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The Human Immunodeficiency Virus Type 1-Specific Protein *vpu* Is Required for Efficient Virus Maturation and Release

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A deletion mutation affecting *vpu* was introduced into an infectious molecular clone of human immunodeficiency virus type 1, and the resultant phenotype was examined after infection of human T lymphocytes. The absence of *vpu* resulted in an accumulation of cell-associated viral proteins and impaired the release of progeny virions. Both electron microscopic and biochemical analyses indicated that a large proportion of the mutant particles was attached to the surface of infected cells. Significant variation in the size and shape of these progeny virions was observed. In addition, intracytoplasmic particles, some of which formed aberrant budding structures, were visualized in T cells infected with the *vpu* mutant. Indirect immunofluorescence analyses of cultures inoculated with wild-type virus with use of a *vpu*-specific antiserum demonstrated that *vpu* is mainly localized to a perinuclear region in the cytoplasm of virus-producing cells.

Human immunodeficiency virus type 1 (HIV-1) contains numerous open reading frames (ORFs), many of which are found in other lentiviruses. The *vpu* ORF, however, is unique to HIV-1, not being present in the genomes of the closely related primate lentiviruses HIV-2 and simian immunodeficiency virus (SIV) (10, 12). The *vpu* gene product is a small, 81-amino-acid protein that is expressed in vivo (3, 16, 23). Because of its structural similarity to the membrane protein M_2 encoded by influenza virus and the previously reported 4- to 10-fold reduction in progeny virion production associated with the interruption of the HIV-1 *vpu* ORF (23), we suggested that *vpu* might play a role in virion maturation, efficient particle release from HIV-1-infected T lymphocytes, or both.

In this report, we have extended our analysis of *vpu* function during productive virus infection of human T cells. We show that *vpu* is expressed only in the cytoplasm of infected cells. In the absence of *vpu*, an accumulation of cell-associated virion proteins associated with increased cytopathicity was observed. The results of cell fractionation studies and electron microscopic analyses indicate that the *vpu* mutant phenotype in infected T cells is characterized by a defect in virion release.

MATERIALS AND METHODS

Cells and viruses. The human T-lymphocytic cell line A3.01 (4) was propagated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). Human peripheral blood leukocytes collected from healthy donors were purified by centrifugation in lymphocyte separation medium (Organon Teknika, Durham, N.C.) and stimulated with 0.25 μg of phytohemagglutinin per ml in RPMI 1640 containing 10% FBS 3 days before infection. After HIV infection, the medium was supplemented with 10% interleukin-2. The tissue culture medium was changed every third day or when the cell concentration exceeded 2×10^6/ml. HeLa cells (ATCC CCL2) or HeLa CD4 cells (a gift from R. Axel) were propagated in Dulbecco modified Eagle medium (DMEM) containing 10% FBS. Filtered supernatants, harvested approximately 48 h posttransfection, were used as virus stocks for wild-type or mutant virus. Confluent cell monolayers were routinely split 1 to 5.

Construction of the *vpu* mutant. A deletion mutant of the infectious molecular HIV-1 clone pNL4-3 (1) was constructed by site-specific oligonucleotide-directed mutagenesis of the 2.7-kilobase *Eco*RI-*Bam*HI fragment cloned into M13. A deletion of 48 nucleotides immediately following the initiator methionine codon of the *vpu* ORF plus a 7-nucleotide insertion resulting in a minus-1 frameshift and premature termination after 14 missense codons was introduced (Fig. 1). The mutated *Eco*RI-*Bam*HI fragment was reinserted into the infectious pNL4-3 proviral clone; the mutation was confirmed by sequencing, using the dideoxy-chain termination method (19).

Transfection of HeLa cells. HeLa cells were grown to about 70% confluency in a 25-cm^2 flask. Before transfection, the medium was replaced with 5 ml of DMEM containing 10% FBS. Calcium phosphate-precipitated plasmid DNA (20 μg) was added to the cells for 4 h, after which the medium was removed and the cells were subjected to a glycerol shock for 2 min (9). The cultures were then washed twice in DMEM and maintained in 5 ml of DMEM supplemented with 10% FBS.

RT assay. Reverse transcriptase (RT) assays on cell-free medium samples (8) were performed as previously described (24). Samples for intracellular RT activity were prepared as follows. Infected-cell suspensions (1 ml) were pelleted and suspended in 1 ml of phosphate-buffered saline (PBS). Cells were disrupted by three cycles of freezing on dry ice and thawing in a 37°C water bath. After vortexing and sedimentation of cell debris, 10-μl samples of supernatant were subjected to RT assay.

Western immunoblot analysis. Cells were pelleted and lysed in CHAPS buffer (50 mM Tris hydrochloride [pH 8], 5 mM EDTA, 100 mM NaCl, 0.5% CHAPS [Behring Diagnostics, La Jolla, Calif.]) containing 0.5% sodium deoxycholate. Insoluble material was removed by centrifugation, the lysates were boiled for 5 min in an equal volume of sample buffer (14), and the proteins were separated on 10 to 20% sodium dodecyl sulfate-polyacrylamide gradient gels. After

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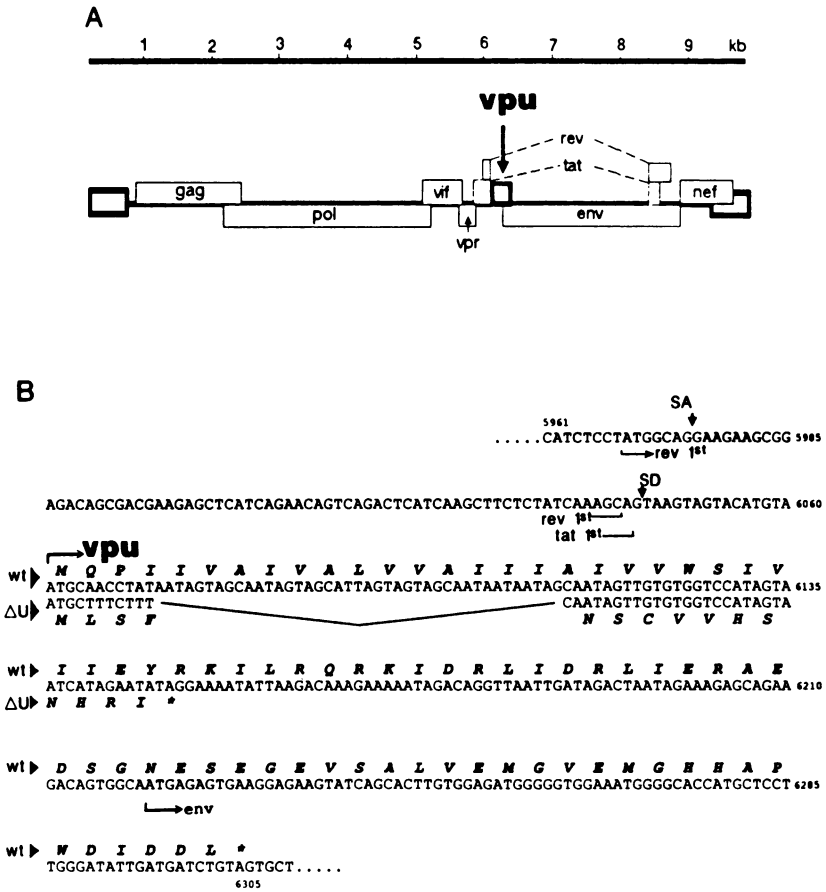


FIG. 1. (A) Genetic map of HIV-1 indicating the position of the *vpu* gene in the viral genome. (B) Nucleotide sequence of the *vpu* gene present in the parental wild-type (wt) plasmid pNL4-3 and the deletion mutant vpuDEL-1 (ΔU). The beginning of the first coding exon of *rev* and the splice donor site (SD) at the end of the first exons of *rev* and *tat* are indicated. SA marks the putative splice acceptor for *vpu*. The deduced amino acid sequences for wild-type and deleted *vpu* are also shown. The beginning of the *env* coding region is marked. Numbers indicate the nucleotide distance from the beginning of the 5′ long terminal repeat.

transfer to 0.22-μm-pore-size nitrocellulose membranes, the filters were incubated with specific antisera and ^{125}I-labeled protein A and then subjected to autoradiography (21).

Immunohistochemistry. In a modification of a previously described method (18), cells (3×10^5) were washed in PBS and centrifuged onto glass slides (5 min at $250 \times g$), using a Cytospin centrifuge (Shandon Inc., Pittsburgh, Pa.). The slides were air dried, and the cells were fixed either in ice-cold acetone for 5 min or in 3.7% paraformaldehyde for 10 min. The dried samples were blocked for 1 h in PBS containing 5% bovine serum albumin. The sera used for the primary antibody reaction were preadsorbed with acetone-fixed uninfected A3.01 cells and cleared by centrifugation ($9{,}000 \times g$ for 1 min). The anti-*vpu* serum raised in rabbits against a *vpu* fusion protein synthesized in *Escherichia coli* (F. Maldarelli, manuscript in preparation) and serum from an HIV-1 seropositive individual were each diluted 1:50. After reaction with the primary antibodies, the cells were extensively washed for 1 h with PBS containing 1% bovine serum albumin. Fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (IgG) (Organon Teknika, Malvern, Pa.) for the anti-*vpu* reaction or tetramethylrhodamine isothiocyanate-coupled anti-human IgG antibodies, developed in goat (Organon Teknika), were used in concentrations recommended by the manufacturer as secondary antibodies. After a 40-min incubation, the slides were washed twice in PBS, rinsed in distilled water, and analyzed by using a fluorescence-activated light microscope (Zeiss, Oberkochen, Federal Republic of Germany).

Electron microscopy. At different times after infection, samples of the infected lymphocyte cultures containing

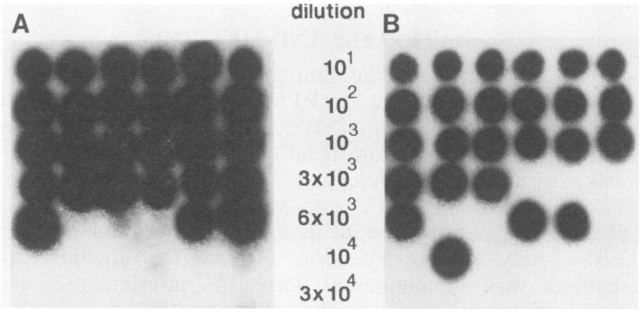


FIG. 2. Endpoint dilution titration of wild-type (A) and vpuDEL-1 (B) virus. Filtered culture medium from wild-type- and vpuDEL-1-infected lymphocyte cultures was adjusted to equivalent RT activity (approximately 6×10^6 cpm/ml). The indicated dilutions of supernatant were used to infect A3.01 cells in sixfold replicas. An autoradiogram of a ^{32}P-RT assay performed on cell-free supernatants 16 days postinfection is shown.

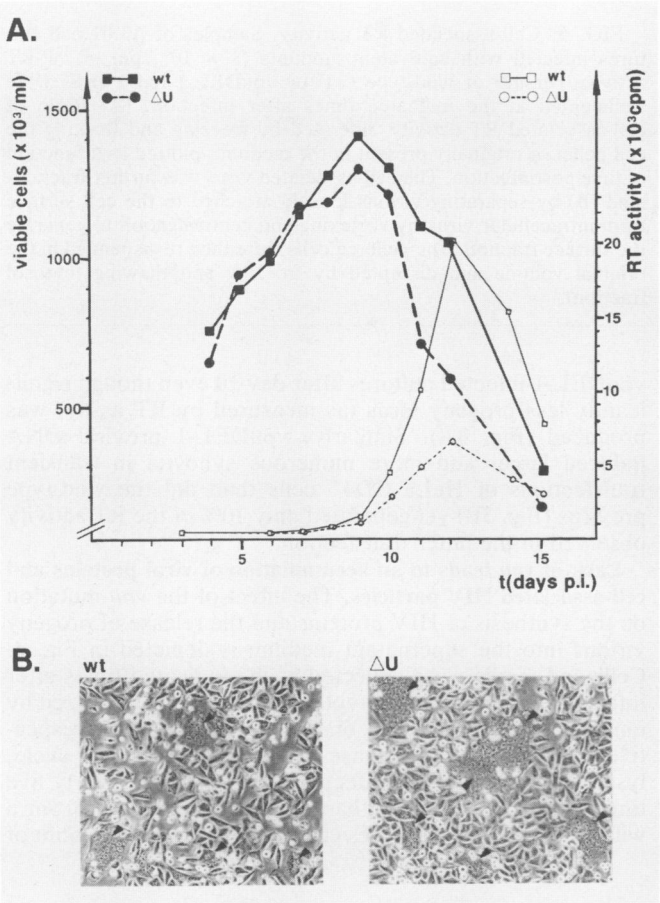


FIG. 3. Cytopathicity of wild-type (wt) and vpuDEL-1 (ΔU) virus. (A) Cell viability of A3.01 cells after infection with wild-type pNL4-3 or vpuDEL-1 was monitored at the indicated times by trypan blue exclusion. RT activity in corresponding supernatant samples was also determined. (B) Cell fusion was assayed in HeLa CD4 cells transiently transfected with equivalent amounts of wild-type or vpuDEL-1 plasmid DNA as described in Materials and Methods. The formation of syncytia was scored under a phase-contrast light microscope 20 h after transfection (magnification, ×62). Syncytia are marked with arrowheads. Supernatant RT activity for vpuDEL-1 was only 1/10 the amount produced in the transfection with wild-type virus.

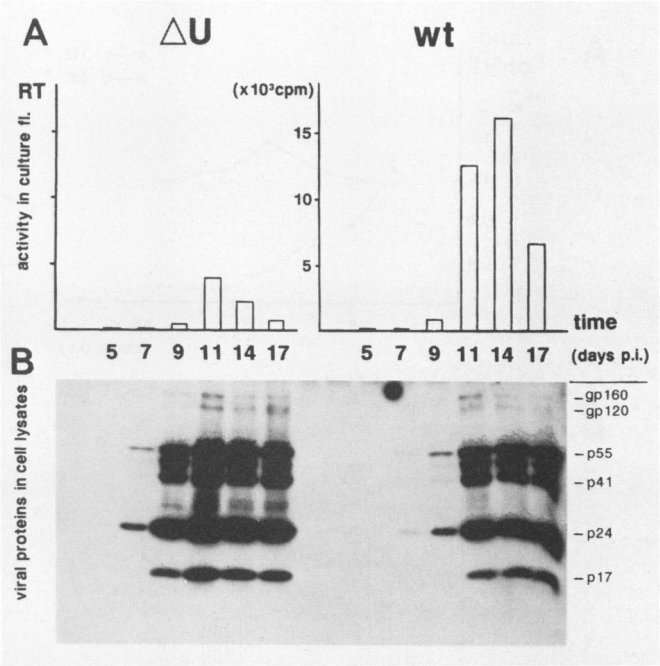


FIG. 4. Accumulation of HIV proteins in lymphocytes infected with the *vpu* mutant. At the indicated times, cells and culture medium were separated by centrifugation (9,000 × *g* for 3 min). The supernatant fraction was assayed for RT activity, and the lysed cell pellets were electrophoretically separated and analyzed by immunoblotting, using human anti-HIV-1 serum. *vpu* is not identified in the blot since the predominant p17 *gag* protein comigrates with *vpu* and obscures it (23). wt, Wild type; ΔU, vpuDEL-1 mutant.

approximately 10^6 cells were pelleted by low-speed centrifugation (320 × *g* for 10 min) and fixed for 15 min at room temperature in 5 ml of a 2.5% glutaraldehyde solution in 100 mM cacodylate buffer as previously described (17, 20). Samples were stored at 4°C until they were processed for ultrastructural studies.

RESULTS

Molecular characteristics of the *vpu* deletion mutant. A *vpu* deletion mutation (vpuDEL-1), identical to that present in a naturally occurring HIV-1 isolate designated NY-5 (2), was introduced into the infectious molecular clone pNL4-3 (1) as described in Materials and Methods. Unlike our previously described linker insertion mutant, which had the potential to encode the amino-terminal 32 residues of *vpu* (23), the deletion present in vpuDEL-1 allowed only the synthesis of a 15-residue missense peptide.

The deduced missense amino acids of the mutated *vpu* lack any homology with the authentic protein (Fig. 1). The splice donor sites for the first coding exons of *tat* and *rev* immediately 5′ of the *vpu* gene are intact, and the *env* gene located 3′ from the *vpu* mutation is preserved (Fig. 1). Western analysis of transfection lysates or radioimmunoprecipitated lysates of vpuDEL-1-infected T cells indicated that none of the other HIV ORFs is affected by the *vpu* deletion. The banding patterns and relative band intensities of wild-type and mutant proteins were indistinguishable (data not shown).

The infectivity of mutant virions is unaffected by the deletion. The infectivity of vpuDEL-1 virus preparations was compared with that of the isogenic wild-type virus in end-point titration experiments. Wild-type and vpuDEL-1 virus stocks were prepared by filtering supernatants from HeLa cells 48 h after transfection. After normalization for RT levels, A3.01 cells in 96-well microdilution plates (10^3 cells per well) were infected with serial dilutions of each virus stock; each dilution was plated in six replicas. The infectious titer of each stock was determined by RT assay of the culture supernatant. Wild-type and vpuDEL-1 HIV-1 had similar infectivities, although vpuDEL-1 consistently generated lower virus yields at each dilution (Fig. 2). It should be emphasized that in this infectivity assay, comparable amounts (RT activity) of cell-free filtered virus were examined; aggregated or cell-associated forms of vpuDEL-1 (see below) were probably eliminated from the test inoculum. Furthermore, immunoblot analyses of wild-type or vpuDEL-1 particles, obtained by pelleting virus present in each filtrate by ultracentrifugation, were indistinguishable, indicating similar protein compositions (data not shown).

In contrast, infection of A3.01 cells with vpuDEL-1 resulted in a more rapid onset of cytopathicity in comparison with wild-type virus. Greater cell killing was evident in the

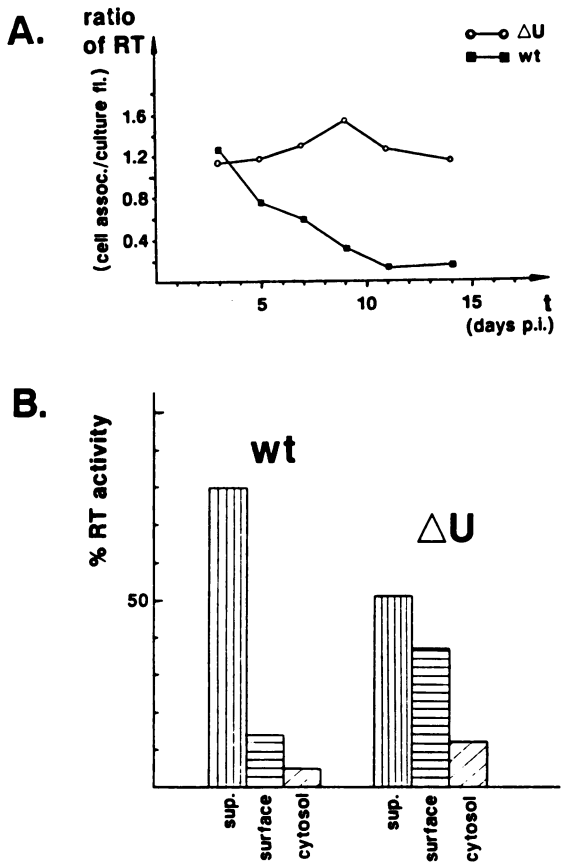


FIG. 5. Cell-associated RT activity. Samples of A3.01 cell cultures infected with equivalent amounts (3×10^6 cpm of ^{32}P-RT activity per ml) of wild-type (wt) or vpuDEL-1 (ΔU) virus were sedimented at the indicated times after infection. (A) Ratio of cell-associated RT activity, liberated by freezing and thawing the cell pellet or originally present in the medium, plotted as a function of time postinfection. The cell-associated virus was further fractionated (B) by separating virions loosely attached to the cell surface from intracellular virus by vortexing and centrifugation to generate the surface fraction. The pelleted cells were then resuspended in the original volume and disrupted by freezing and thawing (cytosol fraction).

vpuDEL-1-infected cultures after day 10 even though significantly less progeny virus (as measured by RT assay) was produced (Fig. 3A). Similarly, vpuDEL-1 proviral DNA induced larger and more numerous syncytia in transient transfections of HeLa CD4$^+$ cells than did the wild-type provirus (Fig. 3B) yet generated only 10% of the RT activity observed in the latter transfection.

Lack of *vpu* leads to an accumulation of viral proteins and cell-associated HIV particles. The effect of the *vpu* mutation on the synthesis of HIV proteins and the release of progeny virions into the supernatant medium is depicted in Fig. 4. Cells and media were collected at the indicated times after infection with wild-type or vpuDEL-1 virus and analyzed by immunoblotting (Fig. 4B) or RT assay (Fig. 4A), respectively. During the early phase (day 9) of the infection cycle, lysates from vpuDEL-1-infected cells contained nearly five times more viral protein than comparable samples from a wild-type virus infection. Even at later times, the amount of

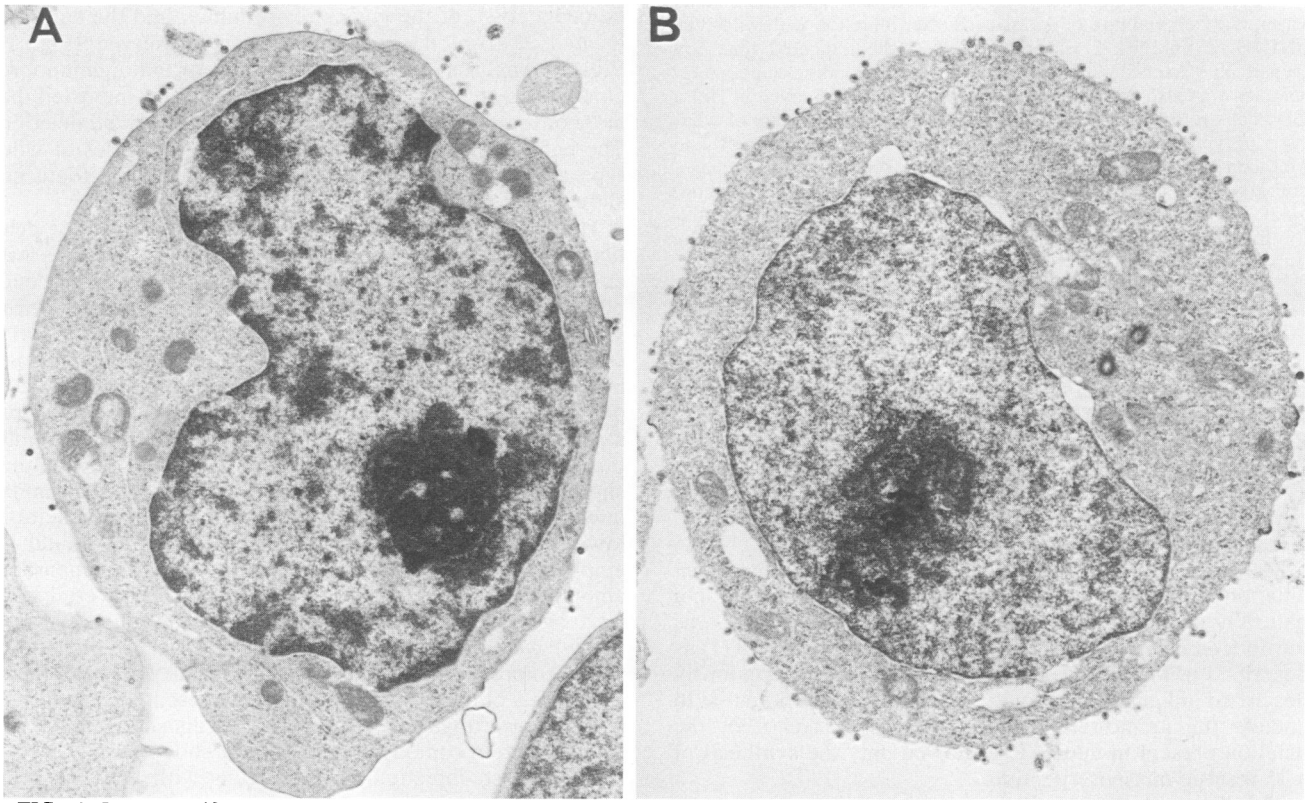
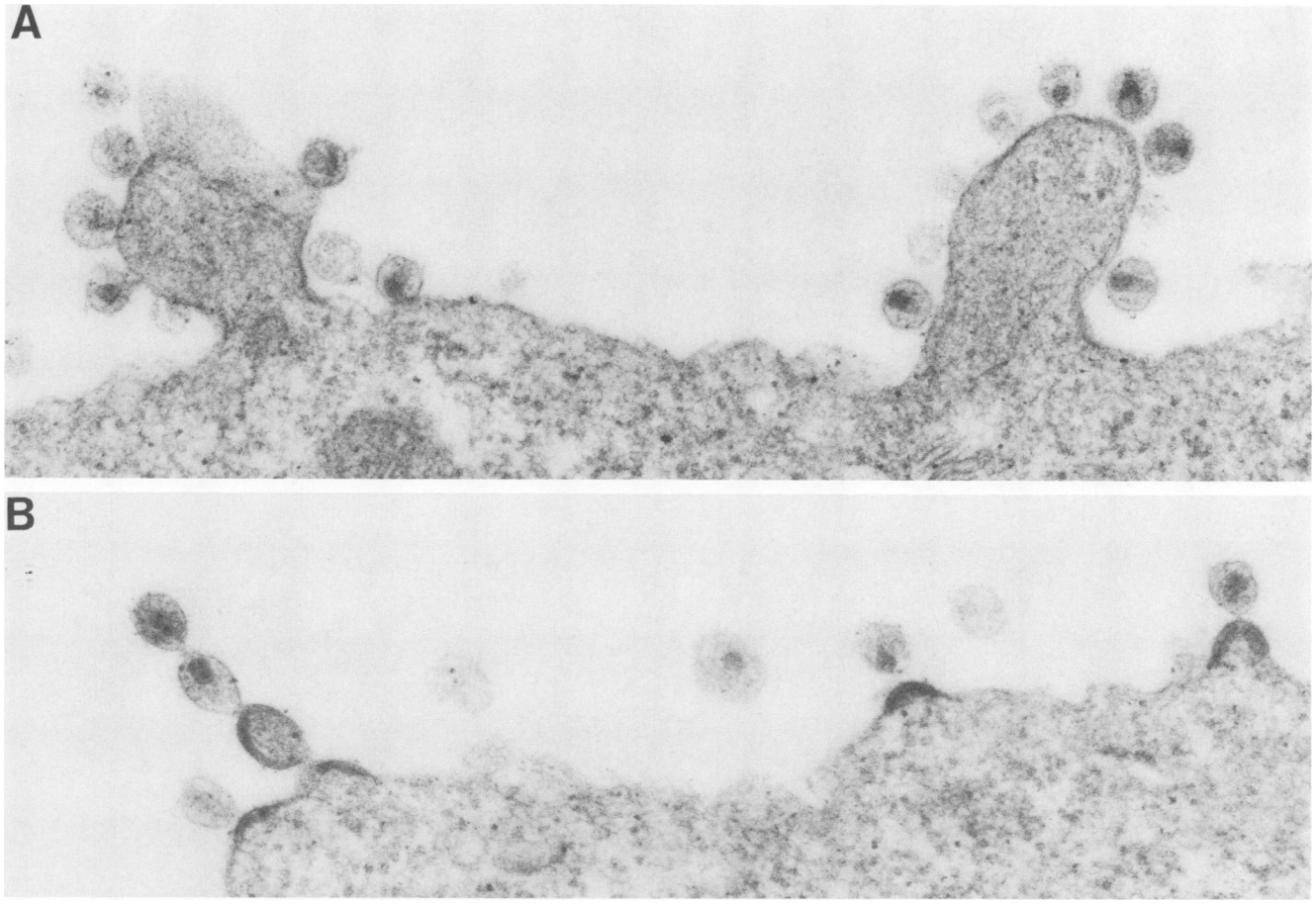


FIG. 6. Low-magnification transmission electron micrographs of A3.01 cells infected with wild-type (A) or vpuDEL-1 (B) virus showing virions associated with the cell surface. Magnification, ×9,000.



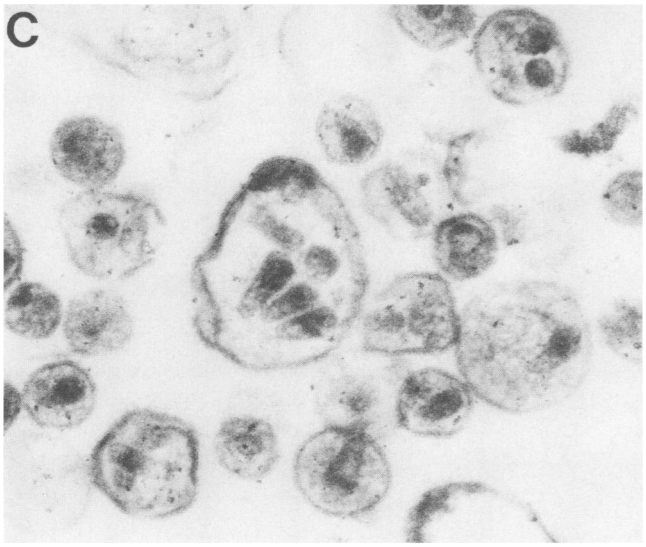
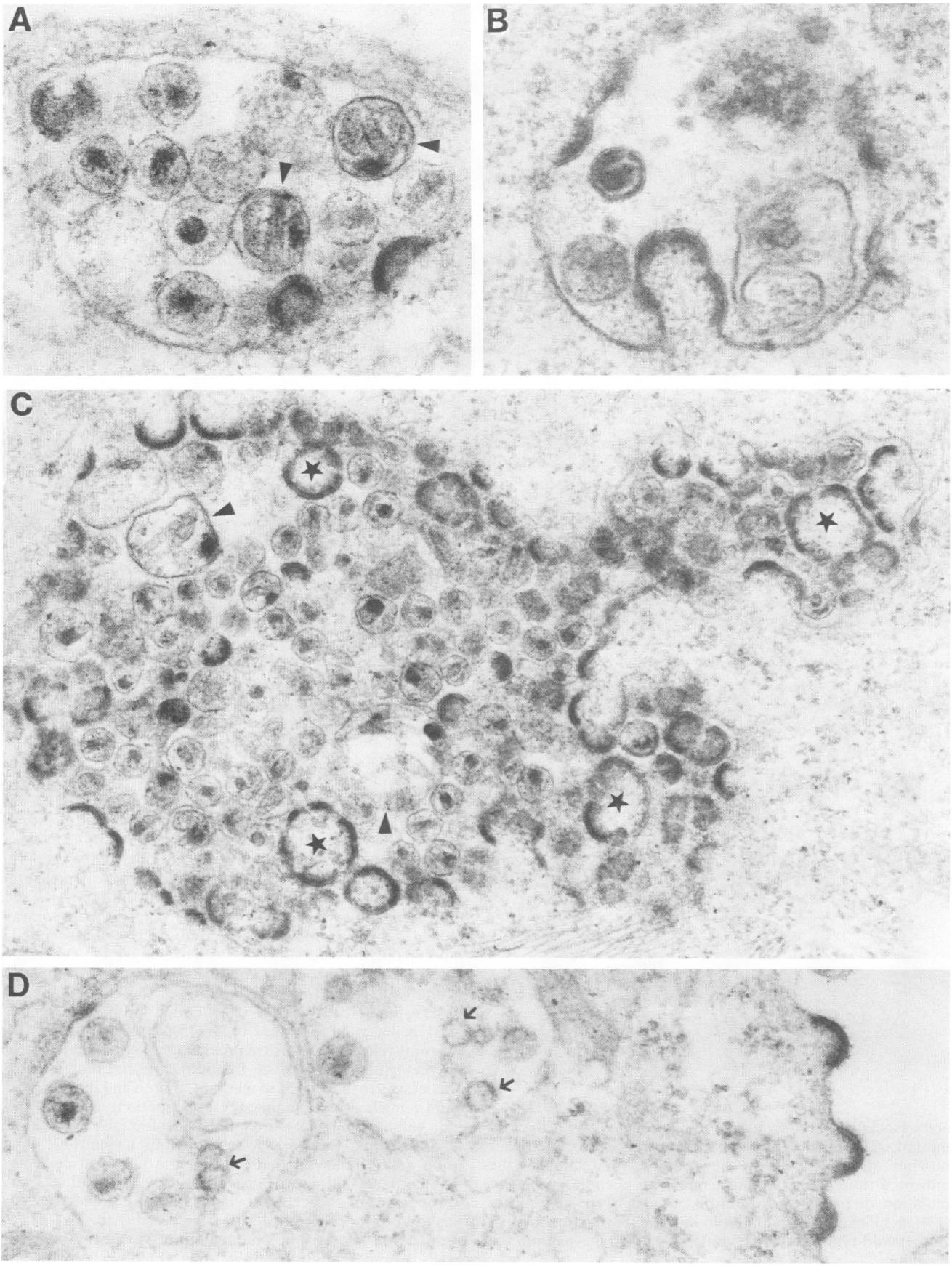


FIG. 7. (A and B) vpuDEL-1 virions closely associated with the plasma membrane of virus-producing cells, particles with condensed nucleoids, while still attached. Magnification, ×65,000. (C) Extracellular cluster of vpuDEL-1 particles of various sizes and shapes, one containing at least five nucleoids. Magnification, ×91,000.

cell-associated HIV proteins in cells infected with the *vpu* mutant was considerably higher. A correction for the lower number of viable cells in the vpuDEL-1 infection at the indicated time points would result in an even greater accumulation of proteins. In contrast, and as reported earlier (23), significantly more (fivefold in the experiment shown in Fig. 4) wild-type virus than vpuDEL-1 was released into the medium.

In a completely independent experiment, cells were separated from the medium by low-speed centrifugation at various times after infection and disrupted by three cycles of freezing and thawing, after which the RT activity present in the medium or released from pelleted cells was measured. Most of the RT activity generated by wild-type HIV was released into the medium by day 11 (Fig. 5A). In contrast, high levels (>50%) of the RT activity were cell associated in the vpuDEL-1 infection. To further analyze the cell-associated progeny virions in vpuDEL-1 infections, samples of cultures exposed to wild-type virus or the *vpu* mutant were harvested before peak RT production, and the resuspended cell pellet was subjected to vigorous vortexing for 10 s. Virions released by this treatment were separated from cells by centrifugation (surface fraction), and the remaining cell pellet was resuspended in medium, frozen and thawed twice, and centrifuged a final time (cytosol fraction). Nearly 75% of the cell-associated RT in vpuDEL-1 infections was released by vortexing (Fig. 5B). A simultaneous trypan blue exclusion assay indicated that most of the vortexed cells were viable (data not shown). These results suggest that a large proportion of the cell-associated vpuDEL-1 particles may have been loosely attached to the cell surface.

Electron microscopic examination of vpuDEL-1-infected T cells. Approximately 10^7 A3.01 cells were infected with equal


A
B
C
D

amounts (based on RT activity) of wild-type or vpuDEL-1 at a multiplicity similar to that used for the experiment shown in Fig. 3; samples were collected on day 7, 9, or 10 and examined by transmission electron microscopy. Although the percentages of virus-producing cells were comparable (by transmission electron microscopy and indirect immunofluorescence) in the two cultures, up to fivefold more particles were associated with the surface of cells infected with vpuDEL-1 (Fig. 6). A characteristic electron microscopic feature of the vpuDEL-1 infection was the presence of progeny particles, physically attached to the plasma membrane of infected cells, that contained prematurely condensed nucleoids (Fig. 7A). In some instances, chains of vpuDEL-1 virions were observed extending from the plasma membrane (Fig. 7B). In addition, a greater proportion of the vpuDEL-1 particles appeared aberrant compared with wild-type HIV, varying in size and shape, and in some instances appeared to harbor multiple nucleoids (Fig. 7C).

Another common feature of vpuDEL-1-infected A3.01 cells was the presence of intracytoplasmic vacuoles containing large numbers of virions in all stages of assembly, including highly aberrant forms (Fig. 8). Atypical detached single, double, and multimeric budding structures that formed rosettelike structures were also found (Fig. 8C). Besides virions, the cytoplasmic vacuoles frequently contained small vesicles similar to multivesicular bodies typically associated with Golgi elements (Fig. 8D). vpuDEL-1-infected peripheral blood leukocytes exhibited a similar phenotype (data not shown).

***vpu* is located in the cytoplasm of virus-producing cells.** Acetone-fixed and permeabilized A3.01 cells, 9 days after infection with wild-type HIV-1, were simultaneously incubated with a monospecific *vpu* antiserum raised in rabbits and human serum with HIV-1 seroreactivity and then examined for the presence of viral proteins by indirect immunofluorescence as described in Materials and Methods. The human anti-HIV-1 serum, which reacted well with HIV *gag* and *env* but not *vpu* in immunoblots (data not shown), gave strong cytoplasmic fluorescence (Fig. 9A). The signal with the anti-*vpu* serum was also strong and could be localized to the perinuclear region of the cytoplasm; no nuclear reactivity was apparent (Fig. 9B). In control infections with vpuDEL-1, the reaction with the serum from the HIV-1-infected individual was similar to the wild-type reaction (Fig. 9C), but no reactivity was observed with the anti-*vpu* serum (Fig. 9D). When cells fixed with paraformaldehyde to study surface reactivity were examined with the same antibodies, intense staining was observed with the human anti-HIV-1 serum (Fig. 8E), presumably because of the presence of *gag* or *env* protein on the plasma membrane, but no reaction above background levels was observed with the *vpu* antiserum (Fig. 8F).

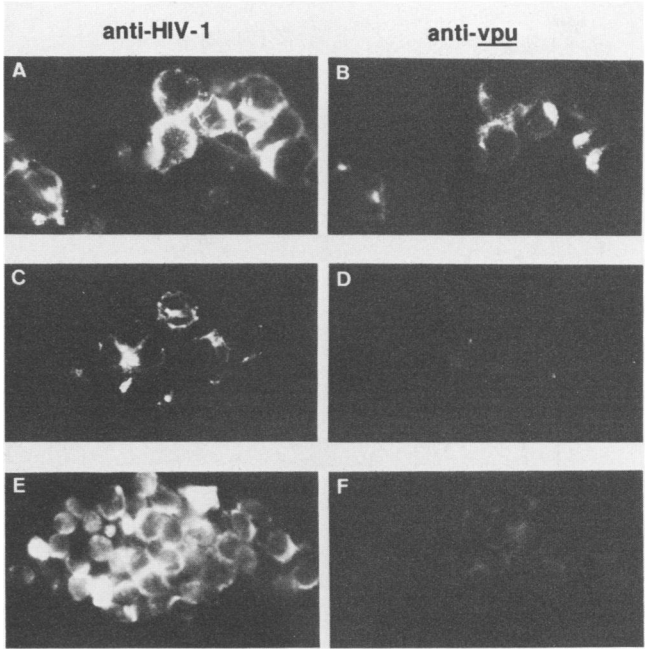


FIG. 9. Localization of *vpu* by indirect immunofluorescence microscopy. Wild-type (A, B, E, and F)- or vpuDEL-1 (C and D)-infected lymphocytes were fixed with acetone (A to D) or in 3.7% paraformaldehyde–PBS (E and F). Samples were simultaneously incubated with human anti-HIV-1 serum and an anti-*vpu* serum raised in rabbits. Tetramethylrhodamine isothiocyanate-conjugated goat anti-human IgG antibody and a fluorescein isothiocyanate-coupled mouse anti-rabbit IgG antibody were used as secondary antibodies, and fluorescence of the same fields was evaluated. Magnifications: A and B, ×504; C to F, ×332.

DISCUSSION

We have recently reported that the HIV-1 *vpu* gene product is an integral membrane protein that is expressed at high levels in virus-producing T lymphocytes but is largely, if not completely, absent from progeny virions (23; this report). Mutations affecting the synthesis of *vpu* lead to a marked reduction in virus particle production and an associated intracellular accumulation of HIV structural proteins (23). In this study, *vpu* was found only in the cytoplasm of productively infected cells by indirect immunofluorescence and appeared to be concentrated in a subcellular organelle, most likely the Golgi complex.

One of the most striking findings of this work came from the electron microscopic analyses, which suggest that *vpu* plays a critical role during the release of virus particles from infected T cells. In the absence of *vpu*, increased numbers of virus aggregates and budding structures, including many mature particles characterized by condensed cores, were found associated with or even attached to the plasma membrane of virus-producing cells. Concomitantly, budding of progeny *vpu* mutant particles from intracytoplasmic membranes into vacuoles was also seen, explaining in part the intracellular accumulation of HIV proteins observed in metabolic labeling (22) and cell fractionation (Fig. 5) experiments. Thus, in addition to resulting in impaired release of

FIG. 8. Intracytoplasmic vacuoles within cells infected with vpuDEL-1-containing particles in various stages of assembly. Shown are budding vacuolar particles (A to D) as well as highly aberrant structures containing multiple nucleoids of aberrant shape (A and C; arrowheads) or inapparent cores (A). Mature and aberrant virions containing eccentric dense material or a nucleoid with a hollow appearance (A and C) can be seen. Incompletely separated adjacent budding units form a stalklike structure (B) or detached rosettelike structures (C; stars) consisting of single and multiple buds located within vacuoles of a multinucleated giant cell. (D) Two vacuoles that contain mature virions as well as small smooth vesicles (arrows). Three typical budding structures are present on the plasma membrane on the right. Magnifications: A and B, ×110,000; C, ×64,000; D, ×80,000.

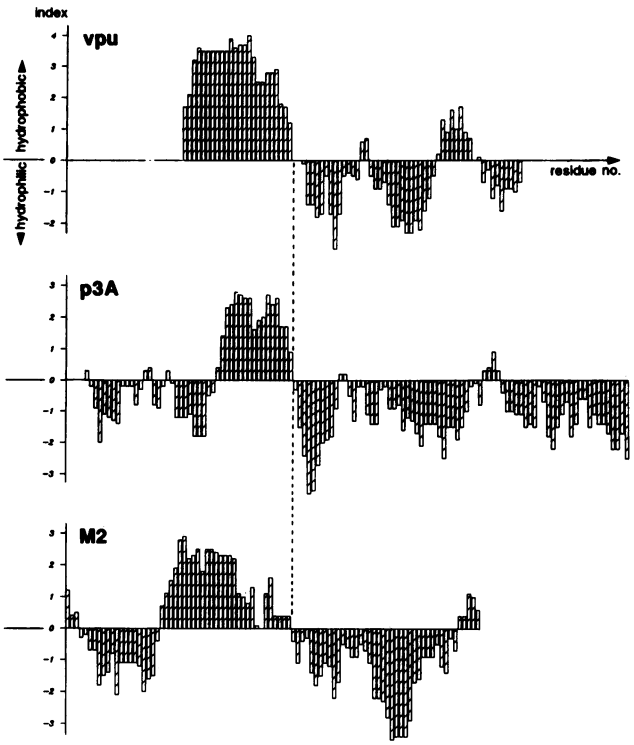


FIG. 10. Hydropathicity profiles of the deduced amino acid sequences for HIV-1 *vpu*, foot-and-mouth disease virus p3A, and influenza virus M_2 proteins. The relative hydropathicity index of each residue is shown as a function of amino acid position in the protein and was calculated by the method of Kyte and Doolittle (13). The border between hydrophobic domain and hydrophilic C terminus of each protein is indicated by the vertical broken line.

virions from the cell surface of lymphocytes, the absence of *vpu* results in the budding and maturation of virus particles at membrane sites ordinarily restricted from such activity. Perhaps the high concentrations of *vpu* associated with the Golgi complex in cells infected with wild-type virus preclude particle production at these intracytoplasmic membranes.

The electron microscopic studies also demonstrated that vpuDEL-1 infections of T lymphocytes were characterized by the production of aberrant virus particles, including some with multiple nucleoids and others that were several times larger than wild-type virions and contained uncondensed nucleoids. This was a prominent feature of intracytoplasmic vpuDEL-1 virus production, in which multimeric budding structures were found. One explanation linking all of these phenomena is that *vpu* mediates a critical and terminal step of the budding process; in its absence, individual immature progeny particles fail to separate from one another and from the plasma membrane. This perturbation of virus release could lead to a retardation of HIV maturation and secondarily result in the accumulation of high intracellular concentrations of viral proteins as well as the diversion of virus production to intracellular sites. A paradox of vpuDEL-1 infections was the earlier appearance of virus-induced cytopathicity that was accompanied by significant reductions in progeny virion production. The intracellular accumulation of potentially toxic viral proteins in vpuDEL-1-infected T lymphocytes might explain the observed reduced cell viability. Similarly, high concentrations of virion structures associated with essential cellular membranes could alter their composition and impair function by altering permeability and transport properties. In addition, the potentially increased concentrations of gp120 and gp41 on the surface of vpuDEL-1-infected T lymphocytes could contribute to the more rapid killing of other $CD4^+$ cells in the culture by cell fusion.

The evolution of small viral proteins like *vpu* may not be unique to HIV-1, since a number of other RNA viruses encode proteins that appear to function during final steps of virion production and show similarity to *vpu*. For example, the size, hydropathicity, and domain structure of the membrane-associated influenza virus M_2 protein (15, 26) and the foot-and-mouth disease virus protein p3A (5) are strikingly similar to those of *vpu* (Fig. 10). A hydrophobic N-terminal anchor is followed by a very hydrophobic C terminus, with at least 18 charged residues in each case. Other possible homologs of *vpu* include the small NB protein of influenza B virus (25) and the SH protein of simian virus 5 (11). These proteins have been suggested to be involved in the process of virus assembly (11, 25, 26). In contrast, neither HIV-2 nor SIV encodes a structural homolog of *vpu* (10, 12). It remains to be shown whether these two primate lentiviruses utilize another viral protein to functionally replace *vpu*. Alternatively, a putative host cell protein may serve as a virus release factor in HIV-2 or SIV infections and obviate the requirement for a virus-encoded *vpu*-like protein.

The intracytoplasmic production of virus particles characteristic of a wild type HIV-1 infection of monocytes-macrophages (6, 7, 17) is reminiscent of the vpuDEL-1 infection of T cells described above. What is puzzling, however, is that HIV-1 and HIV-2 both produce intracytoplasmic virus particles in infected monocytes even though only HIV-1 has the capacity to encode *vpu*. Possible explanations include (i) the presence of an inhibitor of *vpu* in monocytes-macrophages or (ii) the fact that *vpu* function in human T cells requires the synthesis of or the interaction with a cellular cofactor that is not expressed in monocytes. Experiments are in progress to more fully dissect the role of *vpu* in both cell systems and to develop antagonists that specifically abrogate *vpu* function during the acute infection of T lymphocytes.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59:**284–291.
2. **Benn, S., R. Rutledge, T. Folks, J. Gold, L. Baker, J. McCormick, P. Feorino, P. Piot, T. Quinn, and M. Martin.** 1985. Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. Science **230:**949–951.
3. **Cohen, E. A., E. F. Terwilliger, J. G. Sodroski, and W. A. Haseltine.** 1988. Identification of a protein encoded by the *vpu* gene of HIV-1. Nature (London) **334:**532–534.
4. **Folks, T., S. Benn, A. Rabson, T. Theodore, M. D. Hoggan, M. Martin, M. Lightfoote, and K. Sell.** 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA **82:**4539–4543.
5. **Forss, S., K. Strebel, E. Beck, and H. Schaller.** 1984. Nucleotide sequence and genome organization of foot-and-mouth disease virus. Nucleic Acids Res. **12:**6587–6601.
6. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear

phagocytes in HTLV-III/LAV infection. Science **233**:215–219.

7. **Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. E. Burke, D. Skillman, and M. S. Meltzer.** 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. **167**:1428–1441.
8. **Goff, S., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38**:239–248.
9. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–457.
10. **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.
11. **Hiebert, S. W., C. D. Richardson, and R. A. Lamb.** 1988. Cell surface expression and orientation in membranes of the 44-amino-acid SH protein of simian virus 5. J. Virol. **62**:2347–2357.
12. **Hirsch, V., N. Riedel, and J. I. Mullins.** 1987. The genome organization of STLV-3 is similar to that of the AIDS virus except for a truncated transmembrane protein. Cell **49**:307–319.
13. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Lamb, R. A., S. L. Zebedee, and C. D. Richardson.** 1985. Influenza virus M_2 protein is an integral membrane protein expressed on the infected cell surface. Cell **40**:627–633.
16. **Matsuda, Z., M. J. Chou, M. Matsuda, J. H. Huang, Y. M. Chen, R. Redfield, K. Mayer, M. Essex, and T. H. Lee.** 1988. Human immunodeficiency virus type 1 has an additional coding sequence in the central region of the genome. Proc. Natl. Acad. Sci. USA **85**:6968–6972.
17. **Orenstein, J. M., M. S. Melzer, T. Phipps, and H. E. Gendelman.** 1988. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. J. Virol. **62**:2578–2586.
18. **Raff, M. C., K. L. Fields, S.-I. Hakomori, R. Mirsky, R. M. Pruss, and J. Winter.** 1979. Cell-type-specific markers for distinguishing and studying neurons and the major classes of glial cells in culture. Brain Res. **174**:283–308.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
20. **Simionescu, N., and M. Simionescu.** 1976. Galloylglucoses of low molecular weight as mordant in electron microscopy. J. Cell Biol. **70**:608–621.
21. **Symington, J., U. Green, and K. Brackmann.** 1981. Immunoautoradiographic detection of proteins after electrophoretic transfer from gels to diazo-paper: analysis of adenovirus encoded proteins. Proc. Natl. Acad. Sci. USA **78**:177–181.
22. **Strebel, K., T. Klimkait, F. Maldarelli, and M. A. Martin.** 1989. Molecular and biochemical analysis of human immunodeficiency virus type 1 *vpu* protein. J. Virol. **63**:3784–3791.
23. **Strebel, K., T. Klimkait, and M. A. Martin.** 1988. A novel gene of HIV-1, vpu, and its 16 kilodalton product. Science **241**: 1221–1223.
24. **Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. J. Capon, and M. A. Martin.** 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J. Virol. **62**:139–147.
25. **Williams, M. A., and R. A. Lamb.** 1986. Determination of the orientation of an integral membrane protein and sites of glycosylation by oligonucleotide-directed mutagenesis: influenza B virus NB glycoprotein lacks a cleavable signal sequence and has an extracellular NH_2-terminal region. Mol. Cell. Biol. **6**:4317–4328.
26. **Zebedee, S. L., and R. A. Lamb.** 1988. Influenza A virus M_2 protein: monoclonal antibody restriction of virus growth and detection of M_2 in virions. J. Virol. **88**:2762–2772.