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Human Immunodeficiency Virus Type 1 Vpr Arrests the Cell Cycle in G_2 by Inhibiting the Activation of $p34^{cdc2}$-Cyclin B

FABIO RE,[1] DOUGLAS BRAATEN,[1] ETTALY KARA FRANKE,[2] AND JEREMY LUBAN[1,2]*

Departments of Microbiology[1] and Medicine,[2] Columbia University College of Physicians and Surgeons, New York, New York 10032

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Human immunodeficiency virus type 1 (HIV-1) *vpr* inhibits the replication of tumor cell lines and peripheral blood mononuclear cells. Here it is demonstrated that expression of *vpr*, either in the context of a provirus or from an independent genetic element, induces a discrete cell cycle arrest, with cells containing 4*N* DNA. Low cyclin B-associated kinase activity, as well as the status of $p34^{cdc2}$ and cdc25C phosphorylation, indicates that the cascade of reactions which drives the cell into mitosis has not been initiated. The phosphatase inhibitor okadaic acid releases the block, suggesting that Vpr perturbs upstream regulators of the G_2-M transition. These studies demonstrate that HIV-1 *vpr* has profound effects on the cellular factors which control entry into mitosis and indicate *vpr*'s potential contribution to the cellular pathology associated with HIV-1 infection.

Infection with human immunodeficiency virus (HIV) leads inexorably to the destruction of the host immune system: AIDS. Initially, there is loss of $CD4^+$ helper T-cell memory function (21, 26, 37), and with time, there is progressive decline in the number of circulating $CD4^+$ T cells (31). Upon infection of a host, the virus replicates continuously in lymphoid tissues (30), with greater than 10^9 $CD4^+$ cells destroyed and replenished each day (15, 38). This number is similar to estimates of the total number of HIV-expressing $CD4^+$ cells in the body (8, 11), suggesting that AIDS results from the continuous lytic replication of HIV.

A number of theories have been proposed to account for the cytotoxicity associated with HIV-1 infection (31, 39). Among the HIV-1 genes disruptive of cellular function is *vpr*, which encodes a 15-kDa virion-associated protein (5, 42) that is transported to the nucleus of newly infected cells as part of the preintegration complex (14). *vpr* has been associated with the inhibition of replication of cell lines and peripheral blood mononuclear cells and with an accumulation of cells bearing 4*N* DNA (23, 34). Also, disrupted *vpr* coding sequences have been observed in HIV type 1 (HIV-1) proviruses cloned from chronically infected cell lines (33, 34), suggesting that *vpr* expression is selected against in tissue culture.

Here we confirm that *vpr* expression in the context of a provirus prevents cell proliferation and that *vpr* expression independent of other viral elements is sufficient to arrest cells with 4*N* DNA. In addition, we demonstrate that cells expressing *vpr* are arrested with all the biochemical hallmarks of G_2 and that Vpr is most likely interacting with upstream regulators of the cyclin-associated kinase which regulates the G_2-M transition.

MATERIALS AND METHODS

Plasmid DNAs. pNLpuro (23), from D. Weiner, is the complete HIV-1 provirus pNL4-3 (1), in which *env* has been replaced with the selectable marker SV40-*puro*. pNLpuro^{vpr-} contains a frameshift mutation which shortens the 96-codon *vpr* open reading frame to 27 codons. It was engineered by standard methods (35) by filling in an *Afl*II site with the Klenow fragment of DNA polymerase. pHXB2-env (29), from N. Landau, expresses HIV-1 *env* from the simian virus 40 promoter.

HIV-1_{NL4-3} *vpr* (1) was amplified by PCR (35) with oligonucleotides 5′-CGCGCTCGAGGATCCGAGAGATGGAACAAGCCCCAGAAGACC-3′ and 5′-GCGCGGATCCCTAGGATCTACTGGCTCCATTTCTTGC-3′. Simian immunodeficiency virus strain MAC239 (SIV_{MAC239}) *vpx* (18) was amplified with oligonucleotides 5′-CGCGGATCCAGATGTCAGATCCCAGGGAGAGAATC-3′ and 5′-GCGCGGATCCTTATGCTAGTCCTGGAGGGGGAG-3′. Products were digested with *Bam*HI and cloned into pET-11d (Novagen) so that they were 3′ and in-frame with the gene *10* leader peptide. Products were digested with *Nco*I and *Esp*I and transferred to a modified pBluescript II KS$^-$ (Stratagene) which contains consensus eukaryotic ribosome initiation sequences (20) 5′ of an *Nco*I site. These plasmid DNAs were digested with *Spe*I and *Xho*I and ligated to the *Nhe*I and *Xho*I sites of pCEP-4 (Invitrogen) to express either *vpr* or *vpx* fused to the gene *10* leader peptide from the cytomegalovirus immediate-early promoter.

Cells, transfections, viral stocks, and protein expression. Plasmid DNAs were transfected into mammalian cells by calcium phosphate precipitation (7). Viral stocks infectious for a single round of infection were produced by transfection of 293T cells with 10 μg of pHXB2-env and 10 μg of either pNLpuro or pNL puro^{vpr-}. Virion-containing supernatant was harvested at 48 h, filtered, and normalized for virion content by reverse transcriptase activity and Western (immunoblot) analysis for p24 as previously described (9). Rabbit anti-Vpr antibody was a gift from Lee Ratner. Infection, selection, fixation, and staining of $CD4^+$ HeLa cells (from T. Livelli and R. Axel) were carried out as described before (29) except that selection was done with 1 μg of puromycin per ml.

To assay protein expression, HeLa cells were transfected with 20 μg of the pCEP-4-based expression plasmid DNAs. Cells were lysed with IP buffer (25 mM Tris-HCl [pH 8.0], 50 mM NaCl, 5 mM EDTA, 10 mM EGTA [ethylene glycol tetraacetic acid], 1% Triton X-100, 1% sodium deoxycholate, 50 mM NaF, 20 mM β-glycerolphosphate, 100 μM Na_3VO_4, 10 μg of soybean trypsin inhibitor per ml, 1 μg of leupeptin per ml, 50 of phenylmethylsulfonyl fluoride per ml) at 48 h or at the indicated time following selection with hygromycin (750 μg/ml). Lysates were subjected to Western blot analysis as previously described (9) with a monoclonal T7-Tag antibody (Novagen).

Flow cytometry. HeLa cells were placed under selection (750 μg of hygromycin per ml) 12 h posttransfection with the pCEP-4-based expression plasmids. At 72 h, plates were trypsinized to remove dying cells, and viable cells were replated under selection. Cells to be arrested with aphidicolin or nocodazole were first transfected with pCEP-4 DNA, then trypsinized at 48 h, and replated under selection; at 72 h, either aphidicolin (5 μg/ml) or nocodazole (0.4 μg/ml) was added to the medium. At 96 h, 1×10^6 to 2×10^6 cells from each transfection were fixed with 80% ethanol and stained with a solution containing 0.05 mg of propidium iodide per ml, 0.3% Nonidet P-40, and 1 mg of RNase A per ml in phosphate buffered saline (PBS). Cells were analyzed by flow cytometry with a FACStar Plus (Becton Dickinson). Doublets were excluded from the analysis based on pulse width measurements. Equivalent numbers of cells sorted from the 2*N* and 4*N* peaks were subjected to Western blot analysis.

Cell cycle biochemistry. HeLa cells were transfected, selected, and treated with aphidicolin and nocodazole as described for flow cytometric analysis. Cells were lysed in IP buffer, and samples were normalized for total protein by the Bradford dye-binding procedure (Bio-Rad). The lysate was probed directly in Western blots with monoclonal anti-cyclin B1 antibody or rabbit anti-cdc25C (both from Santa Cruz Biotech) as primary antibodies. The same cell lysate was

* Corresponding author. Mailing address: Departments of Microbiology and Medicine, Columbia University College of Physicians and Surgeons, 701 W. 168th St., New York, NY 10032. Phone: (212) 305-8706. Fax: (212) 305-8706. Electronic mail address: Luban@cuccfa.ccc.columbia.edu.

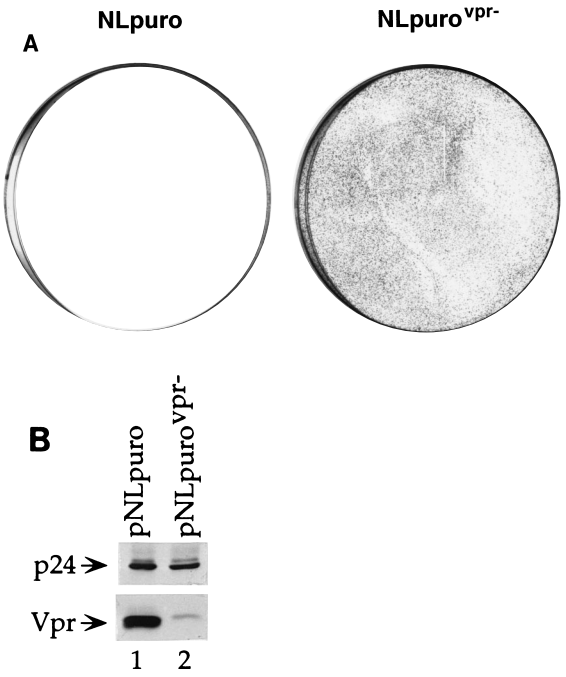

FIG. 1. Cell proliferation is blocked by infection with HIV-1 expressing *vpr*. (A) $CD4^+$ HeLa cells were infected with virions competent for a single round of infection produced by proviral DNAs conferring resistance to puromycin. The *vpr* open reading frame is complete in pNLpuro and disrupted by a frameshift in pNLpuro^{vpr-}. Cells were selected in puromycin for 4 days following infection, and proliferating colonies were detected by staining with crystal violet. (B) Virions purified from the supernatant of cells transfected with pNLpuro or pNLpuro^{vpr-} (lanes 1 and 2, respectively) were probed in Western blots with primary antibodies against p24 and Vpr, as indicated.

immunoprecipitated by standard methods (12) with 0.5 μg of the anti-cyclin B1 antibody and protein A-Sepharose beads (Sigma). Cyclin B-associated proteins were probed in Western blots with either rabbit anti-cdc2 (Oncogene Science) or monoclonal antiphosphotyrosine antibody (Santa Cruz Biotech), or they were used in kinase assays with histone H1 as the substrate, as previously described (16). Briefly, proteins associated with protein A-Sepharose were incubated at 30°C for 20 min in 80 mM β-glycerolphosphate–10 mM $MgCl_2$–1 mM dithiothreitol–20 mM EGTA–10 μM ATP–100 μg of histone H1 (Boehringer) per ml–5 μCi of [γ-^{32}P]ATP (6,000 Ci/mmol)–5 μM peptide inhibitor of cyclic AMP-dependent protein kinase (Sigma). Histone H1 kinase activity was quantitated on a PhosphorImager (Molecular Dynamics). In the indicated experiments, okadaic acid (Sigma) was added to tissue culture medium to a final concentration of 0.5 μM.

RESULTS

Expression of *vpr* within the context of a provirus blocks cell proliferation. To establish that *vpr* inhibits the replication of HIV-infected cells, $CD4^+$ HeLa cells were placed under selection following infection with virus containing a selectable marker in place of *env*. The virus stock used for infection was produced by expressing *env* in *trans* and therefore was competent for only a single round of infection (29). A virus stock from pNLpuro (23), a provirus which contains a complete *vpr* coding sequence, produced fewer than 10 colonies per ml (Fig. 1A). Closer examination of the cells infected by NLpuro demonstrated that puromycin-resistant cells were present but that the majority were unable to proliferate and form colonies. To determine if *vpr* accounted for the inability of these cells to proliferate, a modified pNLpuro in which a frameshift mutation disrupted the *vpr* coding sequence, rendering *vpr* expression undetectable by Western blot, was engineered (Fig. 1B). When viral particle number was normalized to that of the parent virus, the virus stock from pNLpuro^{vpr-} produced 10^4 colonies per ml (Fig. 1A). Thus, *vpr* expression from an integrated provirus precludes host cell proliferation.

Vpr is sufficient to block cell proliferation. To evaluate the effect of its expression independent of other viral elements, *vpr* and the genetically related *vpx* from SIV_{MAC239} (36) were expressed as fusion proteins with a common epitope tag, enabling comparison of expression with a single antibody. At 48 h following transfection of expression plasmids into HeLa cells, *vpr* and *vpx* were found to be expressed at comparable levels (Fig. 2A). When the transfected cells were placed under selection, large numbers of proliferating colonies which expressed *vpx* were obtained (Fig. 2B); in contrast, while many hygromycin-resistant cells were observed following transfection with the *vpr* expression plasmid, few of these cells were able to proliferate and form colonies (Fig. 2B). The nonproliferating cells retained viability for more than 2 weeks; the cells reattached to plastic after release by trypsin and continued to increase in size (Fig. 2C). By immunofluorescence, the nuclei of *vpr*-expressing cells had a strong signal for Vpr, as reported by others (24). This was true of all the nonproliferating cells, while those rare cells which did replicate had no *vpr* expression detectable (data not shown). Differences in cell proliferation were not due to differences in the level of *vpx* expression, since *vpr* and *vpx* were expressed at comparable levels in cells under selection (Fig. 2A). Thus, expression of *vpr* is sufficient for blocking cellular replication.

Cells arrested by Vpr contain 4*N* DNA. To pinpoint which phase of the cell cycle was blocked by *vpr* expression, the DNA content of transfected HeLa cells placed under selection for 3 days was quantitated by flow cytometry. Cells transfected either with vector or with the *vpx* expression plasmid showed a DNA profile similar to that of unsynchronized, cycling cells grown without hygromycin (Fig. 3A). Cells transfected with vector and subsequently subjected to cell cycle blocks with aphidicolin or nocodazole showed the patterns expected of cells blocked in S phase and mitosis, respectively (Fig. 3A). A majority of the cells transfected with the *vpr* expression plasmid had 4*N* DNA content (Fig. 3A). To determine if the 2*N* cells remaining in the population failed to express *vpr*, the 2*N* and 4*N* peaks were sorted and examined by Western blot for *vpr* expression (Fig. 3B). Cells with 4*N* DNA expressed *vpr* to high levels, while *vpr* expression was almost undetectable in cells from the 2*N* fraction. This indicates that *vpr* expression leads to an absolute block in either G_2 or M. Staining of *vpr*-expressing cells with rabbit anti-lamin A and DAPI (4′,6-diamidino-2-phenylindole) demonstrated that the nuclear lamina was intact and no mitotic figures were evident (data not shown), consistent with a block in G_2.

Cell cycle arrest due to Vpr has all the biochemical characteristics of G_2. Progression from the G_2 phase of the cell cycle into mitosis is regulated by a complex consisting of the protein kinase $p34^{cdc2}$ and the positive regulatory subunit cyclin B (19, 27). $p34^{cdc2}$ is synthesized constitutively, but cyclin B levels oscillate, accumulating during the S and G_2 phases. Once the $p34^{cdc2}$-cyclin B complex assembles, its kinase activity is inactivated by a network of kinases and phosphatases, including Wee1, which phosphorylates Tyr-15. When DNA replication and other preparations for mitosis are completed, the phosphatase cdc25C is activated by multiple phosphorylations and in this state removes the inhibitory phosphate from $p34^{cdc2}$. Activation of $p34^{cdc2}$ then begins a cascade of reactions which render mitosis inevitable.

To obtain biochemical evidence that *vpr* expression arrests the cell cycle in G_2, the cell populations previously analyzed for DNA content were also examined for cyclin B-associated protein kinase activity, with histone H1 as a substrate. Cells

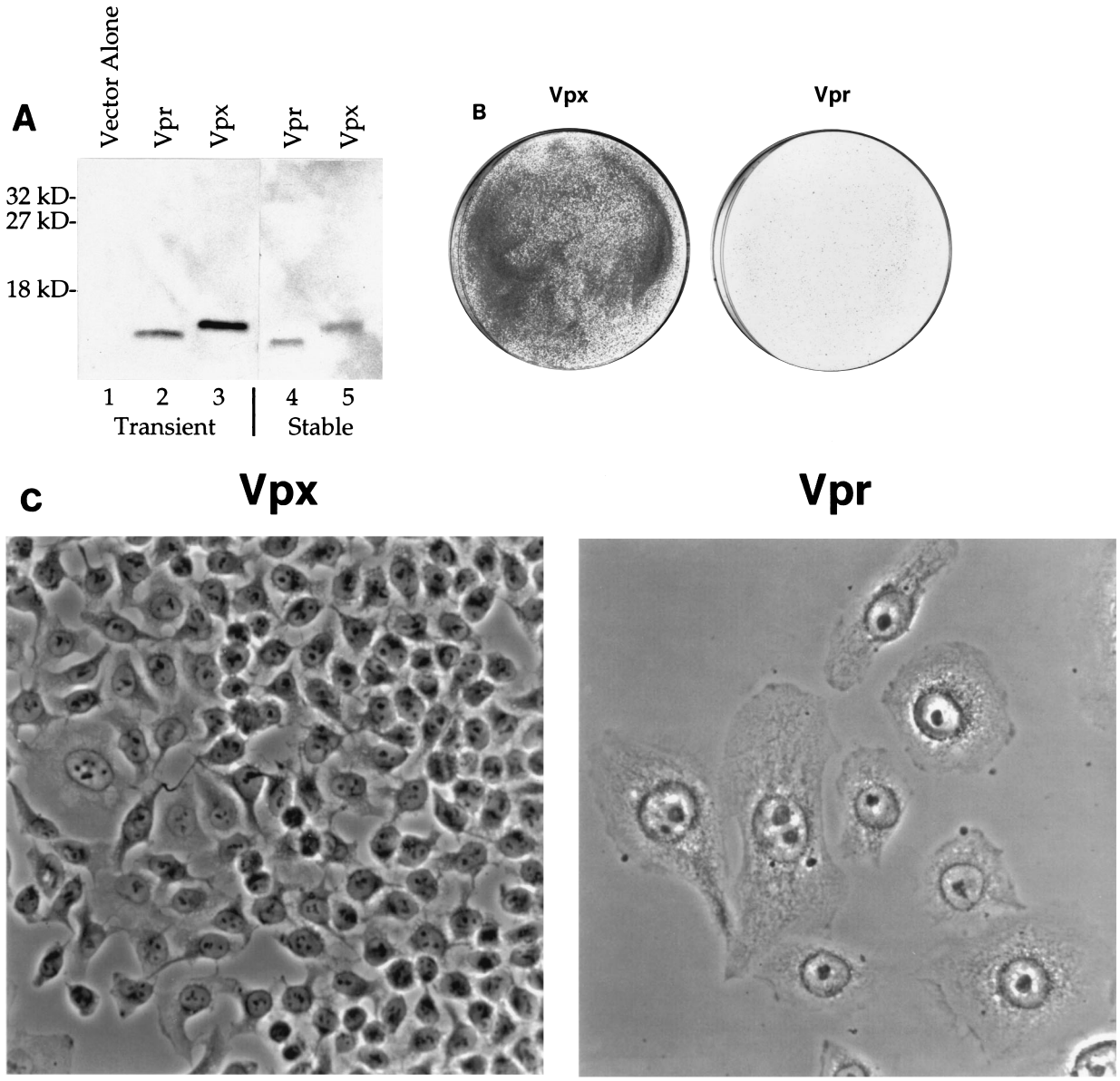

FIG. 2. Expression of HIV-1 *vpr* independent of other viral elements blocks cell proliferation. HeLa cells were transfected with *vpr* or *vpx* expression vectors encoding a common epitope tag. (A) Cell lysate was subjected to Western blot analysis 48 h posttransfection (lanes 1 to 3) or after 7 days under hygromycin selection (lanes 4 and 5). Hygromycin-selected cells were either stained with crystal violet to detect proliferating colonies (B) or fixed in methanol and examined by phase-contrast microscopy (C). The two phase-contrast micrographs were photographed at the same magnification.

blocked in mitosis with nocodazole had very high kinase activity, 100 times greater than that of an unsynchronized cycling population (data not shown) or cells blocked in S phase (Fig. 4A). Cells expressing *vpr* had low kinase activity, comparable to that of cells blocked in S phase (Fig. 4A), consistent with a cell cycle block prior to mitosis. The low kinase activity in *vpr*-expressing cells was not due to failure to assemble the $p34^{cdc2}$-cyclin B complex, since cyclin B and cyclin B-associated $p34^{cdc2}$ protein levels were comparable to those of the nocodazole-blocked cells (Fig. 4A). Rather, the cyclin B-associated $p34^{cdc2}$ from the *vpr*-expressing cells was found to be inactive because of tyrosine phosphorylation; this was demonstrated both by the protein's slower mobility compared with $p34^{cdc2}$ from nocodazole-blocked cells and its reactivity with an antiphosphotyrosine antibody (Fig. 4A). In addition, the phosphatase which removes the inhibitory phosphate from $p34^{cdc2}$, cdc25C, which is itself activated by multiple phosphorylations, was only detected in the inactive, unphosphorylated, faster-migrating form in the lysate of *vpr*-expressing cells (Fig. 4A). Thus, cells expressing *vpr* possess 4*N* DNA content, inactive cyclin B-associated $p34^{cdc2}$ kinase activity, and the inactive form of cdc25C, biochemical characteristics of cells blocked in G_2.

Cell cycle arrest due to Vpr is relieved by okadaic acid. Cells arrested in S phase by aphidicolin can be induced to activate $p34^{cdc2}$-cyclin B and enter mitosis by treatment with okadaic acid, despite the block to DNA replication (41). It is believed that okadaic acid, a specific inhibitor of protein phosphatases 1 and 2A, bypasses this cell cycle checkpoint by inhibiting a phosphatase other than cdc25C which acts upstream of $p34^{cdc2}$-cyclin B (17). The end result is that the inhibitory

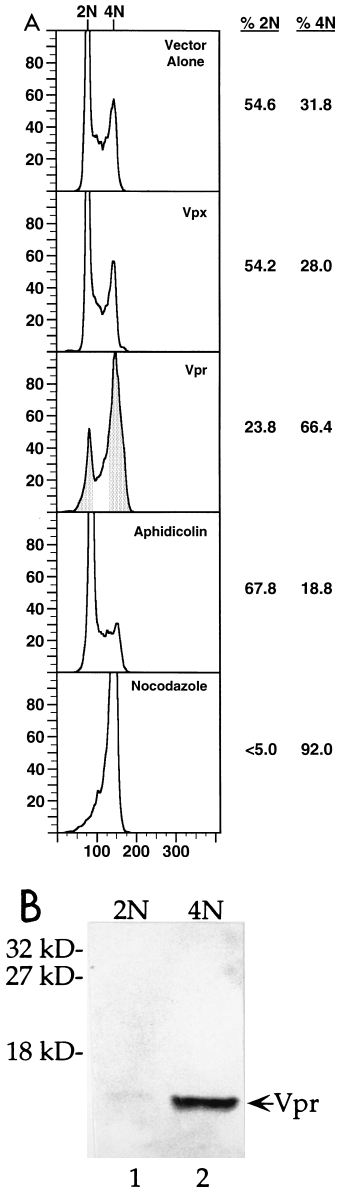

FIG. 3. HeLa cells expressing *vpr* arrest in the cell cycle with 4*N* DNA content. (A) Flow cytometric analysis of HeLa cells stained with propidium iodide (PI). HeLa cells were transfected with *vpr* or *vpx* expression plasmids, or vector alone. As additional controls, cells transfected with vector alone were treated with aphidicolin or nocodazole, which block the cell cycle in the S and M phase, respectively. The abscissa is the relative scale of PI fluorescence, indicating DNA content, and the ordinate shows relative cell number. The percentage of cells containing 2*N* and 4*N* DNA in each population was derived by using the same windows indicated by the shaded regions in the DNA histogram of *vpr*-transfected cells. (B) Western blot demonstrating *vpr* expression in equivalent numbers of cells sorted from the 2*N* and 4*N* peaks of *vpr*-transfected cells as indicated by the shaded regions on the DNA histogram.

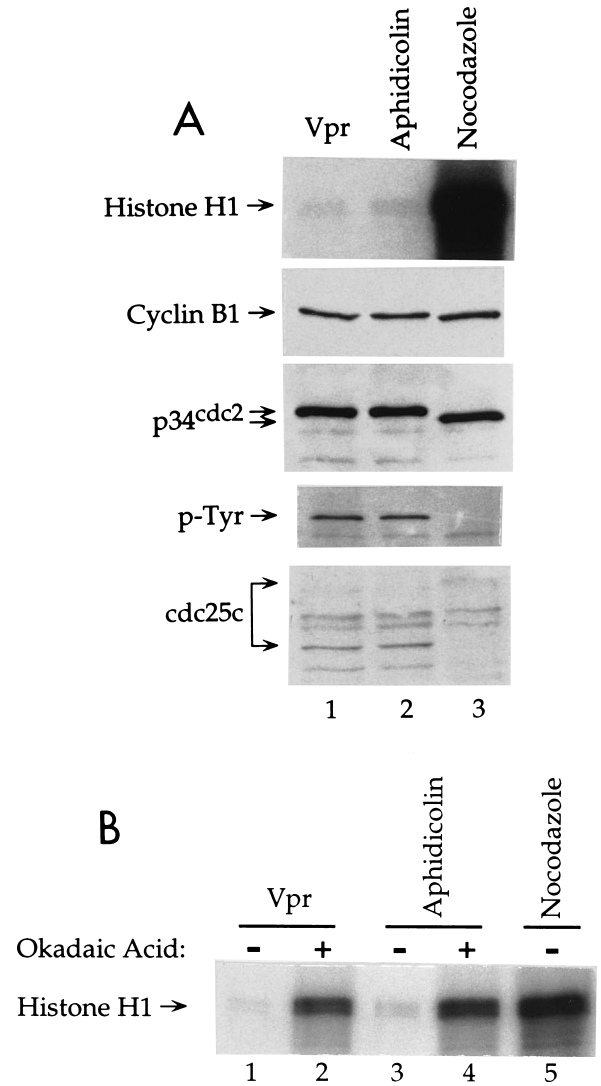

FIG. 4. Expression of HIV-1 *vpr* leads to a reversible block in $p34^{cdc2}$-cyclin B activation. HeLa cells were transfected with a *vpr* expression plasmid or arrested with aphidicolin or nocodazole following transfection with vector alone. (A) Following selection for 72 h, cell lysate was immunoprecipitated with anti-cyclin B antibody and then assayed for cyclin B-associated histone H1 kinase activity or by Western blot for cyclin B-associated $p34^{cdc2}$ or cyclin B-associated phosphotyrosine. Total lysate was probed directly in Western blots for cyclin B or for cdc25C. The arrows show the position of migration of the indicated proteins. The double arrows indicate the position of migration of the unphosphorylated and phosphorylated forms of $p34^{cdc2}$ and cdc25C. (B) The same cell populations were assayed for cyclin B-associated histone H1 kinase activity before and 1 h after treatment with okadaic acid.

phosphate is removed from $p34^{cdc2}$, leading to the activation of its kinase activity and entry into mitosis. To test whether Vpr blocks factors acting upstream of $p34^{cdc2}$, *vpr*-expressing cells and aphidicolin-blocked cells were treated with okadaic acid. In both cases, within 1 h, the majority of cells rounded up, indicating entry into mitosis. Examination of cyclin B-associated histone H1 kinase activity in both cell populations showed levels approaching those of cells blocked with nocodazole (Fig. 4B). The fact that okadaic acid relieves the cell cycle block makes it unlikely that Vpr functions as a direct inhibitor of $p34^{cdc2}$, analogous to the cyclin-dependent kinase inhibitors p21 and p16 (32). In addition, attempts to coimmunoprecipitate Vpr with $p34^{cdc2}$ or cyclin B were unsuccessful. More likely, *vpr* blocks $p34^{cdc2}$-cyclin B activation via interaction with an upstream regulator, perhaps a protein which monitors the DNA replication machinery.

DISCUSSION

We have confirmed previous reports that HIV-1 *vpr* has effects on cell proliferation and that *vpr*-expressing cells arrest with 4*N* DNA. These observations were extended by demon-

strating that cells expressing *vpr* arrest with the biochemical characteristics of cells arrested in G_2. Vpr-arrested cells have very low cyclin B-associated kinase activity. The low kinase activity is not due to a block in cyclin B synthesis, since levels of this protein are comparable to those found in cells blocked in mitosis by nocodazole. Rather, $p34^{cdc2}$ is in the inactive, tyrosine-phosphorylated form.

Several of our experiments suggest that Vpr does not directly inhibit the kinase activity of the $p34^{cdc2}$-cyclin B complex. First, the upstream regulator of the $p34^{cdc2}$-cyclin B complex, cdc25C, is itself in the inactive, unphosphorylated state. Second, okadaic acid releases the Vpr block to $p34^{cdc2}$-cyclin B activation, indicating that the complex is not permanently inactivated by Vpr. Lastly, we have been unable to detect direct biochemical interaction between Vpr and cyclin B or $p34^{cdc2}$ by coimmunoprecipitation.

G_2 cell cycle arrest occurs rarely in normal cellular physiology. More commonly, the cell cycle arrests prior to a commitment by the cell to synthesize DNA. The G_2-M checkpoint is thought to monitor the status of DNA replication, and DNA-damaging agents, such as gamma irradiation, induce a G_2 arrest (2). Perhaps Vpr arrests the cell cycle in G_2 by perturbing the same factors which monitor the status of chromosomal DNA.

The experiments presented here were performed with a tissue culture cell line in order to facilitate detailed biochemical analysis of the Vpr-induced cell cycle arrest. Others have reported similar effects of *vpr* on the growth of primary human mononuclear cells (34), indicating that the Vpr-induced G_2 arrest may be relevant in vivo. The fact that a similar cell cycle arrest has been observed in *Saccharomyces cerevisiae* expressing *vpr* suggests that Vpr most likely disrupts a highly conserved mechanism in eukaryotic physiology (25).

All primate immunodeficiency viruses possess either *vpr* or the highly homologous *vpx*, and some viruses, such as SIV_{MAC239}, possess both genes (36). Thus, *vpr/vpx* must play an important role in viral replication. Perhaps the fact that SIV_{MAC239} possesses both *vpr* and *vpx* explains why Vpx alone did not arrest the cell cycle in our assay. This would be consistent with the recent report that SIV_{MAC239} proviruses lacking either *vpr* or *vpx* are capable of producing AIDS in rhesus monkeys, while the *vpr vpx* double mutant is severely attenuated in pathogenicity (10).

HIV-1 gene expression (6, 23) and replication (3, 4, 13, 22, 28, 40) are stimulated by *vpr*. Perhaps by arresting the infected cell in the G_2 phase of the cell cycle, Vpr optimizes conditions for viral expression. Alternatively, the G_2 arrest might not be directly relevant for viral replication but simply an epiphenomenon. In either case, the cell cycle arrest would still have profound effects on the function of replicating cells of the immune system. Clarification of the precise mechanism by which Vpr disrupts the cell cycle machinery should provide valuable information about the signals which regulate entry into mitosis and will undoubtedly contribute to our understanding of HIV replication and pathogenicity.

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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. **Bacchetti, S., and W. K. Sinclair.** 1970. The relation of protein synthesis to radiation-induced division delay in Chinese hamster cells. Radiat. Res. **44:** 788–806.
3. **Balliet, J. W., D. L. Kolson, G. Eiger, F. M. Kim, K. A. McGann, A. Srinivasan, and R. Collman.** 1994. Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes *vpr*, *vpu*, and *nef*: mutational analysis of a primary HIV-1 isolate. Virology **200:**623–631.
4. **Ballota, C., P. Lusso, R. Crowley, R. C. Gallo, and G. Franchini.** 1993. Antisense phosphorothioate oligodeoxynucleotides targeted to the *vpr* gene inhibit human immunodeficiency virus type 1 replication in primary human macrophages. J. Virol. **67:**4409–4414.
5. **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.
6. **Cohen, E. A., E. F. Terwilliger, Y. Jalinoos, J. Proulx, J. G. Sodroski, and W. A. Haseltine.** 1990. Identification of HIV-1 *vpr* product and function. J. Acquired Immune Defic. Syndrome **3:**11–18.
7. **Cullen, B.** 1987. Use of eukaryotic expression technology in the functional analysis of cloned genes. Methods Enzymol. **152:**684–704.
8. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature (London) **362:**359–362.
9. **Franke, E. K., H. E. H. Yuan, K. L. Bossolt, S. P. Goff, and J. Luban.** 1994. Specificity and sequence requirements for interactions between various retroviral Gag proteins. J. Virol. **68:**5300–5305.
10. **Gibbs, J. S., A. A. Lackner, S. M. Lang, M. A. Simon, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers.** 1995. Progression to AIDS in the absence of a gene for *vpr* or *vpx*. J. Virol. **69:**2378–2383.
11. **Haase, A. T.** 1994. The role of active and covert infections in lentivirus pathogenesis. Ann. N. Y. Acad. Sci. **724:**75–86.
12. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Hattori, N., 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 macrophages. Proc. Natl. Acad. Sci. USA **87:**8080–8084.
14. **Heinzinger, N. K., M. I. Bukrinsky, S. A. Haggerty, A. M. Ragland, V. Kewalramani, M.-A. Lee, H. E. Gendelman, L. Ratner, M. Stevenson, and M. Emerman.** 1994. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. Proc. Natl. Acad. Sci. USA **91:**7311–7315.
15. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature (London) **373:**123–126.
16. **Hoffmann, I., P. R. Clarke, M. J. Marcote, E. Karsenti, and G. Draetta.** 1993. Phosphorylation and activation of human cdc25c by cdc2-cyclin B and its involvement in the self-amplification of MPF at mitosis. EMBO J. **12:**53–63.
17. **Hunter, T.** 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell **80:**225–236.
18. **Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, and R. Desrosiers.** 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science **248:**1109–1112.
19. **King, R. W., P. K. Jackson, and M. W. Kirschner.** 1994. Mitosis in transition. Cell **79:**563–571.
20. **Kozak, M.** 1991. An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell Biol. **115:**887–903.
21. **Lane, H., J. Depper, W. Greene, G. Whalen, T. Waldmann, and A. Fauci.** 1985. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **313:**79–84.
22. **Lang, S. M., M. Weeger, C. Stahl-Hennig, C. Coulibaly, G. Hunsmann, J. Muller, H. Muller-Hermelink, D. Fuchs, H. Wachter, M. M. Daniel, R. C. Desrosiers, and B. Fleckenstein.** 1993. Importance of *vpr* for infection of rhesus monkeys with simian immunodeficiency virus. J. Virol. **67:**902–912.
23. **Levy, D. N., L. S. Fernandes, W. V. Williams, and D. B. Weiner.** 1993. Induction of cell differentiation by human immunodeficiency virus 1 *vpr*. Cell **72:**541–550.
24. **Lu, Y.-L., P. Spearman, and L. Ratner.** 1993. Human immunodeficiency virus type 1 viral protein R localization in infected cells and virions. J. Virol. **67:**6542–6550.
25. **Macreadie, I. G., L. A. Castelli, D. R. Hewish, A. Kirkpatrick, A. C. Ward, and A. A. Azad.** 1995. A domain of human immunodeficiency virus type 1 Vpr containing repeated H(S/F)RIG amino acid motifs causes cell growth arrest and structural defects. Proc. Natl. Acad. Sci. USA **92:**2770–2774.
26. **Murray, H. W., J. K. Hillman, R. Y. Rubin, C. D. Kelly, J. L. Jacobs, L. W.**

Tyler, D. M. Donelly, S. M. Carriero, J. H. Godbold, and R. B. Roberts. 1985. Patients at risk for AIDS-related opportunistic infections: clinical manifestations and impaired γ interferon production. N. Engl. J. Med. **313:**1504–1510.

27. **Nurse, P.** 1994. Ordering S phase and M phase in the cell cycle. Cell **79:** 547–550.
28. **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.
29. **Page, K. A., N. R. Landau, and D. R. Littman.** 1990. Construction and use of a human immunodeficiency virus vector for analysis of virus infectivity. J. Virol. **64:**5270–5276.
30. **Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature (London) **362:**355–358.
31. **Pantaleo, G., C. Graziosi, and A. S. Fauci.** 1993. The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. **328:**327–335.
32. **Peter, M., and I. Herskowitz.** 1994. Joining the complex: cyclin-dependent kinase inhibitory proteins and the cell cycle. Cell **79:**181–184.
33. **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, 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.
34. **Rogel, M. E., L. I. Wu, and M. Emerman.** 1995. The human immunodeficiency virus type 1 *vpr* gene prevents cell proliferation during chronic infection. J. Virol. **69:**882–888.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Tristem, M., C. Marshall, A. Karpas, and F. Hill.** 1992. Evolution of the primate lentiviruses: evidence from *vpx* and *vpr*. EMBO J. **11:**3405–3412.
37. **van Noesel, C. J. M., R. A. Gruters, F. G. Terpstra, P. T. A. Schellekens, R. A. W. van Lier, and F. Miedema.** 1994. Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. J. Clin. Invest. **86:**293–299.
38. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. H. Hahn, M. S. Saag, and G. M. Shaw.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature (London) **373:**117–122.
39. **Weiss, R. A.** 1993. How does HIV cause AIDS? Science **260:**1273–1279.
40. **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.
41. **Yamashita, K., H. Yasuda, J. Pine, K. Yasumoto, H. Nishitani, M. Ohtsubo, T. Hunter, T. Sugimura, and T. Nishimoto.** 1990. Okadaic acid, a potent inhibitor of type 1 and type 2A protein phosphatases, activates cdc2/H1 kinase and transiently induces a premature mitosis-like state in BHK21 cells. EMBO J. **9:**4331–4338.
42. **Yuan, X., Z. Matsuda, M. Matsuda, M. Essex, and T.-H. Lee.** 1990. Human immunodeficiency virus *vpr* gene encodes a virion-associated protein. AIDS Res. Hum. Retroviruses **6:**1265–1271.