Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2433–2437, April 1989
Medical Sciences

Molecular and biological characterization of a replication competent human immunodeficiency type 2 (HIV-2) proviral clone

(human immunodeficiency virus types 1 and 2/simian immunodeficiency virus/human T-cell leukemia virus)

GENOVEFFA FRANCHINI*†, KATHLEEN A. FARGNOLI*, FABRIZIO GIOMBINI*, LINDA JAGODZINSKI‡, ANITA DE ROSSI§, MARNIX BOSCH*, GUNNEL BIBERFELD¶, EVA M. FENYO‖, JAN ALBERT‖, ROBERT C. GALLO*, AND FLOSSIE WONG-STAAL*

*Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; ‡Biotech Research Laboratory, Three Taft Court, Rockville, MD 20850; §Laboratory of Oncology, University of Padova, Padua, Italy; and ¶National Bacteriology Laboratory and ‖Department of Virology, Karolinska Institute 10401, School of Medicine, Stockholm, Sweden

Contributed by Robert C. Gallo, January 3, 1989

ABSTRACT **We obtained complete genomic clones of human immunodeficiency virus type 2 (HIV-2) from the DNA of the neoplastic human cell line HUT 78 freshly infected with a HIV-2 isolate, strain SBL6669. The recombinant phage DNA was transfected into the lymphocytes of CD4-positive HUT 78 cell line to test the replication competence of the proviral DNA. One genomic clone, designated HIV-$2_{SBL/ISY}$, yielded retroviral particles after a few weeks of culture of the transfected cells. The HIV-$2_{SBL/ISY}$ clone contained a complete provirus and cellular flanking sequence. We obtained the DNA sequence of the provirus and compared it with the published sequence of two other HIV-2 isolates. The degree of variability among HIV-2 isolates is comparable to that observed among African HIV-1 isolates sequenced to date. Immunologically, HIV-$2_{SBL/ISY}$ is similar to the parental virus (HIV-$2_{SBL6669}$) but differs in the envelope transmembrane protein that is truncated (gp32–34) in the parental virus and not in HIV-$2_{SBL/ISY}$ (gp41). Both the parental and the cloned viruses are infectious and cytopathic for some human T-cell lines, induce syncytia, and infect a human macrophage cell line (U937) *in vitro*. The availability of a biologically active HIV-2 clone provides the means to study the role and interaction of HIV-2 genes *in vitro* as well as to assess the functional similarities among HIV-1 and HIV-2 genes. Since HIV-$2_{SBL/ISY}$ cloned virus infects fresh peripheral blood T cells from Rhesus macaques *in vitro* and infects the same animal *in vivo*, its use in animals may represent a model for functional study of viral genes *in vivo* as well as for development of experimental approaches to prevent and cure retroviral infection in humans.**

Human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2) are related retroviruses associated with the acquired immunodeficiency syndrome (AIDS) in humans (1–8). A virus closely related to HIV-2, simian immunodeficiency virus $(SIV)_{mac}$, has been isolated from captive macaques with AIDS (9). Other viruses of the same general family have been isolated from both captive and wild Old World monkeys (9–13). The genetic structures of HIV-1 and HIV-2 are more complex than the structure of other animal retroviruses. In addition to the structural genes encoding the core and the envelope proteins, several genes have been described: the *tat* gene (14), a 14-kDa protein that acts in trans to increase the expression of viral genes fron the viral long terminal repeat (LTR); the *rev* gene (19 kDa), which differentially regulates the expression of virion protein (15–17); the *nef* gene (27 kDa), which reduces viral expression; the *vif* gene, which is essential for infectivity of cell-free virus (18, 19); and the *vpr* (20) gene, the function of which is unknown. Another gene, *vpx*, is present in HIV-2 and the related simian virus SIV_{mac} but not in HIV-1 (21, 22). The *vpx* gene protein (p16 for HIV-2 and p14 for SIV) is associated with mature virions, but its function is as yet undefined. The functions of the HIV-1/HIV-2 accessory genes have been studied so far by infection of T cells *in vitro* and it is puzzling that a number of these genes appear dispensable for virus replication. To study the role of these genes *in vivo* and to establish an animal model to test the efficacy of candidate vaccines, we have obtained a biologically active clone of HIV-2, determined its nucleotide sequence, and studied the immunological and biological features of the virus by transfection in human T cells. We also tested the infectivity of the resulting cloned virus by inoculation in rhesus macaques.

MATERIALS AND METHODS

Virus Isolation. HIV-$2_{SBL6669}$ and HIV-2_{NIH-Z} were isolated from the peripheral blood cells of patients by cocultivation with the T cells of the neoplastic cell line HUT 78 (6, 8). Viral production was assessed by reverse transcriptase (RT) activity assay of the infected cells and by electron microscopy.

Molecular Cloning of Proviral DNA. A λ phage library was constructed from the genomic DNA isolated from HUT 78 cells infected with HIV-$2_{SBL6669}$ isolate. The DNA, partially digested with *Sau*3A, was fractionated on a linear 10–40% sucrose gradient and the 20-kilobase (kb) fraction was ligated to EMBL-3 arms (23). The ligated DNA was packaged *in vitro* by using the Stratagene Gigapack. Recombinant clones were obtained from the library by using the SIV *gag* (B16) and envelope (SS35) probes (24). A recombinant clone designated HIV-$2_{SBL/ISY}$ was purified and the insert DNA was used to generate several subclones in the Bluescript vector (23). The subclones were used for dideoxynucleotide chain-termination sequencing with Sequenase (United States Biochemical) (25, 26) and Maxam and Gilbert sequencing (27).

Southern Blot Analysis of HIV-2-Infected Cells. The total cellular DNA from cell lines infected with HIV-$2_{SBL6669}$, HIV-2_{NIH-Z}, and HIV-$2_{SBL/ISY}$ (6, 8) was digested with *Bam*HI, *Xba* I, and *Eco*RI and electrophoresed on 0.8% agarose. As a further control, the DNA of the SIV_{mac}-infected cells was cleaved with the same enzymes. The gel was denatured, neutralized, and blotted to nitrocellulose filters as described (28); the filters were hybridized to the labeled probes B16 or SS35 (24).

DNA Transfection in Neoplastic T Cells. Ten million HUT 78 cells were used for each transfection. Forty million cells

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; FCS, fetal calf serum; RT, reverse transcriptase; LTR, long terminal repeat.

†To whom reprint requests should be addressed.

were resuspended in 40 ml of RPMI 1640 medium with 10% fetal calf serum (FCS) (GIBCO), and incubated at 37°C for 5 hr. After incubation, the cells were washed with RPMI 1640 medium without FCS and aliquoted (10 million cells) into four tubes. The cells were resuspended in 4 ml of RPMI 1640 medium without FCS containing 50 mM Tris·HCl (pH 7.4) plus 10 μg of DNA. Subsequently, 1 ml of 5× DEAE dextran solution (25 mg/ml) in RPMI 1640 medium without FCS in 1 M Tris·HCl (pH 7.4) was added to each tube. The samples were incubated at 37°C for 1 hr with gentle shaking. After incubation, the cells were pelleted at 1500 rpm, in a Sorvall RT6000, and washed twice at room temperature with complete medium (RPMI 1640 medium with 10% FCS). The following day, 10 ml of fresh medium was added. Viral production was monitored by testing for magnesium-dependent RT 1 week posttransfection. For the RT assay, the proteins contained in the supernatant were precipitated with 30% PEG/0.4 M NaCl and the pellets were resuspended in VSB (29). RT was determined by precipitable counts of incorporated ^{3}H.

Immunofluorescence on the Infected Cells. Immunofluorescent staining of infected cells was performed with serum from individuals infected with HIV-2. The cells were pelleted, fixed with 50% methanol/50% acetone for 10 min, and incubated with 15 μl of human serum diluted 1:40 with phosphate-buffered saline (PBS) for 30 min at room temperature. The slides, washed with PBS, were incubated with fluoresceinated anti-human antibodies in the dark for 30 min and positive cells were scored under a UV light microscope. Electron microscopy on the infected cells was performed as described by Biberfeld *et al.* (30).

Infection of Target Cell Lines. Concentrated virus was obtained from the transfected cell line HUT 78 constitutively producing the HIV-$2_{SBL/ISY}$ virus. The equivalent of 1000 $TCID_{50}$ (tissue culture 50% infective dose) infecting virus was used to infect the cell lines H9, MOLT-3, U937, HUT 78, CEM, MT-2, and the T-cell clone 55. Cells (5×10^6) from each culture were treated with Polybrene (Sigma) at 5 μg/ml for 1 hr. The cells were washed with 1× PBS and incubated for 1 hr with the virus. At the end of the incubation period, the cells were washed again and resuspended in RPMI medium/10% FCS. Immunofluorescence and RT activity were measured every 3 days as described above. Cell viability was calculated by subtracting the number of cells incorporating trypan blue color and the number of syncytia was counted under a light microscope.

Western Blot Analysis. Cells from infected cultures were pelleted and the supernatants were centrifuged at 20,000 rpm for 1 hr to pellet the virus. The viral pellet was resuspended in 1× RIPA buffer (5 mM phenylmethylsulfonyl fluoride/75 mM NaCl/25 mM Tris·HCl, pH 7.5/0.5% SDS/5% Triton X-100/5% deoxycholic acid). The lysed virions were loaded on a 10% SDS/polyacrylamide gel and transferred to nitrocellulose filters. The filters underwent reaction with 5% dry milk in PBS for 1 hr (31). The antibodies used are designated in the figure legends. Iodinated *Staphylococcus aureus* protein A was used to detect immunocomplexes.

Immunoprecipitation of Viral Proteins. Cells infected with HIV-2_{ISY} or SIV were incubated in medium supplemented with [^{35}S]methionine and [^{35}S]cysteine (100 μCi/ml; 1 Ci = 37 GBq) for 4 hr, pelleted, and the supernatant was centrifuged at 20,000 rpm for 1 hr, in a Sorvall rotor type SS34, to pellet the labeled virus. The labeled viral lysate was precleared overnight with normal human sera and Sepharose-bound protein A. Aliquots of the precleared cell lysate were incubated with sera from HIV-2-infected humans or SIV-infected monkeys and the immunocomplexes were isolated with *S. aureus* protein A bound to Sepharose. The samples were electrophoresed on a 10% SDS/polyacrylamide gel and the gel was treated with enhancer for 30 min, dried, and autoradiographed.

RESULTS

Molecular Cloning and DNA Sequence of the Complete Provirus HIV-$2_{SBL/ISY}$. A recombinant λ phage containing a complete provirus was obtained from a genomic library constructed from the DNA of the human T-cell line HUT 78 infected with the strain of HIV-$2_{SBL6669}$ (4), using the SIV *gag* and envelope probes as described (24). One positive clone containing the complete proviral DNA was selected and designated HIV-$2_{SBL/ISY}$. The restriction enzyme pattern of the HIV-$2_{SBL/ISY}$ clone, as shown in Fig. 1 (*Upper*) differs considerably from that of previously analyzed HIV-2 provi-

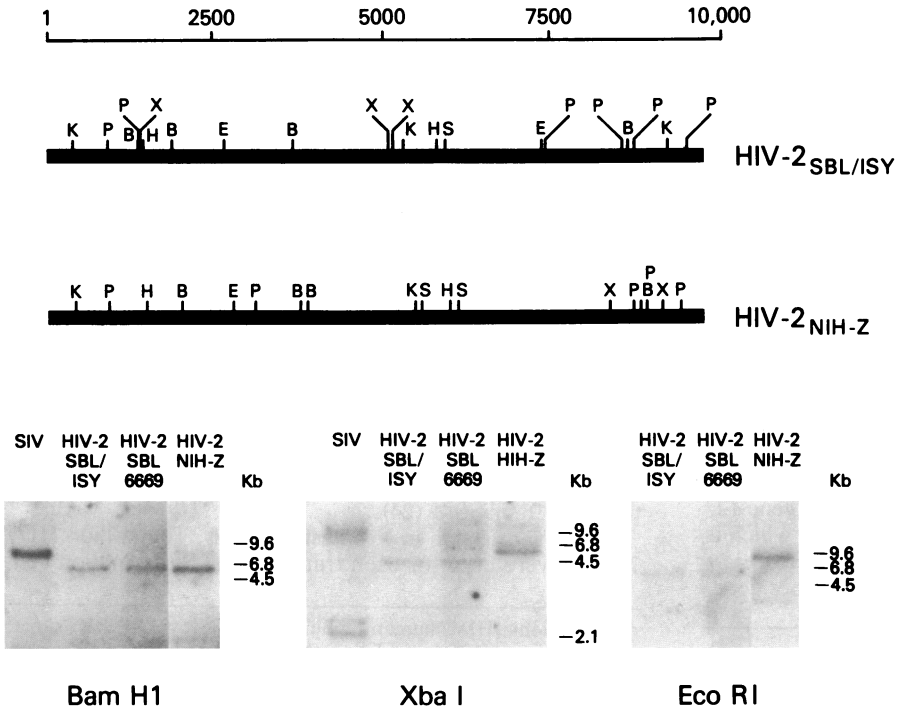

FIG. 1. Restriction enzyme analysis of the genomic DNA of the transfected cell line HUT 78. (*Upper*) Endonuclease restriction maps of the proviral HIV-2 clones obtained from the viral isolates HIV-2_{NIH-Z} and HIV-$2_{SBL6669}$. K, *Kpn* I; P, *Pst* I; B, *Bam*HI; X, *Xba* I; H, *Hind*III; E, *Eco*RI; S, *Sac* I. (*Lower*) Results of *Bam*HI, *Xba* I, and *Eco*RI cleavage of the genomic DNAs of the HIV-2_{NIH-Z}-, HIV-$2_{SBL6669}$-, and SIV_{K6W}-infected cell lines. The second lane of each panel represents the analysis of DNA from the HUT 78 cell line and transfects with the proviral clone HIV-$2_{SBL/ISY}$.

ruses designated HIV-2_{NIH-Z} (8) and HIV-2_{ROD} (32), as well as from SIV_{mac} (33, 34). We obtained the complete DNA sequence of HIV-$2_{SBL/ISY}$ and estimated its similarity with HIV-2_{NIH-Z} and HIV-2_{ROD}.** The overall genomic organization of HIV-$2_{SBL/ISY}$ is similar to the other HIV-2 isolates (8, 32) and nucleotide sequence alignment indicated that HIV-$2_{SBL/ISY}$ is 87% and 89% identical to HIV-2_{NIH-Z} and HIV-2_{ROD}, respectively, which in turn share 88% of their nucleotide sequence.

Comparison of the predicted amino acid sequence of structural and nonstructural protein of the different HIV-2 isolates is presented in Table 1. The results indicate a high degree of amino acid sequence divergence among the HIV-2 isolates in all the viral proteins compared. Interestingly, a similar degree of divergence was described among HIV-1 African isolates but not among HIV-1 American isolates (35).

Viral Production in T Cells Transfected With the HIV-$2_{SBL/ISY}$ Clone. The recombinant phage DNA containing the complete provirus HIV-$2_{SBL/ISY}$ was transfected into the human neoplastic cell line HUT 78. The supernatant of the cell culture was found positive for Mg^{2+}-dependent RT 1 week posttransfection. Viral expression was confirmed by immunofluorescent staining of the infected cells with serum from an individual infected with HIV-2. Southern blot analysis of the total DNA from the transfected cell line after 2.5 months of culture indicated the presence of viral sequences (Fig. 1 *Lower*). Hybridization of *Xba* I- and *Eco*RI-cleaved DNAs to a probe (B16) derived from the SIV *gag* gene revealed the same internal bands for the uncloned parental HIV-$2_{SBL6669}$ and the HIV-$2_{SBL/ISY}$ proviral DNA, indicating that HIV-$2_{SBL/ISY}$ is representative of the majority of genotypes present in the cell line infected with the parental virus. Different restriction patterns were observed with the genomic DNA from the SIV and the HIV-2_{NIH-Z}-infected cell lines. DNAs cleaved with *Bam*HI and hybridized with a SIV envelope probe (SS35) yielded the same internal bands in both HIV-2s (5 kb) and the expected 8.5-kb band in SIV. Electron microscopy analysis performed on the HIV-$2_{SBL/ISY}$-transfected cells revealed the presence of mature virions with the expected cylindrical-shaped core typical of lentiviruses (Fig. 2) and budding particles from the cell membrane (Fig. 2 *Inset*), indicating that transfection of the HIV-$2_{SBL/ISY}$ DNA induced a productive infection of the HUT 78 cell line.

Immunological Characterization of the HIV-$2_{SBL/ISY}$. We performed Western blot analysis and radioimmunoprecipitation on the viral particles obtained from the HUT 78 T cells infected with the replication competent HIV-2 proviral DNA and SIV_{mac} (Fig. 3). The nitrocellulose strips containing unlabeled virion proteins underwent reaction with sera from a SIV_{mac} experimentally infected monkey (lane 1), a HIV-2-infected individual (lane 2), and a normal donor (ND) as well as a mouse monoclonal antibody directed against the HIV-2/SIV_{mac} major *gag* gene protein (p24–26), and control ascitic fluid (C). The most reactive and apparently most abundant viral proteins detected in the HIV-$2_{SBL/ISY}$ and SIV_{mac} virions were the *gag*-encoded p24–26 and p15 proteins (Fig. 3 *Left* and *Center*). Similar results were obtained when radiolabeled HIV-$2_{SBL/ISY}$ virion proteins were used in radioimmunoprecipitation (Fig. 3 *Left*). The envelope glycoprotein gp120 was barely detected by immunoprecipitation and not at all by Western blots (Fig. 3). The DNA sequence of the replication competent proviral clone lacks a termination codon in the transmembrane portion of the envelope gene and should yield a transmembrane envelope glycoprotein of ≈40 kDa. Indeed a very faint band around 40 kDa could be detected in radioimmunoprecipitation or Western blot assays of HIV-$2_{SBL/ISY}$ with positive human sera. However, a well-characterized specific antiserum will be needed to clearly define this protein band. A smear, probably representing proteins with different relative migration rates, was detected around 30 kDa in SIV_{mac}. This smear has been interpreted as the truncated form of the transmembrane protein (6), although the amino acid sequence after the termination codon is expressed in infected animals (37).

Host Range and Cytopathic Effect of HIV-2. The HUT 78 cell line producing the HIV-$2_{SBL/ISY}$ was expanded and virus was concentrated from the supernatant (10 liters) as described (38). We used the HIV-2_{NIH-Z} isolate in a parallel experiment to infect the same cell lines. Equal amounts of concentrated virus were then used to infect several human cell lines and replication and propagation of the virus were monitored by RT assay of the culture supernatant and immunofluorescence on fixed cells. The biological effect exerted by the HIV-2 isolates on the infected cells was

Table 1. Amino acid sequence comparison of different HIV-2 isolates

	gag	*pol*	*vif*	*vpx*	*env*	*nef*
HIV-$2_{SBL/ISY}$/HIV-2_{ROD}	11	9	16	13	20	21
HIV-$2_{SBL/ISY}$/HIV-2_{NIH-Z}	12	10	18	19	20	*
HIV-2_{NIH-Z}/HIV-2_{ROD}	8	9	14	17	20	*
HIV-$2_{SBL/ISY}$/SIV_{mac}	18	26	37	16	31	*
HIV-2_{NIH-Z}/SIV_{mac}	18	25	36	24	30	*
HIV-2_{ROD}/SIV_{mac}	13	17	27	15	30	*

Numbers represent percentage amino acid divergence among the proteins of HIV-2s and SIV_{mac} isolates.
*Analysis was not meaningful because of the presence of either large deletion or premature termination of the amino acid sequence for the *nef*-encoded protein.

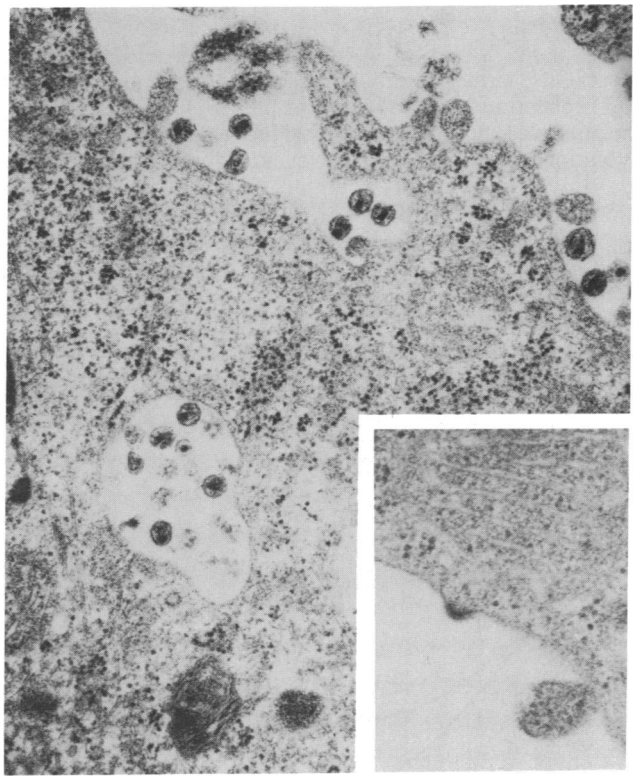

FIG. 2. Electron micrograph of the HIV-$2_{SBL/ISY}$. (*Inset*) Section in which a budding viral particle from a HUT 78 cell can be detected. Several mature virions with dense cylindrical or round core (depending on the plane of the section) can be seen in the remainder of the figure.

**The sequence reported in this paper has been deposited in the EMBL/GenBank data base (accession no. J04498).

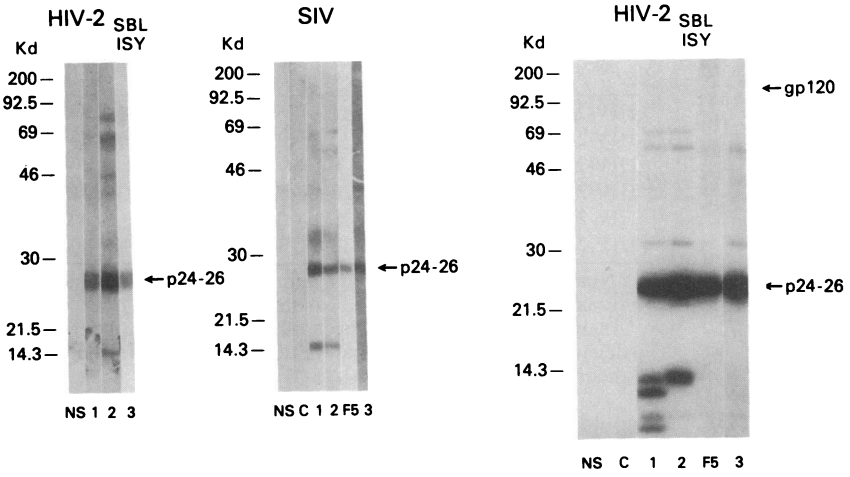

FIG. 3. Analyses of viral proteins. (*Left* and *Center*) Western blot analyses of total viral proteins obtained from disrupted HIV-$2_{SBL/ISY}$ and SIV virions, respectively. (*Right*) Immunoprecipitation of metabolically labeled HIV-$2_{SBL/ISY}$ virions. NS, normal human serum. Lanes: 1, serum from a macaque experimentally infected with SIV; 2 and 3, sera from human infected with HIV-2; c, control mouse ascite; F5, mouse monoclonal antibody directed against the p24 of SIV (36). The molecular mass of the proteins was calculated with respect to the relative migration of the protein marker (Rainbow, BRL).

measured by counting the number of viable cells and syncytia at different time intervals. The results, in detail, on the infectivity of the two HIV-2 isolates are shown only for the H9 and MOLT-3 cell lines (Fig. 4). HIV-$2_{SBL/ISY}$ as well as HIV-2_{NIH-Z} infected the human T-cell lymphotropic virus type I (HTLV-I)-transformed T-cell line MT-2; the T-cell clone 55 immortalized by a single defective copy of HTLV-I (39); and the CEM, HUT 78, MOLT-3, H9, and U937 neoplastic cell lines. The highest cytopathic effect exerted by both HIV-$2_{SBL/ISY}$ and HIV-2_{NIH-Z} was observed in the HTLV-I-infected cells and in the H9 cells, and it is coincident with the highest number of syncytia present in the cell culture (Fig. 4). The parental virus HIV-$2_{SBL6669}$ also infects HUT 78, U937 clone 16, CEM, and Jurkat T cells, and the highest cytopathic effect is observed on the Jurkat and U937-16 cell lines.

DISCUSSION

Most of the viral gene products that regulate viral expression and replication of HIV-1 are also present in HIV-2. In fact, the putative functional domains of the regulatory proteins are evolutionarily conserved. However, differences in the overall structure of the HIV-2 LTRs, which are larger than HIV-1 LTRs, account for a variation in the responsive region to the viral transactivator gene (*tat*) (40). More genetic information is also apparently needed to encode the tat and rev proteins as reflected by the study of functionally active SIV and HIV-2 cDNAs (41). However, the major structural differences appear to be in the presence of a gene (*vpu*) that is present only in type 1 virus (42) and another gene (*vpx*) that is present only in type 2 virus (21, 22). The amino acid sequences of these two genes are not significantly similar and whether they are functionally equivalent is still an open question.

The comparison of the predicted amino acid sequence of the viral proteins of HIV-$2_{SBL/ISY}$ revealed a divergence comparable to the divergence observed among HIV-1 Zairian isolates, which is higher than the divergence described among American isolates. The patients from whom the HIV-2 isolates were obtained originated from geographically neighboring African countries of Guinea Bissau (HIV-2_{NIH-Z}), Cape Verde Islands (HIV-2_{ROD}), and Gambia (HIV-$2_{SBL/ISY}$). The considerable degree of divergence among these HIV-2 isolates would suggest that HIV-2 has been present in the African population for a time similar to HIV-1.

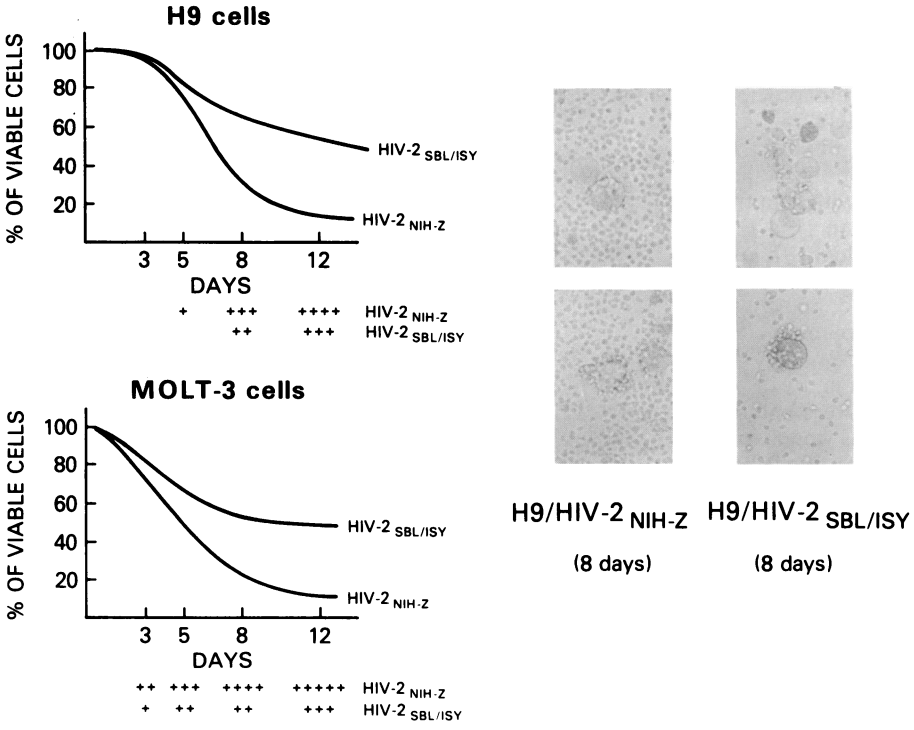

FIG. 4. (*Left*) Graph showing reduction of the number of viable cells in culture after infection with the HIV-2_{NIH-Z} and HIV-$2_{SBL/ISY}$ isolates. + to +++++, relative scale of number of syncytia seen in the cell culture at 3, 5, 8, and 12 days. (*Right*) One example of the size of syncytia serum at day 8 with both the viral isolates.

The availability of a replication competent HIV-2 proviral clone provides the tools to study the newly identified gene (*vpx*) as well as the role of the "nonessential" accessory viral genes in regulating viral replication as well as their role of the viral host range. The value of an infectious HIV-2 clone in developing an animal model for HIV vaccine and therapy studies should also be emphasized. In fact, the infectivity of HIV-$2_{SBL/ISY}$ is not restricted to human cells, but we have shown in a parallel study that this virus infects and kills fresh peripheral blood T cells from rhesus macaques *in vitro* and infects the same animals *in vivo*. Rapid progress in the development of a protective vaccine against HIV has been impaired by the lack of a suitable and cost-effective animal model. Successful infection of non-human primates has been achieved only in chimpanzees (43) and gibbons (P. Markham, personal communication), which are scarce, and to date these animals have not developed AIDS. Since the parental HIV-$2_{SBL6669}$ and the molecular clone HIV-$2_{SBL/ISY}$ productively infect rhesus macaques (unpublished results) and since macaques are sensitive to the pathogenic effect of SIV_{mac}, the development of an animal model using a highly related human virus will obviously be very valuable. Finally, we have recently shown that individual isolates of HIV-1 are composed of microvariants with distinct biological properties and susceptibility to given neutralizing sera (44). The composition of this population presumably drifts because of new mutations and selection in response to changes in available target cells and host immunity. The availability of an infectious molecular clone will allow us to measure the genetic evolution of the viral genome and its immunological consequences in the infected host.

We thank Dr. F. D. Veronese for the mouse anti-SIV p24 monoclonal antibody, J. Lemp for cultivating cell lines, and Dr. Kramarsky for electron microscopy on viral particles. Some of this work was supported by the U.S. Army Medical Research Acquisition Activity Contract DAM D17-86-C-6287. M.B. is a Fellow of the Dutch Organization for the Advancement of Pure Research ZWO.

1. Gallo, R. C. (1987) *Sci. Am.* **256**, 46–56.
2. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Brun-Vezinet, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220**, 868–870.
3. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224**, 500–503.
4. Sarngadharan, M. G., Popovic, M., Bruch, L., Schupbach, J. & Gallo, R. C. (1984) *Science* **224**, 506–508.
5. Clavel, F., Guetard, D., Brun-Vezinet, F., Chamaret, S., Rey, M. A., Santos-Ferreira, M. O., Laurent, A. G., Dauguet, C., Katlama, C., Rouzioux, C., Klatzmann, D., Champalimaud, J. L. & Montagnier, L. (1986) *Science* **233**, 343–346.
6. Albert, J., Bredberg, U., Chiodi, F., Bottiger, B., Fenyo, E. M., Norrby, E. & Biberfeld, G. (1987) *AIDS Res. Hum. Retroviruses* **3**, 1–10.
7. Gottlieb, M. S., Schroff, R., Schauher, H. M., Weisman, J. D., Fan, P. T., Wolf, R. A. & Saxon, A. (1981) *N. Engl. J. Med.* **305**, 1425–1431.
8. Zagury, J. F., Franchini, G., Reitz, M., Collalti, E., Starcich, B., Hall, L., Fargnoli, K., Jagodzinski, L., Guo, H.-G., Laure, F., Arya, S. K., Josephs, S., Zagury, D., Wong-Staal, F. & Gallo, R. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5941–5945.
9. Daniel, M. D., Letvin, N. L., King, N. W., Kannagi, M., Sehgal, P. K., Hunt, R. D., Kanki, P. J., Essex, M. & Desrosiers, R. C. (1985) *Science* **228**, 1201–1204.
10. Benveniste, R. E., Arthur, L. O., Tsai C.-C., Sowder, R., Copeland, T. D., Henderson, L. E. & Oroszlan, S. (1986) *J. Virol.* **60**, 483–490.
11. Fultz, P. M., McClure, H. M., Anderson, D. C., Brent-Swenson, R., Anand, R. & Srinivasan, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5286–5290.
12. Murphey-Corb, M., Martin, L. N., Rangan, S. R. S., Bashin, G. B., Gormus, B. J., Wolf, R. H., Andes, W. A., West, M. & Montelaro, R. C. (1986) *Nature (London)* **321**, 435–437.
13. Fukasawa, M., Miura, T., Hasegawa, A., Morikawa, S., Tsujimoto, H., Miki, K., Kitamura, T. & Hayami, M. (1988) *Nature (London)* **333**, 457–461.
14. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229**, 69–73.
15. Sodroski, J., Goh, W. C., Rosen, C., Dayton, A., Terwilliger, E. & Haseltine, W. (1986) *Nature (London)* **321**, 412–417.
16. Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46**, 807–817.
17. Knight, D. M., Flowerfelt, F. A. & Ghrayeb, J. (1987) *Science* **236**, 837–840.
18. Fisher, G. A., Ensoli, B., Ivano, H. L., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R. C. & Wong-Staal, F. (1987) *Science* **237**, 888–893.
19. Strebel, K., Daugherty, D., Clouse, K., Cohen, D., Folks, T. & Martin, M. A. (1987) *Nature (London)* **328**, 728–730.
20. Wong-Staal, F., Chanda, P. K. & Ghrayeb, J. (1987) *AIDS Res. Hum. Retroviruses* **3**, 33–40.
21. Franchini, G., Rusche, J. R., O'Keeffe, T. J. & Wong-Staal, F. (1988) *AIDS Res. Hum. Retroviruses* **4**, 243–250.
22. Henderson, L. E., Sowder, R. C., Copeland, T. D., Benveniste, R. E. & Oroszlan, S. (1988) *Science* **24**, 199–201.
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 282–285.
24. Franchini, G., Collalti, E., Arya, S. K., Fenyo, E. M., Biberfeld, G., Zagury, J. F., Kanki, P. J., Wong-Staal, F. & Gallo, R. C. (1987) *AIDS Res. Hum. Retroviruses* **3**, 11–17.
25. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
26. Sanger, F. Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
27. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–563.
28. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
29. Rosenberg, N. & Baltimore, D. (1978) *J. Exp. Med.* **147**, 1126–1141.
30. Biberfeld, P., Kramarsky, B., Salahuddin, S. Z. & Gallo, R. C. (1987) *J. Natl. Cancer Inst.* **79**, 933–941.
31. Towbin, H. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4353.
32. Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L. & Alizon, M. (1987) *Nature (London)* **326**, 662–669.
33. Franchini, G., Gurgo, C., Guo, H. G., Gallo, R. C., Collalti, E., Fargnoli, K. A., Hall, L. F., Wong-Staal, F. & Reitz, M. S. (1987) *Nature (London)* **328**, 539–543.
34. Chakrabarti, L., Guyader, M., Alizon, M., Daniel, M. D., Desrosiers, R. C., Tiollais, P. & Sonigo, P. (1987) *Nature (London)* **328**, 543–547.
35. Alizon, M., Wain-Hobson, S., Montagnier, L. & Sonigo, P. (1986) *Cell* **46**, 63–74.
36. Veronese, F. D., Joseph, B., Copeland, T. D., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1989) *J. Virol.*, in press.
37. Franchini, G., Kanki, P. J., Bosch, M., Fargnoli, K. A. & Wong-Staal, F. (1988) *AIDS Res. Hum. Retroviruses* **4**, 251–258.
38. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D. & Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7415–7419.
39. Aldovini, A., DeRossi, A., Feinberg, M., Wong-Staal, F. & Franchini, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 38–42.
40. Arya, S. K. (1988) *AIDS Res. Hum. Retroviruses* **4**, 175–186.
41. Colombini, S., Arya, S. K., Reitz, M. S., Jagodzinski, L., Beaver, B. & Wong-Staal, F. (1989) *Proc. Natl. Acad. Sci. USA*, in press.
42. Cohen, E. A., Terwilliger, E. G., Sodroski, J. G., & Haseltine, W. A. (1988) *Nature (London)* **334**, 532–534.
43. Alter, J. H., Eichberg, J. W., Masur, H., Saxinger, W. C., Gallo, R. C., Macher, A. M., Lane, H. C. & Fauci, A. S. (1984) *Science* **226**, 549–552.
44. Looney, D., Fisher, A. G., Putney, S. D., Rusche, J. R., Redfield, R. R., Burke, D. S., Gallo, R. C. & Wong-Staal, F. (1988) *Science* **241**, 357–359.