Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11889–11893, December 1995
Cell Biology

Human immunodeficiency virus 1 envelope-initiated G_2-phase programmed cell death

VINCENT KOLESNITCHENKO*, LARRY M. WAHL†, HUAN TIAN‡, INKE SUNILA§, YOSHIHIKO TANI‡, DAN-PAUL HARTMANN¶, JEFFREY COSSMAN¶, MARK RAFFELD‖, JAN ORENSTEIN§, LAWRENCE E. SAMELSON*, AND DAVID I. COHEN*,**

*Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, †Laboratory of Immunology, National Institute of Dental Research, ‡Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, ‖Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; §Department of Pathology, George Washington University, Washington, DC 20037; and ¶Department of Pathology, Georgetown University School of Medicine, Washington, DC 20052

Communicated by Robert C. Gallo, National Institutes of Health, Bethesda, MD, August 18, 1995

ABSTRACT **Despite intensive investigation, no clearly defined mechanism explaining human immunodeficiency virus (HIV)-induced cell killing has emerged. HIV-1 infection is initiated through a high-affinity interaction between the HIV-1 external envelope glycoprotein (gp120) and the CD4 receptor on T cells. Cell killing is a later event intimately linked by *in vitro* genetic analyses with the fusogenic properties of the HIV envelope glycoprotein gp120 and transmembrane glycoprotein gp41. In this report, we describe aberrancies in cell cycle regulatory proteins initiated by cell–cell contact between T cells expressing HIV-1 envelope glycoproteins and other T cells expressing CD4 receptors. Cells rapidly accumulate cyclin B protein and tyrosine-hyperphosphorylated p34cdc2 (cdk1) kinase, indicative of cell cycle arrest at G_2 phase. Moreover, these cells continue to synthesize cyclin B protein, enlarge and display an abnormal ballooned morphology, and disappear from the cultures in a pattern previously described for cytoxicity induced by DNA synthesis (S phase) inhibitors. Similar changes are observed in peripheral blood mononuclear cells infected *in vitro* with pathogenic primary isolates of HIV-1.**

Both the tropism of human immunodeficiency virus (HIV) 1 for $CD4^+$ T cells (1, 2) and the relative cytopathicity of individual HIV isolates (3, 4) have been mapped to the envelope glycoproteins (gp120/41). The *nef* (5) and *vpr* (6) accessory genes also appear to contribute to HIV pathogenicity. The most virulent isolates of HIV-1 have the capacity to induce intercellular fusion (syncytium induction) (7), a process that has been associated with rapid disease progression and with rapid loss of $CD4^+$ cells from infected cultures (8). Nevertheless, single cell killing predominates even during infections with syncytium-inducing HIV-1 variants (8), emphasizing the importance of single cell death during HIV infection. Cell killing begins late in the course of *in vitro* HIV infections (9), after envelope glycoproteins have been reexpressed at the surface of infected cells.

Programmed cell death (PCD) is a normal process in organogenesis (10), where it is utilized in lumen formation (11) and in the elimination of unnecessary cells, including the majority of immature T cells of the human thymus (12). PCD is an active process that may be triggered by ligation of receptors such as fas/apo-1 (13) and that is associated with intracellular signal transduction (14). Certain genes, such as *myc* (15), p53 (16), and pRB (17), that regulate the cell cycle can also induce PCD leading to a hypothesis that some types of PCD may result from cell cycle disregulation. Most forms of PCD described in developing T cells result in cell death in the cell cycle near the G_1/S-phase boundary (18). We (19) and others (20) have shown that direct $CD4^+$ T-cell killing by surface-expressed HIV envelope glycoproteins is an active process requiring protein tyrosine kinase stimulation. We demonstrate here that HIV infection, unlike most forms of developmental T-cell death, can initiate cell cycle arrest and cell death at G_2 phase.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Culture. Cell killing was induced through a 1:1 coculture between HIVenv cells and $CD4^+$ T cells as described (21). Control cultures were the relevant $CD4^+$ indicator line and HIVenv cells grown independently and fixed prior to mixing. Protein controls were lysates prepared individually from the two cell lines and then mixed in a 1:1 protein ratio prior to loading.

Cell Cycle Analysis. Cells were fixed in ethanol/PBS, 3:1 (vol/vol), containing 1% calf serum and stained with propidium iodide (50 μg/ml), and relative fluorescence was analyzed by flow cytometry to measure DNA content. Percentages of cells in G_1 or G_2 phase of the cell cycle were determined using the program IMAGE 1.57 to integrate the flow cytometric profiles. As controls (normal), cells were first ethanol-fixed and then mixed with an equal number of ethanol-fixed HIVenv cells prior to analysis for DNA content.

Counterflow Centrifugal Elutriation (CCE). Cells were separated on a Beckman elutriation system at a flow rate of 6–19 ml of phosphate-buffered saline (PBS) per min and a constant rpm of 1500 (300 × *g*). The largest cell fraction isolated from the elutriation chamber was too large to be present in cultures of normal T cells.

Generation of Primary HIV-1 Isolates. Ten primary isolates of HIV-1 (CI 0–9) were generated by coculture of cryopreserved patient peripheral blood mononuclear cells (PBMCs) with phytohemagglutinin-activated (72 h in 2 μg/ml) mononuclear cells from an HIV-negative donor as described (22). Isolates were propagated from patients with various rates of disease progression (23, 24). Cytopathicity of each of the viruses was evaluated from its ability to deplete $CD4^+$ T cells (25, 26) averaged from at least four infections of PBMCs from different donors, with higher numbers (for example, CI-9) indicating the greatest cytopathicity.

HIV Infections. Infections of activated PBMCs (72 h in phytohemagglutinin at 2 μg/ml) were performed with primary isolates of HIV-1 using equal amounts of input virus, normalized by supernatant reverse transcriptase. For cyclin B determinations, 2.5×10^4 cells were cytocentrifuged onto glass

Abbreviations: HIV, human immunodeficiency virus; PCD, programmed cell death; PBMC, peripheral blood mononuclear cell; cdk, cyclin-dependent kinase; CCE, counterflow centrifugal elutriation; CPE, cells with cytopathic effect.

**To whom reprint requests should be addressed.

slides at 700 rpm in a Shandon Cytospin III rotor for 5 min. Cells were fixed for 2 min at room temperature in methanol/acetone, 1:1 (vol/vol), and permeabilized for 15 min with 0.2% Triton/PBS prior to staining with a 1:100 dilution of a rabbit polyclonal anti-cyclin B antibody (27). Stained populations of cells were photographed by a second observer (M.R.), and at least four equivalent fields were scored for positive cells by a third observer (L.M.W.), both of whom were blinded for the original input material.

RESULTS

Cell Cycle Disregulation Induced by HIV Envelope in a Model System. HIV-initiated cytopathicity was induced by cell–cell contact between a transfected cell line (HIVenv) lacking CD4 but expressing envelope glycoproteins (gp120, gp160, and gp41), tat, and rev (21) and several CD4$^+$ human T-cell lines. As described (19), dying cells rapidly accumulated a 34-kDa tyrosine-phosphorylated substrate. The size of this protein and its phosphoamino acid analysis suggested it might be a cyclin-dependent kinase (cdk), which was confirmed by immunoblot analysis with monoclonal (data not shown) and polyclonal antibodies specific for the C terminus of cdk1 (p34cdc2) (Fig. 1*A*) (28). Within 2 h (Fig. 1*A*, lane 3), populations of cells expressing HIV envelope (env) glycoproteins and CD4 had more of the slowest migrating tyrosine-phosphorylated form of cdk1 (29), whose accumulation gradually continued over the subsequent 14 h of culture (Fig. 1*A*, lanes 4–6). The amount of tyrosine-phosphorylated cdk1, measured on a phosphotyrosine immunoblot, increased between 3.4- and 19-fold in overnight cultures of dying cells (data not shown).

Since tyrosine phosphorylation of p34 occurs at G_2 phase (28, 30), this observation suggested that after HIVenv–CD4 interaction, cells were arresting during G_2 phase. To examine this possibility, cells were analyzed for cyclin B protein, which is synthesized in late S phase in our T-cell lines, utilized during G_2/M-phase transitions, and rapidly degraded (31, 32). By 2 h

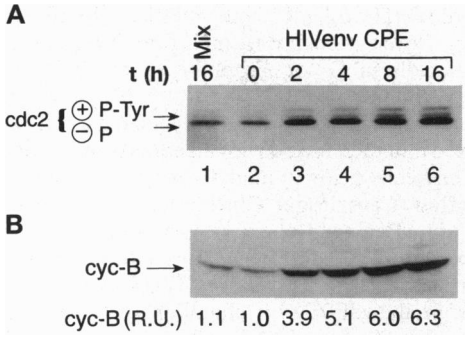
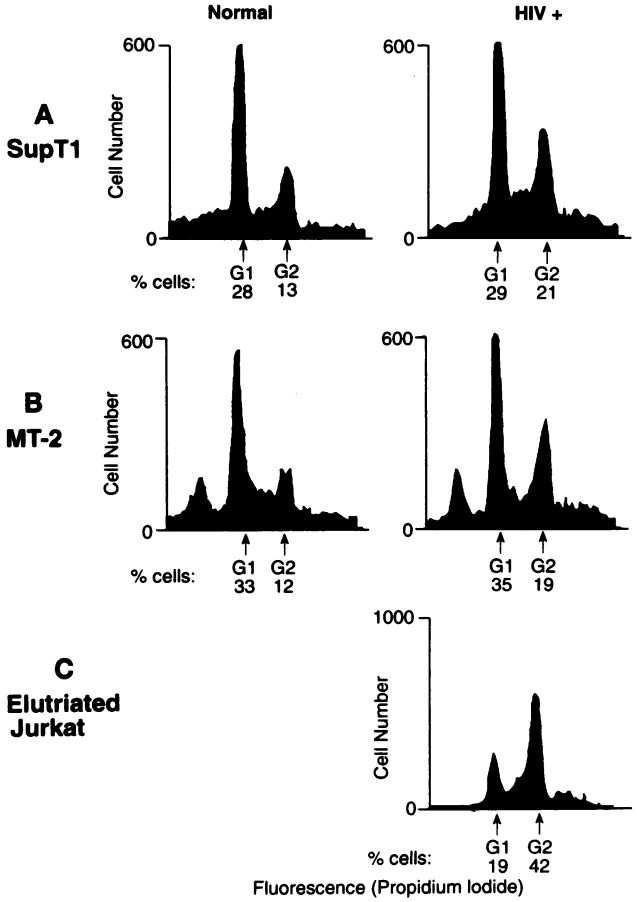


FIG. 1. HIV-1-induced disregulation of mitotic regulatory proteins p34cdc2 and cyclin B. (*A*) Gel shift analysis of cdc2 protein phosphorylated during HIVenv-induced cell death (CPE). Control lysate reconstituted as a 1:1 mixture of proteins from HIVenv and Jurkat cells cultured separately and without cytopathicity (lane 1) or equal amounts of protein lysate from CD4$^+$ Jurkat cells induced to undergo HIVenv cytopathicity for the indicated length of time (lanes 2–6) were resolved by SDS/PAGE on 11% gels prior to analysis of immunoblots with a polyclonal anti-cdc2 antibody (27). Relative positions of the more slowly migrating tyrosine-phosphorylated (P-Tyr) and more rapidly migrating unphosphorylated form of cdc2 are indicated. Assignment of these cdc2 forms was confirmed by coelectrophoresis with tyrosine-hyperphosphorylated cdc2 protein isolated from orthovanadate-arrested cells and with dephosphorylated cdc2 protein isolated from a population of G_1 cells purified by CCE. (*B*) Cyclin B levels. A 1:1 mixture of proteins from control cells (lane 1) or an equivalent amount of proteins isolated from cells at increasing times after CPE induction (lanes 2–6, the time of harvest for each lane corresponding to the matched lane in *A*) was resolved by gradient SDS/PAGE (5–15% gel). The immunoblot was analyzed with a rabbit polyclonal anti-cyclin B antibody (27). The autoradiogram was scanned, and the relative intensities of the cyclin B bands (cyc-B, R.U.) were quantified using the program NIH IMAGE 1.57. Expected migration of cyclin B protein is indicated by the arrow to the left.



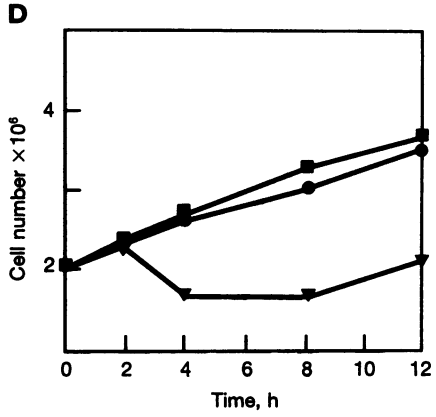


FIG. 2. Characterization of HIVenv-exposed cultures. (*A–C*) Cell cycle profiles. Two hours after HIVenv CPE was initiated (HIV+) in human CD4$^+$ T-cell lines SupT1 (*A*) or MT-2 (*B*) by 1:1 coculture with the HIVenv transfectant, cell cycle analysis was performed and compared to 1:1 mixtures (Normal) of HIVenv and SupT1 (*A*) or MT-2 cells (*B*). (*C*) Human CD4$^+$ Jurkat cells induced for HIVenv CPE were size-fractionated by CCE, and an aberrantly large fraction was analyzed as above for relative DNA content. A peak with less than G_1 DNA, contributed from conventional apoptotic cells (34), was either not detected (*A* and *C*) or not increased (*B*) during HIVenv CPE in multiple experiments. (*D*) Viability analysis. Viability of equivalent cultures of HIVenv cells (●), Jurkat cells (■), or cells induced for HIVenv CPE (Jurkat+HIVenv) (▼) was determined by counting cells excluding trypan blue in a hemocytometer chamber. No more than 5% of cells ever entered into multicellular syncytia, as quantitated either by direct inspection in the hemocytometer or by CCE isolation.

(Fig. 1*B*, lane 3) cells contained 3.9 times more cyclin B protein than control cells (Fig. 1*B*, lane 1) or than cells at the initiation of culture (lane 2). Cells continued to accumulate cyclin B protein over the first 8 h of HIVenv exposure (lanes 3–5), after which cyclin B levels plateaued at 6.3 times their initial level (lane 6). No consistent alterations in cdk2 or cyclin D1 protein were observed in HIVenv-exposed cultures (data not shown).

Cell cycle analysis of SupT1 (Fig. 2*A*) or MT-2 (Fig. 2*B*) $CD4^+$ T cell lines (33) exposed to HIVenv showed a normal number of G_2 cells at 1 h (data not shown) and an increased number of G_2 cells at 2 h (Fig. 2), after which the number of G_2 cells declined to baseline, suggesting that G_2-arrested cells were disappearing from the cultures either due to release from cell cycle blockade or due to cell death. Prior to their disappearance, cells in the HIVenv-exposed cultures dramatically enlarged, permitting their size fractionation from normal cells, including even normal G_2/M cells, by CCE. CCE-fractionated aberrantly large cells (fraction of cells with cytopathic effect, CPE-F) were also predominantly in G_2 (Fig. 2*C*). To learn whether G_2-arrested cells were disappearing from the HIVenv-exposed cultures due to cell death, we performed serial analysis of cell viability over time. Cell number initially increased in these cultures but began to decline between 2 and 4 h (Fig. 2*D*), immediately after G_2 arrest was attained (Fig. 1, lanes 3). In some cultures, >50% of the cells died over the first 24 h, suggesting that both the env-expressing transfectants and $CD4^+$ T cells exposed to HIVenv were dying. Some (at least one-third) of the cells in the CPE-F could be stained with polyclonal anti-HIV antibodies (data not shown), consistent with the participation of HIV-expressing cells in the G_2 death process. Little contribution to overall cell death was made by cells undergoing apoptosis from G_1 phase or by cells entering multinucleated syncytia (Fig. 2).

Enrichment for Large Dying Cells. CCE enrichment was employed to further characterize the dying cells. Light microscopy of the abnormal cells (CPE-F) revealed marked cytoplasmic enlargement ("balloon cells"), an open nucleus, and a prominent nucleolus (Fig. 3*A*). Upon reculture, CPE-F cells were not viable but continued to enlarge and were lysed within 2–4 h, frequently forming anucleate ghosts. Light microscopy of the control mononuclear cells illustrated their relatively small size, moderate nuclear/cytoplasmic ratios, and prominent heterochromatin (Fig. 3*B*). Degenerative changes, including vacuolated cytoplasm, hyperdense or swollen mitochondria, and damaged plasma membranes, were demonstrated by transmission electron microscopy in dying balloon cells (Fig. 3*C*) but not in control cells (Fig. 3*D*).

Cyclin B accumulation, G_2 cell-cycle arrest, and cytoplasmic enlargement have been closely linked to cytotoxicity that is induced when DNA synthesis inhibitors damage cells during S phase of the cell cycle (35). Supranormal levels of cyclin B protein accumulation have been proposed to directly participate in this cell death. To learn whether cyclin B protein accumulated to abnormally high levels during HIVenv-initiated cell death, as in cell death after S-phase DNA damage, we CCE-fractionated cells and analyzed the fractions for cyclin B protein content (Fig. 4). Large dying cells (lane 4, CPE-F) contained 2.4 times more cyclin B protein than normal G_2/M-phase T cells (lane 3), and 29 times more cyclin B than S-phase T cells (lane 2). G_1 cells (lane 1) contained no detectable cyclin B protein.

G_2-Phase and Cell Death During Acute HIV Infection. To investigate whether T-cell killing during HIV-1 infection proceeded by a cytopathic process similar to that observed in the HIVenv model system, we studied phytohemagglutinin-activated PBMCs infected with 10 primary clinical isolates of HIV-1. After infection with either highly or lowly cytopathic variants of HIV-1 (Table 1), PBMCs were stained with polyclonal (Fig. 5*A*) (27) or monoclonal (data not shown) anti-cyclin B antibodies. In the HIV-infected cultures, single aberrantly large cells and syncytia (Fig. 5*A*) reacted strongly with the anti-cyclin B antibodies. Most commonly, staining was observed in a cytoplasmic and perinuclear distribution that prominently highlighted the centrosome, defining the stained

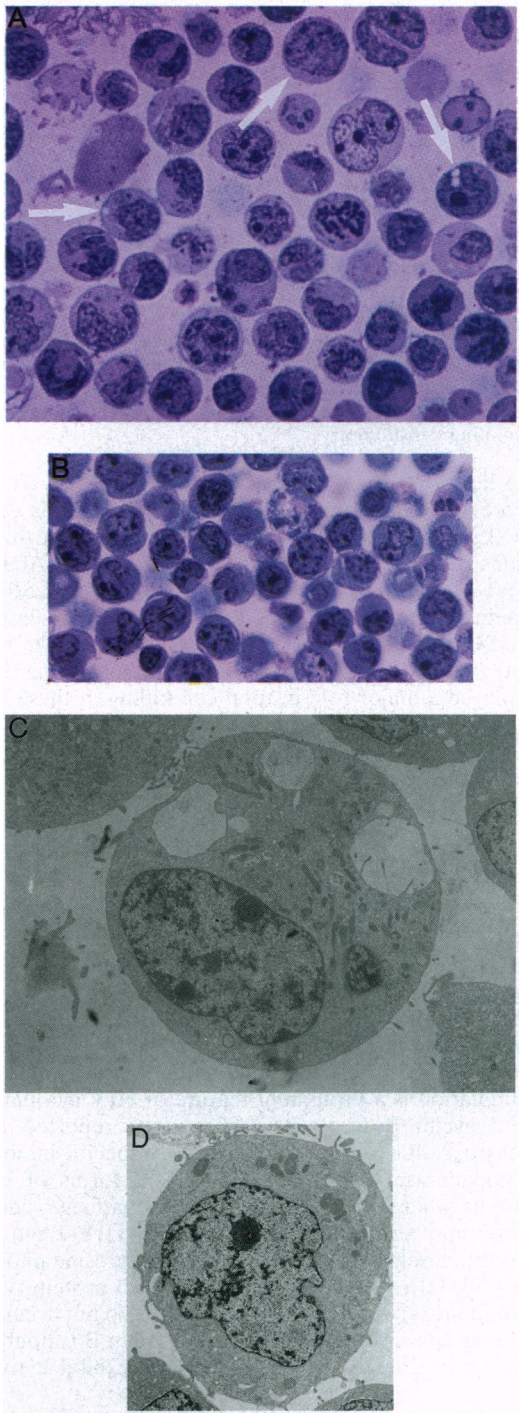


FIG. 3. Morphological characterization of dying cells from HIVenv-exposed cultures. (*A*) Light micrograph of CCE-enriched fraction (CPE-F) showing abnormal large cells (arrows) each having an open nucleus and a prominent nucleolus. (Semi-thick plastic section. Toluidine blue stain.) (*B*) Light micrograph of normal $CD4^+$ Jurkat cells (same magnification as in *A*). (*C*) CPE-F analyzed by transmission electromicroscopy showing large (≈1.5× diameter of control cells) cells with a round nucleus having little heterochromatin, a vacuolated cytoplasm, and degenerating dense mitochondria and damaged microvillus-free plasma membrane. (*D*) Transmission electron microscopy of a typical relatively small control cell with irregular nucleus, prominent heterochromatin, normal appearing mitochondria, and villous plasma membrane.

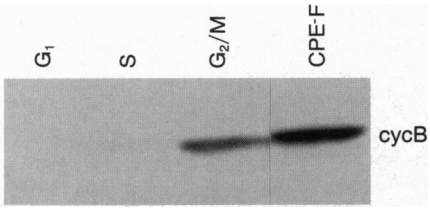


FIG. 4. Cyclin B overexpression in dying cells. After induction of HIVenv CPE, cultures were separated by CCE into G_1-enriched (78.7% G_1) (lane 1), S-phase enriched (32.8% S phase) (lane 2), G_2-enriched (77.0% G_2) (lane 3), or aberrantly large cell fractions (lane 4, CPE-F). Equivalent amounts of protein lysate prepared from each of the fractions was loaded on a 5–15% gradient PAGE gel prior to immunoblot analysis with a polyclonal anti-cyclin B antibody. Relative cyclin B expression (cycB) was quantified for each lane by laser scanning densitometry.

cells to be in G_2 phase (36, 37). These G_2 cells were vastly overrepresented in cultures infected with cytopathic variants of HIV-1 (Fig. 5*A Lower*) when compared with noncytopathic infections or uninfected PBMCs (Fig. 5*A Upper*). Abnormal cells were not highlighted by control antibodies (Fig. 5*B*) or by anti-cyclin D1 antibodies (data not shown). HIV isolates that depleted $CD4^+$ T cells most rapidly also induced the highest percentage of G_2-arrested cells (Table 1), suggesting that G_2 arrest played a major role in total cell killing in these HIV-1 infections. Cyclin B immunoblots also demonstrated a correlation between total cyclin B protein levels in infected PBMCs and HIV cytopathicity (data not shown).

DISCUSSION

Our observations establish that one mechanism of HIV-directed $CD4^+$ T-cell death is triggered after G_2 arrest and suggest further that this process makes a substantial contribution to the cytopathic effect in HIV infections. Cell killing is initiated when T cells expressing HIV envelope glycoproteins contact other $CD4^+$ T cells (8, 21). Cell cycle damage apparently occurs during late S phase, since cells enter G_2 arrest and begin to die within 2 h after HIVenv–CD4 interaction. Cyclin B accumulation is a prominent feature of HIV cytopathicity. Elevated cyclin B levels have also been reported during cytoxicity in cells exposed to aphidicolin, a specific inhibitor of DNA polymerase α (35), and in certain forms of mitotic catastrophe where, like HIV-induced cytopathicity, cdc2 disregulation may also be observed (38). The HIV-1 cytopathic effect is intimately associated with protein tyrosine phosphorylation (19). HIV-exposed cells treated with protein-tyrosine kinase inhibitors such as herbimycin A (19) do not accumulate tyrosine-phosphorylated cdk1 (19) and cyclin B (unpublished results), do not arrest at G_2, and do not exhibit cytopathic changes (19). The HIV-triggered pathway of cell death contrasts most forms of apoptosis or PCD that affect developing thymocytes or mature T cells early in the cell cycle near the G_1/S transition (18, 39).

Table 1. Cyclin B increase and $CD4^+$ cell death in HIV infection

Virus	% decrease in $CD4^+$ cells	% increase in cyclin B^+ cells
Uninfected	0	0 (6.1)
CI-2	0	0 (3.9)
CI-4	9	16.0 (5.4)
CI-9	23	23.5 (5.3)

PBMCs were either sham-infected (uninfected) or HIV-infected with equivalent amounts of input virus (CI-2, CI-4, or CI-9, the higher number indicating higher relative cytopathicity). At 72 h, aliquots of PBMCs were analyzed by flow cytometry for depletion of $CD4^+$ T cells ($CD4^+$ killing *ex vivo*) or stained with anti-cyclin B antibodies to quantitate the increase in cyclin B-producing cell (cyclin B increase). The percentage of cyclin B-staining cells in the uninfected PBMC population was relatively assigned to zero, corresponding on average to 18% of input cells. Results presented are the mean of three experiments. Data in parentheses are SD.

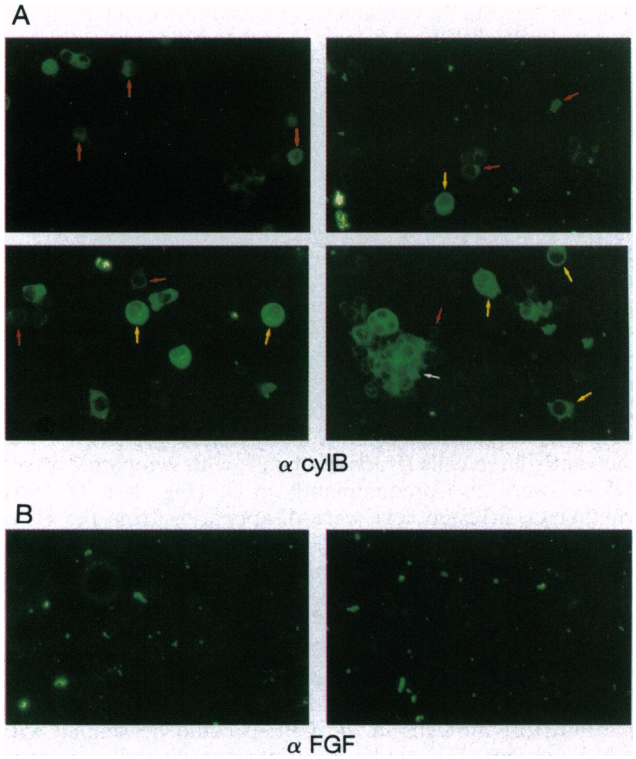


FIG. 5. G_2 arrest of primary HIV-infected PBMCs. (*A*) Immunofluorescence with polyclonal rabbit anti-cyclin B antibody. Seventy-two hours after sham infection (*Upper Left*) or infection with HIV clinical viruses CI-2 (*Upper Right*), CI-4 (*Lower Left*), or CI-9 (*Lower Right*), approximately equivalent fields, each containing 50–100 peripheral blood lymphocytes, were stained with a rabbit polyclonal anti-cyclin B antibody (α cylB) (27), prior to incubation with a secondary biotinylated anti-rabbit serum (Tago) and fluorescein isothiocyanate-avidin (Vector Laboratories) by the manufacturer's specifications. Examples of normal mitoses (red arrows), aberrant individual cells (yellow arrows), and syncytia (white arrows) are indicated. (*B*) Immunofluorescence of sham-infected (*Left*) or HIV-1 (CI-9)-infected (*Right*) PBMCs stained with an irrelevant matched polyclonal rabbit antibody directed to bovine acidic fibroblast growth factor (α FGF) (Upstate Biotechnology).

Characteristic biochemical and morphological changes occur in HIV-1-infected PBMCs or in populations of T cells expressing HIV envelope (tat and rev) proteins and CD4. The single cell killing we observe in PBMCs begins several days after HIV infection, when envelope glycoproteins can be detected on the surface of infected cells (M.R., unpublished observations). This killing appears to be a direct property of the virus (3, 4, 25). Several recent studies have highlighted the ability of HIV-1 to directly and efficiently kill infected $CD4^+$ T cells (25, 40, 41) and have established an important link among viral replication, viral killing, and the development of AIDS (40, 41). The mechanisms of this *in vivo* killing pathway are undefined but are consistent with a process triggered by contact between an infected HIVenv-expressing $CD4^+$ T cell and another (infected or uninfected) $CD4^+$ cell, which might potentially kill either cell in the process.

Our findings with the HIVenv transfectant and the observation that tat- and rev-transfected cells do not induce similar cytopathic changes in $CD4^+$ T cells (Y.T., unpublished observations) imply that HIVenv is primarily responsible for inducing this pathway of cytotoxicity. This conclusion is consistent with a large body of genetic evidence mapping the major

cytopathic determinant within HIV-1 to the envelope gene (3, 4). These results do not preclude a contribution from the *tat* and *rev* genes or HIV accessory genes including *nef* or *vpr* in either promoting or sustaining the G_2 arrest process.

Biological processes capable of triggering cell death have been extensively described, but the mechanisms regulating these phenomena remain mostly unknown. Pathogenic human viruses have been shown to encode proteins capable of interfacing with cell cycle regulation to promote cellular transformation (42) or interrupt PCD (43). This report demonstrates that a pathogenic human virus triggers abnormalities in cell cycle progression and thereby initiates cell death.

We thank Mary Dasso, George Shaw, and Paul Russell for critical review of this manuscript prior to publication; Anthony S. Fauci for guidance and encouragement; Claire McGowan for anti-cyclin B polyclonal antibodies; and John Newport for yeast p13 beads. Ms. Kiki Angelis assisted in preparation. HIV-1 isolates CI 6–9 were derived from patient PBMCs donated by the University of Alabama Center for AIDS Research Core Facility, which is supported by National Institutes of Health Program Grant P30-AI-27767.

1. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312,** 763–767.
2. Klatzman, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. D. & Montagnier, L. (1984) *Nature (London)* **312,** 767–768.
3. Cheng-Mayer, C., Quiroga, M., Tung, J. W., Dina, D. & Levy, J. A. (1990) *J. Virol.* **64,** 4390–4398.
4. Ghosh, S. K., Hahn, B. H. & Shaw, G. M. (1994) *J. Cell Biochem.,* Suppl. 18B, 132 (abstr.).
5. Kestler, H. W., Ringler, D. J., III, Mori, K., Panicali, D. L., Sehgal, P. K., Daniel, M. D. & Desrosiers, R. C. (1991) *Cell* **65,** 651–662.
6. Lang, S. M., Weeger, M., Stahl-Henning, C., Coulibaly, C., Hunsmann, G., Muller, J., Muller-Hermelink, H., Fuchs, D., Wachter H., Daniel, M. M., Desrosiers, R. C. & Fleckenstein, B. (1993) *J. Virol.* **67,** 902–912.
7. Sodroski, J., Goh, W. C., Rosen, C., Campbell, K. & Haseltine, W. A. (1986) *Nature (London)* **332,** 470–474.
8. Connor, R. I., Mohri, H., Cao, Y. & Ho, D. D. (1993) *J. Virol.* **67,** 1772–1777.
9. Leonard, R., Zagury, D., Desportes, I., Bernard, J., Zagury, J.-F. & Gallo, R. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3570–3574.
10. Kerr, J. F. R., Wyllie, A. H. & Currie, A. R. (1972) *Br. J. Cancer* **26,** 239–257.
11. Williams, L. & Bell, L. (1991) *Anat. Embryol.* **183,** 573–578.
12. Wyllie, A. H. (1980) *Nature (London)* **284,** 555–556.
13. Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S. I., Sameshima, M., Hase, A., Seto, Y. & Nagata, S. (1991) *Cell* **66,** 233–243.
14. Uckun, F. M., Tuel-Ahlgren, L., Song, C. W., Waddick, K., Myers, D. E., Kirihara, J., Ledbetter, J. A. & Schieven, G. L. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9005–9009.
15. Evan, G. I., Wyllie, A. H., Gibert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z. & Hancock, D. C. (1992) *Cell* **69,** 119–128.
16. Yonish-Rouach, E., Resnitsky, D., Lotem, J., Sachs, L., Kimchi, A. & Oren, M. (1991) *Nature (London)* **352,** 345–347.
17. Wu, X. & Levine, A. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3602–3606.
18. Mercep, M., Noguchi, P. D. & Ashwell, J. D. (1989) *J. Immunol.* **142,** 4085–4092.
19. Cohen, D. I., Tani, Y., Tian, H., Boone, E., Samelson, L. E. & Lane, H. D. (1992) *Science* **256,** 542–545.
20. Yoshida, H., Koga, Y., Moroi, Y., Kimura, G. & Nomoto, K. (1991) *Int. Immunol.* **4,** 233–242.
21. Tani, Y., Tian, H., Lane, H. C. & Cohen, D. I. (1993) *J. Immunol.* **151,** 7337–7348.
22. Tani, Y., Donoghue, E., Sharpe, S., Boone, E., Lane, H. C., Zolla-Pazner, S. & Cohen, D. I. (1994) *J. Virol.* **68,** 1942–1950.
23. Pantaleo, G., Menzo, S., Vaccarezza, M., Granziosi, C., Cohen, O. J., Demarest, J. F., Montefiori, D., Orenstein, J. M., Fox, C., Schrager, L. K., Margolick, J. B., Buchbinder, S., Giorgi, J. V. & Fauci, A. S. (1995) *N. Engl. J. Med.* **332,** 209–216.
24. Clark, S. J., Saag, M. S., Decker, W. D., Campbell-Hill, S., Roberson, J. L., Veldkamp, P. J., Kappas, J. C., Hahn, B. H. & Shaw, G. M. (1991) *N. Engl. J. Med.* **324,** 954–960.
25. Yu, X., McLane, M. F., Ratner, L., O'Brian, W., Collman, R., Essex, M. & Lee, T.-H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10237–10241.
26. Pantaleo, G., Butuni, L., Graziosi, C., Poli, G., Schnittman, S. M., Greenhouse, J. J., Gallin, J. J. & Fauci, A. S. (1991) *J. Exp. Med.* **173,** 511–514.
27. McGowan, C. H. & Russell, P. (1993) *EMBO J.* **12,** 75–85.
28. Draetta, G. & Beach, D. (1988) *Cell* **54,** 17–26.
29. Gu, Y., Rosenblatt, J. & Morgan, D. O. (1992) *EMBO J.* **11,** 3995–4005.
30. Gould, K. L. & Nurse, P. (1989) *Nature (London)* **342,** 39–45.
31. Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J. & Beach, D. (1989) *Cell* **56,** 829–838.
32. Pines, J. & Hunter, T. (1989) *Cell* **58,** 833–846.
33. Harada, S., Koyanagi, Y. & Yamamoto, N. (1985) *Science* **229,** 563–566.
34. Nicolete, I., Migliorati, G., Pagliacci, M. D., Grignani, F. & Riccardi, C. (1991) *J. Immunol. Methods* **139,** 271–279.
35. Kung, A. L., Sherwood, S. W. & Schimke, R. T. (1993) *J. Biol. Chem.* **268,** 23072–23080.
36. Pines, J. & Hunter, T. (1991) *J. Cell Biol.* **115,** 1–17.
37. Bailly, E., Pines, J., Hunter, T. & Gornens, M. (1992) *J. Cell Sci.* **101,** 529–545.
38. Heald, R., McLoughlin, M. & McKeon, F. (1993) *Cell* **74,** 463–474.
39. Banda, N. K., Bernier, J., Kurahara, D. K., Kurrle, R., Haigwood, N., Sekaly, R. P. & Finkel, T. H. (1992) *J. Exp. Med.* **176,** 1099–1106.
40. Wei, X., Ghosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E. A., Deutsch, P., Lifson, J. D., Bonhoeffer, S., Nowak, M. A., Hahn, B. H., Saag, M. S. & Shaw, G. M. (1995) *Nature (London)* **373,** 117–122.
41. Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowski, M. (1995) *Nature (London)* **373,** 123–126.
42. Nicholas, J., Cameron, K. R. & Honess, R. W. (1992) *Nature (London)* **355,** 362–365.
43. Gregory, C. D., Dive, C., Henderson, S., Smith, C. A., Williams, G. T., Gordon, J. & Rickinson, A. B. (1991) *Nature (London)* **349,** 612–614.