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Recovery of Infectious Human Immunodeficiency Virus Type 1 after Fusion of Defectively Infected Clones of U-937 Cells

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Polyethylene glycol was used to induce polykaryon formation among U-937 cell subclones carrying defective human immunodeficiency virus (HIV) type 1 proviral DNA. Fusion of cells which produced gp120-defective virions (UHC15.7) with cells unable to generate reverse transcriptase (RT) activity (UHC8 and UHC18) yielded polykaryons which made infectious viral progeny that showed normal protein profiles. Southern blot analysis of proviral DNA of cells infected with such fusion-derived virus revealed a restriction map identical to that of cells harboring infectious parental-type HIV type 1 (U-937/UHC1). These results suggest that repair mechanisms involving genetic recombination(s) play a role in the generation of infectious virus after fusion of cells which harbor defective HIV.

The replicative machinery of human immunodeficiency virus type 1 (HIV-1) is responsible for the generation of variants which differ in tropism, virulence, or infectivity (5). Along with other retroviruses, HIV-1 is characterized by the production of defective viral particles. The biological relevance of these defective viruses has, however, not yet been established.

HIV-1 mutations, many of which can affect infectiousness, have been commonly observed in the *env* gene, the most variable region of which is characterized by high levels of genetic diversity (11, 19). Mutations in other genes such as *pol* may affect viral enzyme activity and also generate particles lacking infectivity (10, 15). The point mutations, deletions, and repetitions which account for retroviral defectiveness are due, at least in part, to the error-prone activity of viral reverse transcriptase (RT) (20).

It would be useful to have a rapid, reproducible procedure to test whether complementation among different HIV replication-incompetent cell lines is possible. Accordingly, we wished to determine whether fusion of different cells, each of which harbored defective HIV-1, could give rise to infectious progeny. We relied on a number of clonal derivatives of chronically HIV-1-infected U-937 cells (4) which did not themselves produce infectious viruses. Two of these clones produced particles which apparently lacked RT activity, while a third produced particles devoid of surface gp120. The use of polyethylene glycol to fuse cells carrying these defective yet complementary phenotypes (RT^- and $gp120^-$) yielded hybrid cells that generated infectious virus.

HIV-1-infected U-937 clones (UHC) carrying defective HIV-1 were previously generated in our laboratory (4) and were used in the current experiments. They can be distinguished by the amount of RT produced in the supernatant, the infectivity potential of the virus synthesized in these cultures, and the ability to induce syncytium formation in vitro by coculture with MT-4 indicator cells (Table 1). Of the two RT^- virus-producing cell lines used in this study, UHC8 is characterized by an inability to produce virus with a fully mature core, a phenotype characteristic of viruses lacking a functional protease (15) or grown in the presence of protease inhibitors (1). In addition to a possible defect in protease activity, no RT (complex of p66 and p51) protein or endonuclease (p33) could be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4). Pelleted virus showed large amounts of the $p55^{gag}$ precursor but undetectable amounts of cleaved p24 and p17 subunits. The other RT^- virus produced by clone UHC18 was found to process the $p55^{gag}$ precursor into p24 and p17. In contrast to UHC18, clone UHC8 was unable to induce syncytium formation in vitro despite the presence of normal levels of gp120 on the viruses themselves. Clone UHC15.7 produced noninfectious viral structures devoid of gp120 because of an inability to cleave the envelope precursor protein, gp160 (4). Chronically infected U-937 cells that possessed typical immunoblot patterns for HIV-1 proteins and produced fully infectious HIV-1 (U-937/UHC-1) served as a positive control (4).

Five million UHC15.7 cells were mixed at a 1:1 ratio either with parental U-937 cells or with cells of clone UHC8 or UHC18. The mixtures were washed twice by centrifugation in serum-free RPMI 1640 medium and gently resuspended in 0.7 ml of prewarmed polyethylene glycol 1500 diluted 1:1 in medium. The cells were mixed for 60 s with large-bore transfer pipettes, and then the polyethylene glycol 1500 was sequentially diluted in stepwise fashion by adding double amounts of medium. After this treatment, the cells were washed by centrifugation, resuspended, and aliquoted in fresh medium in a 24-well plate. After 24 h, the culture fluids were harvested, clarified, and assessed for infectiousness.

Viral protein preparations were prepared as follows. Four hundred milliliters of infected cell culture medium was first clarified by centrifugation at 2,000 × *g* for 30 min and then centrifuged at 65,000 × *g* for 60 min. The resulting viral pellet was resuspended in 2 ml of TNE buffer (10 mM Tris-HCl [pH 7.4], 100 mM NaCl, 1 mM EDTA) and further purified by centrifugation onto a sucrose cushion. A viral lysate was obtained by resuspending this virus in TNE buffer containing 1% Triton X-100 and various protease inhibitors and incubating the virus suspension for 30 min at 37°C with frequent agitation. Immunoblotting and visualization of relevant bands were performed on electrophoresed material (20 ng per sample) as previously described (4). Southern blot analysis of integrated HIV-1 provirus, using the PBH-10 plasmid (kindly provided by R. C. Gallo, National Cancer Institute, Bethesda, Md.), was carried out with high-molec-

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TABLE 1. Properties of viral isolates derived from UHC clonal derivatives

Cell type	RT activity[a] (cpm/ml)	p24[a]	Syncytium-inducing activity[a]	Infectiousness of culture fluids	Observed deficit(s) in viral protein production
U-937/UHC1	$>10^5$	Yes	Yes	Yes	
UHC8	0	Yes	No	No	p66, p33, p24, p17
UHC15.7	$>10^5$	Yes	No	No	gp120
UHC18	0	Yes	Yes	No	p66

[a] RT activity, p24 antigen capture, and syncytium-inducing activity were determined as described previously (4).

ular-weight DNA according to a previously described procedure (4).

When polyethylene glycol was used to fuse cells of the RT^- phenotype (UHC8 and UHC18) in a 1:1 ratio with clone UHC15.7, large polymorphonuclear cells were seen within 1 min of contact, indicating that fusion had occurred. The cells were gently washed by centrifugation and seeded into 24-well plates. The infectiousness of the supernatants was tested 24 h later with a variety of target cell types (Table 2). Only clarified culture fluids derived from the various hybrid cell preparations (UHC8 × UHC15.7 and UHC18 × UHC15.7) were able to infect all of the following types of cells: MT-4 cells, H-9 cells, and peripheral blood mononuclear cells. The viruses thus generated could subsequently be passaged onto U-937 cells as well as on each of the two lymphocyte cell lines and peripheral blood mononuclear cells. In contrast, virus particles derived from the fusion of UHC8 and UHC18 were not infectious, indicating that the deficits in each of these clones were not complementary. As a further control to ensure that the cell fusion process was itself not inducing UHC15.7 cells to produce infectious progeny, we prepared hybrids of UHC15.7 and uninfected parental U-937 cells. No infectious viruses were recovered from the culture fluids of such fused cells, suggesting that the block in gp120 maturation characteristic of UHC15.7 was not a cell-dependent phenomenon.

TABLE 2. Infectiousness of viral particles present in culture fluids of fused and parental cells

Cell type	Amt of virus recovered in culture fluids ($TCID_{50}$/ml)[a]	Infectiousness of second-passage virus on indicated cells[b]		
		H-9	Peripheral blood mononuclear	U937
U-937/HIV-1[c]	$>10^5$	+	+	+
U-937/UHC1	$>10^5$	+	+	+
UHC8	0	−	−	−
UHC15.7	0	−	−	−
UHC18	0	−	−	−
UHC8 × UHC15.7	$10^{4.25}$	+	+	+
UHC18 × UHC15.7	$10^{5.0}$	+	+	+
U-937 × UHC15.7	0	−	−	−
UHC8 × UHC18	0	−	−	−

[a] Clarified culture fluid (100 μl) was seeded in quadruplicate into 96-well culture plates containing 10^5 MT-4 cells. After 4 h, the cells were washed and fresh medium was added. Cytopathic effect, syncytium formation, and p24 immunofluorescence were studied to monitor wells in which infection had occurred. Infectious U-937 and U-937/UHC1 culture fluids served as positive controls. $TCID_{50}$, 50% tissue culture infective doses.

[b] The infectiousness of virus obtained after amplification on MT-4 cells was further assessed with H-9 cells, peripheral blood mononuclear cells, and U-937 cells.

[c] U-937/HIV-1, parental U-937 cells chronically infected with HIV-1.

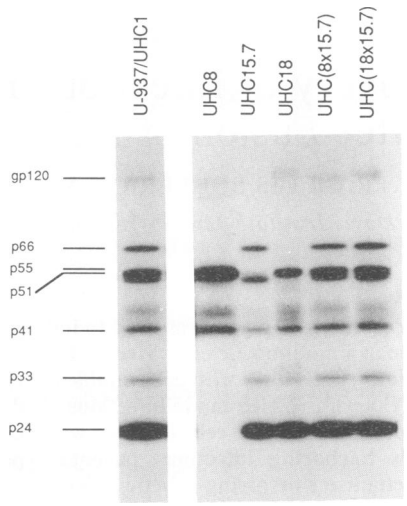

FIG. 1. Immunoblot of purified concentrated virus from infectious U-937/UHC1 cells, UHC clones, and virus derived from UHC8 × UHC15.7 [UHC(8×15.7)] and UHC18 × UHC15.7 [UHC(18×15.7)] hybrids. Lysates (see text) containing 20 μg of total protein were electrophoresed on SDS–12% polyacrylamide gels and immunoblotted with pooled sera from HIV-1-infected individuals.

To show that complementation had occurred, hybrid viruses grown on U-937 cells were banded by sucrose gradient and analyzed by SDS-PAGE. As shown in Fig. 1, the viruses generated by the hybrid cells (UHC8 × UHC15.7 and UHC18 × UHC15.7) possessed protein profiles similar to those of wild-type viruses produced by infected U-937 parental cells (Fig. 1, lane U-937/UHC1). The absence of gp120 in UHC15.7-derived virus and deficits in reverse transcriptase activity in viruses derived from UHC8 and UHC18 were all reversed in the recombinant isolates obtained from these hybrids.

To compare our various isolates, total DNA was extracted from cells and digested with enzymes recognizing one restriction site each in *gag* (*Pst*I), *pol* (*Bcl*I), and *env* (*Bam*HI); in one case, an enzyme cutting at two restriction sites (*Eco*RI) was employed. The three defective clones (UHC8, UHC15.7, and UHC18) each contained a distinct proviral DNA pattern which could further be distinguished from that of the parental, HIV-1-infected U-937 cells. In contrast, DNA obtained from U-937 cells infected with the recombinant viruses, UHC8 × UHC15.7 and UHC18 × UHC15.7, showed patterns identical to that of DNA obtained from wild-type HIV-1-infected U-937 cells (Fig. 2).

The defective producers chosen for this study were selected because they possessed a complementary phenotype. Hybrid cells obtained by fusion of RT^- and $gp120^-$ cells yielded fully infectious virus with a wild-type protein profile. The likelihood of a genetic recombination in our system is suggested by Southern blot reaction digest experiments on the various progeny as well as the fact that these viruses could be propagated over several generations.

The relatively short time period between polyethylene glycol 1500-induced cell fusion and production of infectious viral progeny argues for a two-stage process in the events described. The initial infectious particles produced by the

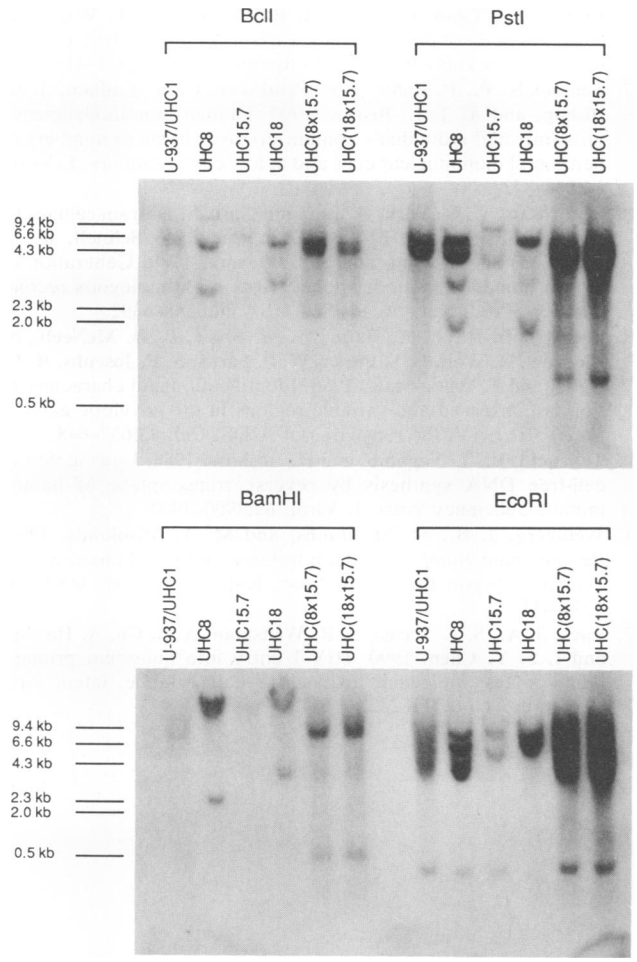

FIG. 2. Southern blot analysis of viral genomic DNA. Twenty micrograms of DNA isolated from U-937 cells infected with infectious HIV-1 (U-937/UHC1) and U-937 cells infected with virus derived from fused cells, i.e., UHC8 × UHC15.7 [UHC(8×15.7)] and UHC18 × UHC15.7 [UHC(18×15.7)], and 30 μg of DNA each from UHC8, UHC15.7, and UHC18 cells were cleaved with *Bcl*I, *Pst*I, *Bam*HI, and *Eco*RI. The DNA was hybridized with PBH-10 and washed as described in the text, and blotted onto nitrocellulose filters. The nitrocellulose filters were exposed for 72 h. *Hin*dIII-cleaved lambda DNA fragments were used as molecular size markers, as indicated on the left.

fused cells may have contained different cRNA molecules within the same viral package. This complementation step may have been followed by genetic recombination during subsequent viral replication. The precise mechanism of recombination is still under investigation and will necessitate knowing how many copies of proviral DNA are present in each of the cloned cell lines.

The generation of defective viral particles occurs widely among retroviruses and has been studied in detail with a number of animal systems. In some cases, defective particles themselves may be responsible for pathophysiologic phenomena and severe immunosuppression, which are characteristic of human AIDS, as has been observed in mice injected with *gag*-defective strains of murine leukemia virus (2, 6). In one study, HIV-1 recovered from asymptomatic men was shown to contain relatively little gp120, p24, and p55 (3), suggesting that defective strains of HIV-1 occur naturally. A large portion of these isolates carried a unique *Bgl*II restriction site in the *gag* region. The isolation of a *tat*-defective strain of HIV-1 has also been reported (12). HIV-1 isolates may also differ in virulence (5, 10), host range (8), and susceptibility to antiviral drugs (13, 16), suggesting extensive heterogeneity at a variety of genetic loci.

Homologous recombination may be used to generate infectious HIV-1 from replication- or infection-defective material. One group of researchers obtained infectious virus after transfection into HeLa cells of two exogenous pieces of HIV DNA (18). Similar data were obtained after transfection of A3.01 cells, which carry only a single copy of defective HIV provirus, with a complementary plasmid (7).

The recombination capacity of defective proviruses may also have clinical relevance, in view of the finding that quiescent or defective proviruses or both may be present at high frequency in infected cells that do not express viral antigens (17, 22). In tissue culture, fusion between HIV-infected and noninfected cells has been commonly observed and is attributable to CD4-gp120 interactions (9). In contrast, virus-mediated formation of syncytia does not seem to occur with high frequency in circulating cells but may be more common at the level of certain organs, such as lymph nodes. Fusion may also occur independently of viral antigen expression, and both interleukin-4 in mouse cells (14) and gamma interferon in human cells (21) have been reported to induce polykaryon formation among monocytes/macrophages. Since these cells harbor large quantities of virus, it is conceivable that genetic mixing may occur in hybrid cells, leading to the formation of viral hybrids with novel properties.

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