November 1993

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

Zheng-Bo Huang  
*St. Lukes/Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University*

Mary Jane Potash  
*St. Lukes/Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University*

Malgorzata Simm  
*St. Lukes/Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University*

Wei Chao  
*St. Lukes/Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University*

Howard Gendelman  
*University of Nebraska Medical Center & Nebraska Center for Virology, hegendel@unmc.edu*

*See next page for additional authors*

Follow this and additional works at: [http://digitalcommons.unl.edu/virologypub](http://digitalcommons.unl.edu/virologypub)

Part of the Virology Commons

Huang, Zheng-Bo; Potash, Mary Jane; Simm, Malgorzata; Chao, Wei; Gendelman, Howard; Edin, Edward; and Volsky, David J., "Infection of Macrophages with Lymphotropic Human Immunodeficiency Virus Type 1 Can Be Arrested after Viral DNA Synthesis" (1993). Virology Papers. 96.  
[http://digitalcommons.unl.edu/virologypub/96](http://digitalcommons.unl.edu/virologypub/96)

This Article is brought to you for free and open access by the Virology, Nebraska Center for at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Virology Papers by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Authors
Zheng-Bo Huang, Mary Jane Potash, Malgorzata Simm, Wei Chao, Howard Gendelman, Edward Edin, and David J. Volsky
Infection of Macrophages with Lymphotropic Human Immunodeficiency Virus Type 1 Can Be Arrested after Viral DNA Synthesis

ZHENG-BO HUANG,1 MARY JANE POTASH,2 MALGORZATA SIMM,2 MUHAMMAD SHAHABUDDIN,2 WEI CHAO,3 HOWARD E. GENDELMAN,3 EDWARD EDEN,1 AND DAVID J. VOLSKY2*  

Division of Pulmonary Medicine1 and Molecular Virology Laboratory,2 St. Lukes/Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University, New York, New York 10019, and Departments of Medicine and Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 681983

Received 23 February 1993/Accepted 26 July 1993

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, NIT-A, also leads to viral DNA synthesis. However, circular viral DNA was detected during strain ADA infection but not during HTLV-IIIB or NIT-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

* Corresponding author.
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 donors and were purified by adherence to plastic. The AM populations were routinely more than 95% positive for nonspecific esterase.

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, NIT (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.
Viral DNA present was not prescribed in single-copy cellular 15 infection of ADAM (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.

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 NIT-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 NIT-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 NIT-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 NIT-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. Schmidt-mayerova 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.

We thank Galina Bentsman for skilled technical assistance.

This work was supported by PHS grants HL43628 and CA37465 to D.J.V. and AmFAR grant 1806-13 to H.E.G. H.E.G. is a Carter-Wallace Fellow of the University of Nebraska Medical Center.

REFERENCES


