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The Human Immunodeficiency Virus Type 1 *vpr* Gene Prevents Cell Proliferation during Chronic Infection†

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Human immunodeficiency virus type 1 (HIV-1) is a retrovirus that can cause extensive cytopathicity in T cells. However, long-term productive infection of T-cell lines has been described. Here we show that although Vpr has no effect on the initial cytopathic effect of HIV-1, viruses that contain an intact *vpr* gene are unable to establish a chronic infection of T cells. However, virus with a mutated *vpr* gene can readily establish such long-term cultures. The effect of Vpr is independent of the *env* gene and the *nef* gene. Furthermore, expression of Vpr alone affects the progression of cells in the cell cycle. These results suggest that HIV-1 has evolved a viral gene to prevent chronic infection of T cells.

AIDS is characterized by a progressive depletion of CD4-positive lymphocytes. Immune system-mediated mechanisms of cell killing were proposed to explain the depletion of CD4 cells because the number of cells in the peripheral blood that were productively infected with human immunodeficiency virus (HIV) was not initially thought to be large enough to account for the cell loss. However, the large viral burden in lymph nodes (11, 28) and the fact HIV is very cytopathic in this setting (6) suggest that direct cell killing by the virus itself could play a considerable role in CD4 depletion.

Infection of susceptible cells by the cytopathic oncoretroviruses has been divided into an acute stage and a chronic stage (reviewed in reference 35). In the acute stage, these viruses cause extensive cytopathic effect that is accompanied by high levels of proviral DNA and superinfection of cells. After this, a chronic infection characterized by immunity to superinfection, no signs of cytopathic effect, and small numbers of proviruses per cell is established (36). HIV also causes cytopathic effect during acute infection and under some conditions can cause chronic infections. Cell death in the acute stage of infection is due to the envelope gene products (16, 21, 33, 39) and appears to be mediated by apoptosis (17, 21, 22, 37).

A previous report suggested that the ability of HIV-infected cell cultures to survive the acute infection and establish cell lines that produce viruses chronically is not mediated by the *env* gene but, rather, by the activity of accessory genes including *vpr* (25). Vpr is a 97-amino-acid protein that associates with the virion via interactions with the 3′ end of Gag (9, 18, 29, 40) and also accumulates in the nucleus of infected cells (20). Mutations in the HIV-1 *vpr* gene significantly reduce infection of terminally differentiated macrophages but have little or no effect on infection of rapidly dividing cells (4, 13, 38). Vpr and the matrix protein (MA) appear to be part of the preintegration complex that allows HIV proviral DNA to enter the nucleus in the absence of mitosis in some nonproliferating cells, such as macrophages (13).

Here, we show that Vpr of HIV-1 essentially prevents the chronic stage of infection of cells by HIV-1 but has no effect on the cytopathicity of the acute infection. Cell cultures infected with an HIV-1 viral stock that contains a full-length *vpr* gene eventually die out after infection. However, the same cultures infected with virus that is mutated in *vpr* recover from the initial cell death in the culture and eventually begin to replicate with the same doubling times as uninfected cells. Cells that eventually grew out of cultures infected with wild-type virus contained a mutated *vpr* open reading frame. In addition, we show that the effect of Vpr is independent of multiplicity of infection and, in contrast to a previous report (25), is independent of the presence of Nef. Furthermore, we show that the effect of Vpr late in the virus life cycle is independent of expression of the envelope gene products in peripheral blood mononuclear cells (PBMC). Transfections with plasmids that express Vpr alone indicate that Vpr expression can alter the progression of cells in the cell cycle and therefore may mediate its effect through prevention of cell proliferation.

MATERIALS AND METHODS

Cells. MT4 and SupT1 cells were obtained from the American Type Culture Collection and were grown in RPMI with 10% cosmic calf serum (Gibco). Peripheral blood lymphocytes were purified from buffy coat preparations, stimulated for 4 days with 0.9 μg of PHA-P (Difco) per ml, and grown in RPMI with 10% fetal bovine serum and 10% interleukin-2 (Cellpro).

Plasmids. All viruses are based on pLai (31), which contains open reading frames for all of the accessory proteins. Plasmid pLai was modified by addition of the simian virus 40 origin of replication in the plasmid backbone to increase virus production after transduction of cells containing the simian virus 40 T antigen (not shown). The following proviral mutants were used in this study: Vpr (insertion of 4 nucleotides at the *Nco*I site at position 5207), Nef (insertion of 4 nucleotides at the *Xho*I site at position 8490), MA NLS mutant (change of amino acids 26 and 27 of MA from Lys-Lys to Thr-Thr) (4), and Env (deletion between *Bgl*II sites at positions 6634 and 7214). Combinations of Vpr, Nef, Env, and MA NLS mutants were made by exchanging DNA fragments between the single mutants.

Virus and infections. Virus stocks were prepared by transfection of 293T cells (30) with 2.5 μg of proviral DNA per well of a six-well dish by the modified calcium phosphate method (8). Cell-free supernatant was collected 1 or 2 days after transfection, and the infectious titer was determined by the MAGI assay as described previously (15). Titers were typically 5×10^5 to 2×10^6 infectious units/ml.

Equal numbers of infectious units of virus were used to infect lymphoid cells. Typically, 1.5×10^6 cells were resuspended in 500 μl of media containing 1.5×10^5 infectious units of virus and 5 μg of Polybrene (Sigma). After 2 h at 37°C, the cells were pelleted, washed three times to remove residual virus, and resuspended in 2 ml of RPMI containing 10% fetal bovine serum. Viable cells were counted every 2 to 4 days based on trypan blue exclusion. $p24^{gag}$ was measured by enzyme-linked immunosorbent assay (ELISA; Coulter) with standards provided by the manufacturer.

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† Dedicated to the memory of Howard M. Temin.

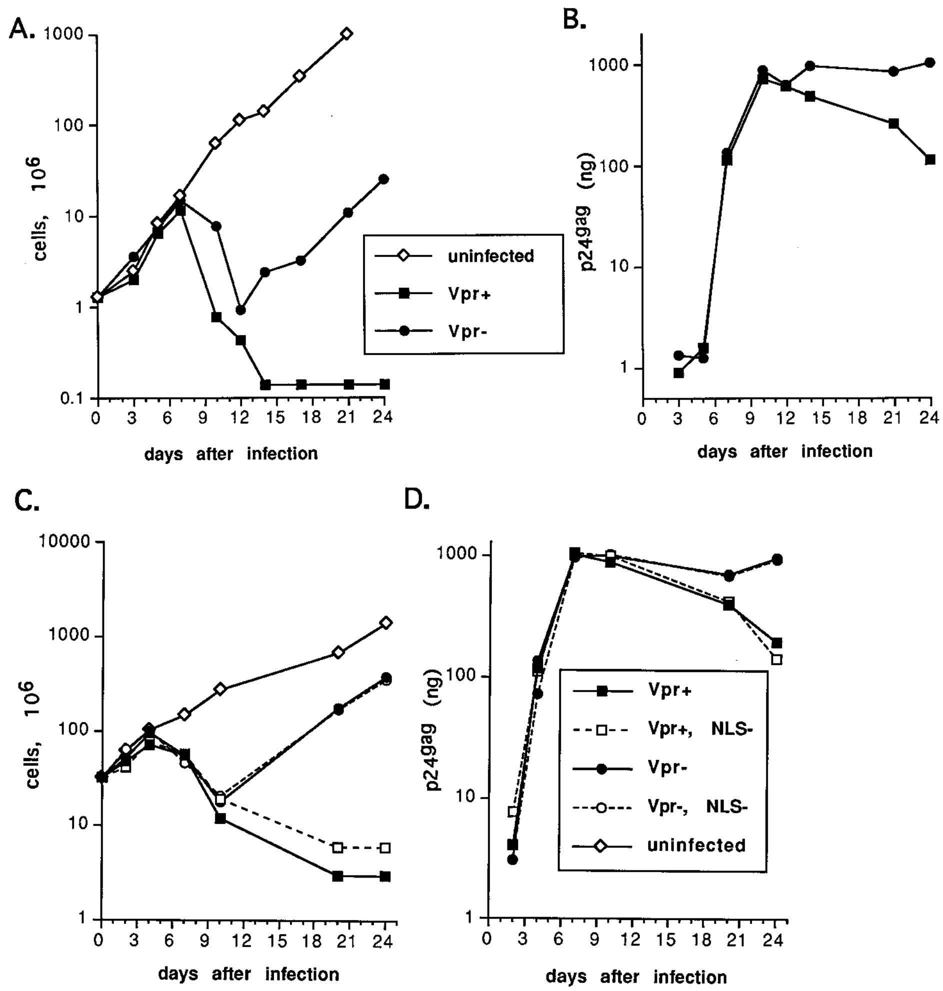

FIG. 1. Chronic infection is prevented in the presence of an intact *vpr* gene. SupT1 (A and B) or MT4 (C and D) cells were infected with HIV-1_{lai} with (Vpr^+) or without (Vpr^-) a full-length *vpr* gene at a multiplicity of about 0.1. The number of viable cells was determined by counting cells that excluded trypan blue (A and C), and the amount of $p24^{gag}$ was determined by ELISA (B and D). The number of cells is cumulative and was corrected for cells discarded when the culture was split (typically 1 to 2 every 3 days). Symbols as indicated in the figure are for panels A and B. The symbols for panels C and D are the same as in panels A and B, except that open squares are Vpr^+ NLS^- and open circles are Vpr^- NLS^-. NLS^- is the mutation in the nuclear localization sequence in MA as described previously (13).

Transient expression of Vpr and flow cytometry. Flow-cytometric analysis of whole cells was done as described previously (2) with some modifications. 293T cells were seeded to 1.5×10^5 per well in six-well plates. Transfections were carried out 2 days later with 5 μg of plasmid LTR-GFP (7), 0.6 μg of cytomegalovirus Tat, and 1 μg of either long terminal repeat (LTR) Vpr or LTR–NLS–β-galactosidase (15). At 48 h later, the cells were washed once with phosphate-buffered saline (PBS), removed from the plate with trypsin, diluted into 5 ml of PBS, and pelleted. They were then fixed in 1 ml of 2% formaldehyde at room temperature for 10 min, pelleted, and resuspended in 80% ethanol for 30 min on ice. After being washed, the cells were treated with 180 U of RNase A per ml in 1 ml of PBS at 37°C for 25 min, washed again, and then passed through a Nytex filer. Propidium iodide was added to 50 μg/ml, and the cells were incubated on ice for 1 h in the dark before they were analyzed by cell sorting.

Green fluorescent protein (GFP) expression was determined by fluorescence emission at 530 nm after excitation at 488 nm (7). Cells were gated positive for GFP expression on an FL2-versus-FL1 graph if the FL1 value was greater than the background determined by using mock-transfected cells after elimination of doublets. The DNA profiles and inferred G_1, S, and G_2 peaks were analyzed by the Multicycle AV program (Phoenix Flow Systems, San Diego, Calif.).

RESULTS

The presence of the Vpr prevents recovery of susceptible T-cell lines from the initial cytopathic effect of HIV-1 infection. To determine the long-term effect of Vpr expression on cells, we infected SupT1 cells (14), a T-cell line that is highly susceptible to the cytotoxic effect of HIV-1, with isogenic strains of HIV_{lai} that contain either a full-length *vpr* gene (called Vpr^+ here) or a truncated *vpr* gene that could encode a 45-amino-acid protein (called Vpr^- here). The number of viable cells in the culture were counted every 2 to 4 days for about 3 weeks, and cell-free supernatant was collected to determine the amount of virus produced.

In the initial week after infection, there was little difference between cells infected with Vpr^+ or Vpr^- virus in either virus growth or cell number (Fig. 1A and B). Large syncytia were observed in both cultures. Between days 7 and 12 after infection, the cell number declined dramatically in both infected-cell populations compared with the uninfected cells (Fig. 1A). However, after 12 days of infection there was a remarkable difference in the number of cells in the Vpr^+ infected culture and the number in the Vpr^- culture. By this time, cultures infected with Vpr^- virus had recovered from the initial cell death and began to grow with about the same doubling time as the uninfected cultures, while the cultures infected with wild-type virus did not recover (Fig. 1A). The amount of virus in the Vpr^- culture also remained at a plateau level, while the amount of virus in the Vpr^+ culture declined (Fig. 1B). This

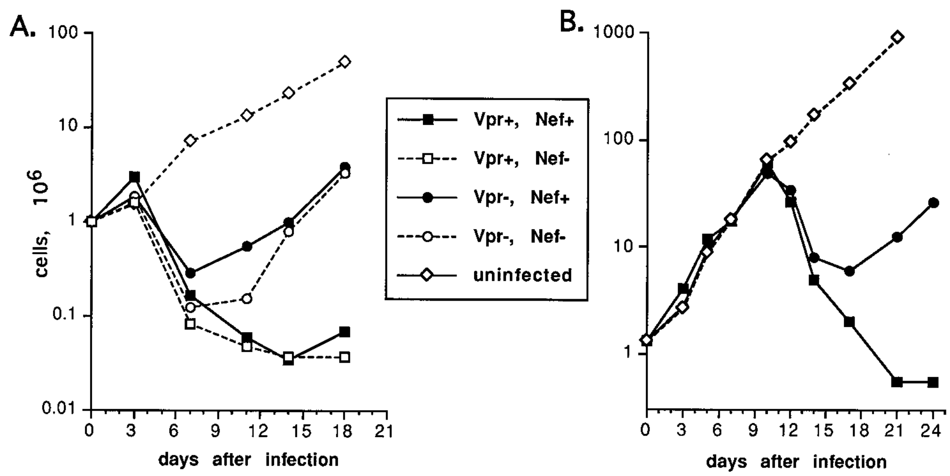

FIG. 2. The phenotype of Vpr late in infection is independent of the multiplicity of infection and of Nef. SupT1 cells were infected with HIV-1_{lai} at a multiplicity of infection of either 1.0 (A), or 0.01 (B). Cells were counted by trypan blue exclusion every 2 to 4 days. Symbols are as indicated in the figure.

result suggests that the presence of Vpr prevents establishment of chronic infection in SupT1 cells.

MT4 cells are another T-cell line that is very sensitive to the cytopathic effects of HIV-1. MT4 cells differ from SupT1 cells in that unlike SupT1 cells, they do not form large syncytia after infection by the Lai strain of HIV-1. Therefore, we repeated the infections with MT4 cells and the same stocks of Vpr^+ and Vpr^- virus and counted cells periodically. The results with MT4 cells were very similar to those with SupT1 cells (Fig. 1C). That is, there was little difference in virus production between Vpr^+ and Vpr^- virus early after infection (Fig. 1D), and cells in cultures infected with either Vpr^+ or Vpr^- viruses began to die at similar rates up to 10 days after infection (Fig. 1C). However, by day 14, there were few cells remaining in the cultures infected with wild-type virus whereas the cultures infected with Vpr^- virus began to divide with the same doubling time as the uninfected cells (Fig. 1C).

Mutations in the MA NLS (4) have a similar phenotype to *vpr* mutants in that both the MA NLS and Vpr play a role in the efficient replication of HIV in macrophages (13). Therefore, to determine if mutations in MA acted similarly to mutations in *vpr* with respect to cell death, we also infected MT4 cells with Vpr^+ and Vpr^- viruses that contained a mutation in the MA NLS (Fig. 1C). Mutations in the MA NLS alone had no effect on the ability of Vpr to suppress cell growth late in infection and did not accentuate the Vpr^- phenotype (Fig. 1C). This indicates that cell death late in infection is not a property of genes that target the preintegration complex to the nucleus (13) but, rather, is specific to Vpr.

The experiments in Fig. 1 were done at a multiplicity of infection of about 0.1. By increasing or decreasing the multiplicity of infection, we could change the shape of the cell growth curve but could not change the final outcome (Fig. 2). That is, when the multiplicity was raised to about 1, cells began to die almost immediately after infection, although, again, eventually the cultures infected with Vpr^- virus recovered (Fig. 2A). The presence of an intact *nef* gene had no effect on this phenotype at either the higher multiplicity (Fig. 2A) or a lower multiplicity (data not shown).

When the multiplicity of infection was lowered to about 0.01, the infected cultures continued to divide at the same rate as uninfected cells for a longer period. However, eventually both Vpr^+ and Vpr^- cultures began to die, and this was followed by a period when only the cultures infected with Vpr^- virus grew out (Fig. 2B). These results indicate that the presence of the *vpr* gene in a provirus prevents cell growth during the chronic stages of virus infection in several cell lines and at different multiplicities of infection. In the absence of the *vpr* gene, cells that are normally highly susceptible to the cytotoxic effects of HIV-1 recover from the acute infection and establish chronically infected cultures that divide at nearly the same rate as uninfected cells.

Growth of cells infected with Vpr^+ virus is accompanied by mutation of the *vpr* open reading frame. In some cultures infected with Vpr^+ virus, cells eventually began to grow out and continued to produce virus. However, the recovery of the culture was always more slow than the recovery of cultures infected with Vpr^- virus (data not shown). We hypothesized that cells infected with Vpr^+ virus had incurred a mutation of the *vpr* reading frame that allowed the infected cells to recover. Therefore, MT4 cells that were infected with Vpr^+ virus (Fig. 1C) were kept in culture for an additional 2 months. Although there were few cells in the culture at the end of 3 weeks (Fig. 1C), by the end of 2 months the culture had recovered. Supernatant from the cultures were collected, and virus was partially purified by ultracentrifugation through a 20% sucrose cushion. Reverse transcriptase PCR with primers that flanked the *vpr* gene was then performed, and the PCR product was cloned and sequenced.

Two types of *vpr* clones were recovered from the chronically infected MT4 cells. In one class (half of the clones), the ATG start codon had been mutated to ACG. Because there is no other in-frame ATG codon in *vpr*, this mutation would effectively eliminate Vpr expression. The *vif* gene overlaps with *vpr* at the 5′ end of *vpr*. Therefore, it is noteworthy that the mutation of the start codon at the second base of the codon occurs in the third base of a *vif* codon and therefore would not change the *vif* reading frame. The second class of mutants contained point mutations in conserved regions of *vpr* (26), and the effects of these mutations on Vpr function is under investigation. In addition, it should be noted that the *vpr* alleles of two isolates of HIV-1 that had been recovered from chronically infected cells, the MFD isolate (34) and the HXB2 isolate (32), both contain frameshift mutations that cause premature truncations of Vpr after 72 and 42 amino acids, respectively (12). These data suggest that mutation of Vpr is required for con-

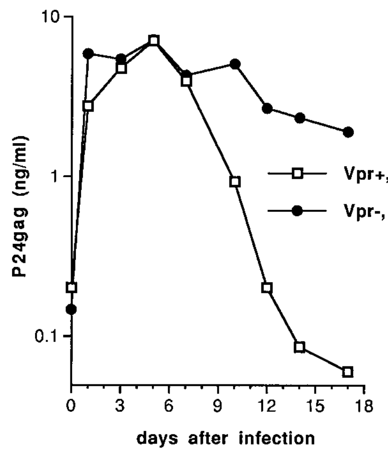

FIG. 3. Infection of PBMC with an HIV pseudotype. PBMC were infected with stocks of HIV that were created by transient cotransfection of an amphotropic envelope-expressing plasmid and an envelope mutant HIV provirus. The medium was completely changed every 2 to 4 days, and the level of p24^{gag} was measured.

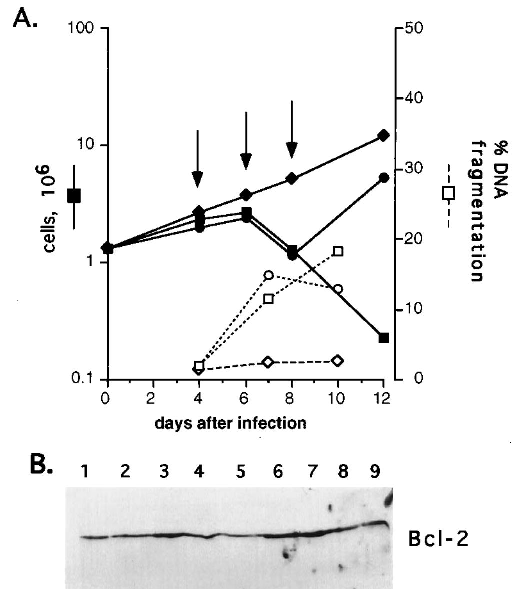

FIG. 4. Bcl-2 levels and apoptosis do not account for the Vpr phenotype. MT4 cells were infected with Vpr$^+$ or Vpr$^-$ virus at a multiplicity of infection of about 0.1. (A) Viable cells were counted on days 4, 6, 8, and 12 by trypan blue exclusion (solid symbols, left *y* axis). About 10^5 cells were taken at days 4, 7, and 10 to determine the amount of intracellular fragmented DNA (open symbols, right *y* axis). Symbols: diamonds, uninfected cells; squares, cells infected with Vpr$^+$ virus; circles, cells infected with Vpr$^-$ virus. (B) On days 4, 6, and 8 after infection (indicated by the arrows in panel A), equal cell equivalents of protein were loaded on a 12.5% polyacrylamide gel, blotted, and probed with antibody to Bcl-2. Lanes: 1, 2, and 3, extracts from cells infected with Vpr$^+$ virus at days 4, 6, and 8 days after infection, respectively; 4, 5, and 6; extracts from cells infected with Vpr$^-$ virus at days 4, 6, and 8 days after infection, respectively; 7, 8, and 9, extracts from uninfected cells taken in parallel with those of the infected cells.

tinued growth of infected cells, although we cannot yet rule out additional changes in the provirus.

Vpr-induced cell death in the absence of Env in the context of a provirus. We wished to determine if Vpr expressed in the context of a provirus had an effect in PBMC cells in addition to its effect on T-cell lines. However, because it is difficult to culture primary T cells for long periods, we wished to carry out these experiments in the absence of spreading virus so that the effect of Vpr could be assessed without the necessity to establish a chronic infection. Therefore, HIV-1 pseudotypes that contained either a wild-type or a truncated *vpr* gene were prepared by cotransfection of HIV-1 molecular clones and an expression vector for the murine amphotropic envelope gene (27). In each case, the HIV proviruses contained a large deletion in the envelope gene such that gp120/gp41 could not be expressed (see Materials and Methods).

The titers of both Vpr$^+$ Env$^-$ and Vpr$^-$ Env$^-$ viruses pseudotyped with amphotropic envelope was 2×10^5 infectious units per ml on HeLa-CD4/LTR–β-gal cells (15). PBMC were stimulated with phytohemagglutinin for 4 days to produce primarily activated T cells. Then, 2×10^6 cells were infected with 1 ml of virus, washed three times, and resuspended in new medium. Because the viruses used for these infections have a deletion in the envelope gene and are therefore capable of only one round of infection, the maximum number of cells infected is a small percentage of the population. Although we would be unable to detect a 10% decrease in cell number, we predicted that if proviruses with Vpr were cytotoxic in the absence of the envelope, the expression of the viral *gag* gene would decrease over time because of loss of infected cells from the culture.

Cell-free supernatant was collected from the infected PBMC 1 day after infection and every 2 or 3 days thereafter (Fig. 3). The day 0 collection was done immediately after the cells were washed after infection, and therefore it represents the residual input virus in the medium (Fig. 3). Each time the supernatant was collected, the cells were centrifuged, washed once, and resuspended in new medium. As expected, p24^{gag} was found in the supernatant as early as 1 day after infection. The amount of p24^{gag} in both Vpr$^+$- and Vpr$^-$-infected cultures was stable for at least 1 week. However, by day 10, the expression of p24^{gag} decayed significantly more rapidly in cultures infected with the Vpr$^+$ pseudotype than in cultures infected with the Vpr$^-$ pseudotype (Fig. 3). These results indicate that viral expression is sustained for longer periods in the absence of Vpr than in the presence of Vpr in PBMC and are consistent with the hypothesis that the presence of Vpr inhibits the proliferation of infected primary cells.

The effect of Vpr is not mediated through Bcl-2 levels. Other investigators have noted that acute HIV-induced cytopathic effect is accompanied by markers of apoptosis (6, 17, 22, 37), and it has been suggested that HIV-1 infection can regulate Bcl-2 levels in B cells (10). Therefore, one hypothesis to explain the recovery from initial cell death of cultures of cells infected with Vpr$^-$ virus is that levels of Bcl-2 were modified by Vpr, thereby affecting cell death later in infection.

To test this hypothesis, we examined both DNA fragmentation, a marker of apoptosis (23), and the amount of Bcl-2 protein in the infected cultures after infection (Fig. 4). As in previous experiments, the number of cells in the culture that had been infected with Vpr$^-$ virus first decreased and then began to increase by day 12 (Fig. 4A). The presence of fragmented DNA on days 6 and 8 suggested that death occurred by apoptosis and correlated with decreases in cell number (Fig. 4A, open symbols). However, the amount of fragmented DNA did not predict which culture would recover from acute cell death.

There is a high level of endogenous Bcl-2 in MT4 cells (Fig. 4B, lanes 7 to 9), as well as in other T-cell lines that we examined (data not shown). On days 4, 6, and 8 after infection (arrows in Fig. 4A), equal amounts of total protein from the cell cultures were probed with Bcl-2 specific antibody. Although there is extensive cell death in the infected cultures, the

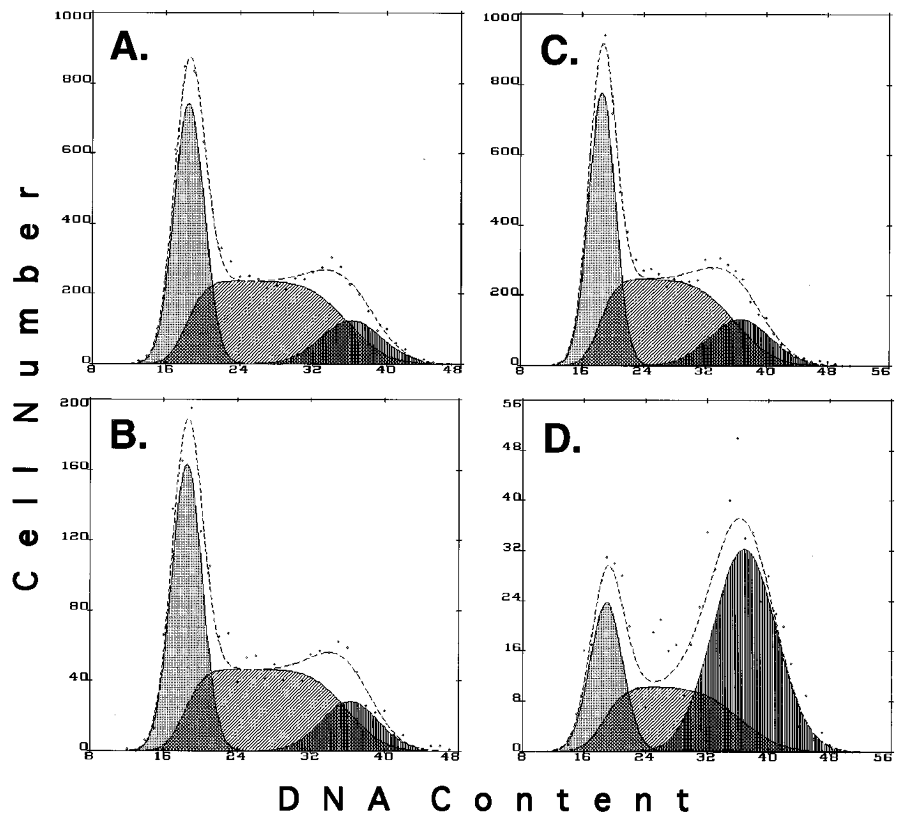

FIG. 5. Vpr transient transfection alters the cell cycle distribution. 293T cells were transfected with a plasmid that expresses the GFP and either Vpr (C and D) or a nucleus-localized β-galactosidase (A and B) from the HIV-1 LTR. The DNA profile of the cells that were GFP positive was analyzed separately from the DNA profile of the cells that were GFP negative. (A) GFP-negative population transfected with NLS–β-galactosidase. (B) GFP-positive population transfected with NLS–β-galactosidase. (C) GFP-negative population transfected with Vpr. (D) GFP-positive population transfected with Vpr. The G_1, S, and G_2 peaks are shown underneath the DNA profile.

amount of Bcl-2 does not significantly increase or decrease relative to that in the uninfected controls, except perhaps for a slight increase on day 8 (Fig. 4B, lanes 3 and 6). Furthermore, there was no difference in levels of Bcl-2 between the cultures that would recover from the initial cell death (the cultures infected with Vpr^- virus [lanes 4 to 6]) and the cultures which did not recover from the initial cell death (the cultures infected with the Vpr^+ virus [lanes 1 to 3]). Reprobing the blot with HIV serum revealed that the Vpr^- and Vpr^+ cultures contained equal amounts of viral protein on days 6 and 8 after infection (data not shown). These results indicate that the difference between culture death (Vpr^+) and culture survival (Vpr^-) is not modulated by differences in Bcl-2 expression. Furthermore, forced expression of Bcl-2 or Bcl-X_L (3) via retroviral vectors did not rescue cultures infected with wild-type Vpr^+ virus (data not shown). Therefore, these results do not support the hypothesis that the presence of Vpr in the provirus exerts its effect on viability of infected cells by modulating Bcl-2.

Expression of Vpr alone alters the distribution of cells in the cell cycle. We wished to determine if Vpr expression could affect cells directly by analyzing the effect of Vpr on cell cycle distribution of transfected cells. Therefore, we transiently cotransfected 293T cells with a plasmid that expresses Vpr from the HIV-1 LTR and with a plasmid that expresses GFP from the same promoter (the HIV-1 LTR) in order to mark successfully transfected cells. Transfected cells were fixed and stained with propidium idodide as whole cells to determine the DNA content and GFP expression simultaneously. As a control for the effects of high-level expression of an exogenous nuclear protein, cells were also cotransfected with GFP and an NLS β-galactosidase gene (15) under control of the HIV-1 LTR. All transfections included the Tat protein to achieve high-level expression from the HIV-1 LTR. Cells that expressed GFP (and therefore were presumed to express the cotransfected marker) were gated on the fluorescence-activated cell sorter, and the DNA profiles of both the GFP-positive populations (Fig. 5B and D) and the GFP-negative populations (Fig. 5A and C) were determined to compare transfected and nontransfected cells in the same culture.

The DNA profiles of GFP-negative populations transfected with either Vpr or β-galactosidase were nearly identical (Fig. 5, compare panels A and C). These cells are presumably nontransfected cells in the culture. The DNA profile of the cells that specifically took up and expressed DNA was then examined by gating the analysis on cells that expressed GFP (Fig. 5B and D). In the control, the GFP-positive cells in the culture cotransfected with both GFP and NLS–β-galactosidase had a DNA profile similar to that of the GFP-negative population (Fig. 5, compare panels A and B). In both populations, about 12% of the cells were in G_2. This indicates that expression of a transfected gene alone does alter cell cycle progression.

On the other hand, the GFP-positive cells that were also transfected with a Vpr expression vector had a DNA profile that was markedly skewed toward G_2 accumulation of DNA (Fig. 5, compare panels C and D). About 52% of the cells were in G_2 in the GFP-positive population, compared with about 13% of the cells in the GFP-negative population in the same culture. These results demonstrate that expression of Vpr in those cells is capable of altering their cell cycle distribution.

DISCUSSION

We have shown that in the presence of an intact *vpr* gene, HIV-1 will kill an entire culture of infected cells. On the other hand, when *vpr* is mutated, the virus will initially kill a large proportion of cells, but a small proportion of the culture will eventually survive and become a chronic producer of virus. Selection for mutations of the *vpr* gene occurs in long-term cultures of cells initially infected with wild-type virus. These results indicate that Vpr has a role in preventing the establishment of cell cultures that chronically produce virus.

The effect of Vpr is observed late after infection even in peripheral blood lymphocytes that were infected with a Vpr^+ provirus that could not encode envelope and therefore could not spread (Fig. 3). This result suggests that the effect of Vpr results from accumulation of newly synthesized Vpr from the integrated provirus rather than from Vpr that is brought into the infected cells in the virion (9) and present in the preintegration complex (13). In addition, it is doubtful that Vpr is responsible for the acute cell death, since cultures infected with either Vpr^+ or Vpr^- virus initially die at equal rates (Fig. 1 and 2) with signs of apoptosis (Fig. 4). We presume that gp120/gp41 is responsible for the acute cytopathicity. A minor effect of Nef on cytopathic effect (25) also cannot be excluded from our data.

The mechanism of action of Vpr is unclear. Vpr itself could be cytotoxic late in infection. However, the effect of Vpr was independent of Bcl-2 and BclX. Furthermore, it had previously been shown that Vpr could cause terminal differentiation of rhabdosarcoma cells (19). Our results are therefore probably more consistent with a hypothesis that Vpr is cytostatic and therefore prevents cells from continued proliferation (or causes their terminal differentiation). On the other hand, Vpr could be specifically toxic to cycling cells. The effects of transfection of Vpr alone (Fig. 5) are consistent with the hypotheses that it produces an arrest of cell cycle progression so that cells accumulate in G_2. A cytotoxic effect of Vpr only in cycling cells would explain why primary macrophages, which are withdrawn from cycling because of differentiation, are capable of long-term expression of HIV-1 without major cytotoxic effects (24), whereas PBMCs are rapidly killed by the virus in a productive infection.

The major reservoir of infected cells in vivo contains latent rather than active proviruses (1, 5, 11). Therefore, the fact that Vpr prevents establishment of chronic infections in T cells may have only a minor effect on total virus burden, although it may contribute to the ultimate decline in CD4 cells. Therefore, the importance of Vpr in macrophages (13, 38) alone could ensure its continued selection in vivo. On the other hand, there may be an evolutionary advantage for HIV to prevent replication of infected T cells. For example, $CD4^+$ cell clones that are specific for HIV antigens would be prevented from expansion and participation in cytotoxic T-lymphocyte responses after infection. The effect of Vpr on the cell cycle also raises the possibility that it plays a role in the differentiation of cells in vivo.

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