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Attenuation of Human Immunodeficiency Virus Type 1 Cytopathic Effect by a Mutation Affecting the Transmembrane Envelope Glycoprotein

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The cytopathic effects of human immunodeficiency virus type 1 (HIV-1) infection are specific for cells that express the CD4 viral receptor and consist of syncytium formation and single-cell lysis. Here we report that a mutation (517A) affecting the amino terminus of the HIV-1 gp41 transmembrane envelope glycoprotein resulted in a virus that was markedly less cytopathic than was wild-type HIV-1. In systems in which cell-to-cell transmission of HIV-1 occurred, the replication ability of the 517A virus was comparable with that of the wild-type virus. Even though the levels of viral protein expression, virion production, and interaction of the envelope glycoproteins with CD4 were similar for the 517A and wild-type viruses, both syncytium formation and single-cell lysis were attenuated for the 517A mutant virus. These results demonstrate that an envelope glycoprotein region important for mediating post-receptor binding events in cell membrane fusion is important for the induction of cytopathic effects by HIV-1. These results also indicate that levels of HIV-1 viral proteins or viral particles produced in infected cells are in themselves not sufficient to induce cytopathic effects.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (5, 13, 33). AIDS is characterized by compromised cellular immunity, in part due to depletion of CD4-positive lymphocytes (15, 28). HIV-1 is directly cytopathic to CD4-positive lymphocytes (5, 33), and recent estimates of viral burden in HIV-1-infected individuals are consistent with the possibility that virus-mediated cell killing contributes to lymphocyte depletion in vivo (8, 19, 21, 40).

The tropism of HIV-1 for CD4-positive cells is due to a specific interaction between CD4, the viral receptor, and the gp120 exterior envelope glycoprotein (9, 23, 24, 31). Following receptor binding, the viral envelope glycoproteins, gp120 and gp41 (1, 38, 52), mediate the fusion of the viral and host cell membranes to allow viral entry (48).

The in vitro cytopathic effects of HIV-1 infection, which consist of the formation of syncytia and the lysis of single cells (30, 33, 44, 45), occur specifically in cells expressing high levels of CD4 receptor (3, 25). Syncytia are formed by the fusion of cells expressing surface HIV-1 envelope glycoproteins with CD4-positive cells (30, 44). Syncytium formation requires the proteolytic processing of the gp160 envelope precursor, stable association of the gp120 and gp41 subunits, cell surface expression, CD4 binding, and membrane fusion events that follow CD4 binding (27). Membrane fusion events are disrupted by changes in the hydrophobic amino terminus of the gp41 transmembrane glycoprotein (27), which bears distant sequence similarity to the fusion regions of paramyxovirus and orthomyxovirus envelope glycoproteins (12, 14). Here we report the effects of changes in this gp41 amino terminal region on HIV-1 replication and cytopathogenicity.

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MATERIALS AND METHODS

Plasmids. Plasmids p517A and p517B were made by cloning the *Kpn*I-*Bam*HI fragments of the pIIIenv517A and pIIIenv517B plasmids, respectively (27), into the infectious proviral clone pHXBc2 (35). The 517A mutation results in an insertion of Gly-Ile-Pro-Ala immediately carboxyl to Ala-517, while the 517B mutation results in an insertion of Ile-His-Arg-Trp-Ile-Ala at the identical site. The pΔ(5928-8595) plasmid contains a replication-defective provirus that has a deletion of *rev* and *env* sequences (43). The pHIV-CAT plasmid contains a HIV-1 provirus with a chloramphenicol acetyltransferase gene replacing the *nef* gene (51). Each plasmid contains a simian virus 40 origin of replication.

Virus replication studies in Jurkat lymphocytes. In initial studies, Jurkat lymphocytes were transfected by using the DEAE-dextran technique (34) with 10 μg (each) of pΔ(5928-8595), pHXBc2, p517A, and p517B. In subsequent studies, Jurkat transfections were done with 20 μg of pΔ(5928-8595), 10 μg of pHXBc2, 10 μg of X10-1 (11), 20 μg of p517A, and 20 μg of pHIV-CAT. Following transfection, viable cells were counted daily by trypan blue dye exclusion. Cultures were resuspended daily in the volume of fresh medium (RPMI 1640 plus 10% fetal calf serum) required to maintain equivalent densities of viable cells (1.5×10^6 to 2×10^6 cells/ml) among cultures.

Reverse transcriptase activity of pelleted virions was measured as described previously (36). For measurement of viral protein expression, equal numbers (1×10^6 to 5×10^6) of viable cells were metabolically labeled with 100 μCi of [^{35}S]-cysteine per ml overnight at different times following transfection. Cells were lysed in 1.0 ml of lysis buffer (31), and HIV-1 proteins were precipitated from cell lysates and supernatants with excess serum from a patient with AIDS (RV119) as previously described (27). Proteins were analyzed on 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gels prior to autoradiography. The relative amounts of the HIV-1 $p24^{gag}$ protein were determined by densitometry.

Live-cell immunofluorescence for HIV-1-specific proteins was performed on each culture at day 6 after transfection by using a 1:100 dilution of RV119 serum from a patient with AIDS and a 1:50 dilution of fluorescein isothiocyanate-conjugated goat $F(ab)_2$ anti-human immunoglobulin (Tago) as previously described (42). Cells were analyzed by fluorescence-activated cell sorting.

To examine complexes of envelope glycoproteins and CD4, radiolabeled cell lysates from transfected Jurkat cultures (day 6 posttransfection) were precipitated with OKT4 monoclonal antibody (Ortho) (20, 31). The 58-kDa CD4 protein and the complexed HIV-1 envelope glycoproteins were visualized on 12.5% SDS-polyacrylamide gels.

The amount of virion-associated envelope glycoproteins was measured by Western immunoblot of the pelleted virus particles. Supernatants from Jurkat cultures exhibiting peak reverse transcriptase levels for HXBc2 and 517A virus and supernatants from a mock-transfected control culture were subjected to low-speed centrifugation (1,500 × *g*) to remove cell debris. The supernatants were filtered (0.22-μm-pore-size membrane filters; Millipore) and centrifuged for 90 min at 35,000 rpm in an SW50.1 rotor (Beckman). The pelleted virions were resuspended in 50 μl of lysis buffer and Western blotted as previously described (7). Envelope glycoproteins were visualized by using RV119 serum from patients with AIDS or an anti-gp41 human monoclonal antibody provided by Susan Zolla-Pazner (32).

Southern blot of infected cells. DNA was prepared from 2×10^7 Jurkat cells, which were either uninfected or infected with wild-type or mutant viruses, according to the procedure of Hirt (18) with modifications of Gowda et al. (16). Cells were lysed at room temperature before NaCl was added to a final concentration of 1 M. After overnight incubation at 4°C, the extract was centrifuged at 30,000 × *g* for 30 min at 4°C. The supernatant was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) followed by two extractions with chloroform-isoamyl alcohol (24:1). The Hirt supernatant DNA was precipitated with 2.5 volumes of ethanol, pelleted, washed in 70% ethanol, and resuspended in 300 μl of a solution containing 10 mM Tris (pH 7.5), 100 mM NaCl, and 1 mM EDTA. The samples were treated with RNase A (200 μg/ml) for 3 h at 37°C, which was followed by proteinase K treatment in the presence of 1% SDS for another 3 h at 37°C. Following final phenol and chloroform extractions, the DNA was ethanol precipitated.

For Southern blotting, Hirt DNA derived from 2×10^7 cells was digested with *Xho*I and subjected to electrophoresis. The DNA was Southern blotted (47) as previously described. The probe consisted of an approximately 9-kb *Mro*I fragment of the HXBc2 provirus. Hybridization and washing conditions were as previously described (42).

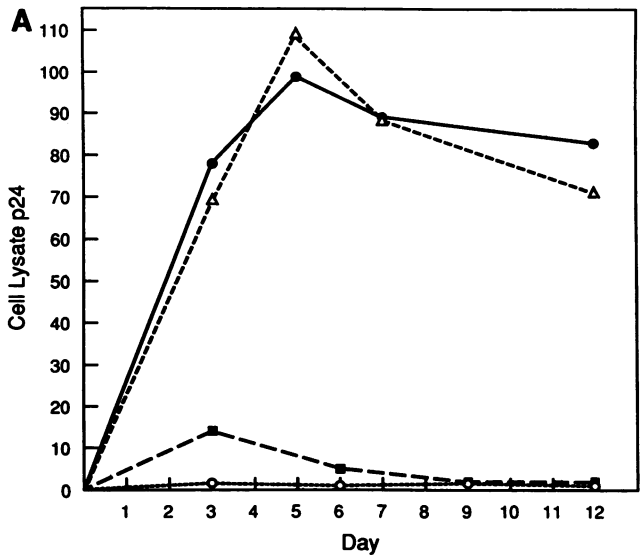
Viral cytopathic effects. Total viable cell counts in Jurkat cultures were determined by counting the number of cells that excluded trypan blue dye. Cultures were examined by light microscopy and syncytia were scored by counting the number of syncytia per high-power field. To quantitate single-cell lysis, more than 200 single cells from each culture were scored for viability by trypan blue dye exclusion. The values for single-cell killing obtained from the pΔ(5928-8595)-transfected cultures were subtracted from the values for the wild-type and mutant virus-infected cultures to obtain values for specific single-cell lysis.

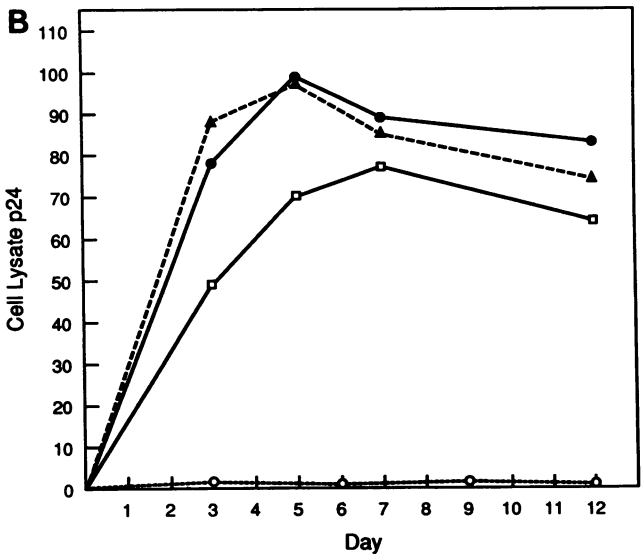
Viral replication in primary human peripheral blood mononuclear cells. Peripheral blood mononuclear cells were harvested by Ficoll-Paque gradient separation (Pharmacia) from pooled donor leukocytes from the Dana-Farber Cancer Institute blood bank. Peripheral blood mononuclear cells were stimulated with 1% phytohemagglutinin and cultured in RPMI 1640 with 20% heat-inactivated fetal calf serum and 30 U of recombinant interleukin-2 per ml. After 5 days in culture, 2.5×10^7 primary cells were infected by cocultivation with 5×10^6 mitomycin C-treated (100 μg/ml, 1 h) Jurkat cells that had been transfected with the pΔ(5928-8595), pHXBc2, or p517A plasmids 6 days earlier. At the time of cocultivation, the HXBc2- and 517A-infected cells were producing similar levels of viral particles, as measured by reverse transcriptase determination (36). Pilot experiments indicated that the mitomycin C-treated Jurkat cells were nonviable by day 4 following treatment. Viral protein expression in the peripheral blood mononuclear cells was examined by radiolabeling equal numbers of viable cells at several times after infection and precipitating viral proteins with RV119 serum from a patient with AIDS as described for the Jurkat cultures above. The depletion of CD4-positive cells from the peripheral blood mononuclear cultures was measured by determining the percentage of cells positive for OKT4 monoclonal antibody (Ortho Diagnostics) by fluorescence-activated cell sorting.

RESULTS

Replicative and cytopathic properties of gp41 mutants. To examine the effects of changes in the gp41 amino terminus on the replicative and cytopathic properties of HIV-1, two mutations (517A and 517B) were introduced into an infectious HIV-1 provirus on plasmid pHXBc2. The 517A mutation results in the insertion of four uncharged amino acids into the gp41 amino terminus, which decreases the syncytium-forming abilities of the envelope glycoproteins to less than 5% of that of the wild-type glycoproteins (17, 27). The 517B mutation results in the insertion of six amino acids, including a charged residue, into the gp41 amino terminus. This disruptive change results in a complete loss of detectable syncytium-forming ability of the envelope glycoproteins (17, 27).

To examine the effects of the mutations on virus replication, CD4-positive Jurkat lymphocytes were transfected with equal amounts of plasmid DNA containing the wild-type HXBc2, 517A, and 517B proviruses. Under these conditions, if infectious virus is produced in the small percentage of cells that express the transfected DNA, viral transmission will occur by both cell-free and cell-to-cell mechanisms. Viral replication was monitored by measuring viral protein expression in the culture. At various times following transfection, equal numbers of viable cells were radiolabeled and viral proteins were immunoprecipitated from cell lysates and supernatants by using an excess of serum from a patient with AIDS (RV119). The Jurkat cultures transfected with the pHXBc2 plasmid expressed increasing amounts of viral proteins until a steady-state level was reached (Fig. 1A), at which time greater than 90% of the cells were infected, as judged by indirect immunofluorescence assays of live and fixed cells with antiserum from a patient with AIDS and anti-p24 monoclonal antibody, respectively (data not shown). The Jurkat cultures transfected with the p517A plasmid demonstrated only slight lags in the rate of increase of viral protein expression, indicating that the 517A virus replicated with only a marginal delay in kinetics relative to that of the wild-type virus. The p517B-transfected cultures demonstrated transient expression of viral proteins, but an increase in viral protein expression with time did not occur, indicating that the 517B mutation





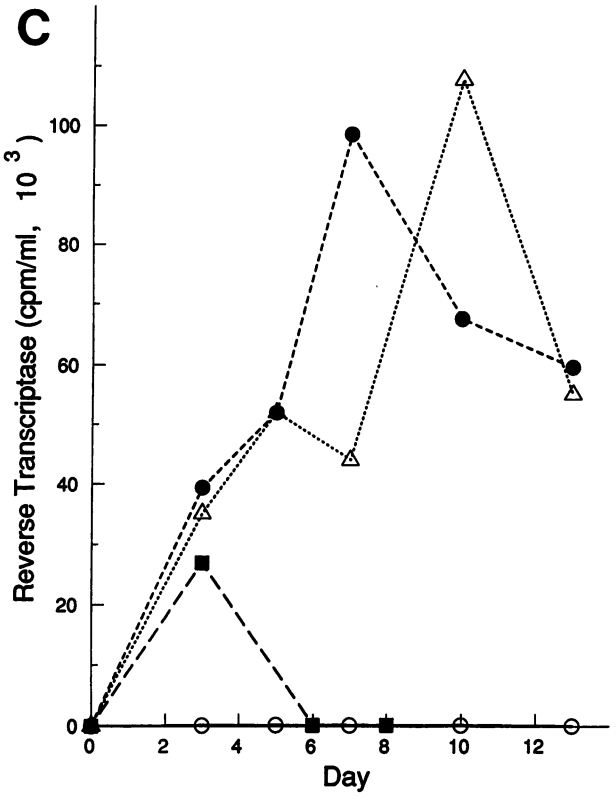


FIG. 1. Replication of wild-type and mutant viruses in Jurkat lymphocytes. (A) The expression of cell lysate p24 antigen, relative to the maximal level attained in wild-type HXBc2-infected cultures, is shown for Jurkat lymphocytes transfected with 10μg of pΔ(5928-8595) (○), pHXBc2 (●), p517A (△), or p517B (■) DNA. (B) Cell lysate p24 expression, relative to maximal p24 expression in HXBc2-infected cultures, is shown for Jurkat cells transfected with 20 μg of pΔ(5928-8595) (○), 10 μg of pHXBc2 (●), 20 μg of p517A (▲), or 20 μg of pHIV-CAT (□) DNA. The results are typical of those observed in five independent experiments. (C) Reverse transcriptase activity in supernatants of Jurkat cells transfected with 20 μg of pΔ(5928-8595) (○), 10 μg of pHXBc2 (●), 20 μg of p517A (△), or 20μg of p517B (■) DNA.

significantly attenuated the ability of the virus to replicate under these conditions.

The cytopathic effects of infection were monitored by counting the total number of viable cells in the cultures transfected with the pHXBc2, p517A, and p517B plasmids. The viability of the p517B-transfected cultures did not significantly differ from that of the mock-transfected cultures, whereas marked decreases in cell viability were noted in the cultures transfected with the pHXBc2 plasmid. Despite the active replication of 517A virus in the Jurkat cells, cytopathic effects in these cultures were markedly reduced relative to those observed in the pHXBc2-transfected cultures (data not shown). In these and subsequent experiments, no cytopathic revertants of the 517A virus were observed.

A possible explanation for the apparent decrease in cytopathogenicity of the 517A virus was that the rate of viral replication and, consequently, the level of viral protein expression were slightly less than those of the culture infected with the wild-type virus. To ensure that differences in the level of viral protein expression were not responsible for observed differences in cytopathic effect, Jurkat cells were transfected with more p517A plasmid DNA than pHXBc2 DNA. In addition, to ensure that variation in cell density secondary to different degrees of cytopathic effect did not contribute to different rates of culture growth or virus replication, all cultures were diluted to the same cell density daily. Under these conditions, the level of viral protein expression was, at all times examined, at least as great for the 517A as for the HXBc2 virus (Fig. 1B). Virion production and release, as measured by immunoprecipitation of labeled cell supernatants and by reverse transcriptase determinations, were comparable for the 517A and wild-type virus-infected cultures (Fig. 1C and 2A.) HIV-specific live-cell immunofluorescence indicated a similar level and distribution of viral proteins on the cell surface of the two cultures (Fig. 2B). Experiments in which radiolabeled cells from the infected cultures were incubated with RV119 serum from a patient with AIDS, washed, lysed, and used for immunoprecipitation by the addition of protein A-Sepharose beads indicated that the envelope glycoproteins were the only viral products accessible to antibody on the surface of live cells (data not shown).

The 517A mutation does not dramatically affect the

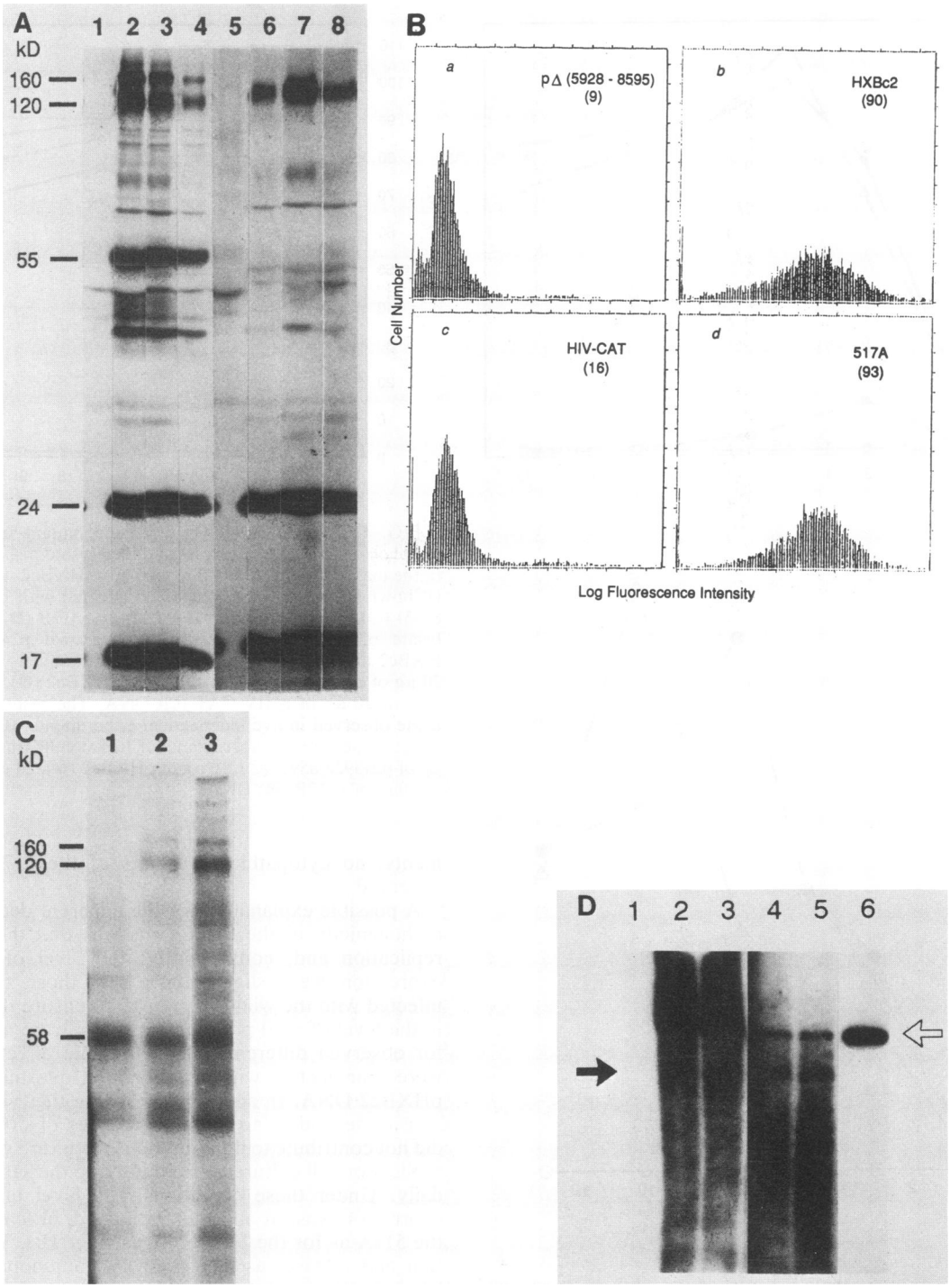


FIG. 2. (A) Immunoprecipitated cell lysates (lanes 1 through 4) and supernatants (lanes 5 through 8) from Jurkat cells on day 5 following transfection with 20 μg of pΔ(5928-8595) (lanes 1 and 5), 10 μg of pHXBc2 (lanes 2 and 6), 20 μg of p517A (lanes 3 and 7), or 20 μg of pHIV-CAT (lanes 4 and 8). The gp160 and gp120 envelope glycoproteins and the $p55^{gag}$, $p24^{gag}$, and $p17^{gag}$ products are marked. kD, Kilodaltons. (B) Live-cell immunofluorescence using antiserum from a patient with AIDS for Jurkat cultures 6 days following transfection with the indicated plasmids. The percentage of cells in the culture exhibiting HIV-specific staining is indicated in parentheses. (C) Complexing of wild-type HXBc2 and 517A viral envelope proteins and CD4. Cell lysates of Jurkat cells 6 days following transfection of pΔ(5928-8595) (lane 1), wild-type pHXBc2 (lane 2), or p517A (lane 3) DNA were immunoprecipitated with OKT4 monoclonal antibody (Ortho). The 58-kDa CD4 protein and the complexed HIV-1 envelope proteins are marked. kD, kilodalton. (D) Detection of unintegrated HIV-1 DNA in Jurkat cells during the course of infection with wild-type HXBc2 virus or mutant 517A virus. Autoradiogram of Southern blots of *Xho*I-digested Hirt DNA prepared from 2×10^7 cells per lane on day 4 following transfection (lanes 1 through 3) and day 8 following transfection (lanes 4 and 5). Jurkat cells were either mock transfected (lane 1) or transfected with pHXBc2 (lanes 2 and 4) or p517A (lanes 3 and 5) DNA. The open arrow indicates 100 pg of *Xho*I-linearized pHXBc2 plasmid DNA that was run in lane 6 as a marker. This 14-kb plasmid band comigrates with a band of residual plasmid DNA seen in each of the infected-cell lanes. The solid arrow indicates the ~9.4-kb fragment derived from linearization of the circular forms of unintegrated HIV-1 viral DNA.

steady-state levels of either cell-associated or supernatant envelope glycoproteins relative to those observed for the wild-type virus in the transfected Jurkat cells (Fig. 2A). Pulse-chase analysis revealed no difference in the rate of gp160 processing or stability of the processed gp120 and gp41 glycoproteins between the two cultures (data not shown). Western blots of pelleted wild-type and 517A virions revealed that the concentration of envelope glycoproteins relative to core proteins in the 517A virions was indistinguishable from the value obtained for the wild-type virions (data not shown). We conclude that the 517A mutation does not appreciably alter the processing, distribution, or steady-state levels of the envelope glycoproteins.

It has been suggested that intracellular complexing of HIV-1 envelope glycoproteins with the CD4 molecule may lead to cytopathic consequences (20). To examine whether the 517A mutation affected the formation of envelope glycoprotein-CD4 complexes, Jurkat cells transfected with the pHXBc2 or p517A plasmids were radiolabeled and cell lysates were precipitated with OKT4 monoclonal antibody, which recognizes a CD4 epitope distant from the gp120-binding site (31). Under the conditions used, most of the immunoprecipitated envelope glycoprotein represents molecules complexed to CD4 prior to the cell lysis (20). The ratio of coprecipitated envelope glycoproteins to CD4 molecules is similar between the 517A virus- and HXBc2 virus-infected cultures (Fig. 2C). Immunoprecipitation of cell lysates with OKT4 monoclonal antibody at different times during the infection also revealed that the decrease in total precipitable CD4 in the infected cultures occurred more rapidly in the cultures infected with the wild-type virus than with the 517A virus (data not shown). The decrease in CD4 expression in cultures infected with natural HIV isolates exhibiting decreased cytopathic effects is reported to be less than that observed in cultures infected with more cytopathic variants (10, 39).

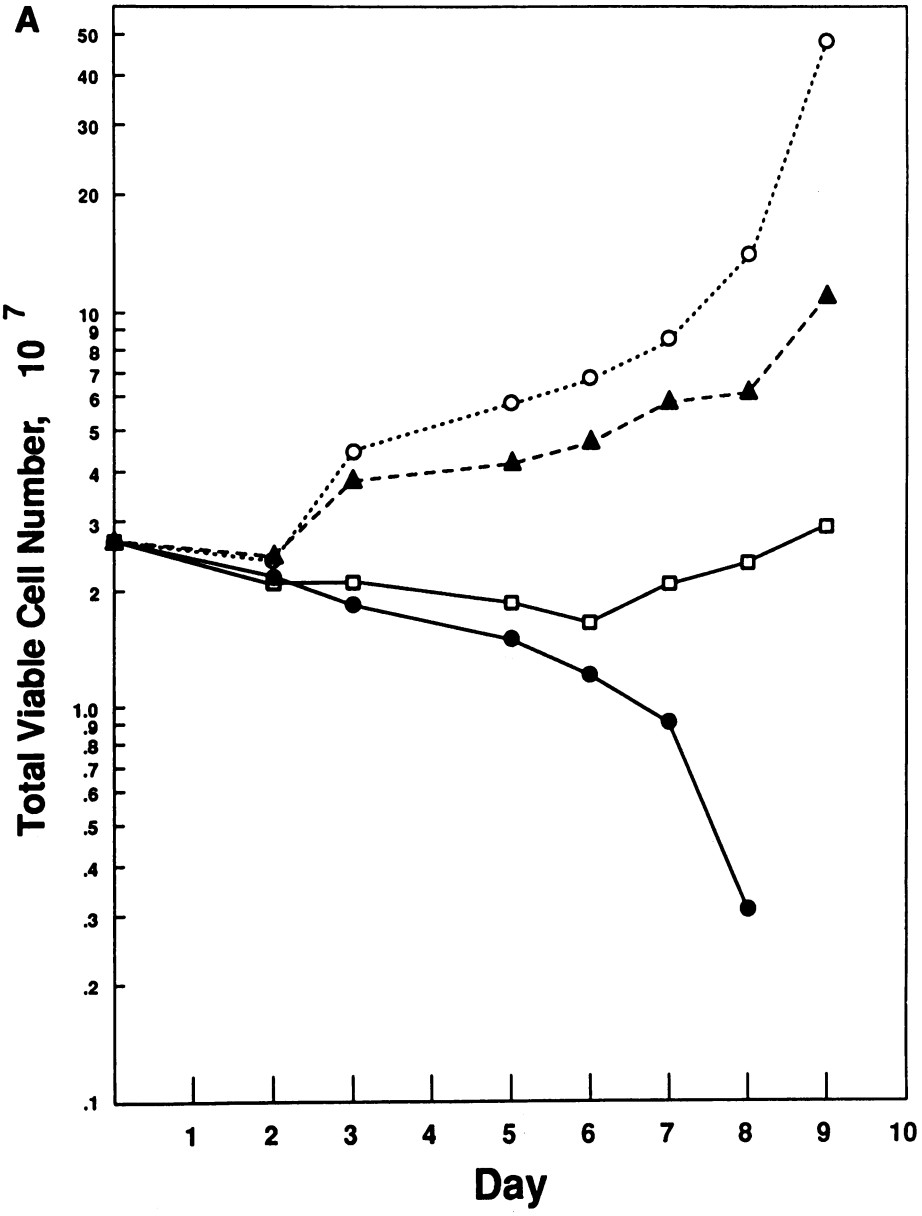
Cytopathic effects associated with animal retroviruses occur concomitantly with superinfection and generation of unintegrated linear viral DNA (53). Hirt supernatants were prepared from the cultures infected with wild-type or 517A viruses and Southern blotted to examine the amount of viral DNA present at various times following transfection. The DNA was digested with *Xho*I to allow a distinction between transfected plasmid DNA and linear proviral DNA. No significant differences were observed between the levels of unintegrated viral DNA for the 517A and the wild-type viruses (Fig. 2D).

A mutant virus derived from the HXBc2 virus was included in the experiments for comparison of replicative and cytopathic properties. The HXB-CAT virus contains a bacterial chloramphenicol acetyltransferase gene replacing the *nef* gene of HXBc2 (51). Under the culture conditions described above, the HXB-CAT virus replicates more slowly than either the HXBc2 or the 517A virus (Fig. 1B).

By using the transfected cultures described above, in which viral protein expression was comparable for the HXBc2 and 517A viruses, the effects of the introduced mutations on cytopathogenicity per se could be directly assessed. The total number of viable cells present in cultures at various times following transfection is shown in Fig. 3A. The number of syncytia and the percentage of single cells unable to exclude the vital dye trypan blue were also measured (Fig. 3B and C). Compared with the mock-transfected cultures, the viability of the pHXBc2-transfected cultures was dramatically decreased. In the wild-type virus-infected culture, syncytium formation was prominent until day 4 following transfection, after which single-cell lysis was the predominant cytopathic effect observed. Following day 10 after transfection, cells emerged that continued to express viral proteins but were resistant to cytopathic effects. The cultures transfected with the p517A plasmid exhibited markedly fewer cytopathic effects than seen in the culture infected with the wild-type HXBc2 virus (Fig. 3A). Syncytium formation was delayed temporally and was lower in magnitude for the 517A virus relative to that for the HXBc2 virus (Fig. 3B and 4). Single-cell lysis was also significantly decreased in the 517A virus-infected culture compared with the wild-type virus-infected culture (Fig. 3C). The cytopathic effects measured in the 517A virus-infected Jurkat cells were less than those seen in the culture transfected with the pHXB-CAT provirus (Fig. 3A, B, and C), which encodes a virus that replicates more slowly than either the 517A or HXBc2 virus. We conclude that the 517A mutation specifically affects the ability of HIV-1 to induce both forms of cytopathic effect, independent of effects of the introduced amino acids on virus replication.

Replicative and cytopathic properties of the X10-1 mutant in Jurkat lymphocytes. A HXBc2 proviral mutant, X10-1, contains a deletion spanning the *env* and *nef* genes and encodes a transmembrane envelope glycoprotein with a deletion of 4 amino acids and an addition of 15 random residues at the carboxyl terminus (11). It has been reported that the X10-1 virus retains wild-type replicative and syncytium-forming abilities but is attenuated for single-cell lysis (11). To examine the replicative and cytopathic properties of this mutant, Jurkat lymphocytes were transfected in parallel with the pHXBc2 and X10-1 plasmids. The X10-1 mutant replicated identically to the wild-type virus in Jurkat lymphocytes (data not shown). The migration on SDS-acrylamide gels of the gp160 and gp41 glycoproteins produced in the X10-1-infected culture differed from that of the HXBc2-infected culture, but the overall level of viral proteins was similar to that seen for the wild-type virus (Fig. 5A). The viability of the X10-1-transfected culture and the amount of syncytium formation and single-cell killing were indistinguishable from those observed for the pHXBc2-transfected culture (Fig. 5B and data not shown).

Replication and cytopathic effects of 517A mutant in primary lymphocytes. To examine whether the decreased cytopathogenicity observed for the 517A virus in Jurkat cells was evident in another cell type, primary human peripheral blood lymphocytes were infected with both the 517A and wild-type viruses by cocultivation with mitomycin C-treated Jurkat lymphocytes. This method results in an efficient, cytopathic infection in primary lymphocytes, which are not as readily transfected as are Jurkat lymphocytes. The levels of viral proteins expressed in the 517A- or HXBc2-infected primary lymphocytes did not differ at any time examined (data not shown). The decrease in CD4-positive cells in the culture was significantly greater for the wild-type virus than for the 517A virus (Fig. 6), suggesting that the induction of cytopathic effects and/or CD4 down-modulation were attenuated by the 517A mutation. Both syncytium formation and single-cell lysis could be observed in the wild-type virus-infected primary lymphocytes. Both forms of cytopathic effect were visibly reduced in the primary lymphocytes infected with 517A virus (data not shown). These results suggest that the 517A virus is less efficient than the HXBc2 virus in induction of cytopathic changes in primary lymphocytes.



DISCUSSION

Here we describe a mutation affecting the HIV-1 gp41 amino terminus that generates a replication-competent virus attenuated in the ability to induce cytopathic effects. The decreased syncytium formation observed in the 517A virus-infected cultures is consistent with the previously reported decrease in the syncytium-forming ability of HIV-1 envelope glycoproteins containing alterations in the gp41 amino terminus (17, 27). The decrease in the amount of single-cell lysis accompanying infection with the 517A virus is noteworthy. Since the 517A mutant does not appreciably differ from the wild-type virus in the generation of extrachromosomal viral DNA, level of viral protein expression, or virion release, these processes by themselves are not sufficient for efficient single-cell lysis by HIV-1. It has been suggested that single-cell killing by HIV-1 occurs secondary to high-level expression of viral proteins and preemption of the host cell synthetic machinery (46). In our study, HIV-1 structural protein synthesis constituted no greater than 3% of the total host cell protein synthesis, even during the period of maximal single-cell lysis (data not shown). We observed no specific or general detrimental effect of HIV-1 gene expression on the synthesis of host cell proteins when the cultures were normalized for viable cells. The observations of extremely high levels of HIV-1 protein synthesis (40% of host cell protein synthesis) by Somasundaran and Robinson (46) may not be general, especially since the cell line used, C8166, is transformed by HTLV-1, which encodes at least two *trans*-activator proteins known to activate HIV-1 gene expression (37, 41). It remains to be demonstrated that HIV-1 protein overexpression per se contributes to cytopathic effects in any circumstances.

The observed decrease in single-cell lysis for the 517A virus relative to the wild-type virus is not readily explained

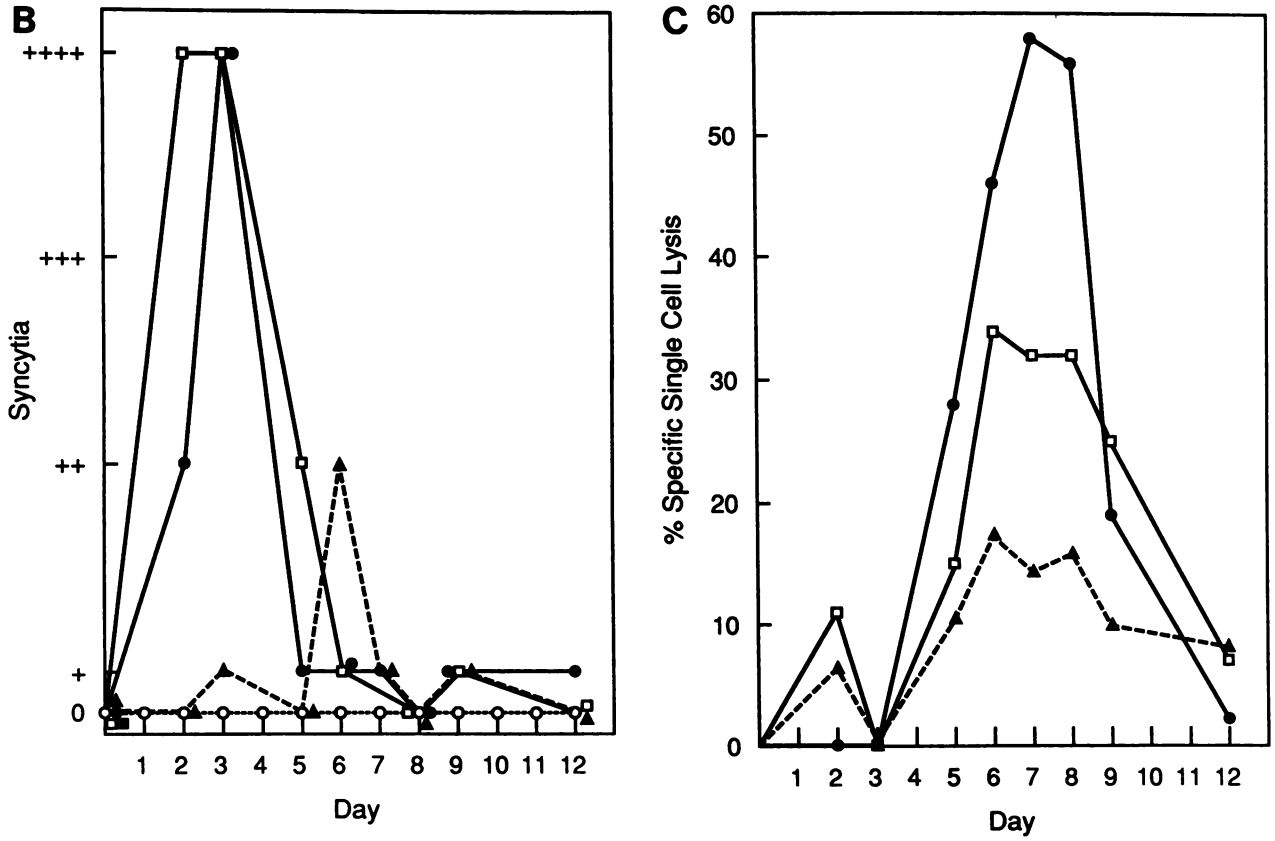


FIG. 3. Cytopathogenicities of wild-type HXBc2, 517A, and HIV-CAT viruses in Jurkat cells. (A) Total viable cell counts in Jurkat cell cultures transfected with 10 μg of pHXBc2 (●), 20 μg of p517A (▲), 20 μg of pHIV-CAT (□), or 20 μg of pΔ(5928-8595) (○). (B) Syncytium formation in Jurkat cell cultures. Symbols for transfected plasmids are as described for panel A. Syncytia formed rarely in some fields (0) or at rates (in syncytia per high-power field) of 1 to 5 (+), 6 to 10 (++), 11 to 20 (+++), or >20 (++++). (C) Single-cell lysis by HXBc2 (●), 517A (▲), and HIV-CAT (□) viruses in the transfected Jurkat cells.

by changes in the distribution, processing, or CD4 complexing of the HIV-1 envelope glycoproteins. Recently, single-cell lysis has been observed in CD4-positive but not CD4-negative lymphocyte lines expressing only the HIV-1 *rev* and *env* genes (25). In this context and in HIV-1-infected cultures, the intracellular interaction of envelope glycoproteins and CD4 has been observed (20, 22). Our data suggest that efficient single-cell lysis requires an envelope glycoprotein function, which is mediated by the gp41 amino terminus, in addition to CD4 binding. The 517A mutation has been shown to affect a postreceptor binding event important for the fusion of membranes during the process of syncytium formation (27). Our results are consistent with a model in which similar fusion events triggered by envelope glycoprotein-CD4 interactions affect the integrity of host cell membranes, leading to loss of single-cell viability.

The effects of the 517A mutation on virus replication in the cells used in these studies were significantly less than the effects of the mutation on induction of cytopathic changes. In sensitive assays measuring the rate of proviral DNA formation following cell-free infection or measuring the ability of envelope glycoproteins to complement a single round of virus entry, delays relative to the wild-type envelope glycoproteins can be detected for the 517A mutant (17, 27a). With the cells and culture conditions used in these experiments, in which the multiplicity of infection following transfection may be saturating and cell-to-cell transmission is possible, any lag in the replicative ability of the 517A mutant exhibits minimal effects on virus spread or protein production.

If the gp41 amino terminus is important for virus entry into the host cell and for cytopathic processes, why does the 517A mutation affect cytopathogenicity dramatically with only minimal effects on virus replication? A likely explanation is that differences exist between the number of successful envelope glycoprotein-receptor interactions required for virus entry and the number required for cytopathic processes. Changes in the efficiencies with which individual envelope glycoprotein-receptor interactions mediate membrane fusion would disproportionately affect processes requiring higher numbers of cooperative successful interactions. On the basis of the phenotype of the 517A mutant, it is likely that virus replication in the culture systems used required fewer successful envelope-receptor events than did cytopathic processes. This would also explain why cells with lower levels of CD4 expression can support HIV-1 replication without demonstrating significant cytopathic effects (3). This model is consistent with the observation that higher titers of anti-CD4 monoclonal antibodies are required to block HIV-1 infection than are required to inhibit syncytia (9) and that some CD4 mutants support virus entry but not syncytium formation (6).

The X10-1 deletion, which affects the gp41 carboxyl terminus, has been reported to result in a replication-com-

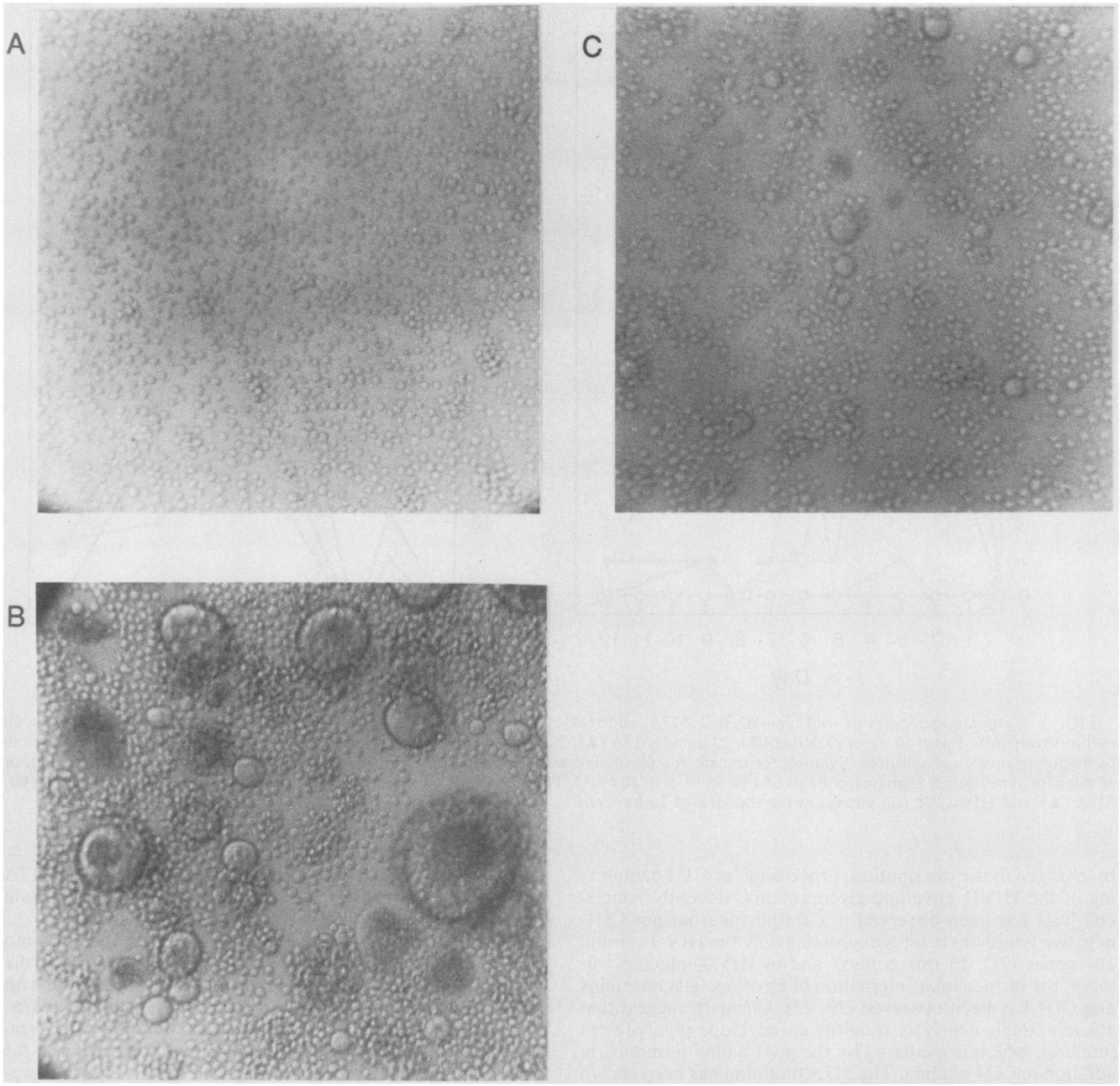


FIG. 4. Jurkat cell cultures transfected with pΔ(5928-8595) (A), pHXBc2 (B), or p517A (C) at the time of peak syncytium formation [day 4 for pΔ(5928-8595)- and pHXBc2-transfected cultures and day 6 for p517A-transfected cultures].

petent HIV-1 exhibiting the ability to form syncytia but not to efficiently lyse single cells (11). In our experiments using Jurkat T lymphocytes, the replication and cytopathogenicity of the X10-1 mutant were indistinguishable from those of the wild-type virus. We have confirmed the presence of the deletion in our X10-1 proviral clone, and the expression of envelope glycoproteins of different apparent molecular weights for X10-1 versus wild-type viruses would argue against the occurrence of a spontaneous reversion of the X10-1 mutation. The reported decrease in single-cell lysis by the X10-1 virus may result from subtle cell-type-specific differences in viral replication and protein production, as changes in the HIV-1 gp41 carboxyl terminus have been demonstrated to result in target cell-specific decreases in viral replication ability (29).

The cytopathogenicity of natural HIV isolates tends to increase in a manner correlated with the stage of disease (4, 49, 50). The molecular basis for these changes is unknown. The work described herein provides an example of a particular mutation that specifically attenuates HIV-1 in vitro cytopathogenicity. It is noteworthy that the phenotypes exhibited by some natural isolates (decreased syncytium formation, decreased single-cell lysis, delayed cell-free viral transmission, decreased CD4 down-modulation) (2, 10, 26)

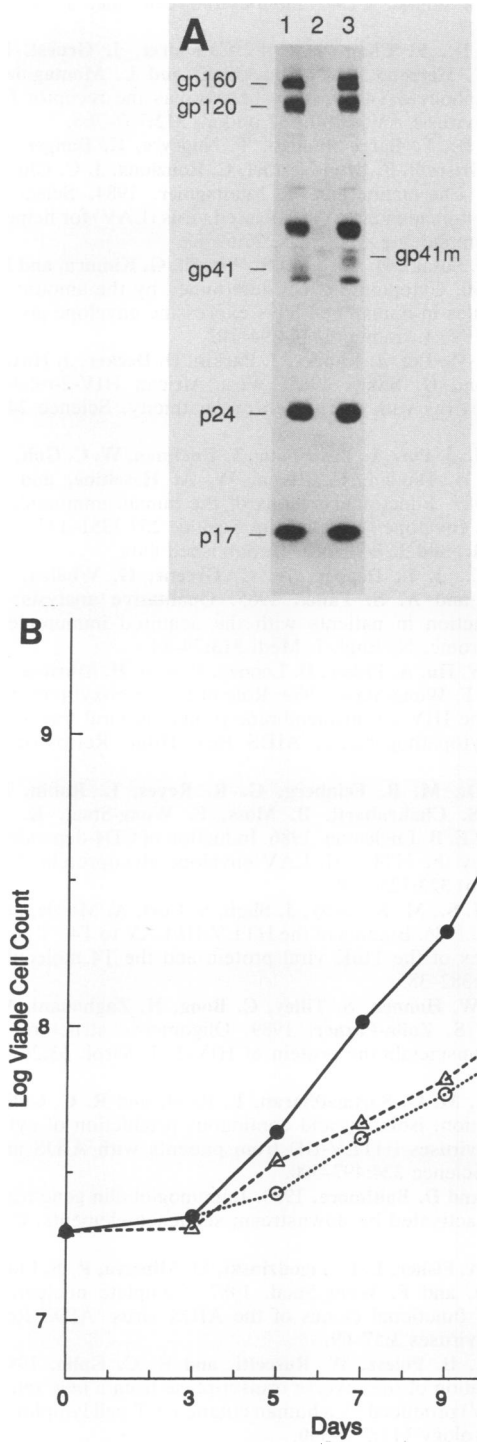


FIG. 5. Replication and cytopathic properties of the X10-1 virus. (A) Immunoprecipitated cell lysates from Jurkat cells on day 7 following transfection with 10 μg of pHXBc2 (lane 1), 20 μg of pΔ(5928-8595) (lane 2), or 10 μg of X10-1 (lane 3). The wild-type gp41 glycoprotein (gp41) and the mutant X10-1 glycoprotein (gp41m) are marked. (B) Total viable cell counts in Jurkat cell cultures transfected with 10 μg of pΔ(5928-8595) (●), 10 μg of pHXBc2 (○), or 10 μg of X10-1 (△).

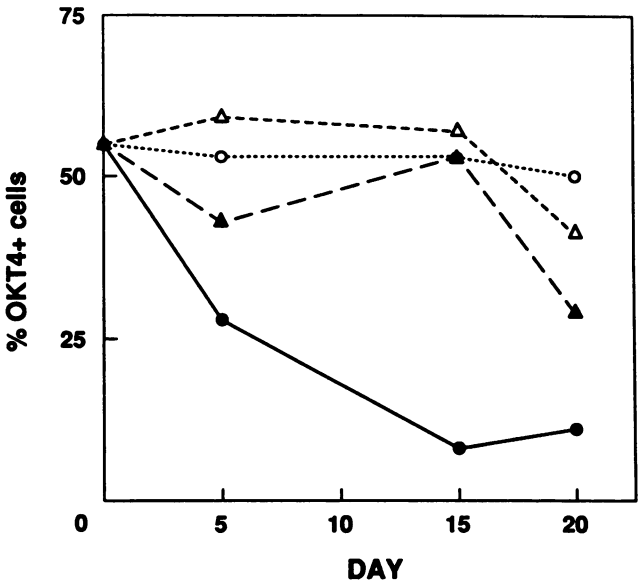


FIG. 6. Loss of CD4-positive cells in peripheral blood mononuclear cultures infected with wild-type and mutant viruses. Percentage of peripheral blood mononuclear cells positive for OKT4-specific cell fluorescence following cocultivation with Jurkat cells transfected with pΔ(5928-8595) (○), pHXBc2 (●), p517A (▲), or p517B (△). Results similar to those shown were observed in two independent experiments.

correspond to those observed for the 517A virus described here. Since the efficiency of HIV-1 envelope glycoprotein-mediated membrane fusion can be affected by changes in gp120 as well as other regions of gp41 (17), it is likely that not all of the changes that account for decreased cytopathogenicity in natural isolates will occur in the gp41 amino-terminal region. The use of mutant viruses exhibiting altered in vitro cytopathogenicity in animal model systems may allow an understanding of the relationship between direct viral cytopathic effects and in vivo CD4-lymphocyte depletion in retrovirus-associated immunodeficiencies.

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