J. Med. **316**, 1180 (1987); M. Guyader *et al.*, *Nature* **326**, 662 (1987).
2. J. Albert *et al.*, *AIDS Res. Hum. Retrovir.* **3**, 3 (1987); S. K. Arya *et al.*, *Nature* **328**, 548 (1987).
3. F. Barin *et al.*, *Lancet* ii, 1387 (1985); P. J. Kanki *et al.*, *Science* **232**, 238 (1986); P. J. Kanki *et al.*, *ibid.* **236**, 827 (1987).
4. The major clinical symptom of the seropositive patients was chronic diarrhea. Twenty HIV-1, all the HIV-2, and 13 dually seropositive individuals had this symptom. Other clinical findings included pulmonary disease in seven individuals seropositive for HIV-1 and seven with dually reactive sera. Neurologic findings were found in six HIV-1 and one HIV-2 (present case) seropositive patients. Other symptoms of the patients included lymphadenopathy and herpes zoster. Four HIV-1 and one dually seroreactive individual were asymptomatic. The seronegative individuals generally had fever or cough, or both. One had carcinoma.
5. L.-Z. Pan *et al.*, *J. Infect. Dis.* **155**, 626 (1986).
6. SIV was obtained as a disrupted cell lysate from P. Marx, University of California, Davis. HIV-1_{SF2} lysates were made from HUT-78–infected cells (*5*). Immunoblot reactivity to the external glycoprotein of either SIV_{mac} or HIV-1_{SF2} was considered indicative of type specificity. Indirect ELISAs were performed using either a 12– or 11–amino acid peptide sequence from the transmembrane protein of HIV-1 and HIV-2, respectively (*7*).
7. J. W. Gnann, Jr., *et al.*, *Science* **237**, 1346 (1987); J. W. Gnann, Jr., *et al.*, *J. Infect. Dis.* **156**, 261 (1987).
8. F. Denis *et al.*, *Lancet* i, 408 (1987).
9. HIV-2_{UC1} was isolated from an individual from Mali who was hospitalized in Abidjan with chronic diarrhea and weight loss. He had intermittent fever, but no lymphadenopathy. His stool specimen indicated infection with strongyloides, cryptosporidia, and *Isospora belli*. His white cell count was 4900 per cubic millimeter with 1960 lymphocytes per cubic millimeter. Prior to death, he developed neurologic findings with ataxia and dementia and had a markedly high fever.
10. L. S. Oshiro, personal communication.
11. C. Cheng-Mayer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2815 (1988).
12. F. Clavel, *AIDS* **1**, 135 (1987); B. Guy *et al.*, *Nature* **330**, 266 (1987).
13. J. Sodroski *et al.*, *Nature* **322**, 470 (1986); J. D. Lifson *et al.*, *ibid.* **323**, 725 (1986).
14. J. A. Levy *et al.*, *Virology* **147**, 441 (1985); J. A. Levy and J. Shimabukaro, *J. Infect. Dis.* **152**, 734 (1985).
15. J. A. Levy *et al.*, *Science* **225**, 840 (1984).
16. HIV-1_{SF471} was isolated from a patient from Burkina Faso who showed serologic evidence of HIV-1 infection only. This patient, hospitalized in Abidjan, had a history of chronic diarrhea (weight loss >40 pounds) with cryptosporidiosis. He had x-ray evidence of pneumonitis compatible with *Pneumocystis carinii* pneumonia but no definite diagnosis could be made. His white cell count was 1900 per cubic millimeter with 720 lymphocytes per cubic millimeter. He died approximately 1 month after this isolate was obtained.
17. J. A. Levy, *Virology* **147**, 441 (1985); L. A. Evans *et al.*, *J. Immunol.* **138**, 3415 (1986); C. Cheng-Mayer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3526 (1987).
18. S. Gartner *et al.*, *Science* **233**, 215 (1986); Y. Koyangai *et al.*, *ibid.* **236**, 819 (1987); C. Cheng-Mayer and J. A. Levy, *Ann. Neurol.* **23**, S58 (1988).
19. L. J. Wysocki and V. L. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2844 (1978); C. M. Walker *et al.*, *Science* **234**, 1563 (1986).
20. A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984); D. Klatzmann *et al.*, *ibid.* **312**, 767 (1984); J. A. Hoxie *et al.*, *Science* **229**, 1400 (1985); J. S. McDougal *et al.*, *ibid.* **231**, 382 (1986).
21. J. A. Hoxie *et al.*, *Science* **234**, 1123 (1986).
22. L. A. Evans, unpublished data.
23. Briefly, 10^5 human PMC (previously stimulated with phytohemagglutinin for 3 days) were incubated with 5 μg of monoclonal antibody (Becton-Dickinson) in 0.1 ml for 30 min at room temperature. Control cultures received no monoclonal antibody. Leu3a recognizes the CD4a antigen and leu4, the CD3 antigen. Virus (0.1 ml; RT activity, 10^6 cpm/ml) was added and the cells were incubated at 37°C for 2 hours before they were washed with PBS and resuspended in 2 ml of medium containing IL-2. At day 3 after inoculation, 2×10^5 fresh PMC were added. The percentage reduction of RT activity in the culture fluid after 9 days was calculated for both leu3a- and leu4-treated PMC using untreated cultures as a reference.
24. R. Anand *et al.*, *Lancet* i, 234 (1987).
25. J. A. Levy *et al.*, *Ann. Inst. Pasteur* **138**, 101 (1987).
26. C. Cheng-Mayer *et al.*, *Science* **240**, 80 (1988).
27. L. A. Evans *et al.*, *J. Immunol.* **140**, 8061 (1988); J. A. Levy *et al.*, in *Vaccines 1987*, H. Ginsberg, R. Lerner, R. Chanock, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988), pp. 168–173.
28. A. Hoffman *et al.*, *Virol.* **147**, 326 (1985).
29. L. Kaminsky *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5535 (1985).
30. We thank A. Gazdar, J. Hoxie, and N. Yamamoto for providing the HUT-78, SupT1 (*21*), and MT4 human T cell lines, respectively, and J. Gnann, Jr., Scripps Clinic and Research Institute, for the ELISA studies with type-specific HIV peptides. This research was supported by NIH grants AI-24499 and PO1 AI-24286.

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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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different HIV-2_{ST} producing cell lines derived from the transmission of cell-free virus from primary ST lymphocyte cocultures showed similar proviral restriction enzyme cleavage patterns but were distinguishable by polymorphic Xba I and Bam HI sites (Fig. 1C). A recombinant DNA λ phage library prepared from one of these cell lines, Hut 78/B12, which represented a biologically cloned high-producer line, yielded three full-length proviral DNA clones (Fig. 1A). Comparative restriction mapping confirmed the genetic similarity between HIV-2_{ST} and HIV-2_{ROD} (10 of 25 restriction sites in common) and the dissimilarity between HIV-2_{ST} and SIV_{mac} (2 of 36 restriction sites in common). Furthermore, one of these proviral DNA clones (λJSP4-27), when transfected into T lymphocyte cell lines, produced virus that was replication competent and infectious and whose biological properties were identical to the parental virus isolate. Thus, HIV-2_{ST} is genotypically more related to HIV-2_{ROD} than to other primate viruses, and within the HIV-2_{ST}

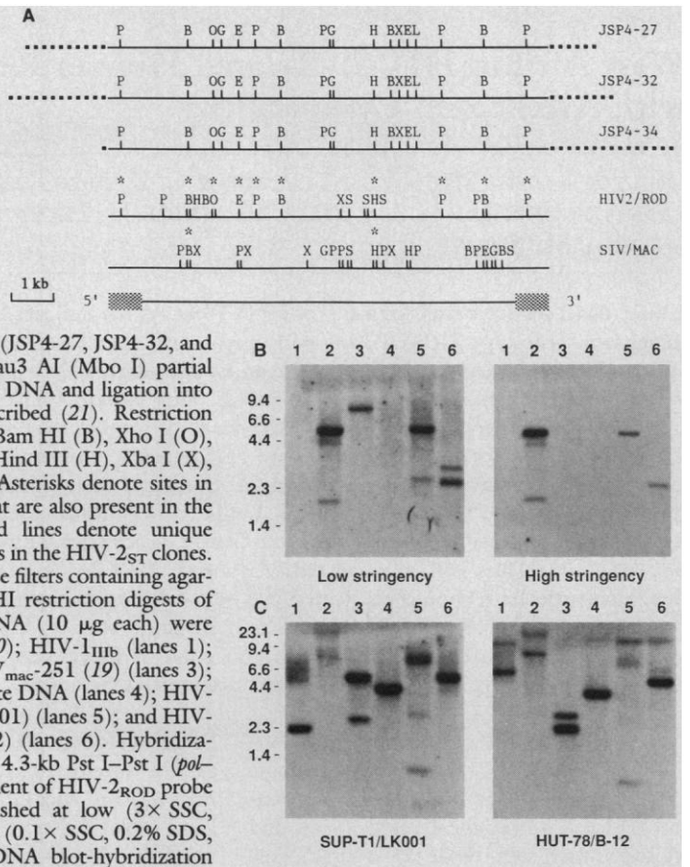

Fig. 1. (**A**) Comparison of full-length HIV-2_{ST}, HIV-2_{ROD}, and SIV_{mac} proviral DNA clones. (**B**) Genetic characterization of HIV-2_{ST} by differential nucleic acid hybridization. (**C**) Restriction enzyme analysis of HIV-2_{ST} DNA. (A) Restriction maps of three full-length recombinant proviral DNA clones of HIV-2_{ST} (JSP4-27, JSP4-32, and JSP4-34) obtained by Sau3 AI (Mbo I) partial digestion of Hut 78/B12 DNA and ligation into J1-lambda phage, as described (*21*). Restriction enzymes were Pst I (P), Bam HI (B), Xho I (O), Bgl II (G), Eco RI (E), Hind III (H), Xba I (X), Sal I (L), and Sst I (S). Asterisks denote sites in HIV-2_{ROD} or SIV_{mac} that are also present in the HIV-2_{ST} clones. Dotted lines denote unique flanking cellular sequences in the HIV-2_{ST} clones. (B) Identical nitrocellulose filters containing agarose gel separated Bam HI restriction digests of virus-infected cellular DNA (10 μg each) were prepared as described (*20*); HIV-1_{IIIb} (lanes 1); HIV-2_{ROD} (lanes 2); SIV_{mac}-251 (*19*) (lanes 3); normal human lymphocyte DNA (lanes 4); HIV-2_{ST} (cell line SupT1/LK001) (lanes 5); and HIV-2_{ST} (cell line Hut78/B12) (lanes 6). Hybridization was to a ^{32}P-labeled 4.3-kb Pst I–Pst I (*pol*–central region–*env*) fragment of HIV-2_{ROD} probe (A) and filters were washed at low (3× SSC, 0.2% SDS, 55°C) or high (0.1× SSC, 0.2% SDS, 65°C) stringency. (C) DNA blot-hybridization restriction cleavage analysis with the same probe as in (B) and performed as described (*20*). SupT1/LK001 and Hut 78/B12 are permanently producing HIV-2_{ST}–infected cell lines, the latter derived by single-cell cloning of a primary Hut 78–infected line. Restriction enzymes were Xba I (lanes 1), Xho I (lanes 2), Bam HI (lanes 3), Eco RI (lanes 4), Hinc II (lanes 5), and Kpn I (lanes 6).

Table 1. Time course of viral infection by HIV-1_{IIIb}, HIV-2_{ROD}, and HIV-2_{ST} assessed by in situ hybridization (*26*). The relative amount of viral mRNA production per cell was scored qualitatively from silver grain densities: −, absent, to ++++, too numerous to count. Although there was a marked delay in the initial development and spread of productive viral infection by HIV-2_{ST}, the few cells that became productively infected at early time points expressed equal amounts of viral RNA on a per cell basis compared with prototype HIV-1 and HIV-2 viruses (ND, not done).

Isolate	Cells expressing viral mRNA (%)	Viral mRNA per cell
	Day 1 after infection	
HIV-1_{IIIb}	3	++++
HIV-2_{ROD}	8	++++
HIV-2_{ST}	0.01	++++
Control	0	−
	Day 3 after infection	
HIV-1_{IIIb}	35	++++
HIV-2_{ROD}	45	++++
HIV-2_{ST}	0.01	++++
Control	0	−
	Day 7 after infection	
HIV-1_{IIIb}	100	++++
HIV-2_{ROD}	100	++++
HIV-2_{ST}	0.5	++++
Control	0	−
	Day 10 after infection	
HIV-1_{IIIb}	100	++++
HIV-1_{ROD}	100	++++
HIV-2_{ST}	10	++++
Control	0	−
	Day 14 after infection	
HIV-1_{IIIb}	ND	ND
HIV-2_{ROD}	ND	ND
HIV-2_{ST}	50	++++
Control	0	−

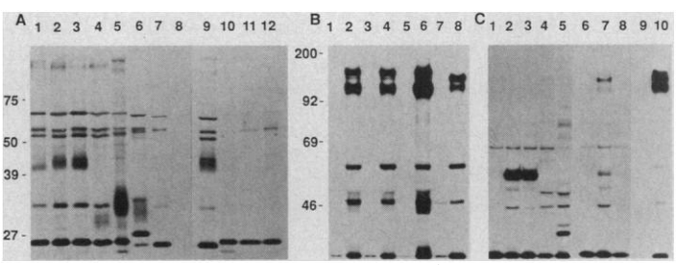

Fig. 2. (**A**) Western immunoblot and (**B** and **C**) RIP analysis of HIV-2_{ST}, HIV-2_{ROD}, SIV_{mac}, and HIV-1_{IIIb}. (A) Immunoblots were performed (*22*) with a human (West African) antiserum to HIV-2 (lanes 1 to 8) and a human antiserum to HIV-1 (lanes 9 to 12). Bound antibody was detected with peroxidase-conjugated goat antibody to human immunoglobulin. Antigen preparations were viral lysates from HIV-2_{ST} line SupT1/LK001 (lanes 1 and 12); single-cell derived clones from SupT1/LK001 designated ST.17 (lane 2), ST.9 (lane 3), ST.24 (lanes 4 and 11); HIV-2_{ROD} (lanes 5 and 10); SIV_{mac} (*19*) (lanes 6); HIV-1_{IIIb} (lanes 7 and 9); uninfected control cells (lane 8). (B) RIP of HIV-2_{ST} proteins from cloned cell lines of infected SupT1 cells shown in (A). Cell lysates from [^{35}S]cysteine– and [^{35}S]methionine–labeled clones were prepared and immunoprecipitated as described (*23*), with either normal human serum (lanes 1, 3, 5, and 7) or serum from a West African patient with HIV-2 infection (lanes 2, 4, 6, and 8). Shown are clones designated ST.9 (lanes 1 and 2), ST.17 (lanes 3 and 4), ST.23 (lanes 5 and 6) that have an *env* precursor molecule of approximately 180 kD, and ST.24 (lanes 7 and 8) that has an *env* precursor molecule of 170 kD. The gp170 of ST.24 corresponds to the truncated transmembrane protein (gp30) of the same virus (A). (**C**) Coprecipitation of HIV-2_{ST} envelope glycoproteins with CD4. Cell lysates from [^{35}S]cysteine– and [^{35}S]methionine–labeled uninfected SupT1 cells (lanes 1 to 5) and HIV-2_{ST}–infected SupT1/LK001 cells (lanes 6 to 10) were prepared and immunoprecipitated (*23*) with OKT3 (lanes 1 and 6), OKT4 (lanes 2 and 7), OKT4A (lanes 3 and 8), normal human serum (lanes 4 and 9), or human serum from a patient with HIV-2 infection (lanes 5 and 10). Both OKT4 and OKT4A immunoprecipitate the 56-kD CD4 molecule from the uninfected cells. However, in the infected cells OKT4 (but not OKT4A) immunoprecipitates CD4 as well as three molecular species of high molecular weight corresponding to viral *env* gene products that are also immunoprecipitated by immune human serum (lane 10).

isolate there are polymorphic genotypes some of which are fully replication competent.

The structural, antigenic, and functional characteristics of HIV-2_{ST} proteins were evaluated by immunoblot and RIP analyses (Fig. 2). The putative major *gag* structural protein and precursor (p26/pr55) of HIV-2_{ST} were comparable in size to p26/pr55 of HIV-2_{ROD}, p27/pr55 of SIV_{mac}, and p24/pr53 of HIV-1. Polymerase and endonuclease proteins of HIV-2_{ST} were tentatively identified as p64 and p34 with equivalent counterparts in HIV-2_{ROD}, SIV_{mac}, and HIV-1. The *env* proteins of HIV-2_{ST} revealed a 140-kD putative extracellular protein similar to that in HIV-2_{ROD}. However, in different subcultures of HIV-2_{ST} that were derived by single-cell cloning of the original SupT1/LK001 cell line, the putative transmembrane proteins (TMP) were either 42 or 30 kD in size. Only smaller sized transmembrane proteins were seen in HIV-2_{ROD} (36 kD) and SIV_{mac} (32 kD) cultures. The HIV-2_{ST} viruses with large and small TMPs could be transmitted cell free, were replication competent, and retained the same size of TMP throughout passage in the same cell targets (SupT1 and CEM×174). This suggests that the observed differences in TMP sizes were due to nucleotide sequence differences in the expressed mRNAs, not to post-transcriptional modifications such as altered glycosylation patterns. Consistent with this suggestion is the finding of translational stop codons in HIV-2_{ROD} and SIV_{mac} viral envelope genes that result in the expression of truncated TMPs (*12*).

Antigenically, HIV-2_{ST} and HIV-2_{ROD} were similar to each other and to SIV_{mac} in their reactivity with HIV-2 antisera and were cross-reactive with HIV-1 only in the major *gag* structural proteins (p24-p27). This was confirmed by bidirectional immunoblotting in which both HIV-1– and HIV-2–specific human antisera were used to probe HIV-1, HIV-2_{ROD}, and HIV-2_{ST} proteins (Fig. 2A). By RIP, we found *env* precursor proteins of approximately 180 or 170 kD in the different HIV-2_{ST} subcultures (reflecting the different sizes of their respective TMPs) and mature extracellular *env* proteins of 140 kD (Fig. 2B). By RIP we also found that, like HIV-1, the HIV-2_{ST} external *env* glycoprotein bound directly to an epitope on CD4 recognized by OKT4A (Fig. 2C), but not by OKT4 (*13*). Other experiments (*14*) with fluorescence activated cell sorting analysis showed that infection of $CD4^+$ cells by HIV-2_{ST} virus down-modulated the expression of $CD4^+$ but not CD8, again analogous with HIV-1 infection (*15*).

Because certain West African human populations infected with HIV-2–related viruses may have less severe immunodeficiency than individuals infected with HIV-1 or some isolates of HIV-2, including HIV-2_{ROD} (*4*, *5*), we evaluated HIV-2_{ST} for cytopathic and cell killing properties in vitro. We first studied the induction of syncytia by HIV-2_{ST}–infected cells cocultured with $CD4^+$ cells. Both HIV-1_{IIIb} and HIV-2_{ROD}, but not HIV-2_{ST}, produced large syncytia when virus-infected cells were cocultured with uninfected $CD4^+$ cells (Fig. 3). Hela-T4, H9, and Hut 78 indicator cells gave identical results. Syncytia induction was inhibited by Leu3a and no syncytia were formed by any HIV-1– or HIV-2–infected cells when they were cocultured with $CD4^-$ cells.

Cytopathicity was also assessed by direct determination of cell killing. HIV-2_{ROD} and two different isolates of HIV-1 (IIIb and BC) caused marked cell killing of SupT1 cells (Fig. 4, A and B), PHA-stimulated normal donor lymphocytes (Fig. 4, C and D), as well as Hut 78 and H9 cells. Equivalent amounts of HIV-2_{ST} showed no detectable cell killing activity against the same cell targets. Even 10- and 100-fold more HIV-2_{ST} virus than three other HIV-1 isolates (WMJ, RH, and BC) led to only transient depression in cell counts (Fig. 4E). These experiments were repeated three times with identical results, each time with the use of newly concentrated virus stocks. Syncytia induction in these experiments was also scored; the syncytia formed were large and numerous in cultures infected by HIV-2_{ROD} and the four HIV-1 isolates, but no syncytia were formed in cultures infected with HIV-2_{ST}. The virus stocks used for these experiments were obtained from the original uncloned HIV-2_{ST} infected line SupT1/LK001 and were infectious and replication-competent as judged by their ability to infect, replicate in, and reinfect $CD4^+$ cells by cell-free passage. That the cell targets shown in Fig. 4 and the virus-infected cells used for the syncytia induction assays in Fig. 3 were comparably infected with HIV-2_{ST}, HIV-2_{ROD}, and HIV-1 was shown by viral-specific immunofluorescence, in situ hybridization, and supernatant particulate reverse transcriptase assays.

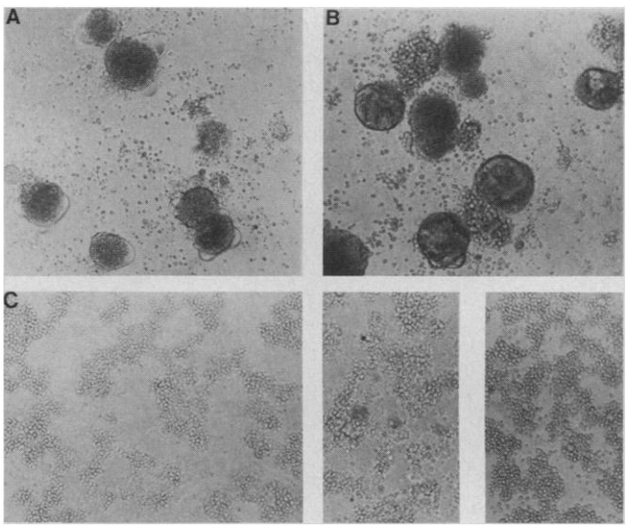

Fig. 3. Syncytium formation by (**C**) HIV-2_{ST}–, (**B**) HIV-2_{ROD}–, and (**A**) HIV-1_{IIIb}–infected cells and $CD4^+$ SupT1 indicator cells. Virus-infected SupT1 cells were mixed 1:5 with uninfected SupT1 cells, incubated for 24 hours, and photographed (*24*, *25*). Results were identical when HeLa-T4 (*24*), H9, and Hut 78 cells were used as uninfected indicators and when HIV-2_{ST}–infected H9 and Hut 78 cells were used as *env* expressing effector cells. The (C) panels show absence of syncytium formation by HIV-2_{ST} strains with large (LK001; ST.9) and small (ST.24) TMPs (×160).

In previous studies of the infectivity and fusion activity of SIV_{mac}, it was observed that the cell line CEM×174 (a somatic cell hybrid between the T cell line CEM and the B cell line B721.174), but neither of its parental cell lines, was exquisitely sensitive to virus infection and syncytia induction (*16*). We therefore used CEM×174 cells in cytopathicity assays with HIV-2_{ST}, and found them highly susceptible to HIV-2_{ST} infection and permissive for permanent, high-titer virus production. Syncytia induction and cell-killing assays showed a modest cytopathic effect but again much less than for prototype strains of HIV-1 and HIV-2. To evaluate the basis for the attenuation of HIV-2_{ST}, we used in situ hybridization to analyze at the single-cell level the rate of virus entry and early gene expression after cell-free viral transmission (Table 1). Compared with prototype strains of HIV-1 and HIV-2, HIV-2_{ST} showed markedly delayed onset of viral RNA production in CEM×174 cells (Table 1) as well as in SupT1 and Hut 78 (*11*) after exposure to cell free virions. However, once infected, CEM×174 cells expressed equivalent amounts of HIV-2_{ST} RNA compared with HIV-2_{ROD} and HIV-1_{IIIb}, and produced equivalent amounts of mature virus as assessed by reverse transcriptase (RT) activity and viral structural proteins. This, and the fact that HIV-2_{ST} is less fusogenic, suggests that the defect in HIV-2_{ST} is at the level of virus entry, not expression.

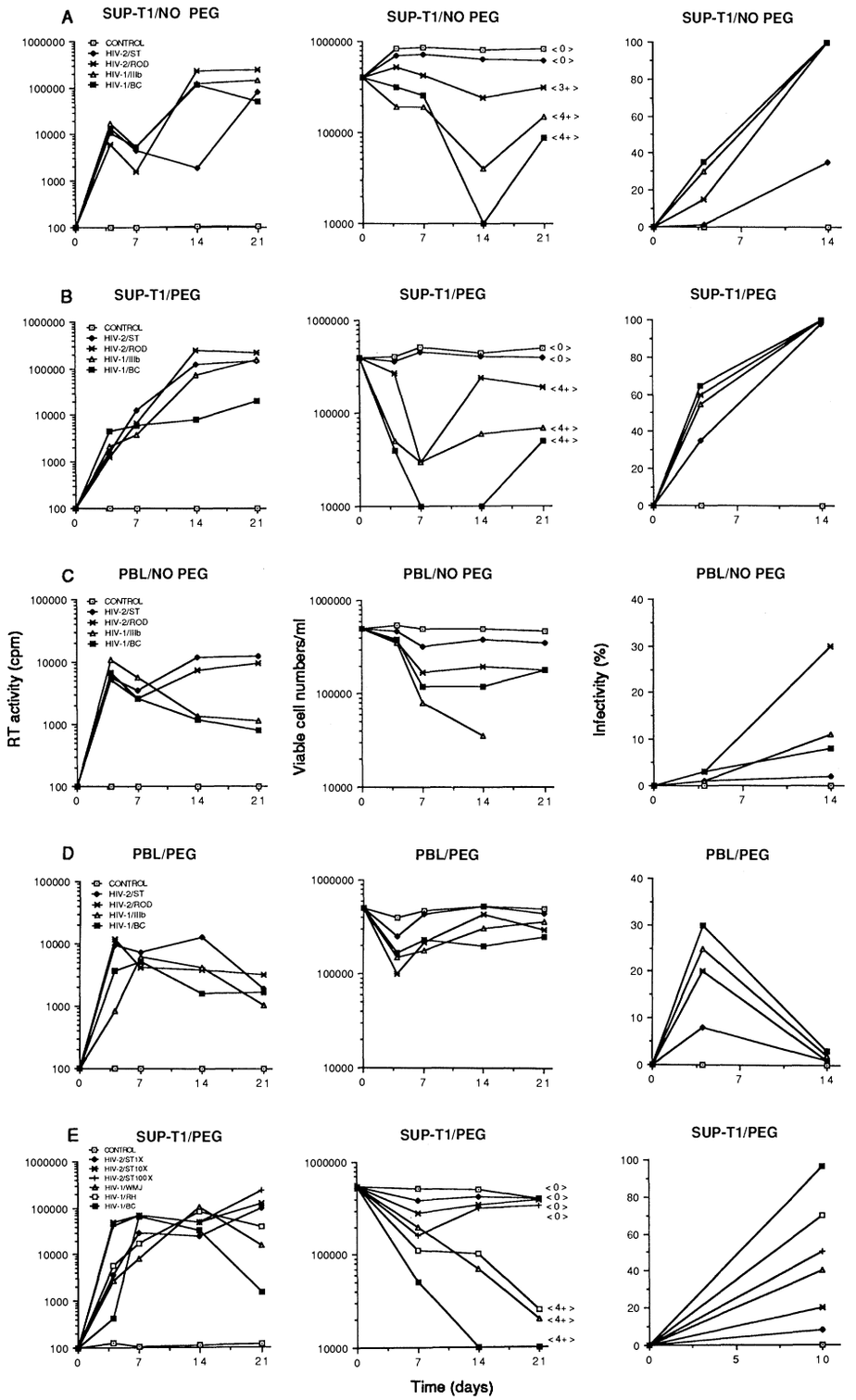

Fig. 4. Cytopathic and cell killing properties of HIV-2_{ST}, HIV-2_{ROD}, and HIV-1 isolates IIIb, BC, WMJ, RH. Each graph summarizes a 21-day experiment in which equivalent amounts of cell-free virus (250,000 cpm of RT activity) were applied to cultures of uninfected SupT1 cells (**A** and **B**) or PHA-stimulated peripheral blood lymphocytes (**C** and **D**). In (**E**) 7500-cpm RT activity of all viruses was used except for HIV-2_{ST}, 10× (75,000 cpm) and HIV-2_{ST}, 100× (750,000 cpm). Shown are supernatant RT activities; percentages of cells infected with virus as determined by indirect immunofluorescence; and cell killing assessed by viable cell counts with trypan blue exclusion, hemocytometry, and automated cell counting. Identical culture splits were done weekly to keep the concentration of control cells between 0.5×10^6 and 1.0×10^6 per milliliter and cell killing was expressed logarithmically as decreases in viable cell numbers. Syncytia were scored from 0 (none) to 4+ (extensive) and are shown in brackets (⟨ ⟩). Mock-infected culture supernatants lacking virus served as controls. In (B), (D), and (E), the final concentration of PEG used to concentrate virus was 10%.

We had considerable difficulty in establishing virus isolates from healthy, antibody-positive Senegalese subjects. Out of four patients studied (*8*), RT activity in lymphocyte coculture supernatants was detected in three, but in only one case was a virus (HIV-2_{ST}) successfully transmitted to permanently producing immortalized T cell lines. These results together with the cytopathicity and infectivity data suggest that certain strains of HIV-2 may be less virulent than others and in turn may be associated with a less severe or altered expression of immune deficiency.

The molecular basis for the attenuated virulence of HIV-2_{ST} in vitro is not known, but likely to involve alterations in the *env* protein. Mullins and co-workers (*17*) have described a replication-defective strain of feline leukemia virus (FeLV) whose variant *env*/LTR region is responsible for enhanced virulence. These workers suggested that biologically important strains of HIVs could have similarly altered genetic and biologic properties.

The significance of different-sized *env* TMPs in HIV-2_{ST} is unknown (*7–9*, *12*, *18*). Biologically cloned cultures of HIV-2_{ST} with either large (42 kD) or small (30 kD) TMPs were both infectious and noncytopathic, as were isolates of SIV_{mac} and HIV-2_{ROD} with only small TMPs. A full-length (gp42) TMP may therefore not be required for these biological functions, although the carboxyl terminus of the TMP reading frame, which is highly conserved among HIV-1, HIV-2, and SIV_{mac}, could potentially encode a separate protein with distinct biologic function.

REFERENCES AND NOTES

1. P. Piot *et al.*, *Science* **239**, 573 (1988).
2. F. Clavel *et al.*, *ibid.* **233**, 343 (1986).
3. F. Clavel *et al.*, *N. Engl. J. Med.* **316**, 1180 (1987).
4. P. Kanki *et al.*, *Science* **236**, 827 (1987); R. G. Marlink *et al.*, *AIDS Res. Hum. Retrovir.* **4**, 137 (1988).
5. G. de Thé, *Proceedings of the Deuxième Colloque Des Cent Gardes*, (Institut Pasteur Paris, 1987).
6. P. Kanki, *et al.*, *Science* **232**, 238 (1986).
7. V. Hirsch *et al.*, *Proc. Natl. Sci. U.S.A.* **83**, 9754 (1986); H. Kornfeld *et al.*, *Nature* **326**, 610 (1987).
8. B. H. Hahn *et al.*, *Nature* **330**, 184 (1987).
9. H. W. Kestler *et al.*, *ibid.* **331**, 691 (1988).
10. HTLV-4 appears to be an accidental laboratory transmission of SIV_{mac}-251 and not a naturally occurring pathogen in West African human populations (*7–9*).
11. L. Kong, S-W. Lee, J. Kappes, unpublished data.
12. F. Clavel, *AIDS* **1**, 135 (1987).
13. J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
14. J. A. Hoxie, unpublished data.
15. J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985).
16. J. A. Hoxie *et al.*, *J. Virol.*, in press.
17. J. Overbaugh *et al.*, *Science* **239**, 906 (1988).
18. J. Albert *et al.*, *AIDS Res. Human Retrovir.* **3**, 3 (1987).
19. We used an SIV_{mac} isolate (*7–9*) originally reported to be HTLV-4 (*6*).
20. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
21. Molecular cloning of DNA from HIV-2_{ST} line

Hut78/B12 in J1-λ phage by Sau3 AI partial digestion, Bam HI ligation, plaque hybridization with a full-length SIV_{mac} probe (*8*), and subsequent restriction enzyme analyses were performed as described (*20*).
22. D. I. Bernstein *et al.*, *J. Gen. Virol.* 67, 1601 (1986).
23. J. A. Hoxie *et al.*, *Science* **234**, 1123 (1986).
24. P. Maddon *et al.*, *Cell* **47**, 333 (1986).
25. J. D. Lifson *et al.*, *Science* **232**, 1123 (1986).
26. Identical amounts of virus (RT activity 100,000 cpm) were concentrated by PEG precipitation of culture supernatants, applied to 5×10^6 CEM×174 cells, and cultured for 14 days. In situ hybridization was performed (*27*) with comparable 4- to 5-kb *pol*–central region–*env* nick-translated DNA probes derived from the homologous proviral genomes [specific activity 2×10^8 dpm/μg (^{35}S)]. Approximately 500,000 cells per slide were examined for viral-specific RNA production. Controls included HIV-1 and HIV-2 probes on uninfected cells and HTLV-1 probe on HIV-1– and HIV-2–infected cells.
27. H. E. Gendelman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7086 (1985).
28. We thank L. Montagnier for $HIV\text{-}2_{ROD}$ virus and clones; P. Kanki and M. Essex for fresh peripheral blood specimens from Senegal; and B. Haggarty, S. Bonser, and J. Holt for technical and editorial assistance. Supported by grants from NIH, the U.S. Army Medical Research Acquisition Activity, the Life and Health Insurance Medical Research Fund, and the Carter-Wallace Corporation. B.H.H. is a Special Fellow and J.A.H. a Scholar of the Leukemia Society of America and G.M.S. is a PEW Scholar of the Biomedical Sciences.

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Two Classes of PDGF Receptor Recognize Different Isoforms of PDGF

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Previous studies involving platelet-derived growth factor (PDGF) have been based on the premise that a single cell-surface receptor binds all three isoforms of PDGF (AA, BB, and AB). It is now shown that two populations of PDGF receptor exist and can be distinguished by their ligand binding specificity. The B receptor binds only the BB dimer, whereas the A/B receptor binds AA, BB, and AB dimers. Human dermal fibroblasts appear to express seven times as much B receptor as A/B receptor. The B receptor is responsible for most PDGF receptor phosphorylation.

PLATELET-DERIVED GROWTH FACTOR is a potent mitogen for various types of mesenchymal cells (*1*). In addition to its mitogenic activities, PDGF stimulates a wide variety of processes, including vasoconstriction (*2*), chemotaxis (*3*), activation of intracellular enzymes such as glycogen synthase (*4*), phosphatidylinositol turnover and Ca^{2+} mobilization (*5*), rearrangement of the cytoskeleton (*6*), and stimulation of tyrosine-specific phosphorylation (*7*). Platelet-derived growth factor was originally identified (*8*) and purified (*9*) from human platelets. Pure PDGF from this source consists of dimers of two polypeptides termed A chain and B chain (*10*). The major form of human platelet PDGF appears to be an AB heterodimer. Naturally occurring homodimeric forms of PDGF (AA and BB) have also been isolated, from U2-OS osteosarcoma cell–conditioned media (*11*) and from porcine platelets (*12*), respectively.

In earlier studies involving PDGF, the existence of a single cell-surface receptor for PDGF was assumed. This receptor was described as a 170- to 180-kD membrane glycoprotein with an extracellular ligand-binding domain and an intracellular split tyrosine kinase domain (*13, 14*). At 37°C, binding of PDGF to this cell-surface receptor stimulates receptor-associated tyrosine kinase activity (*14, 15*) and internalization and degradation of the ligand-receptor complex (*16, 17*). Given the multitude of events that are stimulated after PDGF is added to cells in culture, and the recent finding that the A chain and B chain genes often appear to be independently regulated (*18*), it seemed plausible that the three isoforms of PDGF might stimulate effects unique to each isoform, either through interaction with different classes of PDGF receptor or by differential effects on a single receptor. To investigate these possibilities, we examined each of the three isoforms for their receptor-binding characteristics and their ability to stimulate PDGF receptor downregulation and receptor phosphorylation. For these studies we used recombinant AA and BB homodimers that we produced in a *Saccharomyces cerevisiae* expression system (*19*), AB heterodimer purified from platelet-rich plasma (*20*), and PR7212, a monoclonal antibody to a PDGF receptor, which we recently described (*21*).

Binding of PDGF to cells at 37°C leads to depletion of cell-surface PDGF binding sites resulting from receptor occupation and internalization, a phenomenon known as downregulation (*16*). To study ligand-in-

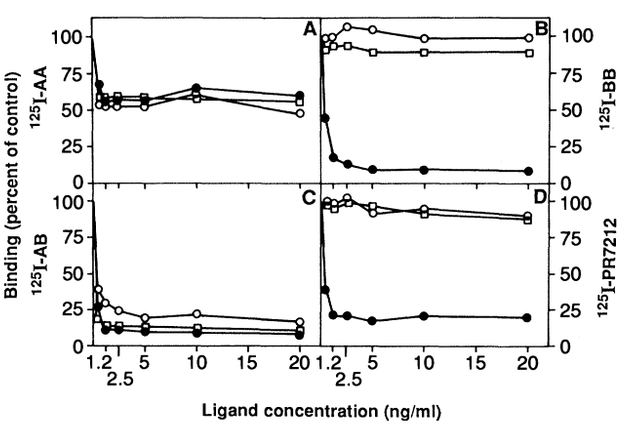

Fig. 1. Receptor downregulation assay. Human dermal fibroblasts grown in 24-well culture dishes (3×10^4 cells per well) were incubated for 2 hours at 37°C with increasing concentrations of the three isoforms of PDGF: (○) AA, (●) BB, or (□) AB. Incubation at 37°C was used to allow ligand-receptor internalization to occur. The cells were washed to remove unbound ligand, then incubated at 4°C for 2 hours with ^{125}I-labeled (**A**) AA, (**B**) BB, (**C**) AB, or (**D**) PR7212. The cells were washed, harvested with Triton X-100 solubilization buffer (*28*), and the total cell-associated ^{125}I was determined. The data were plotted as mean binding as a percent of control binding for determinations made from triplicate wells of a representative experiment. Variation between experiments was less than 10%. At least three experiments were done for each iodinated probe. Nonspecific binding was not subtracted. The inability of any ligand to decrease binding of ^{125}I-labeled AA below 50% reflects the high level of nonspecific binding for this preparation of ^{125}I-labeled AA. AA, AB, and antibody PR7212 were labeled with ^{125}I by use of Iodobeads (Pierce Chemical). BB was labeled with Bolton-Hunter reagent (Du Pont–New England Nuclear).

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