JOURNAL OF VIROLOGY, May 1987, p. 1602–1608
0022-538X/87/051602-07$02.00/0

Vol. 61, No. 5

Differences among Human Immunodeficiency Virus Strains in Their Capacities To Induce Cytolysis or Persistent Infection of a Lymphoblastoid Cell Line Immortalized by Epstein-Barr Virus

KAREN DAHL,[1] KELSEY MARTIN,[2] AND GEORGE MILLER[1,2,3]*

Departments of Pediatrics,[1] Epidemiology and Public Health,[2] and Molecular Biophysics and Biochemistry,[3] Yale University School of Medicine, New Haven, Connecticut 06510-8064

Received 23 October 1986/Accepted 4 February 1987

Four strains of human immunodeficiency virus (HIV) manifest consistent differences in biologic behavior after infection of the X50-7 line of human umbilical cord lymphocytes immortalized by Epstein-Barr virus (EBV). Some dilutions of the first strain examined, human T-cell lymphotropic virus type III B, which is derived from a pool of patient isolates propagated in H9 cells, caused transient cytopathic effects (CPE) followed by recovery of a subpopulation of X50-7 cells which became virus carrier cultures. Other dilutions of the same virus stock completely lysed X50-7 cells. Two other strains, RF2 and YW, both from individual patients with acquired immune deficiency syndrome, always induced complete cytolysis of X50-7 cells at all dilutions which infected the cells. However, RF2 did establish persistent infection of H9 cells. A fourth strain, PH1-MN, from a child with acquired immune deficiency syndrome-related complex, induced only transient CPE in X50-7 and H9 cells, which thereafter always recovered to form carrier cultures. For all four strains, the dilutions of HIV stocks which caused CPE corresponded to dilutions which resulted in the detection of HIV polypeptides by immunoblot. Cytolysis in HIV-infected X50-7 cells was accompanied by a decrease in the amount of EBV nuclear antigen; however, HIV infection did not induce EBV replication. Thus CPE in X50-7 cells is due to replication of HIV per se and not to activation of EBV. The observations indicate that there are differences in the cytolytic properties of HIVs and that these differences are influenced by the target cell.

Although the human immunodeficiency virus (HIV) is an essential element in the causation of acquired immune deficiency syndrome (AIDS), a mystery is presented by the wide spectrum of clinical manifestations, from asymptomatic carrier states to fulminant disease, seen in infected individuals. To account for this wide range in pathology, one theory suggests that activation of the target cells in the host may stimulate viral replication. Another possible, though not mutually exclusive, explanation is that HIV strains may differ in pathogenicity. They may even evolve to increased virulence during the course of infection. Our experiments have bearing on both hypotheses.

The original aim of our experiments was to identify a cell line which might be more sensitive to the infectivity of HIV and thus, perhaps, be useful to isolate HIV from infected patients. To identify such a cell line we initially examined the effects of human T-cell lymphotropic virus type III B (HTLV-III B) on primary lymphocytes and a small selection of lymphoid lines carried in our laboratory. Infection was monitored by performing immunoblots on the cells at intervals after inoculation. We chose the immunoblot (rather than reverse transcriptase or immunofluorescence assays) because of its specificity and potential for distinguishing among subtypes or strains of HIV.

Among the cell lines tested, the most sensitive to HIV infection was the X50-7 line of human umbilical cord lymphocytes immortalized in vitro by Epstein-Barr virus (EBV) (20). Certain dilutions of HTLV-III B supernatants caused complete cytolysis of X50-7 cells, while others induced transient cytopathic effects (CPE) followed by recovery and establishment of an HIV carrier cell line. Since HTLV-III B is known to contain several HIV strains (5, 14, 17), this observation suggested that HTLV-III B stocks might contain more than one biologic variant of HIV, perhaps present in different quantities. This hypothesis was further tested by examining the effects of three other HIV strains on the X50-7 cells.

MATERIALS AND METHODS

Cell lines. X50-7 cells are human umbilical cord lymphocytes immortalized in vitro by EBV. They contain a complete EBV genome, which is tightly latent (20). About 60 to 70% of X50-7 cells bear the CD4 receptor as demonstrated by reactivity with Leu-3a monoclonal antibody (unpublished results). H9 cells, provided by M. Popovic and R. Gallo, are a subclone derived from the HT T-cell leukemia line (15). H9 cells are relatively resistant to HIV-induced CPE, and they readily form persistently infected carrier cultures which contain large amounts of extracellular HIV. BJAB is a B-cell lymphoma line which lacks EBV. Tory are cotton-top marmoset (*Saguinus oedipus*) T cells immortalized in vitro by herpesvirus ateles. Growth medium for the cell lines was RPMI 1640 with 10% fetal bovine serum and antibiotics. Cells were split 1:2 or 1:3 every 4 to 7 days and maintained in 5% CO_2 at 37°C.

HIV strains. Three virus isolates which chronically infect H9 cells were provided by R. Gallo. HTLV-III B was originally derived by incubation of HT cells with pooled concentrated supernatant fluids from cultured lymphocytes of several AIDS patients. Individual proviral DNA clones from HTLV-III B exhibit restriction fragment length

* Corresponding author.

polymorphisms (14, 17). This is evidence that there may be more than one virus strain in HTLV-III B. RF2 was orginally isolated by co-cultivation of primary lymphocytes of an adult Haitian AIDS patient with the H4 clone of HT cells and later propagated in H9 cells (5; personal communication). PH1-MN was obtained by infecting primary lymphocytes with materials from a child with AIDS-related complex, repeatedly passaged in normal phytohemagglutinin-stimulated T lymphocytes from healthy donors, and later adapted to H9 cells.

A fourth strain of HIV, designated YW, was isolated in our laboratory from a patient with AIDS. The patient's mononuclear cells, which had been recovered on a Ficoll-Hypaque gradient, were cocultivated with log-phase X50-7 cells, at 10^6 cells per ml, at ratios of 4:1, 2:1, and 1:1. Cell extracts from these cocultivations were assayed by immunoblotting for HIV polypeptides on day 20, when CPE were first apparent, and on day 34. Only the culture inoculated with a 4:1 ratio of patient cells to X50-7 cells showed viral polypeptides, which were seen faintly on day 20 and more definitively on day 34. On day 45 after co-cultivation, supernatant fluid from the positive flask was filtered through a 0.45-μm-pore-size filter and stored at −70°C.

Assay for virus. Virus stocks were prepared from supernatant fluids of persistently infected H9 cells, X50-7 cells chronically infected with HTLV-III B (designated LL58 in our laboratory), or the stored frozen supernatant from the fresh YW isolate in X50-7 cells. The supernatants, obtained 3 to 5 days after the last cell split, were passed through a 0.45-μm filter. Ten-fold serial dilutions of virus stock were prepared in growth medium.

The indicator cultures, uninfected H9 or X50-7 cells, were initiated at 10^6 cells per ml in a total volume of 9 ml in 25-cm^2 tissue culture flasks. Growth medium (5 ml) was added every 3 to 5 days after inoculation.

Inoculated cells were observed daily for CPE. To detect HIV polypeptides, the culture was agitated and 10-ml samples of cells and supernatant were removed. The cells and cell debris were deposited by low-speed centrifugation and suspended at about 10^6 cells per 10 μl of sodium dodecyl sulfate sample buffer (0.5 M Tris hydrochloride, pH 6.8, 2% sodium dodecyl sulfate, 0.1% 2-mercaptoethanol, 10% glycerol, 0.2% phenylmethylsulfoxide (saturated solution in isopropanol), and bromphenol blue. The samples were boiled for 5 min, sonicated, and stored at −20°C until the immunoblot was run. Each lane of a polyacrylamide gel was loaded with 30 μl of cell extract. Western blots were performed using established methods (2, 18). The blots were blocked with a solution of 50 g of nonfat dry milk per l (6).

Antisera. Two human polyvalent antisera were used to detect antigens on the immunoblots. Antiserum 1 was from an asymptomatic intravenous drug abuser who is the mother of a child with AIDS (11). Her serum contains high titers of antibody to HIV and is also EBV seropositive. Antiserum 2 was from a patient with chronic EBV infection whose serum lacked antibody to EBV nuclear antigen one (EBNA 1) but contained extremely high titers of antibody to EBV replicative antigens when used in Western blots of EBV-producer cells (12, 16). This patient was HIV seronegative. Both sera were used at a 1:400 dilution.

RESULTS

Comparison of sensitivity of X50-7 and H9 cells to infection with HTLV-III B. We measured the infectivity titer of HTLV-III B virus carried by H9 cells in several different cell types. A virus stock consisting of filtered supernatant fluids of H9/HTLV-III B cells was assayed in serial dilutions (from 10^{-2} to 10^{-6}) for its ability to induce HTLV-III polypeptides in primary phytohemagglutinin stimulated adult lymphocytes, uninfected H9 cells, and BJAB, Tory, and X50-7 cells. Three weeks after inoculation, viral polypeptides appeared through the 10^{-3} dilution of the inoculum in primary lymphocytes and H9 cells and through the 10^{-4} dilution in X50-7 cells. Based on immunoblots, the 10^{-5} and 10^{-6} dilutions of HTLV-III B supernatants did not infect the X50-7 cells. BJAB and Tory cells did not become infected by any dilution of HTLV-III B (not shown).

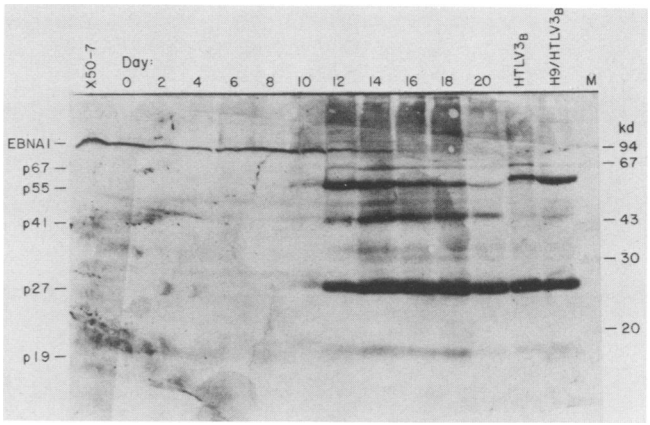


FIG. 1. Time course of appearance of HTLV-III B polypeptides after inoculation of X50-7 cells. A 10^{-2} dilution of H9/HTLV-III B supernatant was incubated with X50-7 cells at room temperature for 4 h, and the inoculum was removed by washing. On day 0 and every other day thereafter a culture sample was analyzed for HTLV-III B polypeptides by immunoblotting. The blot was developed with human antiserum 1, which recognizes many HTLV-III polypeptides and which has antibodies to EBNAs of 78 kilodaltons (EBNA 1) and 43 and 48 kilodaltons (EBNA 4) present in X50-7 cells. HIV proteins were evident by day 10. By day 16, EBNA 1 began to disappear, and it was gone by day 20. Numbers on the right are molecular weight markers. Numbers on the left (p67, etc.) are HIV polypeptides.

In two subsequent experiments, X50-7 cells were reproducibly more sensitive to infection by HTLV-III B than were H9 cells. By day 10, Western blots showed that the titers of the same supernatant were 10^{-4} in X50-7 cells but only 10^{-2} in H9 cells.

Time course of synthesis of HTLV-III B viral polypeptides in X50-7 cells. The kinetics of expression of viral polypeptides in X50-7 cells were examined in two experiments. In one such experiment (Fig. 1), the inoculum was a 10^{-2} dilution of supernatant fluid from H9/HTLV-III B carriers. Viral polypeptides were first evident on day 10 and became maximal on days 12 to 14. In a second experiment, X50-7 cells were inoculated with a 10^{-2} dilution of filtered supernatant fluids from X50-7 cells which had become carriers of HTLV-III B (see below). In this experiment, HIV polypeptides were first seen on day 6 and reached a maximum intensity on day 8. Thus, establishment of HTLV-III B infection in X50-7 cells occurs relatively slowly. The time course of appearance of HIV polypeptides is similar to the kinetics of appearance of reverse transcriptase in acutely infected lymphoid cells (1, 4, 9).

Effect of infection of X50-7 cells with HTLV-III B on expression of EBV proteins. X50-7 cells express the latent life

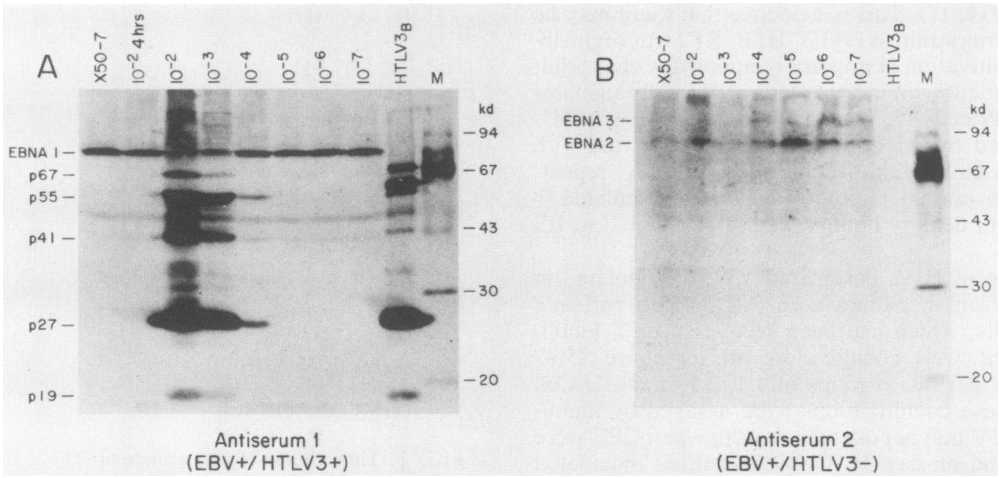


FIG. 2. Titration of H9/HTLV-III B in X50-7 cells. (A) The immunoblot was reacted with antiserum 1 (see legend to Fig. 1). Many HTLV-III polypeptides are seen in X50-7 cells inoculated with 10^{-2} to 10^{-4} dilutions of H9/HTLV-III B, but not in cells inoculated with higher dilutions of virus stock. Culture extracts were prepared 10 days after inoculation of X50-7. (B) A duplicate immunoblot was reacted with a human serum, no. 2, which is HIV seronegative but recognizes EBNA 2 (85 kilodaltons) and EBNA 3 (120 kilodaltons) in X50-7 cells; this serum also contains high titers of antibody to EBV replicative proteins, but these are not demonstrated. X50-7 cells do not replicative EBV after infection with HTLV-III.

cycle of EBV; the principal latent EBV polypeptides recognized by human antisera are the EBNAs. From Fig. 1 it is evident that, beginning about 14 days after infection of X50-7 cells, the content of the EBNA 1 polypeptide begins to decrease. To learn whether HTLV-III B infection caused activation of the EBV replicative cycle, two duplicate immunoblots of HIV-infected X50-7 cells were reacted either with antiserum 1 or 2. Although antiserum 1 demonstrated many HTLV-III B proteins (Fig. 2A), there was no induction of EBV replicative proteins, which are normally recognized by antiserum 2 (Fig. 2B) (12).

CPE and development of carrier cultures in X50-7 cells inoculated with HTLV-III B. The X50-7 cells developed CPE consisting of pyknosis of individual cells and syncytia formation, first noted about day 15 after inoculation (Table 1). In X50-7 cultures inoculated with 10^{-2} and 10^{-3} dilutions of virus, these CPE progressed and the cell culture was eventually destroyed. However, in X50-7 cells which received a 10^{-4} dilution of inoculum the CPE were transient and the culture recovered. Thereafter the cells appeared healthy, but continued to carry HTLV-III B which could be demonstrated by Western blot for at least 12 months. By contrast, all H9 cells which became infected with HTLV-III B showed only transient CPE, and all became virus carriers.

TABLE 1. Outcome of infection of X50-7 cells with serial dilutions of HTLV-III B

Expt	Cell/virus source	Day CPE first noted	Maximum dilution (+) by Western blot		Outcome[a] at dilution:			
			Day 10	Day 21	10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	H9/HTLV-III B	15	10^{-4}	10^{-4}	ND	L	L	C
2	X50-7/HTLV III B	6	10^{-3}	10^{-4}	C	L	L	C
3	X50-7/HTLV III B	7	10^{-3}	10^{-4}	C	L	L	C

[a] C, Carrier; L, lysis. ND, Not done; in experiment 2 X50-7 cells were not inoculated with a 10^{-1} dilution of virus.

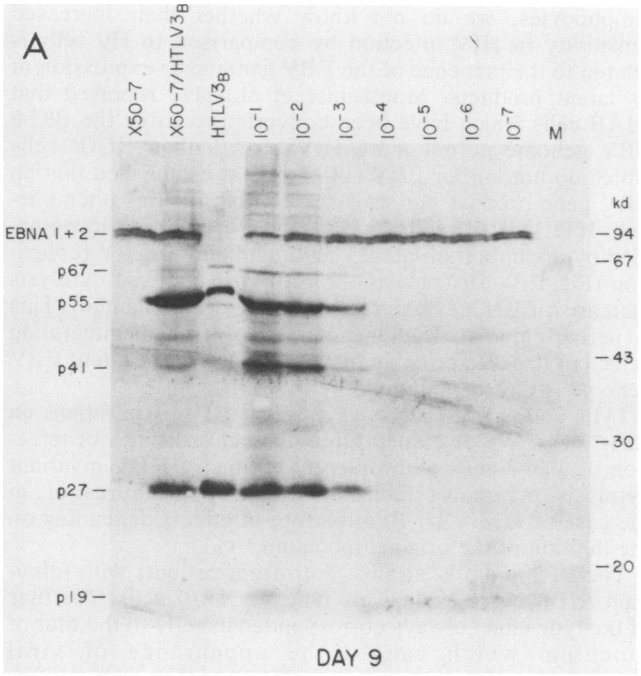
We wondered whether the X50-7 carrier culture which had normal morphology still shed lytic virus. Therefore, we tested supernatants from X50-7/HTLV-III B cultures for their capacity to cause CPE upon passage to uninfected X50-7 cells (Table 1, experiments 3 and 4). In both experiments, CPE were first evident at day 6 or 7. Again, the 10^{-2} and 10^{-3} dilutions of inoculum eventually destroyed the culture, indicating that virus which was lytic for X50-7 cells was still being carried by the X50/HTLV-III B carrier cells. As in the previous experiment, the 10^{-4} dilution caused transient CPE, and the culture recovered and went on to become a carrier. However, paradoxically, in the two experiments in which a 10^{-1} dilution of virus was tested, that dilution caused temporary CPE which resolved; thereafter the cells became HTLV-III B carriers.

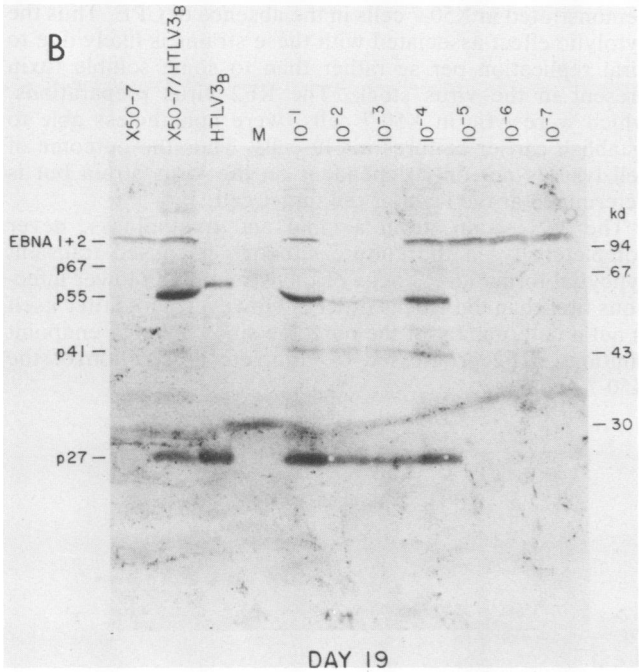
The carrier and lytic effects of different dilutions of HTLV-III B could also be discerned on immunoblots of X50-7 cells. By using the signal of EBNA 1 polypeptide as an index of cell viability, it can be seen that X50-7 cells which received the 10^{-2} and 10^{-3} dilutions of HTLV-III B were progressively lysed, whereas those which received the 10^{-1} and 10^{-4} dilutions of virus were resistant to CPE and became permanent HIV carriers (Fig. 3).

Effects of two other HIV laboratory strains on X50-7 cells. We then studied the effects of two other available HIV strains which were each derived from a single patient. We used RF2 from an adult with AIDS and MN from a child with AIDS-related complex; both strains also chronically infect H9 cells.

By the immunoblot assay, the titers of the RF2 strain were 10^{-3} or 10^{-4} in X50-7 cells (Table 2, Fig. 4). All dilutions of virus stock which caused RF2 polypeptides to appear also lysed the X50-7 cells (Fig. 4). The same results were obtained in three replicate experiments. However, when the H9/RF2 virus was added back to uninfected H9 cells it established carrier cultures (Table 2). Thus the purely lytic behavior of RF2 virus is seen in X50-7 cells, but not in H9 cells.

The MN strain appeared to have a lower titer of infectivity





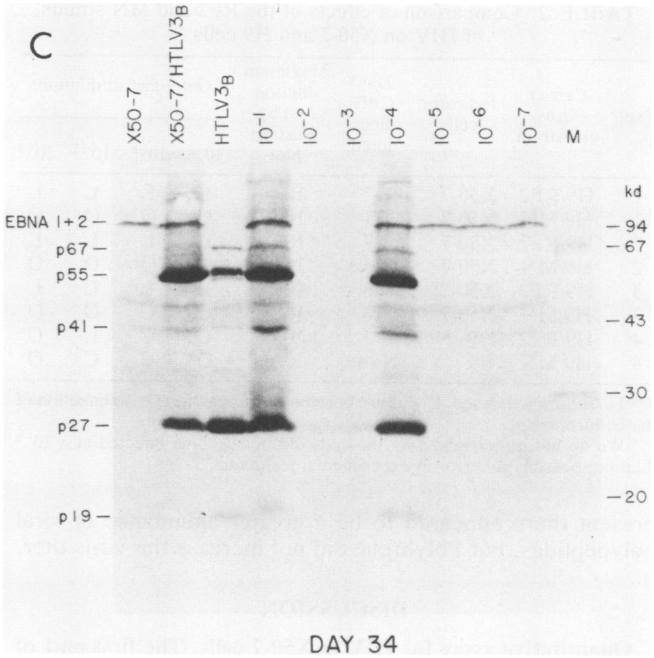


FIG. 3. Outcome of HTLV-III B infection of X50-7 cells. Serial 10-fold dilutions of supernatant from LL58 cells (X50-7 carrier culture of HTLV-III B) were incubated with uninfected X50-7 cells. Cell extracts were prepared on day 9 (A), day 19 (B), and day 34 (C) and analyzed by immunoblotting using antiserum 1. On day 9, the HTLV-III B titer was 10^{-3} and there had been little lytic effect, as judged by the amounts of EBNA 1. On day 19 the titer was 10^{-4}; the cells inoculated with 10^{-2} and 10^{-3} were lysed, and there was no EBNA 1 expression. Some HTLV-III B polypeptides remained. By day 34 the cells which received the 10^{-1} and 10^{-4} dilutions of inoculum were healthy HTLV-III B carrier cultures, and those which received 10^{-2} and 10^{-3} dilutions were dead.

for X50-7 cells than did RF2 (Table 2). However, the content of HIV polypeptides seen on an immublot of H9 cells carrying the MN virus was as great or greater than the amount of HIV poypeptides present in H9/RF2 carrier cells (data not shown). In two of three experiments (Table 2) the MN virus established carrier cultures in X50-7 cells after an initial period of 10 days of CPE. These CPE consisted of giant cell formation, but in contrast to the effects of RF2, there was minimal pyknosis of the individual cells (Fig. 5). By day 23 after inoculation the X50-7 cells carrying MN virus appeared morphologically normal and continued to synthesize EBNA (Fig. 6). By contrast, the lytic effects of RF2 virus were accompanied by loss of EBNA 1 (Fig. 4).

Lytic outcome of infection of X50-7 cells with a virus isolated directly from a patient. In the previous experiments with HTLV-III B, RF2, and MN, we used established H9 or X50-7 carrier cultures as the source of HIV to inoculate X50-7 cells. We wished to know what the outcome of infection of X50-7 cells would be if a virus which had not been previously carried in vitro was used. Washed peripheral mononuclear cells (YW strain) from a woman with AIDS were cocultivated with X50-7 cells at ratios of 4:1, 2:1, and 1:1. Periodically, cell extracts were made from the culture to test for the presence of HTLV-III polypeptides by immunoblotting. CPE consisting of pyknosis were first noted on day 20 in the cultures in which there was the highest ratio of patient cells to X50-7 cells. At this time there was a faint signal of HTLV-III polypeptides in the immunoblot prepared from the culture. By day 34 viral polypeptides were more abundant, and the culture appeared to be entirely lysed by day 45 (not shown). When supernatant from day 45 was added in 10-fold dilutions to fresh X50-7 cells, it caused CPE on day 10 and the virus titer was 10^{-1}. It was completely lytic. In a subsequent experiment, the titer of the same virus stock was 10^{-2} in X50-7 cells and the stock was lytic. In this experiment the effect of 2 μg of Polybrene per ml on virus titer was also evaluated (Fig. 7). When Polybrene was

TABLE 2. Comparison of effects of the RF2 and MN strains of HIV on X50-7 and H9 cells

Expt	Carrier virus cell/strain	Indicator cells	Day CPE first noted	Maximum dilution (+) by Western blot	Outcome[a] at dilution:			
					10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	H9/RF2	X50-7	7	10^{-4}	L	L	L	L
1	H9/MN	X50-7	11	10^{-1}	C	O	O	O
2	H9/RF2	X50-7	7	10^{-4}	L	L	L	L
2	H9/MN	X50-7	None	$<10^{-1}$	O	O	O	O
3	H9/RF2	X50-7	6	10^{-4}	L	L	L	L
3	H9/MN	X50-7	13	10^{-2}	C	C	O	O
4	H9/RF2	H9	5	10^{-3}	C	C	L	O
4	H9/MN	H9	None	10^{-3}	O[b]	C	C	O

[a] L, Culture was lysed; C, culture became a virus carrier; O, no infection of the culture.

[b] We do not understand why the cells did not become infected at a 10^{-1} dilution; possibly an error in experimental technique.

present there appeared to be a greater abundance of viral polypeptides, but Polybrene did not increase the virus titer.

DISCUSSION

Quantitative assay for HIV in X50-7 cells. The first part of our work was directed at developing a sensitive, specific, quantitative infectivity assay for HIV in an established cell line. X50-7 cells immortalized in vitro by EBV proved suitable. Although they are B cells, they express the T4 marker (not shown). They were more sensitive than H9 cells to the same virus stock. They were susceptible to several laboratory strains of HIV (Tables 1 and 2) and to at least one fresh clinical isolate. We have not yet shown, however, that they are equivalent or superior to primary lymphocytes for isolating viruses directly from patients. Using immunoblotting to detect infection of X50-7 cells allows one to measure the content of infectious virus in a stock (Fig. 2) and the kinetics of infection (Fig. 1). Presumably such an assay should also permit distinction to be made among viral strains which differ in the sizes of individual viral polypeptides.

Although X50-7 cells are immortal and therefore activated lymphocytes, we do not know whether their increased sensitivity to HIV infection by comparison to H9 cells is related to the presence of the EBV genome or expression of its latent products. Montagnier et al. have reported that BJAB cells which have been converted to carry the B95-8 EBV genome permit more HIV growth than BJAB cells which do not harbor EBV (13). It is not established that an EBV gene product per se is responsible for this phenomenon. HTLV-III B replication in X50-7 cells is not accompanied by disruption of latency and induction of EBV replication (Fig. 2B). Instead, as the retrovirus-induced cytolysis increases, EBNA expression disappears (Fig. 3 and 4). This is perhaps due to leaching of EBNA from disintegrating cells, but it is also possible that expression of the latent EBV genome is directly inhibited by the retrovirus.

Lytic and carrier effects of different HIV preparations on X50-7 cells. We discerned three distinct outcomes of infection of X50-7 cells with different strains of HIV: invariant cytolysis, invariant establishment of carrier cultures, or, in the case of HTLV-III B, a mixture of effects depending on the dilution of the original inoculum.

The RF2 and YW strains, both from patients with fulminant AIDS, were exclusively lytic for X50-7 cells. The titer of the lytic effect always corresponded exactly to the titer of inoculum which caused the appearance of viral polypeptides; for these strains, HIV proteins were never demonstrated in X50-7 cells in the absence of CPE. Thus the cytolytic effect associated with these strains is likely due to viral replication per se rather than to some soluble toxin present in the virus stock. The RF2 virus preparations, which were lytic in X50-7 cells, were nonetheless able to establish carrier cultures in H9 cells. Thus the outcome of cell lysis is not only dependent on the virus strain but is determined at the level of the target cell.

The MN strain, from a child and mild illness, never completely lysed the culture although it caused transient syncytial formation. Stocks of this strain had a lower infectious titer than did stocks of RF2. However, virus titer itself is not a determinant of the outcome since, even at endpoint dilutions, RF2 progressed to complete destruction of the X50-7 cultures.

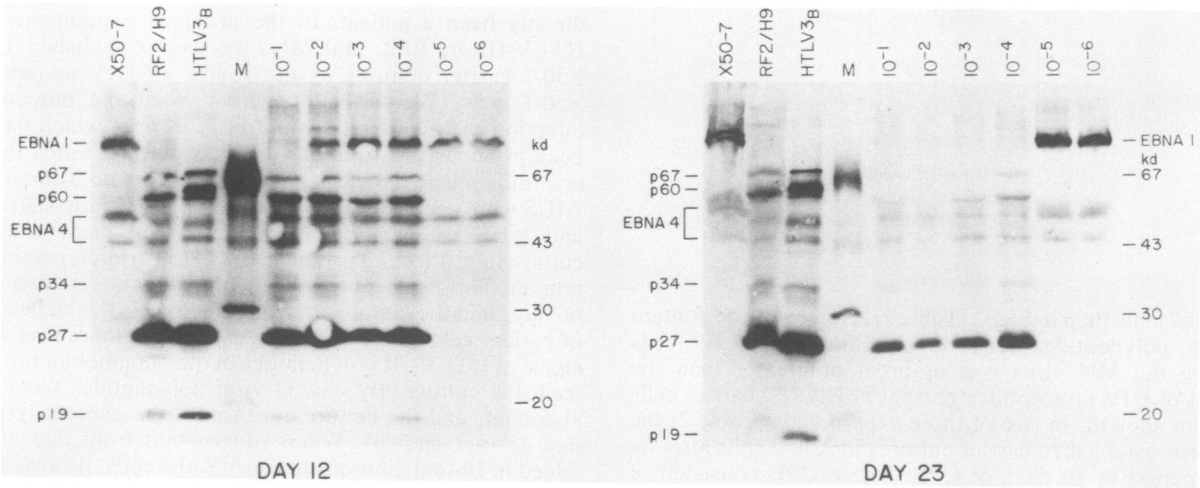


FIG. 4. Lytic effects of the RF2 strain on X50-7 cells. Serial 10-fold dilutions of supernatant fluid from H9/RF2 carrier cultures were incubated with uninfected X50-7 cells. Samples of cell culture were analyzed for viral polypeptides on day 12 and day 23 after infection with antiserum 1. On day 12 the titer of RF2 virus was 10^{-4}, but CPE were most evident at the 10^{-1} dilution with decreased amounts of EBNA 1. By day 23 these CPE had progressed, and no EBNA 1 was seen at any of the dilutions of RF2 stock which had successfully infected X50-7 cells. The virus titer had not changed from day 12.

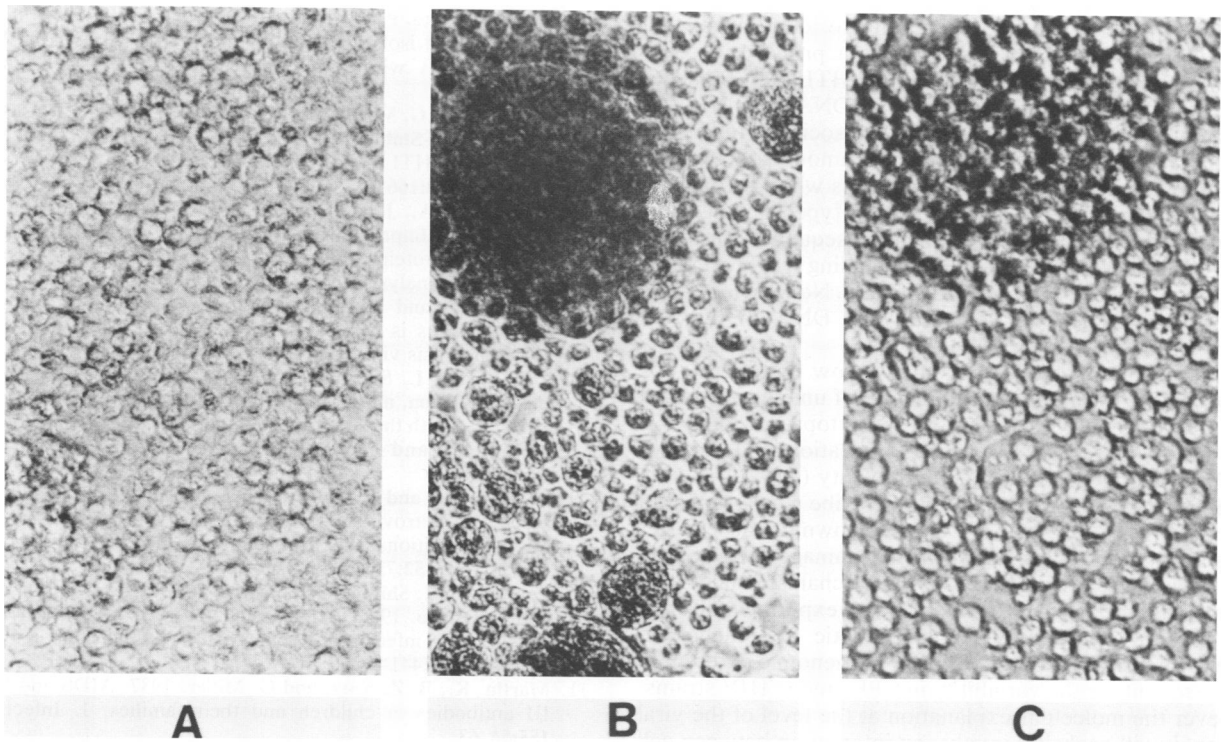


FIG. 5. CPE of RF2 and PH1-MN strains of HIV in X50-7 cells. Unstained. (A) Uninfected X50-7 cells are refractile. (B) X50-7 cells infected with the RF2 strain show giant cell formation, pyknosis, and degradation of individual cells. (C) X50-7 cells infected with PH1-MN show giant cell formation, but individual cells are refractile and healthy.

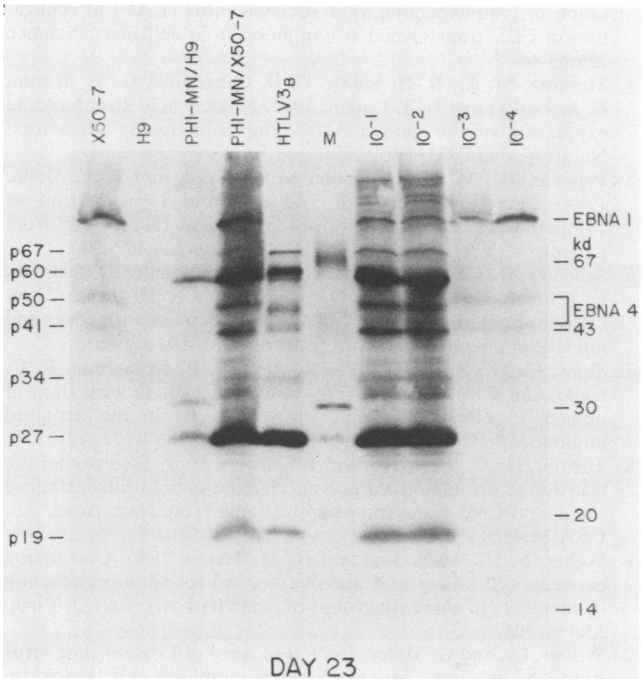


FIG. 6. Establishment of carrier cultures by PH1-MN strain in X50-7 cells. This immunoblot, reacted with antiserum 1 at 1:400 dilution, was prepared with culture samples harvested 23 days after incubation with the PH1-MN strain. The virus infected X50-7 cells at a titer of 10^{-2}. Lane 4 contains an extract of chronically infected X50-7/PH1-MN carrier culture from a previous experiment.

Other examples of lytic and nonlytic interactions by related retroviruses. After infection with certain strains of avian reticuloendotheliosis virus, chick cells exhibit transient lysis which is temporally associated with the accumulation of large amounts of unintegrated viral DNA. Chronically infected cells, which have a normal morphology, have little if

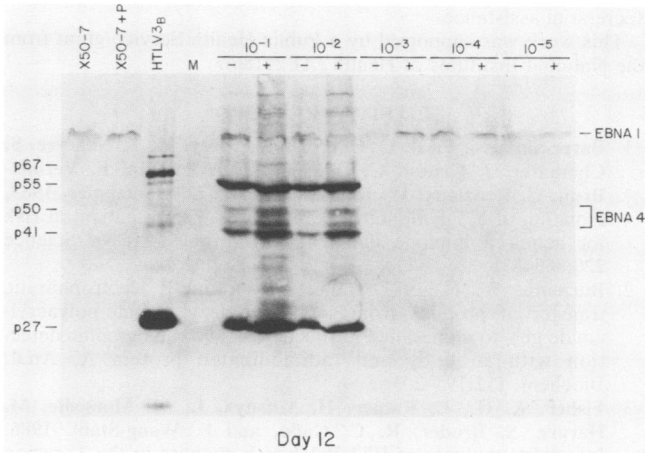


FIG. 7. Isolation of an HIV directly from an AIDS patient in X50-7 cells. Stored supernatant fluid, harvested 45 days after cocultivation of a patient's mononuclear cells with X50-7 cells, was incubated in serial 10-fold dilutions with X50-7 cells. A duplicate culture at each dilution was treated with Polybrene. The titer of HIV (YW) on day 12 was 10^{-2}. The same viral polypeptides were seen in greater quantity in the Polybrene-treated cultures, but the titer of virus was not affected.

any unintegrated DNA (7). The ability to induce lysis and accumulation of unintegrated DNA is a property of only certain serogroups of this virus (19). H9/HTLV-III B carrier cultures also contain unintegrated viral DNA (17). HUT 78 cells acutely infected with the AIDS-associated retrovirus type 2 HIV strain contain significant amounts of unintegrated viral DNA, whereas the same cells when chronically infected with AIDS-associated retrovirus type 2 do not (8). A deletion mutant of HTLV-III B lacking sequences in the 3′ end of the *env* gene and the 3′ open reading frame region is not cytopathic for human T cells (3). Nonetheless, this mutant accumulates unintegrated viral DNA in infected cells.

Thus, related retroviruses may show cytopathic or noncytopathic behavior. Accumulation of unintegrated viral DNA, rather than being a marker for cytopathogenicity, is more likely to reflect successful replication and second-round infection of cells at high multiplicity (19).

Significance of these findings. We describe a cell system in which different strains of HIV can be shown to cause either a totally lytic effect or transient cell damage followed by recovery of the cells. To decipher the mechanism underlying this striking variation in behavior is an experimental challenge. Using classical molecular genetic approaches, it should be possible to identify those viral genomic differences which account for the variability in CPE among HIV strains. Whatever the molecular explanation at the level of the viral genome, it will probably involve an interaction between cell and viral gene products since the CPE of both HTLV-III B and RF2 are more dramatic in X50-7 cells than in H9 cells.

These findings raise the possibility that differences in cytolytic behavior of HIV strains may account for some of the variation in clinical outcome. Previous reports have detected differences in replicative capacity of HIVs from patients with severe or mild disease, but have not directly identified differences in cytopathogenicity (10).

ACKNOWLEDGMENTS

We thank R. Gallo for H9 cells and the HTLV-III B, RF2, and PH1-MN strains of HIV. We are grateful to K. Papov for excellent secretarial assistance.

This work was supported by a Public Health Service grant from the National Institutes of Health (AI 21186).

LITERATURE CITED

1. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
2. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
3. **Fisher, A. G., L. Ratner, H. Mitsuya, L. M. Marseille, M. Harper, S. Broder, R. C. Gallo, and F. Wong-Stahl.** 1986. Infectious mutants of HTLV III with changes in the 3′ region and markedly reduced cytopathic effects. Science **233**:655–659.
4. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Satai, F. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. Science **224**:500–503.
5. **Hahn, B. H., G. M. Shaw, S. K. Arya, M. Popovic, R. C. Gallo, and F. Wong-Stahl.** 1984. Molecular cloning and characterization of the HTLV III virus associated with AIDS. Nature (London) **312**:166–169.
6. **Johnson, D. A., J. W. Gautsch, J. R. G. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Analysis Tech. **1**:3–8.
7. **Keshet, E., and H. M. Temin.** 1979. Cell killing by spleen necrosis virus is correlated with a transient accumulation of spleen necrosis virus DNA. J. Virol. **31**:376–388.
8. **Levy, J. A., L. S. Kaminsky, J. W. Morrow, K. Steimer, P. Luciw, D. Dina, and J. Hoxie.** 1985. Infection by the retrovirus associated with the acquired immunodefiency sydrome: clinical, biological, and molecular features. Ann. Intern. Med **103**:694–699.
9. **Levy, J. A., and J. Shimabukuro.** 1985. Recovery of AIDS-associated retroviruses from patients with AIDS or AIDS-related conditions and from clinically healthy individuals. J. Infect. Dis. **152**:734–738.
10. **Levy, J. A., J. Shimbukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro.** 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human helper T cells. Virology **147**:441–448.
11. **Martin, K., B. Z. Katz, and G. Miller.** 1987. AIDS and HTLV III antibodies in children and their families. J. Infect. Dis. **155**:54–63.
12. **Miller, G., E. Grogan, D. F. Fischer, J. C. Niederman, R. T. Schooley, W. Henle, G. Lenoir, and C.-R. Liu.** 1985. Antibody responses to two Epstein-Barr virus nuclear antigens defined by gene transfer. N. Engl. J. Med. **312**:750–755.
13. **Montagnier, L., J. Gruest, S. Chamaret, S. Dauget, C. Axler, D. Guetard, M. T. Nugeyre, F. Barre-Sinoussi, J.-C. Chermann, J. B. Brunet, D. Klatzmann, and J. C. Gluckman.** 1984. Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. Science **225**:63–66.
14. **Muesing, M. A., D. H. Smith, C. D. Cabradilla, C. V. Benton, L. A. Lasky, and D. J. Capon.** 1985. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. Nature (London) **313**:450–458.
15. **Popovic, M., M. G. Sarngadharan, E. Reed, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic human T lymphotropic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
16. **Schooley, R. T., R. W. Carey, G. Miller, W. Henle, R. Eastman, E. J. Mark, K. Kenyon, E. O. Wheeler, and R. H. Rubin.** 1986. Chronic Epstein-Barr virus infection associated with fever and interstitial pneumonitis. Ann. Intern. Med. **104**:636–643.
17. **Shaw, G. M., B. H. Hahn, S. K. Arya, J. E. Groopman, R. C. Gallo, and F. Wong-Stahl.** 1984. Molecular characterization of human T cell lymphotropic virus type III in the acquired immunodeficiency syndrome. Science **226**:1165–1171.
18. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
19. **Weller, S. K., A. E. Joy, and H. M. Temin.** 1980. Correlation between cell killing and massive second-round superinfection by members of some subgroups of avian leukosis virus. J. Virol. **33**:494–506.
20. **Wilson, G., and G. Miller.** 1979. Recovery of Epstein-Barr virus from non-producer neonatal human lymphoid cell transformants. Virology **95**:351–358.