p17^{Gag}$ antiserum from M. Phelan, and HIV-1 gp41 antiserum from S. Zolla-Pazner.

This work was supported by grants from the National Institutes of Health (R29 AI31816, U01 AI35282, RO1 AI33826, and RO1 AI34748) and by core facilities of the Birmingham Center for AIDS Research (Central Virus Core; P30 AI27767), the Birmingham Comprehensive Cancer Center (Protein Sequencing Core; P30 CA13148), and the Birmingham Veterans Administration Medical Center.

REFERENCES

1. **Akari, H., J. Sakuragi, Y. Takebe, K. Tomoaga, M. Kawamura, M. Fukasawa, T. Miura, T. Shinjo, and M. Hayami.** 1992. Biological characterization

of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. Arch. Virol. **123:**157–167.
2. **Arthur, L. O., J. J. W. Bess, I. R. C. Sowder, R. E. Benveniste, D. L. Mann, J.-C. Chermann, and L. E. Henderson.** 1992. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. Science **258:**1935–1938.
3. **Arya, S. K., and R. C. Gallo.** 1986. Three novel genes of human T-lymphotropic virus type III: immune reactivity of their products with sera from acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83:**2209–2213.
4. **Blanc, D., C. Patience, T. F. Schultz, R. Weiss, and B. Spire.** 1993. Transcomplementation of Vif^- HIV-1 mutants in CEM cells suggests that vif affects late steps in the viral life cycle. Virology **193:**186–192.
5. **Borman, A. M., C. Quillent, P. Charneau, C. Dauguet, and F. Clavel.** 1995. Human immunodeficiency virus type 1 Vif^- mutant particles from restrictive cells: role of Vif in correct particle assembly and infectivity. J. Virol. **69:** 2058–2067.
6. **Courcoul, M., C. Patience, F. Rey, D. Blanc, A. Harmache, J. Sire, R. Vigne, and B. Spire.** 1995. Peripheral blood mononuclear cells produce normal amounts of defective Vif^- human immunodeficiency virus type 1 particles which are restricted for the preretrotranscription steps. J. Virol. **69:**2068–2074.
7. **Dewhurst, S., J. E. Embertson, D. C. Anderson, J. I. Mullins, and P. N. Fultz.** 1990. Sequence analysis and acute pathogenicity of molecularly cloned SIVsmmPBj14. Nature (London) **345:**636–640.
8. **Fan, L., and K. Peden.** 1992. Cell-free transmission of vif mutants of HIV-1. Virology **190:**19–29.
9. **Felber, B. K., C. M. Drysdale, and G. N. Pavlakis.** 1990. Feedback regulation of human immunodeficiency virus type 1 expression by the *rev* protein. J. Virol. **64:**3734–3741.
10. **Fisher, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petteway, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1987. The sor gene of HIV-1 is required for efficient virus transmission *in vitro*. Science **237:**888–893.
11. **Gabuzda, D.** 1995. Personal communication.
12. **Gabuzda, D. H., K. Lawrence, E. Langhoff, E. Terwilliger, T. Dorfman, W. A. Haseltine, and J. Sodroski.** 1992. Role of *vif* in replication of human immunodeficiency virus type 1 in $CD4^+$ T lymphocytes. J. Virol. **66:**6489–6595.
13. **Gabuzda, D. H., H. Li, K. Lawrence, B. S. Vasir, K. Crawford, and E. Langhoff.** 1994. Essential role of vif in establishing productive HIV-1 infection in peripheral blood T lymphocytes and monocyte/macrophages. J. Acquired Immune Defic. Syndr. **7:**908–915.
14. **Ghosh, S. K., P. N. Fultz, E. Keddie, M. S. Saag, P. M. Sharp, B. H. Hahn, and G. M. Shaw.** 1993. A molecular clone of HIV-1 tropic and cytopathic for human and chimpanzee lymphocytes. Virology **194:**858–864.
15. **Gibbs, J. S., and R. C. Desrosiers.** 1993. Auxiliary proteins of the primate immunodeficiency viruses, p. 137–158. *In* B. R. Cullen (ed.), Frontiers in molecular biology: human retroviruses. Oxford University Press, New York.
16. **Goncalves, J., P. Jallepalli, and D. H. Gabuzda.** 1994. Subcellular localization of the Vif protein of human immunodeficiency virus type 1. J. Virol. **68:**704–712.
17. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326:**662–669.
18. **Hahn, B. H.** 1994. Viral genes and their products, p. 21–43. *In* S. Broder, T. Merigan, and D. Bolognesi (ed.), Textbook of AIDS medicine. Williams & Wilkins, New York.
19. **Höglund, S., A. Ohagen, K. Lawrence, and D. Gabuzda.** 1994. Role of vif during packing of the core of HIV-1. Virology **201:**349–355.
20. **Israel, Z. R., G. A. Dean, D. H. Maul, S. P. O'Neil, M. J. Dreitz, J. I. Mullins, P. N. Fultz, and E. A. Hoover.** 1993. Early pathogenesis of disease caused by SIVsmmPBj14 molecular clone of macaques. AIDS Res. Hum. Retroviruses **9:**277–286.
21. **Kan, N. C., G. Franchini, F. Wong-Staal, G. C. DuBois, W. G. Robey, J. A. Lautenberger, and T. S. Papas.** 1986. Identification of HTLV-III/LAV *sor* gene product and detection of antibodies in human sera. Science **231:**1553–1555.
22. **Karczewski, M. K., and K. Strebel.** Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. J. Virol., in press.
23. **Kawamura, M., T. Ishizaki, A. Ishoto, T. Shioda, T. Kitamura, and A. Adachi.** 1994. Growth ability of human immunodeficiency virus type 1 auxiliary gene mutants in primary blood macrophage cultures. J. Gen. Virol. **75:**2427–2431.
24. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spuge, S. R. Conley, J. P. Moore, J. L. Raina, H. Renz, H. R. Gelderblom, and P. L. Nara.** 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology **189:**695–714.
25. **Lebowitz, J., S. Kar, E. Braswell, S. McPherson, and D. L. Richard.** 1994. Human immunodeficiency virus-1 reverse transcriptase heterodimer stability. Protein Sci. **3:**1374–1382.
26. **Lee, T. H., J. E. Coligan, J. S. Allan, M. F. McLane, J. E. Groopman, and M. Essex.** 1986. The new HTLV-III/LAV protein encoded by a gene found in cytopathic retroviruses. Science **231:**1546–1549.
27. **Li, Y., H. Hui, C. J. Burgess, R. W. Price, P. M. Sharp, B. H. Hahn, and G. M. Shaw.** 1992. Complete nucleotide sequence, genome organization, and biological properties of human immunodeficiency virus type 1 in vivo: evidence for limited defectiveness and complementation. J. Virol. **66:**6587–6600.
28. **Li, Y., J. C. Kappes, J. A. Conway, R. W. Price, G. M. Shaw, and B. H. Hahn.** 1991. Molecular characterization of human immunodeficiency virus type 1 cloned directly from uncultured human brain tissue: identification of replication-competent and -defective viral genomes. J. Virol. **65:**3973–3985.
29. **Minassian, A. A., V. S. Kalyanaraman, R. C. Gallo, and M. Popovic.** 1988. Monoclonal antibodies against human immunodeficiency virus (HIV) type 2 core proteins: cross-reactivity with HIV type 1 and simian immunodeficiency virus. Proc. Natl. Acad. Sci. USA **85:**6939–6943.
30. **Oberste, M. S., and M. A. Gonda.** 1992. Conservation of amino acid sequence motifs in lentivirus Vif proteins. Virus Genes **6:**95–102.
31. **Panet, A., D. Baltimore, and T. Hanafusa.** 1975. Quantitation of avian RNA tumor virus reverse transcriptase by radioimmunoassay. J. Virol. **16:**146–152.
32. **Park, I.-W., K. Myrick, and J. Sodroski.** 1994. Effects of vif mutations on cell-free infectivity and replication of simian immunodeficiency virus. J. Acquired Immune Defic. Syndr. **7:**1228–1236.
33. **Reddy, T. R., G. Kraus, O. Yamada, D. J. Looney, M. Suhasini, and F. Wong-Staal.** 1995. Comparative analyses of human immunodeficiency virus type 1 (HIV-1) and HIV-2 Vif mutants. J. Virol. **69:**3549–3553.
34. **Robinson, W. E., M. K. Gorny, W. M. Mitchell, and S. Zolla-Paner.** 1991. Two immunodominant domains of gp41 bind antibodies which enhance human immunodeficiency virus type 1 infection in vitro. J. Virol. **65:**4169–4176.
35. **Saag, M. S., M. J. Crain, W. D. Decker, S. Campbell-Hill, S. Robinson, W. E. Brown, M. Leuther, R. J. Whitley, B. H. Hahn, and G. M. Shaw.** 1991. High-level viremia in adults and children infected with human immunodeficiency virus: relation to disease stage and $CD4^+$ lymphocyte levels. J. Infect. Dis. **164:**72–80.
36. **Sakai, H., R. Shibata, J.-I. Sakuragi, S. Sakuragi, M. Kawamura, and A. Adachi.** 1993. Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. J. Virol. **67:**1663–1666.
37. **Sakai, K., X. Ma, I. Gordienko, and D. J. Volsky.** 1991. Recombinational analysis of a natural noncytopathic human immunodeficiency virus type 1 (HIV-1) isolate: role of the *vif* gene in HIV-1 infection kinetics and cytopathicity. J. Virol. **65:**5765–5773.
38. **Simon, J. H. M., T. E. Southerling, J. C. Peterson, B. E. Meyer, and M. H. Malim.** 1995. Complementation of *vif*-defective human immunodeficiency virus type 1 by primate, but not nonprimate, lentivirus *vif* genes. J. Virol. **69:**4166–4172.
39. **Sodroski, J., W. C. Gob, C. Rosen, A. Tartar, D. Porttelle, A. Burny, and W. Haseltine.** 1986. Replicative and cytopathic potential of HTLV-III/LAV with *sor* gene deletions. Science **231:**1549–1551.
40. **Sova, P., M. van Ranst, P. Gupta, R. Balachandran, W. Chao, S. Itescu, G. McKinley, and D. J. Volsky.** 1995. Conservation of an intact human immunodeficiency virus type 1 *vif* gene in vitro and in vivo. J. Virol. **69:**2557–2564.
41. **Sova, P., and D. J. Volsky.** 1993. Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1. J. Virol. **67:**6322–6326.
42. **Strebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV 'A' (sor) gene product is essential for virus infectivity. Nature (London) **328:**728–730.
43. **Subbramanian, R. A., and E. A. Cohen.** 1994. Molecular biology of the human immunodeficiency virus accessory proteins. J. Virol. **68:**6831–6835.
44. **Trono, D.** 1995. HIV accessory proteins: leading roles for the supporting cast. Cell **82:**189–192.
45. **von Schwedler, U., J. Song, C. Aiken, and D. Trono.** 1993. *vif* is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:**4945–4955.
46. **Wieland, U., J. Hartmann, H. Suhr, B. Salzberger, H. J. Eggers, and J. E. Kühn.** In vivo genetic variability of the HIV-1 vif gene. Virology **203:**43–51.
47. **Wu, X., H. Liu, H. Xiao, J. Kim, P. Seshaiah, G. Natsoulis, J. D. Boeke, B. H. Hahn, and J. C. Kappes.** 1995. Targeting foreign proteins to human immunodeficiency virus particles via fusion with Vpr and Vpx. J. Virol. **69:**3389–3398.
48. **Xu, J.-Y., M. K. Gorny, T. Palker, S. Karawowska, and S. Zolla-Pazner.** 1991. Epitope mapping of ten human monoclonal antibodies to gp41, the transmembrane protein of gp41. J. Virol. **65:**4832–4838.
49. **Yu, X., Z. Matsuda, Q.-C. Yu, T.-H. Lee, and M. Essex.** 1993. Vpx of simian immunodeficiency virus is localized primarily outside the virus core in mature virions. J. Virol. **67:**4386–4390.
50. **Zombek, D., N. R. Landau, and D. Trono.** 1994. Vif is incorporated into HIV-1 particles: mapping of its packaging requirements. *In* Retroviruses '94. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.