phosphorus); UpA, 5′-*O*-adenosyl 3′-*O*-uridyl phosphate; d[Cp(S)T], 5′-*O*-thymidyl 3′-*O*-cytidyl phosphorothioate; d[CpT], 5′-*O*-thymidyl 3′-*O*-cytidyl phosphate; d[Cp(S)G], 5′-*O*-guanosyl 3′-*O*-cytidyl phosphorothioate; 2′,3′-cUMP, uridine 2′,3′-cyclic monophosphate; 2′-UMP, uridine 2′-monophosphate; 3′-UMP, uridine 3′-monophosphate; HPLC, high-performance liquid chromatography; dNTPαS, 2′-deoxyribonucleoside 5′-*O*-(1-thiotriphosphate); and NTPαS, ribonucleoside 5′-*O*-(1-thiotriphosphate).

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17. When the electrophoresis period was short and the primer had not been run off the gel, a series of bands across all four lanes was observed. The uppermost band was unextended primer, whereas the lower bands presumably arose from primer that had been degraded by the 3′ to 5′ exonuclease activity of the Klenow fragment, but not then extended. As a test, we prepared a sequencing primer that contained a phosphorothioate group at the first internucleotide linkage from the 3′ end. Our expectations, based on precedent (*18, 19*) that this primer would be resistant to the exonuclease activity of the Klenow fragment, were confirmed, and only the unreacted primer was observed in all four lanes. However, this partial degradation of the primer does not interfere with reading the sequence.
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23 December 1987; accepted 28 April 1988

Characterization of a Noncytopathic HIV-2 Strain with Unusual Effects on CD4 Expression

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A new isolate of the human immunodeficiency virus type 2, designated HIV-2_{UC1}, was recovered from an Ivory Coast patient with normal lymphocyte numbers who died with neurologic symptoms. Like some HIV-1 isolates, HIV-2_{UC1} grows rapidly to high titers in human peripheral blood lymphocytes and macrophages and has a differential ability to productively infect established human cell lines of lymphocytic and monocytic origin. Moreover, infection with this isolate also appears to involve the CD4 antigen. However, unlike other HIV isolates, HIV-2_{UC1} does not cause cytopathic effects in susceptible T cells nor does it lead to loss of CD4 antigen expression on the cell surface. These results indicate that HIV-2 may be found in individuals with neurologic symptoms and that the biological characteristics of this heterogeneous subgroup can differ from those typical of HIV-1.

TWO PREVIOUSLY DESCRIBED ISOlates of HIV-2, LAV-2 [now called HIV-2_{ROD} (*1*)] and SBL6669 (*2*) obtained from West African patients with AIDS or AIDS-related disease induce cytopathic effects in cultured cells. Here we report the isolation and characterization of a noncytopathic HIV-2–like virus from a patient in the Ivory Coast. Its biologic properties in vitro appear distinct from many iso-

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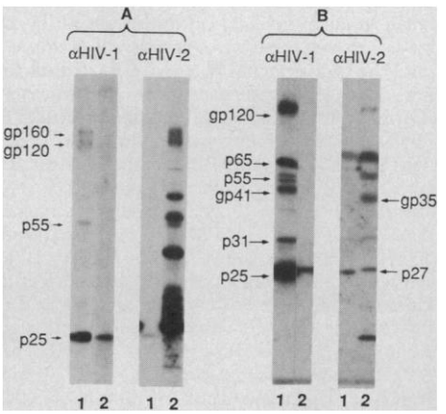

Fig. 1. Immunoblot analysis of HIV viral antigens. (**A**) Sera of the African patients from whom HIV-1_{SF471} (αHIV-1) and HIV-2_{UC1} (αHIV-2) were isolated were tested by immunoblot with electrophoretically separated HIV-1_{SF2}– [also called AIDS-associated retrovirus, ARV-2 (*15*)] (lane 1) and SIV_{mac}– (lane 2) infected HUT-78 cell lysates (*6*). (**B**) Isolates of HIV-1 and HIV-2 were grown to high titer (>1 × 10^6 cpm/ml of RT activity) and centrifuged (20,000*g*) through a layer of 20% glycerol for 2 hours. Viral pellets were suspended in 0.1 ml of PBS and stored at −70°C. Individual proteins were resolved by polyacrylamide gel electrophoresis and transferred to nitrocellulose for immunoblot analysis (*5*). Viral antigens present in the pellets from HIV-1_{SF471}– (lane 1) or HIV-2_{UC1}– (lane 2) infected cultures were reacted with serum from the patient infected with HIV-1_{SF471} (αHIV-1) or from the patient infected with HIV-2_{UC1} (αHIV-2). A similar result to that obtained with HIV-2 serum in lane 2 was observed with an antiserum to SIV_{mac}.

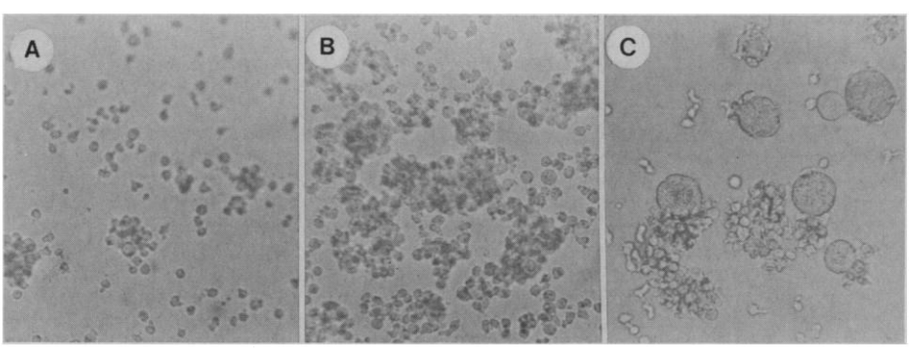

Fig. 2. Induction of cytopathology by cocultivation with uninfected SupT1 cells. (**A**) Uninfected; (**B**) HIV-2_{UC1}–infected (100% HIV antigen-positive); and (**C**) HIV-1_{SF471}–infected (35% HIV antigen-positive) SupT1 cells were mixed with uninfected SupT1 cells (99% $CD4^+$) at a ratio of 1:10 and observed for the formation of multinucleated giant cells and balloon degenerative forms. Note the CPE only in (C) (×400).

lates of HIV-1 and HIV-2 reported to date.

The restricted antigenic cross-reactivity between the *env* proteins of HIV-1 and HIV-2 and the homology between HIV-2 and the simian immunodeficiency virus [SIV_{mac} (*3*)] were used as criteria for classifying HIV infection in the Ivory Coast. Serum samples from 68 patients (*4*) attending the Treichville Hospital in Abidjan were analyzed by immunoblot (*5*) for reactivity to HIV-1 and SIV_{mac} external membrane antigens (*6*) and by ELISA for reactivity to type-specific immunodominant synthetic peptides derived from the transmembrane glycoproteins of either HIV-1 or HIV-2 (*7*). Consistent with recent studies conducted in the Ivory Coast (*8*), serologic evidence of HIV-1 infection (36 patients out of 58), HIV-2 infection (3 of 58), or dual HIV-1/HIV-2 reactivity (19 of 58) was observed in all but ten of the patients. Complete concordance was found with the results obtained from type-specific ELISAs and those observed with immunoblot assays measuring reactivity to the external glycoprotein of either HIV-1 or SIV_{mac}.

By cocultivation with mitogen-stimulated peripheral blood mononuclear cells (PMC) from normal donors, viruses were recovered from the PMC of seven patients with serologic evidence of HIV-1 infection, six patients with evidence of dual reactivity, and one patient with HIV-2 infection. When analyzed by immunoblot, all of the isolates from individuals with serologic evidence of HIV-1 or HIV-1/HIV-2 infection were identified as HIV-1. An HIV-2–like virus ($HIV\text{-}2_{UC1}$) was isolated from the patient with serologic evidence of only HIV-2 infection (*7*) (Fig. 1A). Although the patient presented with normal numbers of peripheral blood lymphocytes, he had evidence of multiple parasitic bowel infections and developed high fever, ataxia, and dementia before death (*9*). Electron microscopic studies revealed that $HIV\text{-}2_{UC1}$ had the morphologic features of a lentivirus (*10*).

Like other HIV-2 isolates (*1, 2*), $HIV\text{-}2_{UC1}$ was more similar antigenically to SIV than HIV-1 (Fig. 1B); only the *gag* and polymerase proteins (p25 and p65, respectively) of HIV-1 were recognized by the sera of the $HIV\text{-}2_{UC1}$–infected individual. Conversely, sera from HIV-1–infected individuals, both from the United States and Africa, detected only the *gag* proteins of $HIV\text{-}2_{UC1}$. Moreover, sera from SIV-infected rhesus monkeys strongly recognized the *env* proteins of $HIV\text{-}2_{UC1}$ but not those of HIV-1. As with $HIV\text{-}2_{ROD}$ (*1*), the external glycoprotein of $HIV\text{-}2_{UC1}$ migrated at approximately 140 kD (Fig. 1B) and not 120 kD as reported for HIV-1. However, several African HIV-1 isolates characterized in this laboratory (including $HIV\text{-}1_{SF471}$; Fig. 1B) also have an external glycoprotein of approximately 140 kD (*11*). The transmembrane glycoprotein of $HIV\text{-}2_{UC1}$ appears to be truncated, with an apparent size of 35 kD (compared to 41 kD for HIV-1). This smaller size of the HIV-2 transmembrane protein compared to that of HIV-1 is reported to be due to a stop codon located within the *env* gene (*12*). Finally, as noted with $HIV\text{-}2_{ROD}$ (*1*), the major core protein of $HIV\text{-}2_{UC1}$ had a slightly higher molecular weight (p27) than that of HIV-1 (p25; Fig. 1B).

The original cultures of all 14 isolates showed peak reverse transcriptase (RT) activity (≥400,000 cpm/ml) within 10 days. Cytopathic effects (CPE), defined as syncytia formation and balloon degeneration (*13*), were observed in all the PMC infected with the Ivory Coast HIV-1 isolates, but were notably absent from the $HIV\text{-}2_{UC1}$ infected PMC culture.

The host range of $HIV\text{-}2_{UC1}$ was studied by using PMC of human and primate origin (*14*) as well as established lines of human T and monocytic cells and peripheral blood macrophages. A comparison was made with two HIV-1 isolates, one from a San Franciscan patient [$HIV\text{-}1_{SF2}$ (*15*)] and the other from a patient in the Ivory Coast [$HIV\text{-}1_{SF471}$ (*16*)]. Like previous isolates of HIV-1 (*14, 17*), $HIV\text{-}2_{UC1}$ varied in its ability to productively infect different cell types (Table

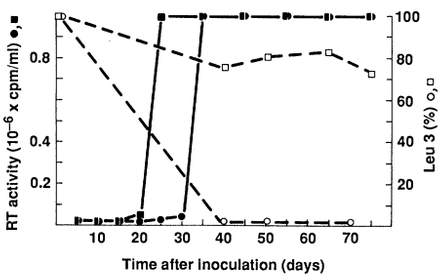

Fig. 3. Comparative cell surface expression of CD4 antigen after infection with $HIV\text{-}2_{UC1}$ or HIV-1. Cultures of the SupT1 cells that were inoculated with either $HIV\text{-}1_{SF471}$ (○ and ●) or $HIV\text{-}2_{UC1}$ (□ and ■) (input inoculum; 200,000 cpm of RT activity) were followed for both virus replication (appearance of RT activity: ● and ■) and the cell surface expression of the CD4 antigen (leu3a FACS: ○ and □). Uninfected cultures of the SupT1 cell line were 99% CD4 antigen positive. Multinucleated giant cell and balloon formation, followed by cell death, was observed only in SupT1 cell cultures inoculated with $HIV\text{-}1_{SF471}$.

Table 1. Comparative host range of HIV-1 isolates and $HIV\text{-}2_{UC1}$. For the infectivity studies, PMC from donors (seronegative for HIV-1 and HIV-2) were prepared by Ficoll/Hypaque separation of heparinized blood (*14*), treated for 3 days with phytohemagglutinin (PHA;3 μg/ml), and cultured in the presence of IL-2 (5%, Electonucleonics). For the preparation of peripheral blood macrophages, PMC were resuspended in RPMI 1640 medium containing 10% fetal calf serum and 5% human serum and dispensed into 25-cm^2 flasks (Falcon) (2×10^7 PMC per flask). The CEM and Jurkat T cell lines and the human monocytic cell line U-937 were purchased from the American Type Tissue Culture Collection. The K4 B cell line was prepared by Epstein-Barr virus transformation (*27*) of peripheral blood B cells from a HIV antibody-negative individual. Productive HIV infection of PMC and peripheral blood macrophages was assessed for 30 days; growth in established human cell lines was followed for 60 days. Virus replication was measured by particle-associated RT activity in the culture supernatants (*28*). Infection of cells was confirmed by indirect immunofluorescent assays for viral antigens (*29*). Symbols: +++, ≥500,000 counts per minute (cpm)/ml; ++, 100,000 to 500,000 cpm/ml; +, 20,000 to 100,000 cpm/ml; ±, 10,000 to 20,000 cpm/ml; −, < 10,000 cpm/ml; ND, not done. The inhibition experiments were performed as described previously and the results are expressed as RT activity (cpm × 10^3 per milliliter; percentage reduction compared to the untreated controls) (*20, 23*).

Cell or antibody	$HIV\text{-}1_{SF2}$	$HIV\text{-}1_{SF471}$	$HIV\text{-}2_{UC1}$
	Infectivity studies		
Cells			
Human PMC	+++	+++	+++
Chimpanzee PMC	+	ND	±
Baboon PMC	ND	ND	±
Rhesus monkey PMC	ND	ND	±
Human peripheral blood macrophages	−	±	+++
HUT-78	+++	+++	+++
SupT1	+++	+++	+++
CEM	+++	+++	−
Jurkat	+++	+++	+++
MT-4	+	+++	++
U-937	++	+++	−
K4 B cell line	−	ND	+
	Inhibition studies		
Monoclonal antibodies			
Leu4	168.9 (0%)	ND	1992.8 (0%)
Leu3a	2.6 (98%)	ND	1.5 (99%)

1). All the PMC cultures and most established human T cell lines were susceptible to the virus, as was the K4 B cell line. However, replication was not observed in the CEM T cell or the U937 monocytic cell lines that supported infection by the HIV-1 isolates. Moreover, unlike the HIV-1 isolates, HIV-2_{UC1} grew well in peripheral blood macrophages; this characteristic has been observed primarily with HIV-1 isolates from the brain (*18*).

Although both HIV-1_{SF2} and HIV-1_{SF471} infection of established T cell lines resulted in dramatic CPE, no such CPE was observed in any of the HIV-2_{UC1} infected cultures despite the rapid release of high titers of infectious virus. Atypical large cells that constituted less than 5% of the culture were seen only transiently in the HIV-2_{UC1}–inoculated established cell lines at the onset of progeny virion production.

The induction of CPE after infection with HIV-2_{UC1} and HIV-1 isolates was further assessed by mixing virus-infected cells with uninfected T cell lines expressing high levels of CD4 antigen. As shown in Fig. 2, the cultures containing HIV-2_{UC1}–infected cells showed no syncytia whereas the HIV-1_{SF471} infected cells produced extensive CPE when mixed with all of the uninfected $CD4^+$ cells. When HIV-2_{UC1}–infected SupT1 cells were mixed with HIV-1_{SF471}–infected cells that had emerged from a cytopathic infection, dramatic CPE was observed. This finding suggested that the CD4 molecule, which appears to be essential for syncytia formation (*13*), is still expressed on the surface of HIV-2_{UC1}–infected SupT1 cells.

Fluorescence-activated cell sorting (FACS) was used to quantitate the expression of CD4 on the surface of T cells infected with HIV-2_{UC1}. In a control experiment in which we used SupT1 cells inoculated with HIV-1_{SF471} (35% of cells were HIV antigen positive by immune fluorescence assay), the surface expression of the CD4 protein declined concomitantly with the appearance of high levels of RT activity in the supernatant. No such reduction in CD4 expression occurred in cells infected with HIV-2_{UC1} (Fig. 3). The same observations were made when $CD4^+$ PMC [obtained by panning (*19*)] were inoculated with HIV-2_{UC1}. Ninety percent of the infected cells, of which 30% were HIV antigen positive by indirect immunofluorescence, still expressed CD4 on the cell surface. These results are markedly different from those reported for HIV-1 infection of $CD4^+$ cells (*20, 21*). Moreover, in both enriched $CD4^+$ PMC and established T cell lines, no reduction in cell viability (determined by trypan blue exclusion) was noted after HIV-2_{UC1} infection. A 20% reduction in the rate of cell division was observed in the HIV-2_{UC1} infected SupT1 cells, but this effect on cell replication has also been noted with isolates of HIV-1 (*22*).

To confirm the presence of cells expressing both HIV-2_{UC1} and CD4 antigens in the infected cell population, we performed double-staining experiments using sera from HIV-infected patients and monoclonal antibodies to the CD4 protein (leu3a, Becton-Dickinson). As shown in Fig. 4A, cells that expressed both antigens on their cell surface were present in the HIV-2_{UC1}–infected cultures. Cells infected with HIV-1_{SF471} were negative for the expression of the CD4 protein (Fig. 4C). From the HIV-2_{UC1}–infected SupT1 cell culture, a clone (C6) was obtained by limiting dilution in which 100% of the cells expressed viral proteins and 80% of the cells expressed CD4 on the cell surface. When these clonal cells were used in cocultivation experiments with uninfected SupT1 cells, no CPE was observed. However, prominent CPE was seen when C6 cells were mixed with HIV-1_{SF471}–infected SupT1 cells.

Experiments were then conducted to determine if HIV-2_{UC1} uses the same cellular receptor as HIV-1 (*20*). Inhibition of infection of PMC (>90%) was observed only when monoclonal antibodies to CD4a and not the CD3 protein (cell surface antigen common to all T cells) were used [Table 1 (*23*)].

HIV-2_{UC1} thus differs from other HIV-2 isolates reported to date with respect to its lack of cytopathogenicity and failure to suppress CD4 antigen expression in infected cells. In our laboratory, all HIV-1 isolates that productively infect established T cell lines induce dramatic CPE and reduce the surface expression of the CD4 antigen. HIV-2_{ROD} and HIV-$2_{SBL6669}$ are both cytopathic in culture (*1, 2*). Moreover, HIV-2 isolates recently obtained in our laboratory from the PMC of other West African patients have been found to induce CPE in vitro and to reduce CD4 antigen expression.

The isolation of HIV-2_{UC1} from an individual with parasitic bowel infections and neurologic abnormalities suggests that this virus could be associated with disease. HIV-1 isolates from AIDS patients with neurologic disorders also replicate efficiently in macrophages (*18*) and may be less cytopathic than other HIV-1 isolates (*24*). HIV-1 has also been recovered from individuals with neurologic symptoms but near normal T helper cell numbers (*25*). However, the possibility that another disease-producing virus strain was present in this Ivory Coast patient cannot be excluded. Recent studies suggest that the CPE-inducing properties of HIV-1 become more pronounced as the disease progresses (*26*). Conceivably, subsequent isolates from this patient before death could have been cytopathic, but no further blood was available.

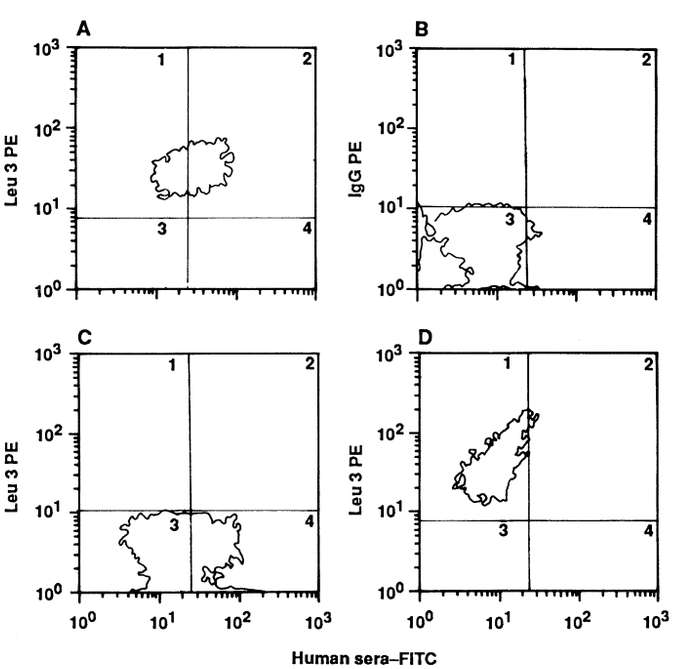

Fig. 4. Detection of viral and CD4 antigens by FACS. HUT-78 cells that were infected with HIV-2_{UC1} (**A**) or HIV-1_{SF471} (**B** and **C**) were analyzed for the surface expression of both HIV (*X* axis) and CD4 antigens (*Y* axis). For the detection of HIV antigens, cells were first mixed with a pool of sera from both HIV-1– and HIV-2–infected individuals (A, C, **D**), and then reacted with FITC-labeled goat antiserum to human IgG (Becton-Dickinson). CD4 antigens were subsequently detected by using a phycoerythrin-labeled murine monoclonal antibody specific for CD4 (leu3a, Becton-Dickinson). Neither of the infected cell lines showed reactivity with sera from antibody-negative individuals or with a phycoerythrin-labeled control mouse monoclonal antibody specific for human IgG (Becton-Dickinson) (B). Uninfected HUT-78 cells showed reactivity only with leu3 (D).

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29 March 1988; accepted 5 May 1988

West African HIV-2–Related Human Retrovirus with Attenuated Cytopathicity

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Clinical and seroepidemiological studies in West Africa indicate that human immunodeficiency virus type 2 (HIV-2) is widespread and associated with immunodeficiency states of variable degree. In this study, an isolate of HIV-2 from a patient in Senegal was molecularly cloned and characterized. This isolate (HIV-2_{ST}) was shown by hybridization and restriction enzyme analysis to be more related to the prototype HIV-2_{ROD} than to other human or primate retroviruses. Cultures of HIV-2_{ST} showed genotypic polymorphism, and clones of the virus had transmembrane envelope glycoproteins of 30 and 42 kilodaltons. Unlike other immunodeficiency viruses, HIV-2_{ST} did not cause cell death or induce cell fusion in peripheral blood lymphocytes or in any of four $CD4^+$ cell lines tested. Although HIV-2_{ST} entered cells by a CD4-dependent mechanism and replicated actively, cell-free transmission of the virus was retarded at the level of cell entry. These findings suggest that immunodeficiency viruses prevalent in West African populations are members of the HIV-2 virus group and that certain strains of this virus have attenuated virulence.

HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV-1) is the etiologic agent of epidemic AIDS in Central Africa, Europe, the United States, and most countries worldwide (*1*). Sporadic cases of AIDS, especially in West Africa, have been attributed to a different but related retrovirus, HIV-2 (*2*, *3*). Although HIV-2 may cause fatal immunodeficiency (*3*), studies (*4*, *5*) have suggested that many West African individuals infected with HIV-2 or a related virus may have less severe immunodeficiency than individuals infected with HIV-1. For those studies, serum samples from several West African populations were tested by immunoblot and radioimmunoprecipitation (RIP) assays with viral-specific target antigens derived from cultures of HTLV-4_{PK82} (*6*), a virus strain indistinguishable from a simian immunodeficiency virus of macaques (SIV_{mac}) (*7*–*10*). We have now characterized an isolate of HIV-2 from a healthy prostitute in Senegal, West Africa, that is less cytopathic than the prototype HIV-2_{ROD}.

HIV-2_{ST} was isolated from peripheral blood mononuclear cells (PBMC) of patient ST as described (*8*). Virus was transmitted to four immortalized T cell lines (Hut78, H9, SupT1, and CEM×174) by repeated PEG (polyethylene glycol) precipitations of primary culture supernatants in an attempt to isolate and amplify viruses with potentially attenuated virulence. The DNA genome of HIV-2_{ST} in these cultures hybridized at low and high stringency to the HIV-2_{ROD} probe (Fig. 1B, lanes 5 and 6), whereas SIV_{mac} DNA hybridized to the HIV-2_{ROD} probe only under conditions of low stringency (Fig. 1B, lanes 3). Conversely, HIV-2_{ROD} and HIV-2_{ST} DNA hybridized with equal intensity to SIV_{mac} probe, but only at low stringency, and a full-length HTLV-I probe showed no hybridization (*11*). Two

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