AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 5, Number 4, 1989
Mary Ann Liebert, Inc., Publishers

Biologic and Molecular Characterization of Producer and Nonproducer Clones from HUT-78 Cells Infected with a Patient HIV Isolate

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ABSTRACT

HUT-78 cells were infected with a reverse transcriptase (RT)-positive supernatant of a culture of peripheral blood lymphocytes (PBL) from an AIDS patient and then cloned. Of these clones, two have been isolated and characterized. Clone D10 is persistently and productively infected with an HIV variant. The clone F12, in spite of the presence of an integrated full-length HIV provirus, does not release virus particles in the medium.

D10 and F12 clones substantially differ in terms of protein pattern; that is, D10 is superimposable to infected HUT-78 cells, whereas F12 exhibits a decreased uncleaved p55 *gag* precursor and the presence of uncleaved gp160 and of a unique p19, although they do not show qualitative or quantitative differences in viral RNA synthesis. Restriction patterns of F12 proviral DNA do not show major genomic deletions. These results indicate that F12 clone cells carry an HIV genome with minor mutations that probably affect the correct production of viral proteins at a posttranscriptional level. In addition, the F12 clone is resistant to high-multiplicity superinfection with HIV-1 or HIV-2.

INTRODUCTION

A PECULIAR FEATURE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS) and associated clinical disorders, is the biologic and genetic heterogeneity among different isolates.

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Since 1985 we have performed a large number of HIV isolations from patients with AIDS or AIDS-related complex.[1] Preliminary characterization suggested that isolates vary both in terms of biologic behavior (i.e., differential ability to productively infect human cells) and of structural components (i.e., viral proteins and restriction patterns of proviral DNA). These results are in agreement with data from different laboratories. Evans et al.[2] found that various $CD4^+$ human cells are differently sensitive to the infection by the same HIV isolate; at the same time, different isolates may or may not infect the same cell type. Also genomic variability of HIV isolates, analyzed via DNA restriction patterns[3-7] or by comparing the sequences of different cloned HIVs[8] has been reported.

Taken together, these data demonstrate that HIV isolates vary remarkably in nucleotide sequence and consequently in protein composition. The most variable sites are located in the *env* gene, which codes for the envelope proteins, and more precisely in a region coding for the extracellular portion of HIV gp120.[9,10] Biologically, the genomic variability of HIV, also typical of other lentiviruses, such as the equine infectious anemia virus (EIAV), can play a role in fighting the host immune defense mechanisms during the course of infection.[11,12]

The great variability of HIV poses the question of whether it is possible to detect in the same subject the simultaneous presence of two or more genotypically distinct HIV variants, derived either by an in vivo superinfection with different HIVs or by the de novo formation of HIV variants during the course of HIV infection. This possibility was raised when the presence of two complete genomic equivalents was recognized by HIV-specific probes both in the DNA of H9/HTLV-IIIB cells (originally infected with a mixture of HIV isolates)[13] and in the DNA of cells infected with a single HIV isolate.[1,3,5] More recently, Koyanagi et al.[14] have isolated two related but genotypically distinct variants of HIV from two different sources (brain tissue and cerebrospinal fluid) of the same patient. In addition, Von Briesen et al.[15] have isolated and molecularly cloned different genetic variants of HIV from peripheral blood lymphocytes (PBL) of a single patient.

In our laboratory we have infected HUT-78 cultures with a reverse transcriptase (RT)-positive supernatant of PBL from an AIDS patient. A subsequent cellular cloning by the limiting dilution method gave rise to several clones, two of which are characterized here. One clone releases HIV virions, and the other exhibits HIV markers but does not release RT-containing virions or viral proteins and cannot be superinfected by other HIV isolates.

MATERIALS AND METHODS

Cell cultures and cloning

Separation, stimulation, and cultivation of PBL from AIDS donors were described elsewhere.[1] All cell cultures (uninfected HUT-78, D10, and F12 clones, HTLV-IIIB–infected HUT-78) were grown in RPMI-1640 medium (Flow Laboratories, Scotland) containing 20% inactivated fetal calf serum (FCS) and split biweekly. Cell cloning was performed by the limiting dilution method in 96-well plates.

HIV infection and detection

HUT-78 cells, previously treated with polybrene (1 μg/ml; Sigma, St. Louis, MO), were infected by an ultracentrifuged (200,000 × *g* for 30 minutes) RT-positive supernatant (about 10,000 cpm per 10^6 cells). Virus was absorbed for 1 hour at 37°C with occasional shaking, and then cells were resuspended in complete medium and split at the concentration of 3×10^5 per ml twice a week. The presence of HIV in the supernatant was monitored by reverse transcriptase assay as described,[1] and/or by antigen capture assay (Cellular Products Inc., Buffalo, NY).

Electron microscopy analysis

Cells were prefixed in suspension with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 hour and then washed twice with phosphate-buffered saline (PBS) plus 3% sucrose. Cells were then postfixed in

1% osmium tetroxide for 45 minutes, dehydrated in graded ethanol, and embedded in Epon-812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 400 transmission electron microscope.

Indirect immunofluorescence assay (IFA)

The presence of cytoplasmic HIV-related antigens was assessed by IFA as described.[16] Briefly, cells were washed twice with and resuspended in PBS at 10^6 per ml. Cell suspension (20 μl) was spotted onto a slide, air dried, and fixed in cold acetone for 10 minutes at room temperature. Patient's serum diluted 1:20 (20 μl) was applied on the fixed cells and incubated for 45 minutes at room temperature. The slides were then washed for 1 hour in PBS, and 20 μl of 1:20 rabbit antihuman IgG conjugated with fluorescein isothiocyanate (FITC, Cappel Lab., Cochranville, PA) was added and incubated at 37°C for 60 minutes. The slides were washed extensively in PBS before microscope examination under ultraviolet (UV) illumination. Uninfected HUT-78 cells were used as negative control.

To assess the presence of CD4 membrane antigen and of HIV-specific membrane antigens, we performed the membrane indirect immunofluorescence as described.[17] Briefly, 5×10^5 cells were washed three times in PBS plus 5% FCS, the pellet was dried, and 10 μl of appropriately diluted anti-CD4 monoclonal or of 1:20 diluted patient's serum was added and incubated for 20 minutes. Cells were then washed three times in PBS plus 5% FCS, and 10 μl of either 1:20 rabbit antimouse $F(ab')_2$ or 1:20 rabbit antihuman IgG, conjugated with FITC, was added at room temperature. Appropriate cell numbers were spotted onto a slide, air dried, and fixed in ethanol-acetic acid (9:1) for 20 minutes at −20°C, and then rehydrated and washed three times in PBS before UV microscope examination.

Radioimmunoprecipitation assay (RIPA)

HIV-infected and uninfected HUT-78 cells were seeded at 1.5×10^6 per ml and labeled with 100 μCi/ml of [^{35}S]cysteine (NEN-DuPont, Boston, MA, specific activity 1008 Ci/mmol) for 6 hours after 2 hours of starvation in cysteine-free medium. For ^{32}P labeling, HTLV-IIIB–infected HUT-78 and F12 cells were seeded at 10^6 per ml in phosphate-free medium and after an overnight incubation labeled with 200 μCi/ml of [^{32}P]orthophosphoric acid (NEN-DuPont, specific activity 3000 Ci/mmol) for 6 hours. The cell pellet was resuspended in radioimmunoprecipitation (RIPA) buffer (50 mM Tris pH 7.4, 0.5% Triton X-100, 100 mM NaCl, and 100 U/ml of aprotinin). The postnuclear supernatant containing about 4×10^6 cpm was exposed overnight to protein-A Sepharose (Pharmacia, Fine Chemicals, AB, Uppsala, Sweden) previously incubated with a positive reference human serum. After three washes in RIPA buffer and one wash in 10 mM Tris-HCl, pH 7.4, and 0.1 M NaCl, the pellet was resuspended in 50 μl double-strength SDS-PAGE sample buffer (10 mM Tris-HCl, pH 8.0, 2% SDS, and 2% β-mercaptoethanol), boiled 5 minutes, and run on a discontinuous 12% or on a 12–20% linear gradient SDS-polyacrilamide gel, both with 3.5% stacking gel. The gel was fixed, stained, and autoradiographed. The experimental procedure for pulse-chase experiments was identical as for the RIPA, except that after 6 hours of labeling the cells were washed three times with PBS and resuspended at 1.5×10^6 per ml in cold complete medium. Cells were then collected and processed as in RIPA at 0, 3, 6, and 9 hours after the removal of [^{35}S]cysteine.

Western blot (WB) analysis

Postnuclear cell lysates (200 μg) were fractionated by electrophoresis on a polyacrylamide-SDS slab gel. Proteins were transferred to a nitrocellulose sheet (0.22 μm; Bio-Rad, Richmond, VA) via electrophoretic blotting, washed, and blocked with nonfat dry milk to minimize nonspecific binding. Nitrocellulose strips were reacted with 1:100 dilution of a positive human serum. After the washing cycles, bound viral proteins were visualized through a peroxidase-labeled goat antihuman IgG (Bio-Rad).

Nucleic acid analysis

DNA. High-molecular-weight DNA was extracted from cell clones, and 15 μg was digested with the appropriate restriction enzymes according to the manufacturer's recommendations. The DNA was then subjected to electrophoresis in 0.8% agarose slab gel and blotted through a high electric field (Transblot, Bio-Rad) onto Gene Screen-Plus filters (NEN-DuPont).

Hybridization was performed at 42°C in a shaking water bath for 24 hours in 50% formamide, 1 M NaCl, 5% dextran-sulfate, 1% SDS, and 100 μg/ml of sonicated salmon sperm DNA. Filters were then washed twice for 30 minutes in double-strength standard saline citrate (SSC) at room temperature, twice in double-strength SSC and 0.1% SDS at 42°C, and finally twice in SSC diluted 0.1× at room temperature. The probes were nick translated at a specific activity of about 2×10^8 cpm/μg DNA using a ^{32}P-labeled deoxicytidine triphosphate (NEN-DuPont; specific activity 3000 Ci/mmol). Probes utilized were the full genomic length (less 180 bp at 5′-LTR) BH10 probe (gift of Dr. R.C. Gallo, Bethesda, MD), a 3.8 kb *gag-pol,* and a 1.9 kb *env* probe (gift of Hoffmann-La Roche, Basel, Switzerland) corresponding to the most conserved sequences overlapping the HIV exones. Filters were finally exposed to Kodak SO-282 films for 24–72 hours in an holder with intensifying screens.

RNA. Total RNA was extracted by the guanidine-isothiocyanate method[18]; the poly-A$^+$ RNA was separated by oligo-dT cellulose (Pharmacia) chromatography. Poly-A$^+$ RNA (3–5 μg) was subjected to formaldehyde-formamide denaturing gel electrophoresis and then blotted and hybridized as described for the DNA procedures.

RESULTS

Isolation and biologic characterization of D10 and F12 clones

HUT-78 cells were infected with an RT-positive supernatant of PBL from an AIDS patient. HIV-infected cultures were maintained by adding weekly fresh HUT-78 cells until cell viability dramatically decreased. Fresh HUT-78 cells were then infected with a 100-fold concentrate supernatant of the former culture and after 16 passages were cloned by the limiting dilution method. Several HIV-infected cell clones were obtained, two of which were expanded and characterized. The first clone (D10) is chronically HIV infected, releases HIV virions at high levels (Table 1), and replicates indefinitely without cocultivation with uninfected cells. The second (F12), albeit persistently HIV infected (Table 1), does not release infectious viral particles in the supernatant, as confirmed by the unsuccessful infection of HIV-susceptible cells (PBL, H9, or HUT-78) with a 500-fold concentrated F12 supernatant. The presence of noninfectious aberrant HIV particles in F12 clone was ruled out by electron microscopic analysis. No mature or budding particles were noticed in F12, whereas mature type C particles were clearly distinguishable in D10 cells (data not shown).

TABLE 1. BIOLOGIC CHARACTERIZATION OF D10 AND F12 HIV-INFECTED CELL CLONES

					Cell membrane IFA[b] *(%)*	
	RT[a]	*HIV antigen*	*Syncytia*	*Cytoplasmic IFA,*[b] *anti-HIV (%)*	*anti-CD4*	*anti-HIV*
HUT-78	–	–	–	—	98.0	—
HUT-78/HTLV-IIIB	+	+	+	93.6	—	ND[d]
D10	+	+	+	91.0	—	73.4
F12	–	–[c]	–	92.3	—	1.6

[a]Mean values at confluence: HUT-78/HTLV-IIIB, 3×10^5 cpm/ml; D10, 5×10^5 cpm/ml; HUT-78 and F12, below the background.
[b]Values of a representative experiment.
[c]Abnormal amounts of cellular debris in F12 supernatants may cause a faint positivity, just above the background.
[d]Not done.

To assess the possible release of viral proteins, F12 cells were labeled with [^{35}S]cysteine, the supernatant was concentrated 500-fold via ultrafiltration (Centricon, Amicon, Danvers, MA; cutoff, 10,000 daltons) and analyzed by RIPA in parallel with a similar preparation from a D10 clone culture. Even loading a 10-fold excess of cpm, no viral products were recognized in F12 supernatant, whereas the typical HIV antigens were remarkably present in D10 supernatant (data not shown).

The growth rate of the two clones is similar, as both reach saturation at about 2×10^6 cells per ml after 4 days of culture. Viral infection and production were assessed by RT assay, by the antigen capture assay, and by cytoplasmic IFA performed with a polyclonal anti-HIV serum. Table 1 shows that the percentage of IFA-positive cells is equally high (80–90%) in F12, D10, and control positive cells (HUT-78/HTLV-IIIB).

The two clones were also analyzed for the presence of HIV-specific antigens on the plasma membrane. A partially purified human anti-HIV serum recognized 73.4% D10 cells and only 1.6% F12 cells. These data as well as those from RIPA experiments (see later) indicate the presence of HIV-specific gp120 antigens on the plasma membrane of the D10 cell clone but not on that of the F12 clone.

Finally, the presence of CD4 receptor sites was tested using a monoclonal anti-CD4$^+$ antibody. As already described for other HIV-infected cell lines,[19] neither D10 nor F12 clones exhibit any CD4 membrane antigen.

All these features are invariably conserved even after 12 months of culture.

Attempts to rescue HIV from F12 clone cells

F12 clone cells have been treated without success with a wide spectrum of doses of fluoro-, bromo-, and iododeoxyuridine for possible rescue of the integrated provirus (data not shown).

Further, we tried to complement possible defects of F12 proviral genome by superinfecting these cells with different HIV-1 or HIV-2 isolates. Even with a high multiplicity of HIVs, every attempt met with failure.

Transcription directed by the HIV LTRs was shown to be *trans*-activated by superinfection with herpes simplex virus type 1 (HSV-1).[20] F12 clone cells were thus infected with 1, 4, or 10 plaque-forming units

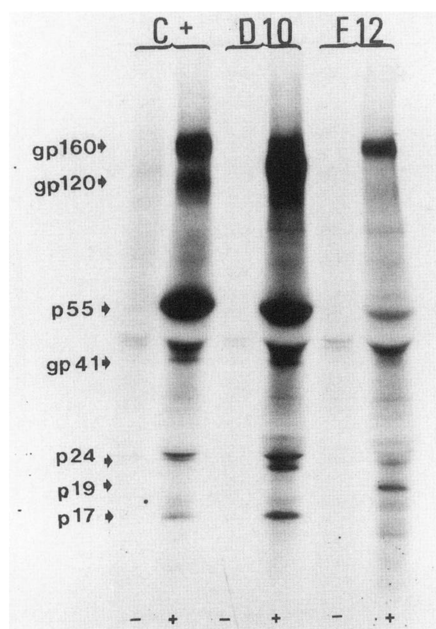

FIG. 1. Radioimmunoprecipitation of [^{35}S]cysteine-labeled cellular lysates of HTLV-IIIB–infected HUT-78 cells (c+) and D10 and F12 clones. About 4×10^6 cpm per condition was incubated with human negative (−) or HIV-positive (+) serum and processed as described. The arrows indicate the major viral antigens detectable in the RIPA test.

(PFU) per cell of HSV-1, but no HIV production was detectable under any conditions, even if the superinfecting HSV-1 did replicate in F12 as well as in HUT-78 control cells (data not shown).

Protein pattern analysis

The productively infected D10 clone shows a viral protein pattern resembling that of HUT-78/HTLV-IIIB cells except for the lower band of the p24–25 doublet and the electrophoretic mobility of gp120, possibly due to a different pattern of glycosylation (Fig. 1).

In contrast, in the F12 cell clone there is a consistent reduction in p55 *gag* precursor, which is poorly cleaved with the consequent almost total absence of p24 and p17 *gag* proteins. Further, only gp160 precursor is present but there is a lack of gp120 and gp41, easily visible in HUT-78/HTLV-IIIB and in D10 cells. Pulse-chase experiments did not show any cleavage of gp160 in F12 clone cells even 9 hours after the removal of radioactive label (data not shown).

In D10 and F12 cells, p64 and p38 proteins (the reverse transcriptase and the viral endonuclease, respectively) are visible by WB analysis while being undetectable by RIPA. In F12 cells there is a prominent band of a protein with an M_r of 19 kDa, also visible by WB analysis (Fig. 2), that may be either an aberrant product of the *gag* gene or the product of the *rev* HIV exone. To define more exactly the nature of this protein species, we have labeled HTLV-IIIB–infected HUT-78 and F12 cells with [^{32}P]orthophosphoric acid (Fig. 3). The failure to reveal substantial differences in the RIPA of ^{32}P-labeled proteins between the positive control and the F12 clone, that is, the absence of any 19 kDa signal, suggests that this protein does not belong to the phosphorylated *gag* antigen family. Conversely, the species most represented is the p27 *nef* protein, which is present at comparable levels in both F12 and HTLV-IIIB–infected HUT-78 cells.

The possibility of a double infection by HTLV-I and HIV-1 in the F12 clone (a p19 protein is a core protein of HTLV-I) has been ruled out by (1) utilizing cytoplasmic IFA with monoclonal antibodies against the HTLV-I p19 core protein, and (2) hybridizing F12 DNA with an HTLV-I probe of full genomic length (data not shown).

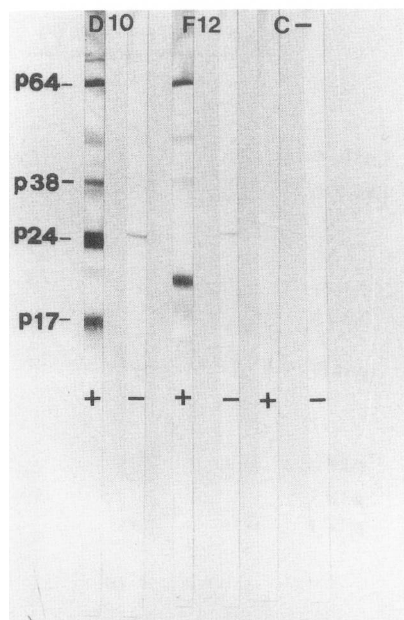

FIG. 2. Western blot analysis of uninfected HUT-78 (C−), D10, and F12 cell lysates. The nitrocellulose strips were incubated with human negative (−) or HIV-positive (+) serum. On the left are indicated the position of the more evident viral antigens, including the *pol*-specific viral proteins p64 (reverse transcriptase) and p38 (endonuclease).

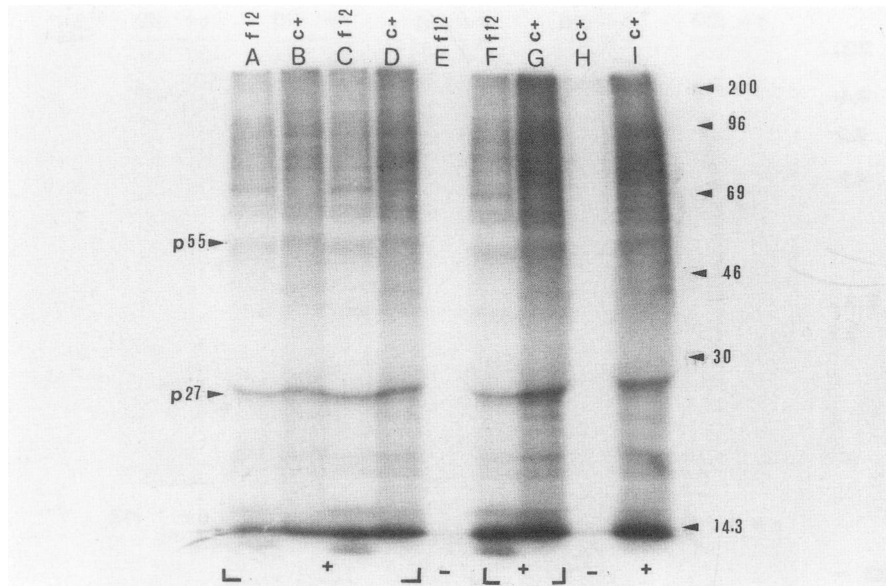

FIG. 3. Radioimmunoprecipitation of [^{32}P]orthophosphoric acid-labeled cellular lysates of HTLV-IIIB–infected HUT-78 cells (c+) and F12 clone. About 3 × 10^5 (lanes A and B), 5 × 10^5 (lanes C and D), or 10^6 (lanes F, G, and I) cpm per condition was incubated with an HIV-positive (+) or negative (lanes E and H, 5 × 10^5 cpm) serum and processed as described. On the left are indicated the major viral antigens detectable in the RIPA test (the p55 *gag* precursor and the p27 *nef* protein); on the right are indicated the M_r of the standards.

DNA analysis

The pattern analysis (Fig. 4) of HIV proviral DNA restricted by the SstI enzyme, which cleaves in the LTR regions,[13] shows that both D10 and F12 clones have an HIV genome of a length similar to that of HTLV-IIIB–infected HUT-78 cells, thus ruling out that the lack of HIV release in F12 cells is due to major genomic deletions. As already demonstrated for HTLV-IIIB–infected H9 cells,[13] the presence of two coexisting HIV genomes in D10 and F12 cells is demonstrated by the SstI and EcoRI patterns.

Most restriction enzymes (six of eight for the D10 clone and six of nine for the F12 clone, not all shown in Fig. 4) generate different cleavage patterns with respect to HUT-78/HTLV-IIIB control cells. In contrast, the heterogeneity is less pronounced between the two clones (e.g., EcoRI) and between each clone and the infected parenteral cell population, from which they derive (data not shown). The XbaI enzyme does not recognize proviral sites in HTLV-IIIB–infected HUT-78 and in D10 cells. That two additional bands of high molecular weight are generated only in F12 clone may be interpreted as the appearance of an XbaI-specific internal site in one of the two coexisting HIV genomes.

Finally, as reported for the H9/HTLV-IIIB cells,[3,10] the restriction pattern of the two clones did not change noticeably even after 12 months of culture (data not shown).

RNA analysis

Several studies of HIV-specific RNAs[21,22] reported the presence in HIV-infected cells of three major bands at 9.3, 4.3, and 1.8–2 kb, representing, respectively, the genomic length transcript, the *env*-specific mRNA, and a family of minor messengers coding for the regulatory HIV proteins. An additional transcript was occasionally detected at 5 kb.[23]

The hybridization of intracellular viral poly-A$^+$ RNA with the full-length genomic BH10 probe reveals the same three major bands in both positive control cells, in D10, and F12 clones (Fig. 5). The evident overproduction of 1.8–2 kb viral RNA in the F12 clone hybridized with a BH10 probe can hypothetically fit with the overexpression of a 19 kb regulatory protein in the F12 clone (Fig. 1). Hybridization of the same RNAs with the *gag-pol* or *env* subgenomic probes fails to show any quantitative (as shown also by

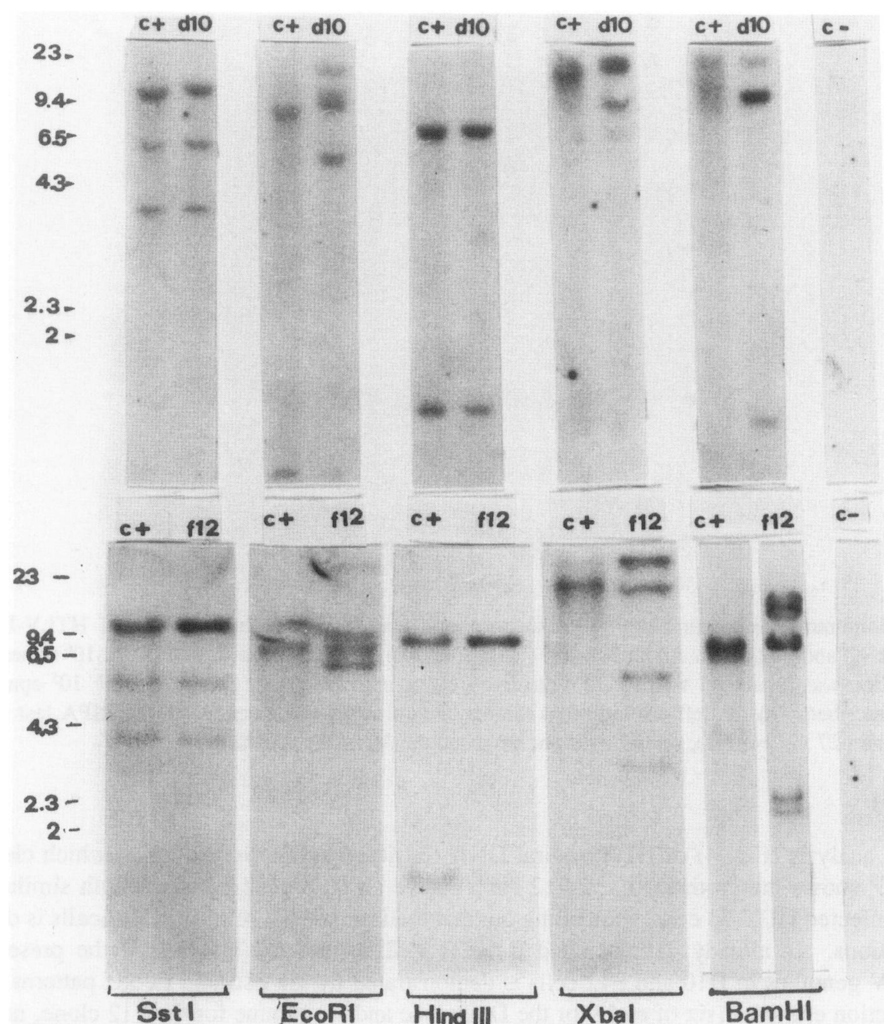

FIG. 4. Restriction enzyme analysis of HIV DNA integrated in D10 and F12 genomes. Each pattern was compared with that of HTLV-IIIB–infected HUT-78 cells (c+); (c−), uninfected HUT-78 cells. At the bottom are indicated the restriction enzymes employed; on the left are indicated the kilobase pairs (kbp) of the λ DNA marker cut by HindIII.

hybridization with the glucose-6′-phosphodehydrogenase probe) or qualitative differences among D10, F12, and HUT-78/HTLV-IIIB cells (Fig. 5).

DISCUSSION

The cloning of HUT-78 cells infected with an RT-positive supernatant of PBL from an AIDS patient led to the isolation of two clones harboring different HIV variants, one (D10) persistently infected by a replication-competent HIV, the other (F12) exhibiting an integrated HIV genome unable to generate infectious viral particles. The isolation of such clones emphasizes the possibility of recovering more than one HIV variant from PBL of the same AIDS patient. More interestingly, from the same virus isolation assay we have detected a defective HIV variant that, once integrated in the host genome, is able to protect the cells from HIV superinfection through a phenomenon of homologous viral interference.

The simultaneous presence of more than one variant in cell populations infected with HIV was first reported by Wong-Staal et al.,[3] who described the coexistence of at least two different genotypes in 2 of 18 AIDS isolates. This evidence came from restriction pattern analysis of integrated viral DNA using enzymes recognizing sites conserved in LTRs (i.e., SstI).

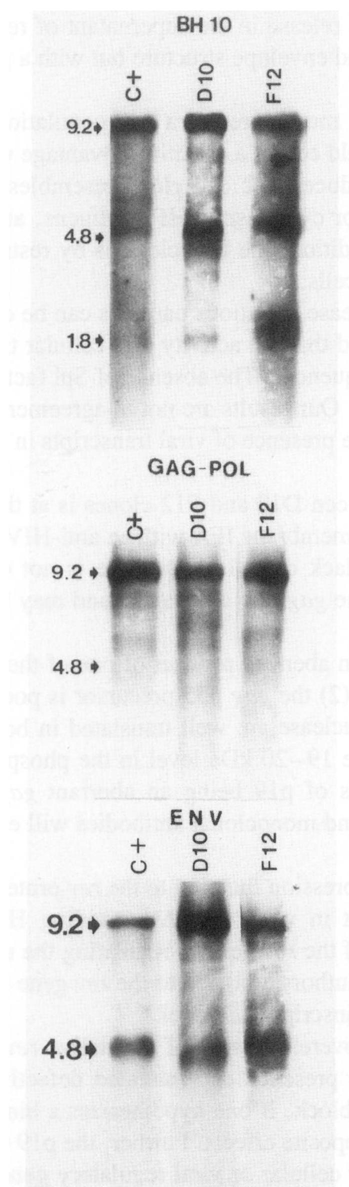

FIG. 5. Northern blot analysis of viral RNA: 3–5 μg poly-A$^+$ RNA of D10, F12, and HTLV-IIIB–infected HUT-78 (c+) cells were hybridized with the nick-translated full genomic length BH10 probe, with subgenomic *gag-pol* or *env* probes, both representing the most conserved sequences of the respective exones. The arrows on the left indicate the kbp of the HIV RNA major signals.

The possibility that proviral genomes present in D10 and F12 clones originated from in vitro mutations cannot be ruled out, since so many culture passages were necessary to establish the clones. However, this hypothesis is strongly in contrast to the in vitro great genomic stability of integrated proviral HIV, as already shown comparing proviral restriction patterns after 3,[10] 9,[3] or even 12 months (our results for D10 and F12 clones) of culture.

The nonproducer HIV-infected F12 clone may have originated from an HIV-defective particle originally present in the AIDS patient and released from the patient's PBL. This virus could have infected CD4$^+$ HUT-78 cells, taking advantage of the presence of a coinfecting replication-competent HIV. Such viral

complementation could in turn lead to release in the supernatant of reinfected fresh HUT-78 cells of viral particles with normal nucleocapside and envelope structure but with a genome unable to code for infectious HIV virions.

Both D10- and F12-infected clones may represent a cell population originally harboring HIV variants with limited cytopathic effects that could confer a selective advantage with respect to cells infected by more cytopathic HIV variants. The nonproducer F12 cell clone resembles the D10 clone in terms of cellular growth, percentage of cells positive for cytoplasmic HIV antigens, and down regulation of CD4 receptor sites and viral RNA transcripts. In addition, the F12 clone is by restriction pattern much more related to D10 than to the HUT-78/HTLV-IIIB cells.

The inability of the F12 clone to release infectious particles can be due to both viral and cellular factors. Among the latter, Jones et al.[24] showed that the activity of a cellular transcription factor (Spl) is necessary for the correct transcription of HIV sequences. The absence of Spl factor leads to a 10-fold reduction in the in vitro HIV transcriptional efficiency. Our results are not in agreement with the hypothesis of a Spl defect in F12 clone because of the comparable presence of viral transcripts in D10, F12, and HTLV-IIIB–infected HUT-78 cells (Fig. 5).

The most important difference between D10 and F12 clones is at the level of viral protein pattern. The very low positivity of F12 cells by the membrane IFA with an anti-HIV antiserum fits well with the absence of cleavage products of gp160. The lack of gp160 cleavage is not due to a defect in the viral-specific protease, which plays a role only in the *gag* p55 cleavage[25] and may be due to a mutation in the cleavage site.

F12 p19 can hardly be considered an aberrant product of one of the three structural genes, since (1) the *env* gp160 is substantially uncleaved, (2) the *gag* p55 precursor is poorly expressed, and (3) the p64 viral polymerase as well as the p34 endonuclease are well translated in both F12 and D10 clones (Fig. 2). In addition, the absence of a signal at the 19–20 kDa level in the phosphorylated protein pattern of the F12 clone does not support the hypothesis of p19 being an aberrant *gag* product (Fig. 3). Experiments in progress utilizing anti-*rev* polyclonal and monoclonal antibodies will establish whether the F12 p19 protein is the product of the *rev* gene.

Sodroski et al.[26] assigned an *anti*-repression function to the *rev* protein to counter the effects of *cis*-acting negative regulatory sequences present in viral mRNAs encoding HIV structural proteins. Conversely, Feinberg et al.[22] hypothesized a role of the *rev* gene in regulating the splicing of viral mRNAs. Regardless of the mechanism of action, all these authors assigned to the *rev* gene a positive regulatory function of *gag* and *pol* protein synthesis at the posttranscriptional level.

It is thus difficult to correlate the overexpression of a putative *rev* p19 protein with reduced *gag* p55 production unless we hypothesize the presence of a mutated defective *rev*. Alternatively, the increased presence of p19 may account for this block, if one hypothesizes a bimodal effect of *rev* protein by which low and high amounts would induce opposite effects. Further, the p19 overproduction may be a response to some undefined block related to either cellular or viral regulatory genes.

An accurate study of the sequences and of the secondary structure of viral mRNA will also be useful to exactly understand both the mechanism(s) of translation inhibition of *gag* specific messengers and the origin of the uncleavage of *env* gp160.

ACKNOWLEDGMENTS

This work was supported in part by grants from AIDS Project 1987–1988 of the Ministry of Health, Rome, from Gruppo di Virologia 87.01640.04 Consiglio Nazionale delle Ricerche, Rome, and from the Associazione Italiana per le Ricerche sul Cancro.

We are indebted to Ms. A. Guderzo for excellent secretarial assistance.

REFERENCES

1. Rossi GB, Verani P, Macchi B, Federico M, Orecchia A, Nicoletti L, Buttó S, Lazzarin A, Mariani G, Ippolito G, and Manzari V: Recovery of HIV-related retroviruses from italian patients with AIDS or AIDS-related complex and from asymptomatic at-risk individuals. Ann NY Acad Sci 1988;511:390–400.

2. Evans LA, McHugh TM, Stites DP, and Levy JA: Differential ability of human immunodeficiency virus isolates to productively infect human cells. J Immunol 1987;138:3415–3418.

3. Wong-Staal F, Shaw GM, Hahn BH, Salahuddin SZ, Popovic M, Markham P, Redfield R, and Gallo RC: Genomic diversity of human T-Lymphotropic virus type III (HTLV-III). Science 1985;2:759–762.

4. Hahn BH, Shaw GM, Taylor ME, Redfield RR, Markham PD, Salahuddin SZ, Wong-Staal F, Gallo RC, Parks ES, and Parks WP: Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science 1986;232:1548–1553.

5. Magasiny S, Spire B, Barré-Sinoussi F, and Chermann JC: Genomic variability of selected LAV-related AIDS retroviruses. AIDS Res 1986;2:19–30.

6. Devare SG, Srinivasan A, Bohan CA, Spira TJ, Curran JW, and Kalyanaraman VS: Genomic diversity of the acquired immunodeficiency syndrome retroviruses is reflected in alteration of its translational products. Proc Natl Acad Sci USA 1986;83:5718–5722.

7. Benn S, Rutledge R, Folks T, Gold J, Baker L, McCormick J, Feorino P, Piot P, Quinn T, and Martin M: Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. Science 1985;230:949–951.

8. Srinivasan A, York D, Ranganathan P, Ferguson R, Butler D Jr, Feorino P, Kalyanaraman V, Jaffe H, Curran J, and Anand R: Transfusion-associated AIDS: Donor-recipient human immunodeficiency virus exhibits genetic heterogeneity. Blood 1987;69:1766–1770.

9. Starcich BR, Hahn BH, Shaw GM, McNeely PD, Modrow S, Wolf H, Parks ES, Parks WP, Josephs SF, Gallo RC, and Wong-Staal F: Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell 1986;45:637–648.

10. Hahn BH, Gonda MA, Shaw GM, Popovic M, Hoxie JA, Gallo RC, and Wong-Staal F: Genomic diversity of the acquired immune deficiency syndrome virus HTLV-III: Different viruses exhibit greatest divergence in their envelope genes. Proc Natl Acad Sci USA 1985;82:4813–4817.

11. Clements JE, Pederson FS, Narayan O, and Haseltine WA: Genomic changes associated with antigenic variation of Visna virus during persistent infection. Proc Natl Acad Sci USA 1980;77:4454.

12. Montelaro RC, Parekh B, Orrego A, and Issel CJ: Antigenic variation during persistent infection by equine infectious anemia virus, a retrovirus. J Biol Chem 1984;259:10539.

13. Hahn BH, Shaw GM, Arya SK, Popovic M, Gallo RC, and Wonġ-Staal F: Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature 1984;312:166–169.

14. Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, and Chen ISY: Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science 1987;236:819–821.

15. Von Briesen H, Becker WB, Henco K, Helm EB, Gelderblom HR, Brede HD, and Rubsamen-Waigmann H: Isolation frequency and growth properties of HIV-variants: Multiple simultaneous variants in a patient demonstrated by molecular cloning. J Med Virol 1987;23:51–66.

16. Robert-Guroff M, Ruscetti FW, Posner LE, Poiesz BJ, and Gallo RC: Detection of the human T-cell lymphoma virus p19 in cells of some patients with cutaneous T-cell lymphoma and leukemia using a monoclonal antibody. J Exp Med 1981;154:1957–1964.

17. Gathings WE, Lawton AR, and Cooper MD: Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. Eur J Immunol 1977;7:804.

18. Chirgwin JM, Przybyla AE, MacDonald RJ, and Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979;18:5294–5299.

19. Hoxie JA, Alpers JD, Rackowski JL, Huebner K, Haggarty BS, Cedarbaum AJ, and Reed JC: Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. Science 1986;234:1123–1127.

20. Mosca JD, Bednarik DP, Raj NBK, Rosen CA, Sodroski JG, Haseltine WA, and Pitha PM: Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. Nature 1987;325:67–70.

21. Muesing MA, Smith DH, Cabradilla CD, Benton CV, Lasky LA, and Capon DJ: Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. Nature 1985;313:450–458.

22. Feinberg MB, Jarrett RF, Aldovini A, Gallo RC, and Wong-Staal F: HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell 1986;46:807–817.

23. Folks TM, Powell D, Lightfoote M, Koenig S, Fauci AS, Benn S, Rabson A, Daugherty D, Gendelman HE,

Hoggan MD, Venkatesa S, and Martin MA: Biological and biochemical characterization of a cloned leu-3^{-} cell surviving infection with the acquired immune deficiency syndrome retrovirus. J Exp Med 1986;164:280–290.

24. Jones KA, Kadonaga JT, Luciw PA, and Tjian R: Activation of the AIDS retrovirus promoter by the cellular transcription factor Spl. Science 1986;232:755–758.
25. Kramer RA, Schaber MD, Skalka AM, Ganguly K, Wong-Staal F, and Reddy EP: HTLV-III *gag* protein is processed in yeast cells by the virus *pol*-protease. Science 1986;231:1580–1584.
26. Sodroski J, Goh WC, Rosen C, Dayton A, Terwilliger E, Haseltine W: A second post-transcriptional trans-activator gene required for HTLV-III replication. Nature 1986;321:412–417.

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2. Stephen J. Wagner, Andrey Skripchenko, Louis Cincotta, Dedeene Thompson-Montgomery, Helen Awatefe. 2005. Use of a flexible thiopyrylium photosensitizer and competitive inhibitor for pathogen reduction of viruses and bacteria with retention of red cell storage properties. *Transfusion* **45**:5, 752-760. [CrossRef]
3. M. Federico, F. Nappi, R. Bona, P. D'Aloja, P. Verani, G.B. Rossi. 1995. Full expression of transfected nonproducer interfering HIV-1 proviral DNAabrogates susceptibility of human He-La CD4+ cells to HIV. *Virology* **206**:1, 76-84. [CrossRef]
4. P. Verani, S. Buttò, B. Taddeo, M. Federico, G.B. Rossi. 1993. HIV variability and perspectives for a vaccine. *Vaccine* **11**:5, 542-544. [CrossRef]
5. A.M. Luciani, A. Rosi, M.T. Maggiorella, M. Federico, N. Sulli, P. Verani, G.B. Rossi, V. Viti, L. Guidoni. 1991. Interaction of HIV-1 with susceptible lymphoblastoid cells 1H NMR studies. *FEBS Letters* **285**:1, 11-16. [CrossRef]