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Infection of Macrophages with Lymphotropic Human Immunodeficiency Virus Type 1 Can Be Arrested after Viral DNA Synthesis

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Lymphotropic strains of human immunodeficiency virus type 1 (HIV-1), including HTLV-IIIB, replicate poorly in macrophages. We have shown previously that lymphotropic HIV-1 fuses equally well with T lymphocytes and macrophages (M. J. Potash, M. Zeira, Z.-B. Huang, T. Pearce, E. Eden, H. Gendelman, and D. J. Volsky, Virology 188:864–868, 1992), suggesting that events in the virus life cycle following virus-cell fusion limit virus replication. We report here that HIV-1 DNA is synthesized efficiently in either ADA or HTLV-IIIB infected alveolar macrophages or monocyte-derived macrophages within 24 h of virus infection, as observed by polymerase chain reaction for amplification of viral DNA sequences from the *gag* gene. Infection by a cloned lymphotropic HIV-1 strain, N1T-A, also leads to viral DNA synthesis. However, circular viral DNA was detected during strain ADA infection but not during HTLV-IIIB or N1T-A infection of monocyte-derived macrophages. These findings indicate that during replication of lymphotropic HIV-1 in macrophages, all steps of the virus life cycle up to and including reverse transcription take place and that defects in later events, including DNA migration to the nucleus, may account for the limited production of viral proteins.

Human immunodeficiency virus type 1 (HIV-1) productively infects both T lymphocytes and monocytes (6, 7, 10, 17, 19, 23), but the extent of replication is strain dependent. To explain the differences in tropism, a defect in virus internalization was suggested in a study of lymphotropic HIV-1 replication in a transformed monocytic cell line (12). In several recombinant viruses, regions of the viral *env* outside the binding site of CD4, but including V3, have been identified as conferring tropism to primary monocytes or to lymphocytes (3, 4, 13, 21, 27, 29). These findings led to the proposition that steps involving virus entry into cells determine tropism of HIV-1. We investigated this issue by measuring HIV-1–macrophage fusion using the membrane fluorescence dequenching assay and found that lymphotropic HIV-1 fused efficiently with alveolar macrophages (AM) (24). However, the fluorescence dequenching assay reflects only an initial phase of the process of virus entry, that is, fusion between the HIV-1 envelope and the cell plasma membrane (15). Therefore, we chose to monitor the next step in virus replication in macrophages which is reliably measured and is dependent upon the completion of HIV-1 entry into cells, the synthesis of viral DNA.

Peripheral blood lymphocytes (PBL) were cultured in medium containing phytohemagglutinin and interleukin 2 and monocyte-derived macrophages (MDM), purified by centrifugal elutriation of blood mononuclear cells, were cultured in 10% GCT-conditioned medium (Sigma, St. Louis, Mo.), a source of several colony stimulating factors. MDM were infected with DNase type IV-treated HTLV-IIIB at a multiplicity of infection (MOI) of one or three, PBL were infected at an MOI of 1, and viral DNA synthesis was monitored by semiquantitative polymerase chain reaction (PCR) amplification as described previously (11) (Fig. 1). Primers SK38 and SK39 (22), which amplify a region of the viral *gag* gene synthesized relatively late in reverse transcription, present in complete and nearly complete HIV-1 DNA molecules (8), were employed for 40 cycles of amplification. These PCR conditions permit the estimation of viral DNA copy numbers in experimental samples in the range from 100 to 10,000 by comparison with the standard cell line, ACH-2, which contains one or two copies of HIV-1 DNA per cell (5). To control the amount of DNA used as substrate for PCR, the single copy cellular gene, the β-globin gene, was amplified in parallel using primers β-3 and β-4 for 30 cycles of amplification (9). Recent studies have pointed out that certain viral stocks contain HIV-1 DNA which is inaccessible to digestion by DNase and may complicate the interpretation of results of PCR (14, 28). To determine specifically whether the viral DNA detected is carried over in the virus stock, is contained in virions (14, 28), or is newly synthesized, an aliquot of the HTLV-IIIB stock was UV-inactivated prior to exposure to MDM. As shown in Fig. 1, no DNA was detected when the virus stock was inactivated prior to infection. In addition, the absence of viral DNA 10 h after infection in this population of MDM indicates that the viral stock itself contained no detectable HIV-1 DNA following DNase digestion. However, viral DNA, which we conclude was newly synthesized, was detected at 24 h after HTLV-IIIB infection of MDM and at 10 h after infection of PBL.

We then analyzed the accumulation of HIV-1 DNA or p24 during the course of infection of a different population of MDM by HTLV-IIIB or by strain ADA (Fig. 2A). No viral DNA was detected at 1.5 h after infection, indicating that any DNA detected at later points during infection was newly synthesized and was not present in the viral stocks. HIV-1 DNA was synthesized within 10 h of infection by both viruses, and the amount of viral DNA increased over the course of

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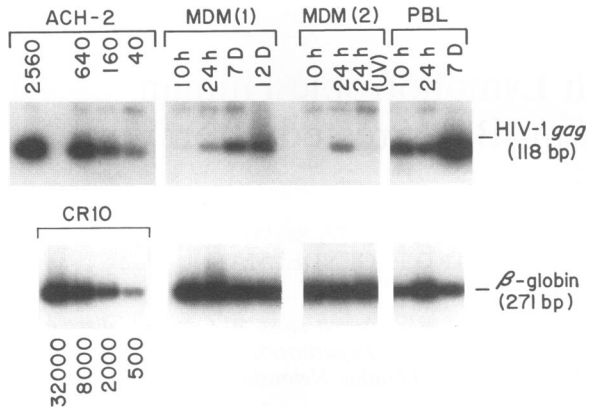

FIG. 1. Synthesis of HIV-1 DNA during HTLV-IIIB infection of PBL or MDM. PBL were isolated and stimulated with PHA-IL2, and MDM were cultured for 3 days prior to infection with DNase treated HTLV-IIIB. MDM (1) were infected at an MOI of 3, and MDM (2) were infected at an MOI of 1. Cells were harvested at the indicated time points and subjected to PCR under standard conditions (9) using primers noted in the text. The bands labeled ACH-2 served as a standard for HIV-1 DNA (3); the subclone of a T-cell leukemia, CR10, served as a control for cellular DNA content, at the numbers of cells indicated.

infection by both viruses. The viral DNA synthesis by HTLV-IIIB-infected MDM was somewhat more rapid in this population of MDM than in the populations illustrated in Fig. 1. However, the production of DNA during HTLV-IIIB infection was lower than or equal to that found during ADA infection in all experiments. As determined by comparing the p24 expression in these infections (Fig. 2B), ADA was 200 times more productive than HTLV-IIIB in MDM at the peak of infection, day 14, validating that the virus stocks employed maintain their previously described tropism (29). Concentrations of HIV-1 p24 detected in the supernatant of HTLV-IIIB-infected MDM increased from less than 10 pg/ml at day 1 after infection to 1,600 pg/ml at day 7, and 3,700 pg/ml at day 14 and then decreased to 1,700 pg/ml at day 21, indicating a low-level virus replication that may permit further cycles of infection and contribute to the observed increase in DNA copy number. This general pattern of infection has been reproduced in MDM derived from three different donors (data not shown). Although the kinetics and extent of infection varied depending on the macrophage population ADA-infected cells produced at least 100-fold more p24 than HTLV-IIIB-infected MDM, but HTLV-IIIB-infected cells generally showed the same levels of or somewhat less viral DNA at the time points tested than ADA-infected macrophages. These findings indicate that the extensive restriction in HTLV-IIIB production during infection of macrophages cannot be attributed to limits on reverse transcription of incoming virion RNA or on any step in the virus life cycle, including entry, which precedes reverse transcription.

Next, we sought to determine the pattern of lymphotropic HIV-1 DNA synthesis in AM. We have shown previously that lymphotropic HIV-1 fuses but does not replicate in AM or produce viral DNA detectable by PCR 21 days after infection (24). We thus compared infection of AM by HTLV-IIIB to infection by ADA by evaluation of viral DNA and p24 synthesis (Fig. 3). AM were obtained by bronchopulmonary lavage of healthy, HIV-1-seronegative volunteers and were purified by adherence to plastic. The AM populations were routinely more than 95% positive for nonspecific esterase.

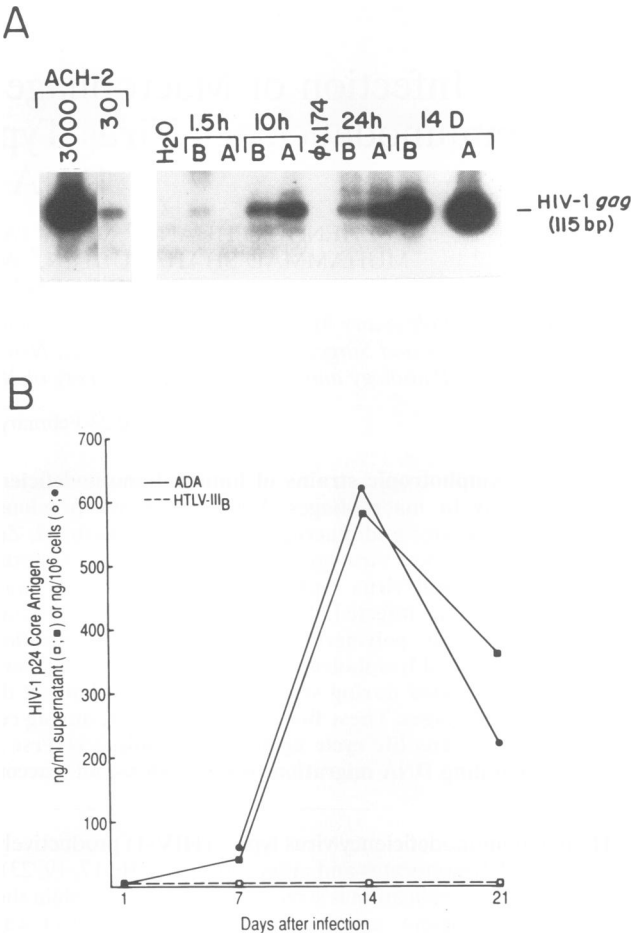

FIG. 2. Synthesis of HIV-1 DNA and p24 during ADA or HTLV-IIIB infection of MDM. (A) MDM cultured for 3 days and infected by ADA (lanes A) or HTLV-IIIB (lanes B) at an MOI of 1 were harvested at the indicated time points after infection for PCR. ACH-2 DNA was amplified in parallel to serve as a standard for HIV-1 DNA. (B) Enzyme-linked immunosorbent assay for intracellular or extracellular p24 core antigen using the HIV Ag kit (Coulter, Hialeah, Fla.) was performed on MDM at the indicated time points.

Similar to the observation during infection of MDM, the absence of viral DNA at 1 h after infection indicated that the DNase treatment of both virus stocks was effective and that the DNA detected later in infection was newly synthesized. AM synthesized viral DNA during either ADA or HTLV-IIIB infection but synthesized less viral DNA and 100-fold less p24 during HTLV-IIIB infection than during ADA infection. The viral DNA copy number declined from about 1 copy per 10 cells to 1 copy per 100 cells during HTLV-IIIB infection. Infection of AM from two other donors produced similar results (not shown). The decline in HTLV-IIIB DNA in AM over time is consistent with our previous report in which no viral DNA was seen 21 days after infection with another lymphotropic HIV-1 strain, N1T (24). These results indicate that the patterns of lymphotropic HIV-1 DNA synthesis in MDM and AM differ. Both cell types synthesize viral DNA within 24 h of infection; the copy number increases or remains stable in MDM but decreases in AM. The cause for these differences is obscure but may derive from the state of differentiation of AM and MDM at the time of infection and during culture.

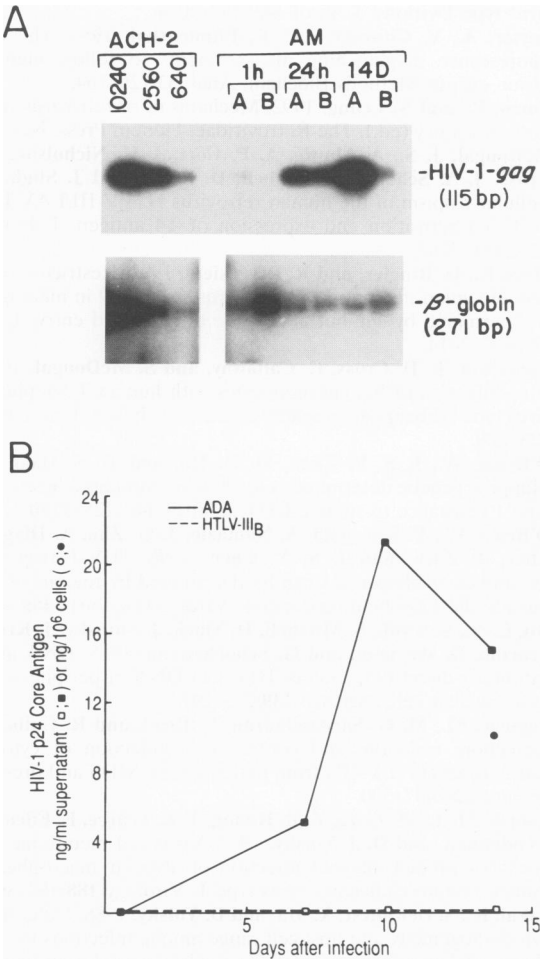

FIG. 3. Synthesis of HIV-1 DNA during ADA or HTLV-IIIB infection of AM. (A) AM were cultured overnight, and the procedures described in the legend to Fig. 2 were followed. Amplification of the single-copy cellular gene, the β-globin gene, was performed as described in the text and served as a control for cellular DNA content. (B) Enzyme-linked immunosorbent assay for extracellular or intracellular p24 core antigen, using the HIV Ag kit, was performed on AM at the indicated times.

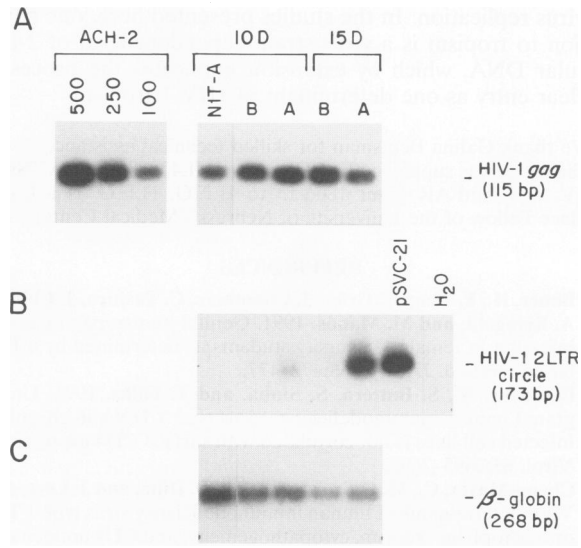

FIG. 4. Synthesis of circular HIV-1 DNA during ADA, HTLV-IIIB, or N1T-A infection of MDM. MDM cultured for 3 days and infected by ADA (lanes A), HTLV-IIIB (lanes B), or N1T-A at an MOI of 1 were harvested at the indicated time points after infection for PCR, using primers for the *gag* region (A), 2-LTR circular DNA (B), and β-globin gene (C). The conditions for amplification of 2-LTR circular DNA (2) and β-globin DNA (1) have been described. The lane labeled pSVC-21 contains a sample from a productively infected culture of T cells which serves as a positive control for circular DNA, and the lane labeled H_2O contains the reagents used for amplification and serves as a negative control for PCR. The levels of extracellular p24 detected at 10 days after infection were 537 pg/ml for ADA-infected cells, 952 pg/ml for HTLV-IIIB-infected cells, and less than 100 pg/ml for N1T-A-infected cells; at 14 days after infection, levels were 8,247 pg/ml for ADA-infected cells and 687 pg/ml for HTLV-IIIB-infected cells.

To determine the fate of the viral DNA synthesized during infection of MDM by lymphotropic HIV-1, we repeated the experiment whose results are shown in Fig. 2 and amplified viral DNA using primers specific for the 2-long terminal repeat (2-LTR) circular DNA form (2), as well as *gag*. We chose to investigate the presence of circular DNA because circularization of retroviral DNA occurs primarily in the nucleus (16), and the detection of circular DNA is a surrogate for assay of nuclear migration. We also infected MDM with N1T-A, a lymphotropic molecular clone of HIV-1 (25), to determine the efficiency of DNA synthesis by a cloned virus replicating in MDM (Fig. 4). Viral DNA was present in ADA-, HTLV-IIIB-, and N1T-A-infected MDM at roughly equivalent levels at 10 or 15 days after infection. However, 2-LTR circular viral DNA was present only in ADA-infected MDM at low levels 10 days after infection and at higher levels 15 days after infection and was not detected in HTLV-IIIB- or N1T-A-infected MDM. These findings suggest that although there is little quantitative difference in viral DNA synthesis by lymphotropic or monocytotropic strains replicating in MDM, there is a marked qualitative difference, which may reside in the entry of the viral DNA into the nucleus.

The results presented here demonstrate that lymphotropic HIV-1 efficiently enters and synthesizes viral DNA during infection of macrophages, indicating that the inefficient HTLV-IIIB expression in these cells must arise from restrictions at later phases of virus replication. This study confirms our previous report on lymphotropic HIV-1 fusion and replication in AM (24). That the cloned lymphotropic HIV-1 strain N1T-A behaved similarly to the HTLV-IIIB isolate excludes the possibility that a cryptic monocytotropic variant within the HTLV-IIIB population accounted for the viral DNA synthesis observed in our experiments.

Our findings are supported by two recent studies. Schmidtmayerova and colleagues showed that infection of MDM by three different lymphotropic HIV-1 strains resulted in a high frequency of cells synthesizing HIV-1 DNA (26). Mori and colleagues found that a lymphotropic strain of simian immunodeficiency virus synthesized viral DNA during infection of simian alveolar macrophages (18). These studies and ours differ from previous reports which showed a deficiency in HIV-1 DNA synthesis during infection of blood monocytes by lymphotropic HIV-1 strains (20, 21). There are several possible explanations for the differences, including the particular strains of HIV-1, the culture conditions, the infection conditions, and the time of sampling employed. Tropism to macrophages has been shown to be controlled by *vpr* and *vpu* (30) in addition to *env* and may devolve from restrictions in more than one stage

of virus replication. In the studies presented here, one contribution to tropism is a virus strain-dependent level of 2-LTR circular DNA, which by extension implicates the process of nuclear entry as one determinant of HIV-1 tropism.

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REFERENCES

1. **Bauer, H., Y. Ting, C. Grier, J. Chambers, C. Tashiro, J. Chimera, A. Reingold, and M. Manos.** 1991. Genital human papiloma virus infection in female University students as determined by a PCR-based method. JAMA **265**:472–477.
2. **Besansky, N., S. Buttera, S. Sinha, and T. Folks.** 1991. Unintegrated human immunodeficiency virus type 1 DNA in chronically infected cell lines is not correlated with surface CD4 expression. J. Virol. **65**:2695–2698.
3. **Cheng-Mayer, C., M. Quiroga, J. Tung, D. Dina, and J. Levy.** 1990. Viral determinants of human immunodeficiency virus type 1 T-cell or macrophage tropism, cytopathogenicity, and CD4 antigen modulation. J. Virol. **64**:4390–4398.
4. **Chesebro, B., J. Nishio, S. Perryman, A. Cann, W. O'Brien, I. S. Y. Chen, and K. Wehrly.** 1991. Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-leukemia cells, and macrophages. J. Virol. **65**:5782–5789.
5. **Clouse, K. A., D. Powell, I. Washington, G. Poli, K. Strebel, W. Farrar, P. Barstad, J. Kovacs, A. S. Fauci, and T. Folks.** 1989. Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone. J. Immunol. **142**:431–438.
6. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1988. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233**:215–219.
7. **Gendelman, H. E., J. Orenstein, M. Martin, G. Gerrua, R. Mitra, T. Phipps, L. Wahl, C. Lane, A. Fauci, D. Burke, D. Skillman, and M. 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.** 1990. Retroviral reverse transcriptase: synthesis, structure and function. J. Acquired Immune Defic. Syndr. **3**:817–831.
9. **Higuchi, R.** 1989. Simple and rapid preparation of samples for PCR. *In* H. Erlich (ed.), PCR technology. Principles and applications for DNA amplification. Stockton Press, New York.
10. **Ho, D., T. Rota, and M. S. Hirsch.** 1986. Infection of monocyte-macrophages by human T lymphotropic virus type III. J. Clin. Invest. **77**:1712–1715.
11. **Inis, M. A., D. Gelfand, J. Suinsky, and T. White (ed.).** 1990. PCR protocols, Academic Press, San Diego.
12. **Kim, S., K. Ikeuchi, J. Groopman, and D. Baltimore.** 1990. Factors affecting cellular tropism of human immunodeficiency virus. J. Virol. **64**:5600–5604.
13. **Liu, Z.-Q., C. Wood, J. Levy, and C. Cheng-Mayer.** 1990. The viral *env* gene is involved in macrophage tropism of a human immunodeficiency virus type 1 strain isolated from brain tissue. J. Virol. **64**:6148–6153.
14. **Lori, F., F. Veronese, A. L. De Vico, P. Lusso, M. S. Reitz, Jr., and R. C. Gallo.** 1992. Viral DNA carried by human immunodeficiency virus type 1 virions. J. Virol. **66**:5067–5074.
15. **Loyter, A., V. Citovsky, and R. Blumenthal.** 1988. The use of fluorescence dequenching measurements to follow membrane fusion events. Methods Biochem. Anal. **33**:129–164.
16. **Luciw, P., and N. Leung.** 1992. Mechanisms of retrovirus replication. *In* J. Levy (ed.), The Retroviridae. Plenum Press, New York.
17. **McDougal, J. S., A. Mawle, A. P. Cort, J. K. Nicholson, G. D. Cross, J. A. Scheppler-Campbell, D. Hicks, and J. Sligh.** 1985. Cellular tropism of the human retrovirus HTLV-III/LAV I. Role of T cell activation and expression of T4 antigen. J. Immunol. **135**:3151–3162.
18. **Mori, K., D. Ringler, and R. Desrosiers.** 1993. Restricted replication of simian immunodeficiency virus strain 239 in macrophages is determined by *env* but is not due to restricted entry. J. Virol. **67**:2807–2814.
19. **Nicholson, J., D. Cross, C. Callaway, and S. McDougal.** 1986. In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus. J. Immunol. **137**:323–329.
20. **O'Brien, W., I. S. Y. Chen, D. D. Ho, and E. S. Daar.** 1992. Mapping genetic determinants for human immunodeficiency virus type 1 resistance to soluble CD4. J. Virol. **66**:3125–3130.
21. **O'Brien, W., Y. Koyanagi, A. Namazie, J.-Q. Zho, A. Diague, K. Idler, J. 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.
22. **Ou, C.-Y., S. Kwok, S. Mitchell, D. Mack, J. Sninsky, J. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **239**:295–297.
23. **Popovic, M., M. G. Sarngadharan, E. Read, and R. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
24. **Potash, M. J., M. Zeira, Z.-B. Huang, T. E. Pearce, E. Eden, H. E. Gendelman, and D. J. Volsky.** 1992. Virus-cell membrane fusion does not predict efficient infection of alveolar macrophages by human immunodeficiency virus type 1. Virology **188**:864–868.
25. **Sakai, K., S. Dewhurst, X. Ma, and D. Volsky.** 1988. Differences in cytopathogenicity and host cell range among infectious molecular clones of human immunodeficiency virus type 1 simultaneously isolated from an individual. J. Virol. **62**:4078–4085.
26. **Schmidtmayerova, H., C. Bolmont, S. Baghdiguian, I. Hirsch, and J.-C. Chermann.** 1992. Distinctive pattern of infection and replication of HIV-1 strains in blood-derived macrophages. Virology **190**:124–133.
27. **Shioda, T., J. 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.
28. **Trono, D.** 1992. Partial reverse transcripts in virions from human immunodeficiency and murine leukemia viruses. J. Virol. **66**:4893–4900.
29. **Westervelt, P., H. E. Gendelman, and L. Ratner.** 1991. Identification of a determinant within the human immunodeficiency virus surface envelope glycoprotein critical for productive infection of primary monocytes. Proc. Natl. Acad. Sci. USA **88**:3097–3101.
30. **Westervelt, P., T. Henkel, D. B. Trowbridge, J. Orenstein, J. Heuser, H. E. Gendelman, and L. Ratner.** 1992. Dual regulation of silent and productive infection in monocytes by distinct human immunodeficiency virus type 1 determinants. J. Virol. **66**:3925–3931.