supply of the mixture never exceeded 100 cm^3/min. The growth resulting from the different methane levels was then measured every 2 days. The methane concentrations in the water were measured by gas chromatography with a Porapak Q column and a flame ionization detector. The oxygen concentrations were monitored with a polarographic oxygen electrode (Strathkelvin, U.K.). During the entire duration of this study the mussels appeared to be in good health, that is, none of the animals died, the siphons were extended, and extensive byssal threads were produced.
21. Monthly Gas Quality Report, Ehrenberg meter station, no. 32002, sampling date, 30 July 1987 (San Diego Gas and Electric, San Diego, August 1987).
22. C. Anthony, *The Biochemistry of Methylotrophs* (Academic Press, New York, 1982).
23. M. C. Kennicutt II *et al.*, *Nature (London)* **317**, 351 (1985).
24. Supported by NSF grants OCE86-10512 (H.F.) and OCE86-10514 (J. J. Childress and C.R.F.). The National Undersea Research Program of the National Oceanic and Atmospheric Administration supported the collecting cruise and the dive time with the RV *Seward Johnson* and the DSRV *Johnson Sealink*, and *NR1* submersible support was provided by the Office of Naval Research. We thank the crew and pilots of the RV *Seward Johnson* and the DSRV *Johnson Sealink* for their assistance in collecting animals, B. Ward for the use of the gas chromatograph, and J. J. Childress for critically reviewing the manuscript.

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Biologic Features of HIV-1 That Correlate with Virulence in the Host

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Individuals infected with the human immunodeficiency virus type 1 (HIV-1) may be asymptomatic or have AIDS-related complex or the acquired immuno deficiency syndrome (AIDS). Little is known about the factors that influence progression of infection to AIDS. In this study of isolates of HIV-1 obtained at intervals during the infection of four individuals, the development of disease was found to be correlated with the emergence of HIV-1 variants that were more cytopathic in vitro as the disease progressed and that replicated more efficiently in a wide variety of different human cells. The biologic properties of HIV-1 in vitro thus appear to reflect its virulence in the host. Further studies of such sequentially isolated viruses may lead to the identification of viral genes that govern pathogenesis.

BIOLOGIC (*1–6*), SEROLOGIC (*7*), and molecular (*8, 9*) studies of the human immunodeficiency virus type 1 (HIV-1) have indicated that this virus is highly heterogeneous. Individual isolates of HIV-1 can be distinguished by their differential capacity to infect and replicate in a wide variety of cultured human cells including T and B lymphocytes, macrophages, and brain-derived cells (*1, 4, 6, 10, 11*). Studies of the varying abilities of HIV-1 isolates to replicate to high titers and to induce cytopathic changes in infected cells have indicated that these characteristics are correlated with the efficiency of the virus to form plaques in the MT-4 cell line (*12*). Differences in the susceptibility of various HIV-1 isolates to neutralization by HIV-1–positive sera have also been observed (*6, 7, 11*).

In the present studies, these biologic and serologic properties of HIV-1 were used to examine the possible changes in the virus over time in the same individuals. Our results from four individuals show that the progression of disease is correlated with the emergence of HIV-1 isolates that, in comparison with the virus isolated initially, are more cytopathic and replicate to higher titers in a wide variety of different human cells. Serologic and genomic restriction analyses indicate that in each individual the sequential isolates are related. The observations suggest that certain changes in the structure of HIV-1 can influence its virulence in the host.

Isolates of HIV-1 were obtained at intervals from peripheral blood mononuclear cells (PMC) of four subjects selected randomly from a group of seropositive individuals whom we have followed for the past 4 years (Table 1). Three of the four subjects progressed to more severe disease during the time of observation; one has remained asymptomatic (Table 1, subject 4). At the time of virus isolation, we noted that HIV-1 was recovered more readily from each individual's PMC as the disease progressed. For example, in subject 2, HIV_{SF216} emerged in the patient's PMC culture within 12 days whereas the previous isolates (HIV_{SF94} and HIV_{SF118}) took close to 1 month to be detected. Similar findings on time of recovery of HIV-1 from PMC have been reported (*12, 13*).

We first determined the abilities of HIV_{SF2} and HIV_{SF13} (from subject 1) to infect established human T cell lines (HUT-78 and MT-4), the U937 monocytic cell line, primary macrophage/monocytes, and certain established B cell lines (*1, 6*). These viruses had been kept in culture for over 3 years. As shown in Table 2, both isolates replicated in HUT-78 and MT-4 cells and the monocytic line, but only HIV_{SF13} productively infected primary macrophages and both of the B cell lines. Furthermore, HIV_{SF13} was more cytopathic as reflected by syncytia formation and balloon degeneration in infected PMC, HUT-78, and MT-4 cells. It also readily induced plaques in MT-4 cells (Table 2).

Subgroups of HIV-1 isolates have been identified on the basis of their patterns of sensitivity to serum neutralization (*7*). When tested with three HIV-1 antibody positive sera, HIV_{SF2} and HIV_{SF13} were neutralized to a similar extent by all three sera at dilutions of 1:100 or greater.

We then studied the biologic and serologic properties of isolates from three other individuals (Table 1). These isolates had been in culture for only 3 to 4 weeks. As the disease progressed in subjects 2 and 3, the HIV-1 isolated had a wider host range and greater cytopathic and replicative properties. In subject 2, for example, the isolate obtained 2 months before the patient died (HIV_{SF216}) replicated quickly and to high titers in all established human cell lines and primary macrophages, and also produced

Table 1. Isolation of HIV-1 from individuals at different stages of infection. The isolates were initially recovered by cocultivation of the individual's PMC with PMC from seronegative donors for 10 to 30 days as described (*13, 16*). The isolates were subsequently passaged onto fresh PMC and when virus titers were high [reverse transcriptase (RT) activity $\geq 10^6$ cpm/ml (*17*)], aliquots of filtered virus stocks were frozen at −70°C until use. KS, Kaposi's sarcoma; PCP, *Pneumocystis carinii* pneumonia; LAN, persistent lymphadenopathy.

HIV-1 isolate	Month and year of isolation	Clinical state
	Subject 1	
SF2	11/83	Oral candidiasis
SF13	4/84	KS, PCP
	11/84	Deceased
	Subject 2	
SF94	3/85	Asymptomatic
SF118	4/85	Asymptomatic
SF216	10/85	LAN, diarrhea
	12/85	Deceased
	Subject 3	
SF73	12/84	Asymptomatic
SF328	3/86	LAN
SF665	9/87	PCP
	Subject 4	
SF341	4/86	Asymptomatic
SF488	4/87	Asymptomatic

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plaques in the MT-4 cells (Table 3). In contrast, the isolate obtained 6 months previously (HIV_{SF118}) replicated in the established human cell lines but showed slower kinetics of replication in the Jurkat, CEM, and U937 cell lines compared with HIV_{SF216}, and the titers of virus produced were lower (Fig. 1). Neither HIV_{SF118} nor an earlier isolate (HIV_{SF94}) replicated in primary macrophages or induced plaques in the MT-4 cell line. HIV_{SF94} replicated in the HUT-78 cells, but with a much slower kinetics (Fig. 1). The isolate HIV_{SF73} from subject 3 did not replicate in any established human cell lines over a 30-day culture period. The sequential isolates HIV_{SF328} and HIV_{SF665} both replicated in the HUT-78 cells with the same kinetics of replication. HIV_{SF665}, however, was more cytopathic in the HUT-78 cells, replicated in primary macrophages, and induced plaques in MT-4 cells. The only difference in the biologic properties of the sequential isolates from subject 4, who remains asymptomatic, was that the earlier isolate, HIV_{SF341}, replicated to a limited extent in primary macrophages whereas HIV_{SF488} did not.

When tested with three HIV-1 antibody–positive sera, each group of isolates from the same individual displayed similar patterns of sensitivity to serum neutralization. These results, although limited, suggest that the serologic properties of the virus remained stable whereas the biologic properties changed. Further studies, however, may reveal some differences in neutralization patterns.

We then purified the isolates and subjected them to immunoblot analyses (Fig. 2). Since differences in processing of viral proteins have been observed when viruses are grown in different cell lines (*14*), all the isolates were grown in PMC from the same seronegative donor. Only the envelope glycoprotein (gp120) displayed variations in size among the HIV-1 isolates from the same individual. This overall change in size of gp120 did not appear to correlate with differences in pathogenic properties since the same variability in gp120 was observed in sequential isolates from subject 4 (HIV_{SF341} and HIV_{SF488}). The changes in size of gp120 may be the result of envelope sequence variations among HIV-1 isolates leading to differences in glycosylation.

We also analyzed infected cellular DNA by the Southern blot technique. Although we detected some variations in restriction enzyme patterns among the sequential isolates from each individual, the differences were limited to the loss or gain of only one restriction site [for example, the loss of a Sac I site and the gain of a Kpn I site in HIV_{SF13} compared to HIV_{SF2} (Fig. 3A) for subject 1; the gain of a Hind III site in HIV_{SF94} and HIV_{SF216} compared with HIV_{SF118}; and the gain of a Bgl II site in HIV_{SF94} and HIV_{SF118} as compared with HIV_{SF216} (Fig. 3B) for subject 2]. The Hind III digestion patterns for isolates from subject 2 suggest that there was a progressive change from the initial (HIV_{SF98}) to the intermediate (HIV_{SF118}) and then to the last isolate (HIV_{SF216}). The differences in the isolates from each of subjects 3 and 4 were also limited. In contrast, we observed marked heterogeneity when we compared isolates among the four different subjects (Fig. 3). Our data, like those of others (*9*), suggest that the sequential isolates from the same individual are closely related variants of one another.

These studies indicate that disease progression correlates with the appearance of variant viruses that are more cytopathic and have a wider host range than the original isolate. The variants we isolated could have coexisted in each host from the time of

Table 2. Comparison of isolates recovered from subject 1. Equal amounts (RT activity ~10^6 cpm/ml) of HIV_{SF2} [formerly AIDS-associated retrovirus, ARV-2 (*16*)] and HIV_{SF13} were used for infectivity studies. The B cell lines were established in our laboratory by EBV transformation of uninfected B cells from HIV-1 seropositive individuals (*6*). The macrophages were maintained in medium containing 5% human serum (*3, 11, 18*). Replication of the virus over a 30-day period was determined as described (*1, 4, 11*). Results represent RT activity in culture supernatant at day 30. Symbols: −, no RT activity in supernatant; +, RT activity of 20,000 to 50,000 cpm/ml; ++, RT activity of >50,000 cpm/ml. Background was generally at <2,000 cpm/ml. Cytopathology was assessed by the presence of syncytia and balloon degeneration in established T cell lines. The plaque assay was conducted with the MT-4 cell line (*19*); + indicates plaque-forming ability. Each assay was performed at least twice and gave similar results.

Properties	HIV_{SF2}	HIV_{SF13}
Replication in		
T cell line (MT-4)	++	++
T cell line (HUT-78)	++	++
B cell line (100)	++	++
B cell line (1000)	−	++
Monocytic cell line (U937)	++	++
Primary macrophages/ monocytes	−	+
Cytopathology	+	++
Plaque formation	−	+

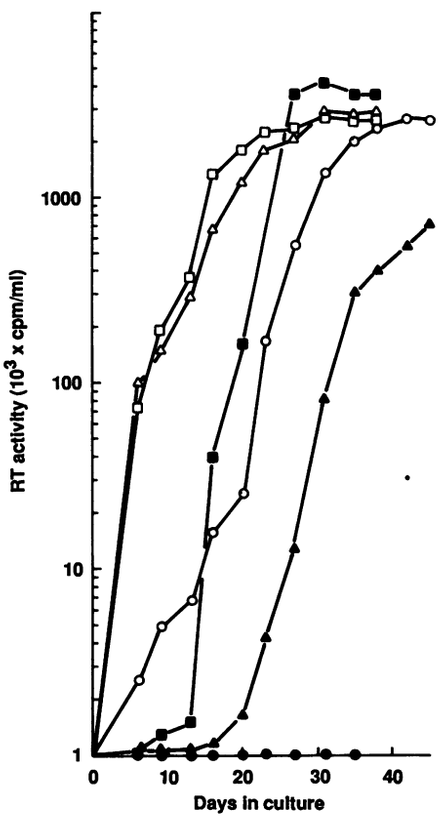

Fig. 1. Kinetics of replication of sequential isolates from subject 2 in HUT-78 and Jurkat cells. HUT-78 or Jurkat (5×10^5) cells were infected with 1 ml of virus-containing fluids (~10^6 cpm/ml of RT activity) as described (*1*). At the time points indicated, samples of medium were assayed for RT activity (*17*). HIV_{SF94} in HUT-78 cells (○), HIV_{SF118} in HUT-78 cells (△), HIV_{SF216} in HUT-78 cells (□), HIV_{SF94} in Jurkat cells (●), HIV_{SF118} in Jurkat cells (▲), and HIV_{SF216} in Jurkat cells (■).

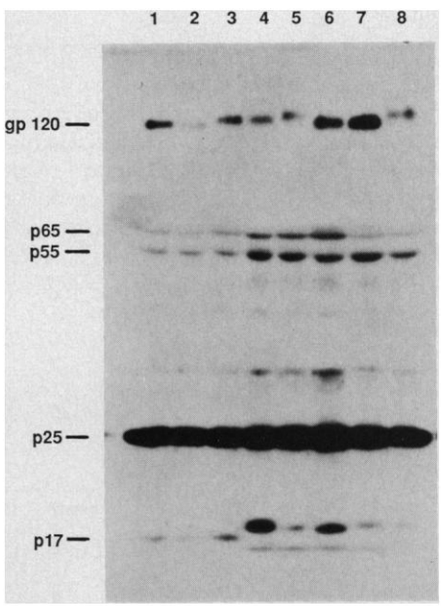

Fig. 2. Immunoblot analyses of sequential isolates. Supernatant fluids from normal PMC infected with HIV_{SF94} (lane 1), HIV_{SF118} (lane 2), HIV_{SF216} (lane 3), HIV_{SF73} (lane 4), HIV_{SF328} (lane 5), HIV_{SF665} (lane 6), HIV_{SF341} (lane 7), and HIV_{SF488} (lane 8) were harvested, filtered, and pelleted over 20% glycerol. The pellets were disrupted in buffer (0.05*M* tris-HCl, *p*H 7.8, 0.15 mg/ml dithiothreitol, 0.1% Triton), and analyzed by immunoblot on a 10% SDS-polyacrylamide gel as described (*20*). The molecular weights of the major viral proteins are noted.

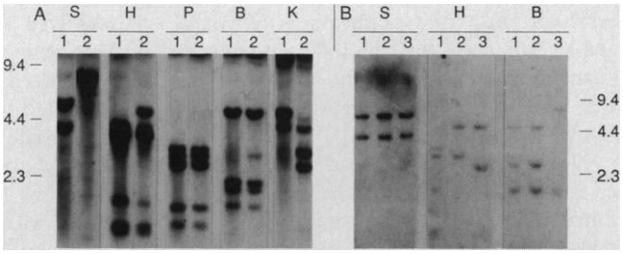

Fig. 3. Southern blot analyses of HIV-infected cell DNA. High molecular weight whole-cell DNA was prepared from HUT-78 cells infected with isolates from subject 1 and normal PMC infected with isolates from subject 2 at 10 to 12 days after infection. Samples (15 μg) of each DNA were digested with the restriction enzymes indicated and subjected to electrophoresis on 0.8% agarose gels. The DNA fragments were blotted onto nitrocellulose membranes, and viral species were detected with a radioactive probe representing the entire HIV-1 genome. (**A**) HIV_{SF2} (lane 1) and HIV_{SF13} (lane 2) from subject 1; (**B**) HIV_{SF94} (lane 1), HIV_{SF118} (lane 2), and HIV_{SF216} (lane 3) from subject 2. Enzymes: S, Sac I; H, Hind III; K, Kpn I; B, Bgl II; P, Pvu II.

Table 3. Biologic properties of sequential isolates from subjects 2, 3, and 4. Equal amounts of each isolate (RT activity ~10^6 cpm/ml) were used to infect the cell types listed. The procedures used were as described in Table 2. Each assay was performed at least twice and gave similar results. For replication: −, no RT activity in culture supernatant; +, RT activity of 20,000 to 50,000 cpm/ml; ++, RT activity of >50,000 cpm/ml. Background was generally at <2,000 cpm/ml. HUT, HUT-78 cell line; JUR, Jurkat cell line; MAC, primary peripheral blood macrophages; CPE, cytopathic effects (syncytia formation and balloon cell degeneration); PMC, peripheral blood mononuclear cells.

HIV-1 isolate	Replication in					CPE in PMC	Plaque formation
	HUT	JUR	CEM	U937	MAC		
				Subject 2			
SF94	++	−	−	−	−	−	−
SF118	++	+	+	+	−	+	−
SF216	++	++	++	++	++	+	+
				Subject 3			
SF73	−	−	−	−	−	−	−
SF328	++	−	−	−	−	−	−
SF665	++	−	−	−	+	+	+
				Subject 4			
SF341	−	−	−	−	+	−	−
SF488	−	−	−	−	−	−	−

infection and had different levels of expression during the course of infection. Alternatively, the virus originally transmitted to the host could have undergone genomic changes during the course of infection. We do not believe these biologic changes reflect the conditions in vitro because, except for isolates from subject 1, all isolates were characterized within 3 to 4 weeks of isolation. If selection of one isolate occurred, then this preferential recovery gave the same results consistently: the three individuals who advanced in disease yielded the more cytopathic viruses than the individual who remained healthy. Finally, most of the isolates were retested after 3 months in culture and showed the same biologic properties. The lack of molecular change in HIV-1 after long-term passage has been reported (*15*).

These results suggest that the development of disease symptoms in HIV-1–infected individuals is associated with the emergence of more pathogenic virus variants. Future studies with these isolates should provide information on the genes that determine the virulence of HIV-1, and identify potential targets for antiviral therapy.

REFERENCES AND NOTES

1. J. A. Levy *et al., Virology* **147**, 441 (1985).
2. B. Asjo *et al., Lancet* **1986-II**, 660 (1986).
3. S. Gartner *et al., Science* **233**, 215 (1986).
4. L. A. Evans *et al., J. Immunol.* **138**, 3415 (1987).
5. K. Dahl *et al., J. Virol.* **61**, 1602 (1987).
6. J. A. Levy *et al., Vaccines* **87**, 168 (1987).
7. A. M. Prince *et al., J. Infect. Dis.* **156**, 268 (1987); C. Cheng-Mayer *et al., Proc. Natl. Acad. Sci. U.S.A.*, in press.
8. F. Wong-Staal *et al., Science* **229**, 759 (1985); M. Alizon *et al., Cell* **46**, 63 (1986); S. Magasiny *et al., AIDS Res.* **2**, 19 (1986).
9. B. H. Hahn *et al., Science* **232**, 1584 (1986).
10. C. Cheng-Mayer *et al., Proc. Natl. Acad. Sci. U.S.A.* **84**, 3526 (1987); J. A. Levy *et al., Ann. Inst. Pasteur* **138**, 101 (1987).
11. C. Cheng-Mayer and J. A. Levy *Ann. Neurol. AIDS Suppl.* **23**, S59 (1988).
12. M. Tateno *et al.*, in preparation.
13. J. A. Levy and J. Shimabukuro, *J. Inf. Dis.* **152**, 734 (1985).
14. C. Cheng-Mayer *et al.*, unpublished observation.
15. M. Robert-Guroff *et al., J. Immunol.* **137**, 3306 (1986); C. Cheng-Mayer and J. A. Levy, unpublished observation.
16. J. A. Levy *et al., Science* **225**, 840 (1984).
17. A. D. Hoffman *et al., Virology* **147**, 326 (1985).
18. D. D. Ho *et al., J. Clin. Invest.* **77**, 1712 (1986); K. A. Nicholson *et al., J. Immunol.* **137**, 323 (1986).
19. S. Harada *et al., Science* **229**, 563 (1985).
20. L.-Z. Pan *et al., J. Infect. Dis.* **155**, 626 (1987).

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