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Dual Regulation of Silent and Productive Infection in Monocytes by Distinct Human Immunodeficiency Virus Type 1 Determinants

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The regulation of human immunodeficiency virus type 1 infection and replication in primary monocytes was investigated by mutagenesis of recombinant proviral clones containing an *env* determinant required for the infectivity of monocytes. Virus replication was assayed by determination of reverse transcriptase activity in culture fluids and by recovery of virus from monocytes following cocultivation with uninfected peripheral blood mononuclear cells. Three virus replication phenotypes were observed in monocytes: productive infection, silent infection, and no infection. Incorporation of the monocytetropic *env* determinant in a full-length clone incapable of infection or replication in primary monocytes (no infection) conferred the capacity for highly efficient virus replication in monocytes (productive infection). Clones with the *env* determinant but lacking either functional *vpr* or *vpu* genes generated lower replication levels in monocytes. Mutation of both *vpr* and *vpu*, however, resulted in nearly complete attenuation of virus replication in monocytes, despite subsequent virus recovery from infected monocytes by cocultivation with uninfected peripheral blood mononuclear cells (silent infection). These findings indicate a central role for the "accessory" genes *vpu* and *vpr* in productive human immunodeficiency virus type 1 replication in monocytes and indicate that *vpu* and *vpr* may be capable of functional complementation.

Human immunodeficiency virus type 1 (HIV-1) infection of macrophages has been demonstrated in brain, spinal cord, lung, lymph node, and skin during subclinical infection and disease and is postulated to underlie important clinical manifestations of HIV-1 infection, including disease latency and development of a spectrum of AIDS-related central nervous system disorders (2, 4, 11, 14, 15, 19, 33, 37). However, detailed molecular analysis of virus-host cell interactions involving monocytes was limited until recently by the restricted tropism of the earliest and most widely studied HIV-1 genetic clones for primary monocytes cultured in vitro (14, 15). Previously, we and others have demonstrated that a discrete *env* determinant, including the V3 loop but not the CD4-binding domain, is necessary and sufficient for HIV-1 infection of monocytes (23, 29, 36). Additionally, we have identified three virus replication phenotypes in monocytes in vitro, using molecularly defined proviral clones (35). These include productive infection, with the generation of high virus replication levels; silent infection, with low to undetectable virus replication in monocytes, despite ultimate virus recovery from infected monocytes following cocultivation with uninfected, phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs [lymphoblasts]); and no infection, with neither virus replication in nor virus recovery from monocytes observed. In the present study, we investigated the roles of the HIV-1 "accessory" genes *vpr* and *vpu*, which are dispensable for virus replication in primary and immortalized $CD4^+$ T lymphocytes. We demonstrate that *vpr* and *vpu* are central to the regulation of virus replication in primary monocytes and together mediate the expression of silent versus productive infection.

To study viral regulation of monocyte infection, we utilized a panel of chimeric HIV-1 clones, constructed from the nonmonocytetropic clone HXB2 and the monocytetropic clone ADA, as previously described (16, 26, 35, 36). To correct a *vpr* defect in each of these clones, the result of a single base insertion in HXB2, 2.7-kb *Sal*I-*Bam*HI HXADA DNA fragments (nucleotides 5785 to 8474) were subcloned into the full-length proviral clone NL4-3, in which the *vpr* open reading frame is intact (1). The resultant NLHXADA clones contained the ADA-derived *env* determinant previously localized to nucleotides 7040 to 7323, flanked by additional ADA- or HXB2-derived sequences encoding other portions of *env* and *vpu* and small portions of *tat* and *rev*. A clone in which the entire 5785-to-8474 sequence was HXB2 derived (thus lacking a monocytetropic *env* determinant) was used as a negative control for these experiments. Because HXB2 lacks a *vpu* initiator methionine codon, clones in which *vpu* was HXB2 derived were defective for that product, in contrast to clones with an ADA-encoded *vpu*. Finally, a *vpr* mutant corresponding to each NLHXADA clone was generated by introducing a frameshift mutation at codon 63.

Virions from the recombinant clones, generated by transfection, were assayed for their ability to infect and replicate in primary monocytes by the presence of reverse transcriptase (RT) activity in culture supernatants (25) and by the ultimate recovery of virus following cocultivation of monocytes with uninfected PBMCs. The results are summarized in Fig. 1. All clones containing the ADA-derived *env* determinants and an intact *vpr* gene generated high virus replication levels in monocytes. Inactivation of *vpr* in these

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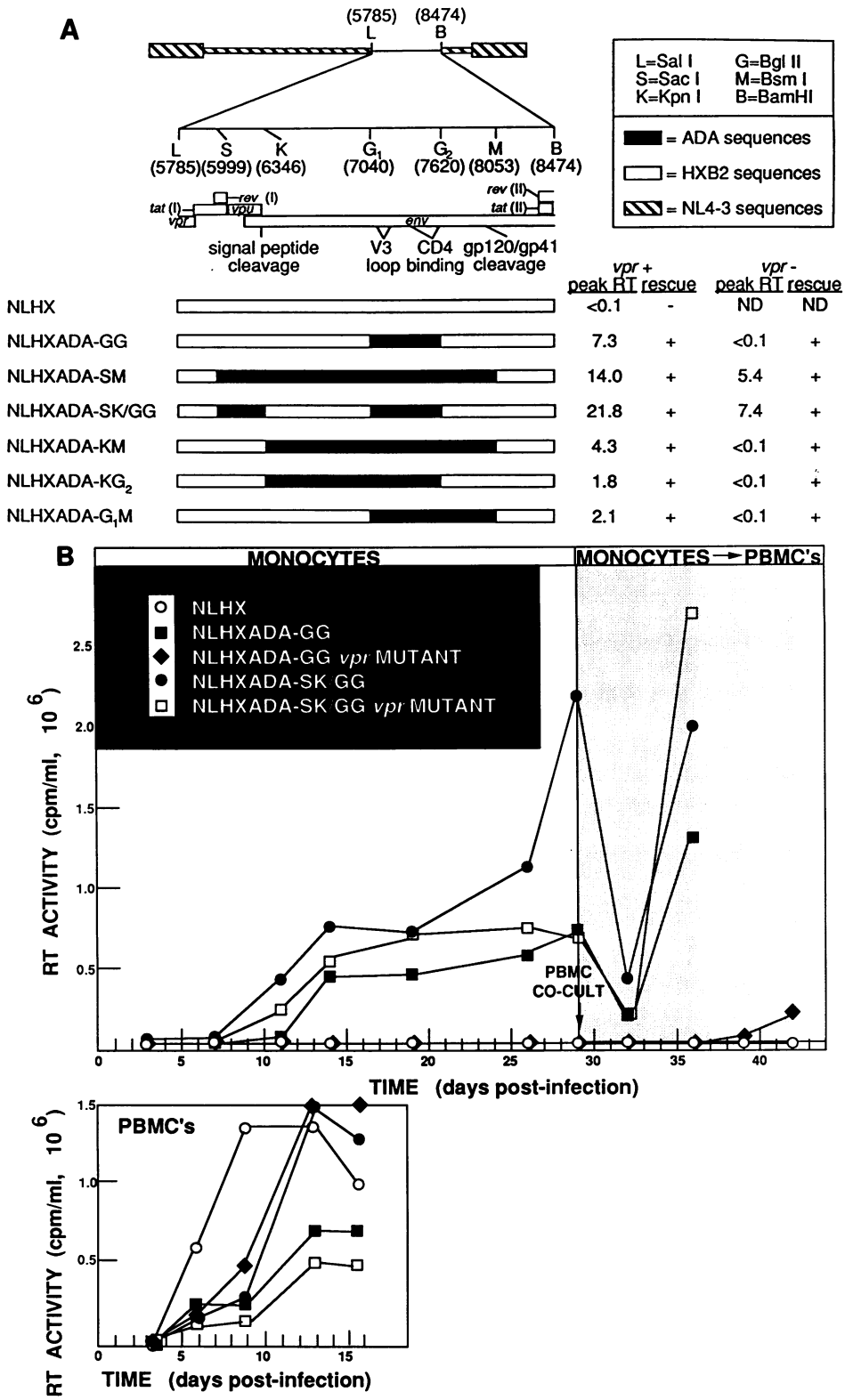

FIG. 1. Replication of recombinant HIV-1 clones with both wild-type and mutant *vpr* genes. (A) The panel of recombinant NLHXADA clones is represented diagrammatically. The region of the genome corresponding to the HXADA fragments (nucleotides 5785 to 8474) is expanded to highlight the relative positions of HXB2- and ADA-derived sequences. The open reading frames in this portion of the genome are represented above. Recombinant clones were generated by reciprocal DNA fragment exchanges of ADA- and HXB2-derived sequences into a *Sal*I-*Bam*HI fragment (5785 to 8474) from HXB2 subcloned into an intermediate shuttle vector, utilizing the restriction enzyme sites indicated on top. The resultant chimeric *Sal*I-*Bam*HI fragments were then subcloned into the clone NL4-3 to generate

clones, however, generated divergent results, depending upon the derivation of nucleotide sequences 5999 to 6345 (SK fragment). Clones in which this portion of the genome was ADA derived generated lower (but readily detectable) virus replication levels than did their wild-type *vpr* counterparts. However, *vpr* mutants in which SK was HXB2 derived typically failed to generate virus replication levels detectable above background in monocytes, despite subsequent virus recovery from these cultures onto uninfected PBMCs. The negative control clone, which carried a wild-type *vpr* but lacked the monocytetropic *env* determinant, generated virions which neither replicated in nor were recovered from monocytes, as previously demonstrated. No significant differences were seen in the replication of each virus strain on PBMCs obtained from several different donors.

Monocytes were infected with recombinant HIV-1 clones containing a functional *vpr* gene, stained with toluidine blue, and examimed by light microscopy (1-μm-thick plastic sections). Cultures infected with a nonmonocytetropic virus, NLHXADA-SK, which contains a functional *vpu* gene, were indistinguishable from uninfected cells, with rare, small multinucleated cells (Fig. 2A). Cultures productively infected with virus containing the monocytetropic *env* determinant and a functional (NLHXADA-SM [Fig. 2B]) or nonfunctional (NLHXADA-GG [Fig. 2C]) *vpu* gene showed characteristic cytopathic effects (15). These consisted of the formation of multinucleated giant cells, often containing 10 or more nuclei per cell, and cell lysis. The frequencies and sizes of these cells were comparable in the NLHXADA-SM- and NLHXADA-GG-infected monocyte cultures. Virus production and cellular degeneration and necrosis were primarily confined to the multinucleated cells. Transmission electron microscopy examination demonstrated typical budding and mature virions in intracellular vacuoles that were associated with the plasma membrane, in both the presence and absence of *vpu*, but not in the NLHXADA-SK-infected cells (Fig. 2D). Freeze fracture scanning electron microscopy demonstrated budding of virion particles from the plasma membrane of monocytes infected with virus which lacked a functional *vpu* (Fig. 2E). No virus could be detected in monocytes infected with recombinant clones lacking both *vpr* and *vpu* (data not shown).

The SK fragment encodes the entire *vpu* gene product, 14 amino acids at the C termini of both the *tat* and the *rev* first exons, and the N-terminal 41 amino acids of *env* (Fig. 3). Although the absence of a *vpu* initiator methionine codon in HXB2 is the most obvious difference between the SK portions of HXB2 and ADA, a role for *tat*, *rev*, or *env* could not be ruled out. The *env* sequences differ at 7 of 41 predicted amino acid positions, not including the nonaligned insertion of 3 residues and deletion of 4 residues in ADA. All but three of these differences are confined to the signal peptide, which varies by up to 30% between different clones (20). Furthermore, *tat* and *rev* both differ at 3 of 14 amino acid positions between the ADA and HXB2 SK fragments, with four of these six changes being conservative in nature. Therefore, it is unlikely that these alterations in *env*, *tat*, or *rev* alter their function. However, to formally determine the specific requirement for *vpu* during HIV-1 infection of monocytes, the *vpu* initiator methionine codon of the silent infection clone NLHXADA-GG (*vpr* mutant) was restored by site-directed mutagenesis. The resultant clone was found to generate virus capable of productive infection of monocytes (data not shown).

HIV-1 and related lentiviruses are distinct from most other retroviruses in that besides the structural *gag*, *pol*, and *env* genes common to all retroviruses, they also encode a number of genes whose functions have been shown or are speculated to be regulatory in nature. In HIV-1, these genes include *tat*, *rev*, *vif*, *nef*, *vpu*, and *vpr* (6–8, 26, 32, 38). While *tat*, *rev*, and *vif* are essential for viral gene expression or virion infectivity, the precise role and overall importance of *vpr*, *vpu*, and *nef* are unclear, since these genes are dispensable for virus infection and replication in $CD4^+$ lymphocytes in vitro (8–10, 12, 13, 22, 24, 30, 32). The availability of molecular HIV-1 clones which infect and replicate in monocytes at levels comparable to those observed with many monocytetropic virus isolates has facilitated investigation of the role that these viral genes may play in regulating the virus life cycle in monocytes. In the present study, we observed moderately decreased levels of virus replication in the absence of either *vpr* or *vpu*, whereas in the absence of both genes, virus replication in monocytes dropped to levels barely at or below the level of detection by the RT assay, such that infection of these cells usually could be detected only by virus rescue onto PBMCs.

The *vpr* open reading frame encodes a protein of 96 amino acids in most HIV-1 clones and is conserved in other lentiviruses, including visna-maedi virus (20, 31). Previous studies have shown that *vpr* is not required for HIV-1 infection or replication in $CD4^+$ lymphocytic cell lines in vitro, although its inactivation led to slower replication kinetics and delayed cytopathogenicity in these cells (6, 10, 24). A recent study involving HIV type 2 (HIV-2) has shown that *vpr* is likewise dispensable during infection of PBMCs and T-cell lines but essential for productive infection of monocytes (17). The *vpr* protein has been demonstrated by radioimmunoprecipitation to be virion associated, and thus it is speculated to function either late in the virus life cycle, during particle assembly or maturation, or early, during the initial stages of infection (6). The *vpu* gene encodes an 80-to-82-amino-acid protein. It has not been reported whether the *vpu* protein is found in virion particles. *vpu* has been shown to augment virion particle release from infected cells without affecting levels of viral RNA or protein synthesis (8, 32). In the absence of *vpu*, a higher ratio of immature to mature particles has been seen, with a shift in capsid

recombinant NLHXADA clones. To inactivate *vpr*, clones were digested with *Eco*RI (nucleotide 5745), treated with Klenow fragment, and religated to generate a 4-bp insertion, as previously described (24). The replication levels of these clones in monocytes are summarized to the right of each clone. Monocytes were infected as previously described (36) by using filtered virus stocks generated by transfection of proviral DNA onto SW480 cell monolayers, and titers were determined by measuring RT activity. Infections were done at low multiplicities of infection (10 to 100 tissue culture infective doses per well) with monocytes plated at an initial density of 2×10^5 cells per well. Virus replication was monitored by serial determinations of RT activity in culture supernatants (25). Peak RT activities (days 26 to 29) are expressed as 10^5 counts per minute per milliliter. To determine virus rescue, fresh, uninfected PBMCs were added to monocyte cultures at 29 days postinfection, cocultivated (co-cult) for 2 days, and maintained separately for up to 12 additional days, while RT activity was monitored. Rescue was scored as positive with two successive RT results that were more than fivefold above background level. (B) The replication kinetics of representative NLHXADA clones is graphed. Similar results were obtained in three to five replicate experiments.

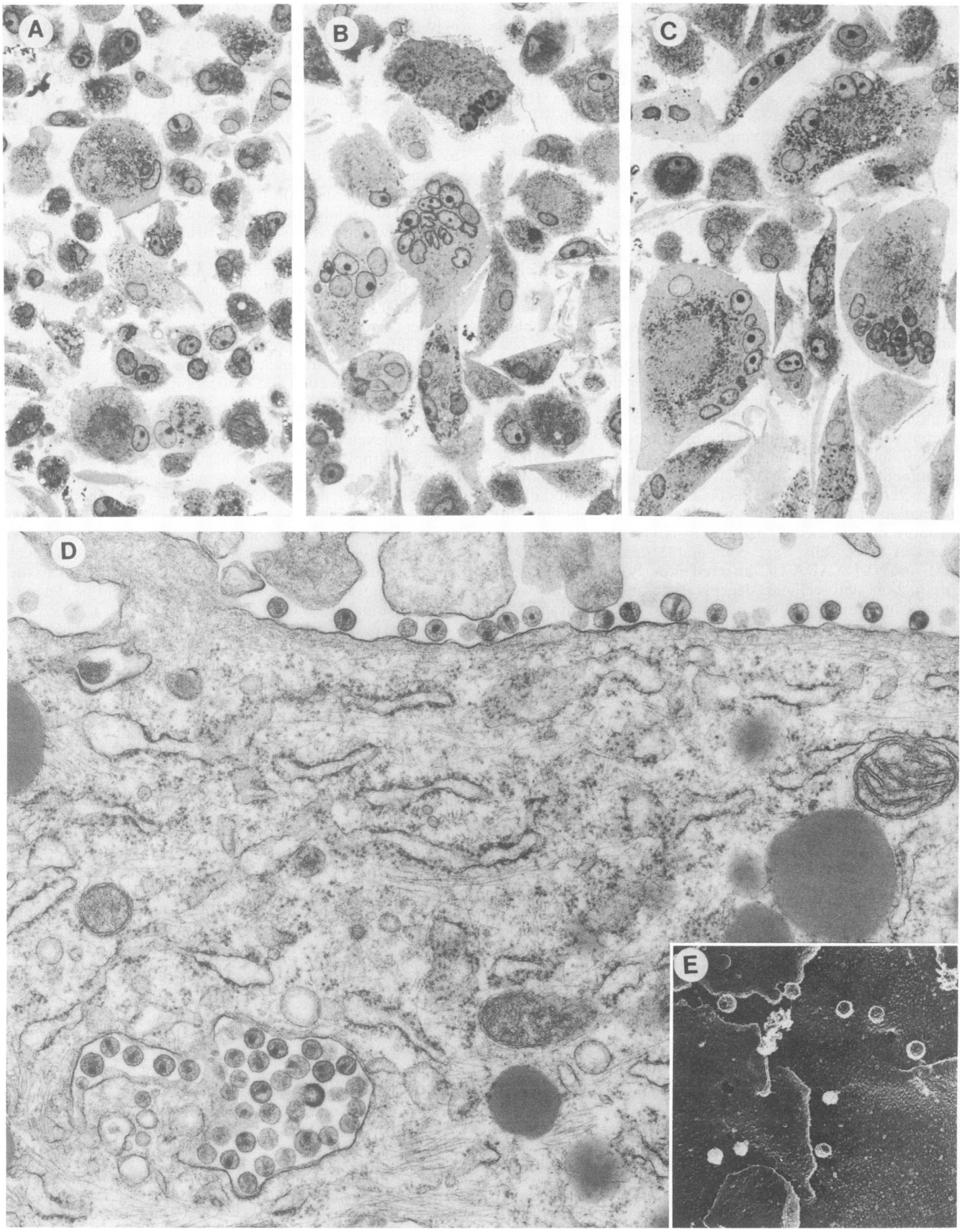
A
B
C
D
E

FIG. 2. Light, transmission, and freeze fracture scanning electron microscopy of infected monocytes. Light micrographs of toluidine blue-stained semithin plastic sections showing typical fields of primary monocytes infected by nonmonocytetropic clone NLHXADA-SK (35) (A) and monocytetropic clones NLHXADA-SM (B) and NLHXADA-GG (C) are shown (15). The multinucleated giant cells were fewer and smaller in panel A than in panels B and C. Magnification, ×480. Infected adherent cultured cells were carefully washed twice with phosphate-buffered saline (PBS), fixed in situ with 2% glutaraldehyde (pH 7.2) in PBS, scraped free with a rubber policeman, transferred to a 15-ml plastic conical tube, and pelleted for 10 min at 600 × *g* centrifugation. The cells were mixed with warm agar, repelleted in the Microfuge for 1 min, and refrigerated overnight to form a firm agar block. The cell block was divided into small pieces and processed into Spurr's plastic, after osmification and block uranyl acetate staining (15). Sections (1 μm thick) were stained with toluidine blue for light microscopy, while thin sections (600 Å [60.0 nm]) were stained with uranyl acetate and lead citrate for transmission electron microscopy. (D) Transmission electron micrograph of a small portion of a multinucleated cell from NLHXADA-GG-infected monocytes showing a cytoplasmic vacuole (lower left) containing immature and mature virions and numerous typical mature particles associated with a stretch of plasma membrane. Magnification, ×34,000. (E) Transmission electron microscopy view of NLHXADA-GG-infected monocytes, stabilized by formaldehyde fixation before quick-freezing, freeze-drying, and platinum replication (18). Budding from the convoluted surface are several 50-nm-diameter brightly outlined spherical virus particles. At higher magnification (not shown), these display characteristic surface coats of gp120 "pegs."

formation from the plasma membrane to intracellular membranes (8). In monocytes, however, particle assembly and release occur both at the plasma membrane and in intracellular vacuoles in the presence or absence of *vpu*, as shown in Fig. 2D.

It is intriguing that HIV-2 and simian immunodeficiency virus lack a *vpu* open reading frame but instead carry a gene designated *vpx*, which encodes a protein of 114 to 118 amino acids in these viruses (20). *vpu* and *vpx* occupy similar positions in their respective viral genomes, between *pol* and *env*, but have only distant amino acid homology. Recently, it has been suggested that *vpx* and *vpr* arose by duplication from a common progenitor in HIV-2 and simian immunodeficiency virus, on the basis of predicted amino acid sequence homology between the genes (34). To investigate the possibility of a similar link between *vpr* and *vpu* in HIV-1, the predicted amino acid sequences of both *vpu* and *vpx* were aligned with that of *vpr* (Fig. 4). Although less compelling that the homology between *vpr* and *vpx*, a 38% identity was observed between *vpr* and *vpu* over a 24-residue overlap at the C terminus of *vpu* and the N terminus of *vpr*. These sequences were particularly rich in acidic residues. Similarity in the hydrophilicity profiles of these portions of the *vpu*, *vpr*, and *vpx* products was also noted. The striking effect on virus replication levels in monocytes observed only when both genes were defective suggests that their gene products may perform similar roles and thus provide partial functional complementation. Alternatively, since lower replication levels were observed in the absence of either gene, the nearly complete attenuation observed in the absence of both may result from a compound effect of the loss of two relatively important but functionally unrelated genes. More detailed studies to determine the precise mechanisms of action of the *vpr* and *vpu* gene products will be required to address these alternatives. In either case, our data indicate that together, *vpr* and a second determinant, *vpu*, are more important for efficient HIV-1 infection and replication in primary monocytes than was observed previously in lymphocytes. These observations provide a rationale for designing potential antiviral therapies to block the action of these gene products during HIV-1 infection of monocytes.

Persistent infection of tissue macrophages plays an important role in the pathogenic effects of other lentiviruses, including equine infectious anemia virus, visna-maedi virus, and caprine arthritis-encephalitis virus, providing a sanctuary for continuous virus replication in the face of a vigorous host immune response (15, 21). The onset of increased virus replication has been correlated with the onset of clinical disease manifestations, such as encephalitis, pneumonitis, arthritis, and hemolytic anemia. Similarly, HIV-1 infection of macrophages generates a reservoir of virus which is present throughout the course of subclinical infection and clinical disease. The existence of poorly replicative HIV-1 variants may be essential for establishment of persistent macrophage infection during the early, asymptomatic stage of disease. Several studies have suggested a relationship

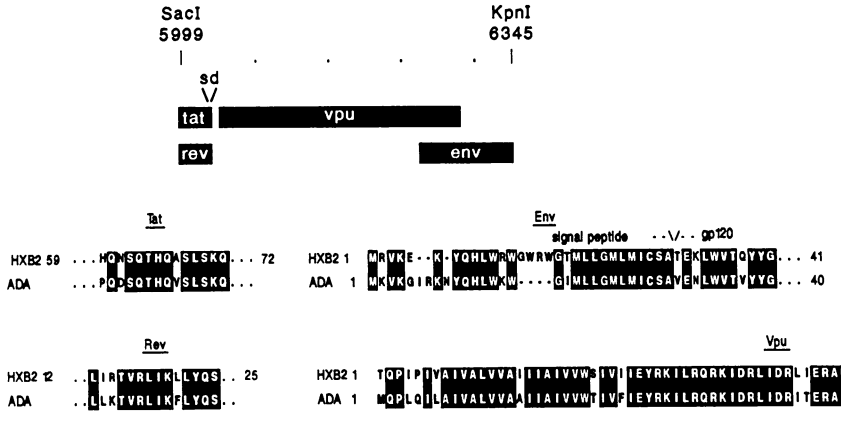

FIG. 3. Comparison of SK virus replication determinant from HXB2 and ADA. The predicted amino acid sequences of *vpu* and the portions of *tat*, *rev*, and *env* which are encoded by nucleotides 5999 to 6345 (SK fragment) from HXB2 and ADA are aligned by using single-letter amino acid designations. Identical residues are indicated within boxes.

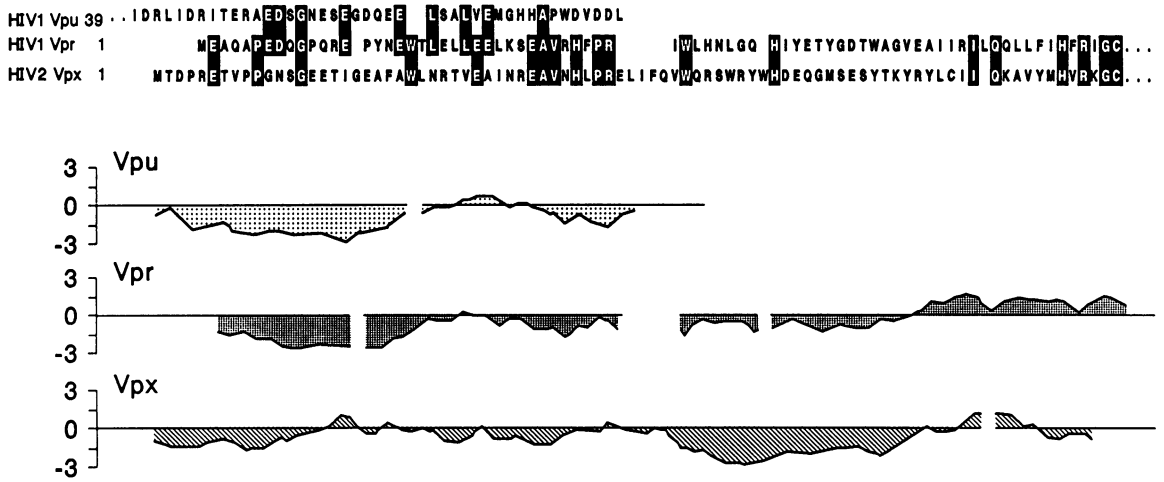

FIG. 4. Predicted amino acid homology between *vpr*, *vpu*, and *vpx*. The predicted amino acid sequence of the NL4-3-derived *vpr* gene is aligned with homologous regions of the ADA-derived *vpu* gene and the *vpx* gene encoded by the HIV-2_{ROD} clone, with single-letter amino acid designations. Identical residues are indicated within boxes. Hydrophilicity profiles for the corresponding segments of each protein are shown at the bottom.

between the in vitro replicative properties of HIV-1 isolates in T lymphocytes and clinical-disease stage, with earlier isolates tending to replicate more slowly and to lower levels ("slow, low") than isolates from later stages of disease ("rapid, high") (3, 5, 27). Nonessential regulatory genes are ideally suited to act as "molecular switches" for control of replication phenotypes by their activation or inactivation, particularly in viruses such as HIV-1, which characteristically generate high levels of sequence diversity. We demonstrate here that discrete genetic alterations in such accessory genes result in profoundly different replication rates in monocytes in vitro, which suggests a mechanism for transition from subclinical to clinical disease in vivo. These findings thus provide a rationale for addressing on a wider scale whether functional status of *vpr* and/or *vpu* correlates with disease stage or serves as a potential prognostic indicator of disease progression and outcome.

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REFERENCES

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. **Armstrong, J. A., and R. Horne.** 1984. Follicular dendritic cells and virus-like particles in AIDS-related lymphadenopathy. Lancet **ii:**370–372.
3. **Asjo, B., L. Manson-Morfeldt, J. Albert, G. Biberfeld, A. Karlsson, K. Ludman, and E. M. Fenyo.** 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet **ii:**660–662.
4. **Chayt, K. J., M. E. Harper, L. M. Marselle, E. B. Lewin, R. M. Rlose, J. M. Oleske, L. G. Epstein, F. Wong-Staal, and R. C. Gallo.** 1986. Detection of HTLV-III RNA in lungs of patients with AIDS and pulmonary involvement. JAMA **256:**2356–2359.
5. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. Science **240:**80–82.
6. **Cohen, E. A., G. Dehni, J. G. Sodroski, and W. A. Haseltine.** 1990. Human immunodeficiency virus *vpr* product is a virion-associated regulatory protein. J. Virol. **64:**3097–3099.
7. **Cohen, E. A., Y. Lu, H. Gottlinger, G. Dehni, Y. Jalinoos, J. G. Sodroski, and W. A. Haseltine.** 1990. The T open reading frame of human immunodeficiency virus type 1. J. AIDS. **3:**601–608.
8. **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.
9. **Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Hoh, and W. A. Haseltine.** 1986. The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. Cell **44:**941–947.
10. **Dedera, D. A., W. Hu, N. Vander Heyden, and L. Ratner.** 1989. Viral protein R of human immunodeficiency virus types 1 and 2 is dispensable for replication and cytopathogenicity in lymphoid cells. J. Virol. **63:**3205–3208.
11. **Eilbott, D. J., N. Peress, H. Burger, D. LaNeve, J. Orenstein, H. E. Gendelman, R. Seidman, and B. Weiser.** 1989. Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: expression and replication in macrophages. Proc. Natl. Acad. Sci. USA **86:**3337–3341.
12. **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 HTLV-III/LAV modulates virus propagation *in vitro*. Science **237:**888–893.
13. **Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, C. Debouk, R. C. Gallo, and F. Wong-Staal.** 1986. The trans-activator gene of HTLV-III is essential for virus replication. Nature (London) **320:**367–371.
14. **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.
15. **Gendelman, H., J. M. Orenstein, L. M. Baca, B. Weiser, H. Burger, D. C. Kalter, and M. S. Meltzer.** 1990. The macrophage in the persistence and pathogenesis of HIV-1 infection. AIDS

3:475–495.
16. **Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, C. H. Lane, A. S. Fauci, and D. S. Burke.** 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. **167:**1428–1441.
17. **Hattori, T., F. Michaels, K. Fargnoli, L. Marcon, R. C. Gallo, and G. Franchini.** 1990. The human immunodeficiency virus type 2 vpr gene is essential for productive infection of human macrophages. Proc. Natl. Acad. Sci. USA **87:**8080–8084.
18. **Heuser, J. E.** 1989. Effects of cytoplasmic acidification on clathrin lattice morphology. J. Cell Biol. **108:**401–411.
19. **Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science **233:**1089–1093.
20. **Myers, G., A. B. Rabson, J. A. Berzofsky, T. F. Smith, and F. Wong-Staal (ed.).** 1990. Human retroviruses and AIDS, 1990. Los Alamos National Laboratory, Los Alamos, N.M.
21. **Narayan, O., and J. E. Clements.** 1989. The biology and pathogenesis of lentiviruses. J. Gen. Virol. **70:**1617–1639.
22. **Niederman, T. M. J., B. J. Thielan, and L. Ratner.** 1989. Human immunodeficiency virus type 1 negative factor is a transcriptional silencer. Proc. Natl. Acad. Sci. USA **86:**1128–1132.
23. **O'Brien, W. A., Y. Koyanagai, A. Namazie, J. Q. Zhao, A. Diagne, K. Idler, J. A. Zack, and I. S. Y. Chen.** 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4 binding domain. Nature (London) **348:**69–73.
24. **Ogawa, K., R. Shibata, T. Kiyomasu, I. Higuchi, Y. Kishida, A. Ishimoto, and A. Adachi.** 1989. Mutational analysis of the human immunodeficiency virus *vpr* open reading frame. J. Virol. **63:**4110–4114.
25. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77:**7415–7419.
26. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313:**277–284.
27. **Schuitemaker, H., N. A. Kootstra, R. E. de Goede, F. de Wolf, F. Miedema, and M. Tersmette.** 1991. Monocytotropic human immunodeficiency virus type 1 (HIV-1) variants detectable in all stages of HIV-1 infection lack T-cell line tropism and syncytium-inducing ability in primary T-cell culture. J. Virol. **65:**356–363.
28. **Schwartz, S., B. K. Felber, E. M. Fenyo, and G. N. Pavlakis.** 1989. Rapidly and slowly replicating human immunodeficiency virus type 1 isolates can be distinguished according to target cell tropism and monocyte cell lines. Proc. Natl. Acad. Sci. USA **86:**7200–7203.
29. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature (London) **349:**167–169.
30. **Sodroski, J. G., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. Haseltine.** 1986. A second post-transcriptional *trans*-activation gene required for HTLV-III replication. Nature (London) **321:**412–417.
31. **Sonigo, P., M. Alizon, K. Staskus, D. Klatzmann, S. Cole, O. Danos, E. Retzel, P. Tiollais, A. Haase, and S. Wain-Hobson.** 1987. Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. Cell **42:**369–382.
32. **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.
33. **Tschachler, E., V. Groh, M. Popovic, D. L. Mann, K. Konrad, B. Safai, L. Eron, F. diMarzo-Veronese, K. Wolff, and G. Stingl.** 1987. Epidermal Langerhans cells—a target for HTLV-III/LAV infection. J. Invest. Dermatol. **88:**233–237.
34. **Tristen, M., C. Marshall, A. Karpas, J. Petrik, and F. Hill.** 1990. Origin of vpx in lentiviruses. Nature (London) **347:**341–342.
35. **Westervelt, P., H. E. Gendelman, Y. Li, and L. Ratner.** 1990. A determinant within the HIV-1 gp120 envelope protein critical for infection of primary monocytes, p. 71–76. *In* R. M. Chanock, H. S. Ginsberg, F. Brown, and R. A. Lerner (ed.), Vaccines 91. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Westervelt, P., H. E. Gendelman, and L. Ratner.** 1991. Identification of a determinant within the HIV-1 surface envelope glycoprotein critical for productive infection of cultured primary monocytes. Proc. Natl. Acad. Sci. USA **88:**3097–3101.
37. **Wiley, C. A., R. D. Schrier, J. A. Nelson, P. W. Lampert, and M. B. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83:**7089–7093.
38. **Wong-Staal, F., P. K. Chanda, and J. Ghrayeb.** 1987. Human immunodeficiency virus: the eighth gene. AIDS Res. Hum. Retroviruses **3:**33–39.