Evidence for a Role of Virulent Human Immunodeficiency Virus (HIV) Variants in the Pathogenesis of Acquired Immunodeficiency Syndrome: Studies on Sequential HIV Isolates

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Sequential human immunodeficiency virus (HIV) isolates, recovered from a panel of longitudinally collected peripheral blood mononuclear cells obtained from 20 initially asymptomatic HIV-seropositive homosexual men, were studied for differences in replication rate, syncytium-inducing capacity, and host range. Eleven individuals remained asymptomatic; nine progressed to acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) at the time point at which the last HIV isolate was obtained. In 16 individuals, only non-syncytium-inducing (NSI) isolates, with a host range restricted to mononuclear cells, were observed. From four individuals, high-replicating, syncytium-inducing (SI) isolates that could be transmitted to the H9, RC2A, and U937 cell lines were recovered. From two of these four individuals, SI isolates were obtained throughout the observation period. In the two others, a transition from NSI to SI HIV isolates was observed during the period of study. Three of these four individuals developed ARC or AIDS 9 to 15 months after the first isolation of an SI isolate. With the exception of the two individuals in whom a transition from NSI to SI isolates was observed, within a given individual the replication rate of sequential HIV isolates was constant. A significant correlation was found between the mean replication rate of isolates obtained from an individual and the rate of CD4+ cell decrease observed in this individual. In individuals with low-replicating HIV isolates, no significant CD4+ cell loss was observed. In contrast, recovery of high-replicating isolates, in particular when these were SI isolates, was associated with rapid decline of CD4+ cell numbers and development of ARC or AIDS. These findings indicate that variability in the biological properties of HIV isolates is one of the factors influencing the course of HIV infection.

The clinical course of infection with the human immunodeficiency virus (HIV) exhibits a remarkable degree of variability with respect to the latency period (16, 25), occurrence of HIV antigenemia (2, 8, 12), and clinical manifestations upon development of disease. In this study, we address the possibility that differences in pathogenic properties between HIV variants may influence the course of HIV infection. Molecular analysis of HIV isolates has demonstrated extensive genomic variability (1, 5), especially in the *env* gene region (14, 33, 38). Although some features of the development of HIV infection have been linked to certain structural and regulatory properties of HIV isolates at the molecular level (11, 21, 31, 32), little is known of the possible consequences of genomic variability for differences in pathogenic properties of divergent HIV strains. It is well known from studies with animal retroviruses, including lentiviruses (9, 19, 23, 27, 35), that virus strains may exhibit such differences in virulence. For these reasons, we and others (3, 4, 7, 10, 34, 37) studied the biological properties of HIV isolates in cell culture. In a previous study on 24 transsectionally obtained HIV isolates (34), we observed clear, reproducible differences between HIV isolates from different individuals with respect to replication rate, syncytium-inducing (SI) capacity, and host range. HIV isolates with high replication rates and SI capacity in primary isolation were found more frequently in individuals with acquired immuno-

deficiency syndrome (AIDS) or AIDS-related complex (ARC) (34).

The present study was undertaken to find out whether changes in biological characteristics of HIV isolates occur in the course of time and to obtain evidence for a possible pathogenic significance of particular viral properties from the way in which recovery of particular HIV isolates is related in time to the clinical course of HIV infection.

MATERIALS AND METHODS

Subjects. HIV isolates were recovered from 20 male homosexuals participating in a large-cohort study (8). All subjects were seropositive and asymptomatic (Centers for Disease Control stage II or III) at the beginning of the observation period.

Virus isolation. Mononuclear cells (MNC) were obtained from heparinized venous blood by isolation on a Percoll density gradient and cryopreserved until use. For HIV isolation, MNC (3.5×10^6) from seropositive individuals were cocultivated with 2.5×10^6 2-day phytohemagglutinin-prestimulated MNC from healthy seronegative blood donors. All isolations were performed in duplicate. To be able to compare data obtained from different isolations, MNC of one blood donor were used in all isolations; duplicate cocultivations were performed with MNC of various blood donors. Cells were cultured under conditions described

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previously (34). Three times a week, cultures were checked for syncytium formation. Each time, at least ten microscope fields (magnification, ×100) per culture were observed. Twice a week, starting on day 4, cells were pelleted and supernatants were polyethylene glycol precipitated (PEG 6000; final concentration, 7.5%) for testing in reverse transcriptase (RT) and HIV p24 antigen capture assays. Once a week, fresh 2-day phytohemagglutinin-stimulated MNC from a healthy blood donor were added. A culture was considered positive for HIV when at least two successive supernatants were found to be positive (i.e., over three times the negative control) in antigen capture or RT assays. Cultures were maintained up to a maximum of 35 days, unless virus was recovered before. RT activity was measured as described previously (34). HIV p24 was detected in a radioimmunoassay (Tersmette et al., submitted for publication). Briefly, HIV p24 was captured by rabbit anti-HIV immunoglobulin G coupled to Sepharose beads and detected by a ^{125}I-labeled monoclonal antibody directed to HIV p24.

Transmission to cell lines. Transmission of HIV isolates to H9, a CD4$^+$ T-cell line, and U937 (26) and RC2A (6), both CD4$^+$ promonocytic cell lines, was attempted by both inoculation with cell-free culture supernatants and cocultivation (34).

Determination of CD4$^+$ cell numbers. CD4^{2+} cell numbers were determined at 3-month intervals by cytofluorography as described previously (22). For the analysis of CD4$^+$ cell decline, the three-point moving average of the absolute CD4$^+$ cell numbers was calculated. The CD4$^+$ cell decline per year was expressed as a percentage of the initial CD4$^+$ cell number. In two persons with biphasic curves (subjects 2 and 10), the highest three-point average, occurring 9 and 12 months, respectively, after the start of the observation period, was used to calculate the subsequent CD4$^+$ cell decline.

Determination of serum HIV antigen. Sera were tested at 3-month intervals in a solid-phase immunoassay for HIV antigen (Abbott Laboratories, Chicago, Ill.) according to the instructions of the manufacturer.

Restriction fragment analysis. High-molecular-weight DNA from continuously infected H9 cells was prepared as described previously (36). DNA samples (10 µg) were digested with restriction enzymes under conditions recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, Md.). Gel electrophoresis on 0.6% agarose gels and transfer to nitrocellulose were carried out as described previously (36). Restriction fragments of phage lambda after digestion with HindIII were used as molecular weight markers. The probes were subcloned from an HIV lymphadenopathy-associated virus HindIII fragment (base 1709 to 8170) in pSP65 (courtesy of M. Martin, National Institutes of Health, Bethesda, Md.) and are depicted in the figure. Hybridization of the nitrocellulose filters was performed as described earlier (28) with 10^6 cpm of ^{32}P-labeled nick-translated probe in the hybridization mix. Routinely, filters were washed in 0.1× SSC–0.1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 42°C for 20 min. Filters were exposed to film (X-Omat R; Eastman Kodak Co., Rochester, N.Y.) with intensifying screens.

RESULTS

Recovery of HIV isolates. The clinical features of the 20 seropositive homosexual men from whom sequential HIV isolates were obtained are summarized in Table 1. The period between the first and last MNC sample studied ranged from 13 to 33 months (average, 22.9 months). Eleven of the subjects had detectable serum HIV antigen at entry, and two other subjects (2 and 10) converted for HIV antigen during the period of observation. Eleven individuals remained asymptomatic throughout the period of observation. Nine of the individuals developed ARC or AIDS at the time the last MNC sample was obtained.

From these 20 individuals, a total of 87 isolates derived from 98 different MNC samples was obtained. Isolation was successful from 35 of 37 (95%) of the MNC samples of individuals who developed ARC or AIDS and from 52 of 61 (85%) of the individuals who remained asymptomatic. Replication of most isolates could be detected by both RT and antigen capture assay. In some MNC samples from individuals who remained asymptomatic (subjects 17 through 20), HIV replication could be demonstrated within 35 days of culture in the antigen capture assay only, probably reflecting the higher sensitivity of this assay compared with the RT assay.

Biological properties of sequential HIV isolates. The biological characteristics of the isolates obtained are summarized in Table 1. In this study, the first day of detection of HIV replication in the antigen capture assay was used as a parameter for viral replication rate. Other parameters, such as the first day of detection in the RT assay or the day of RT peak activity, gave similar results (data not shown). In Table 1, the mean first day of detection in the antigen capture assay of all isolates from one individual is shown. Standard deviation is indicated when three or more isolates were obtained from one person.

A remarkably constant replication rate was observed when isolates from one individual were compared, except for isolates from subjects 1 and 2, which will be dealt with below. The duplicate cocultivations gave comparable results (not shown), in agreement with previous experiments (34). In contrast, clear differences in replication rate were observed between isolates obtained from different individuals (Table 1). Previously, we showed that differences in HIV replication rate in an MNC culture can still be observed when comparable numbers of cells infected with different HIV isolates are used as an inoculum (34). To further exclude that the differences in day of detection of viral replication resulted from differences in number of infected patient cells, we studied the effect of absolute number of infected cells upon the rapidity of virus detection. In two experiments, titration of patient cells in our isolation protocol did not result in a significant decrease of rapidity of virus detection (Table 2), providing further proof that this parameter reflects a specific property of a virus isolate.

Only a minority of HIV isolates was able to induce syncytia. This property was observed for all isolates obtained from subjects 3 and 10 and the last four isolates of subjects 1 and 2 (see below). SI isolates had a significantly higher mean replication rate compared with non-SI (NSI) isolates ($P = 0.005$; Fig. 1A). As observed previously (34), a strong correlation existed between SI capacity and the ability to replicate in the H9 cell line (Tables 1 and 3). In reverse, in repeated attempts none of the NSI isolates could be transmitted to the H9 cell line, nor could any of the NSI isolates tested be transmitted to two CD4$^+$ promonocytic cell lines, U937 and RC2A.

Relation between biological properties of HIV isolates and clinical course. Once we had established that differences in biological properties existed between HIV isolates from different individuals and that within one individual, unless a

TABLE 1. Clinical characterization of 20 seropositive men and biological properties of sequential HIV isolates obtained from these individuals

Subject[a]	Clinical outcome[b]	Period of observation (mos)	Serum HIV antigen	CD4+ cell count at end of observation period	CD4+ cell decrease (%)[c]	No. of isolates	Mean (± SD) day of HIV detection[d]	Syncytium induction	Transmission to H9 cell line
1	OI	22	−	0.1	47	3[e]	4 ± 0.58	−	−
						4[f]		+	+
2	ARC	27	−/+[g]	0.0	71	4[e]	4 ± 0.0	−	−
						4[f]		+	+
3	KS	15	+	0.1	69	5	4 ± 0.0	+	+
4	OI	21	+	0.1	29	2	4	−	−
5	ARC	33	−	0.1	31	3	6 ± 1.73	−	−
6	OI	24	−	0.3	36	3	4 ± 0.0	−	−
7	OI	23	+	0.3	17	2	6	−	−
8	OI	13	+	0.4	15	2	9	−	−
9	OI	17	+	0.2	27	6	13 ± 3.43	−	−
10	AS[h]	32	−/+[g]	0.2	67	5	4 ± 1.22	+	+
11	AS[h]	32	+	0.2	23	3	5 ± 1.73	−	−
12	AS	30	+	0.2	33	3	5 ± 1.73	−	−
13	AS	22	+	0.4	13	8	9 ± 1.46	−	−
14	AS	18	−	0.6	0	7	10 ± 2.49	−	−
15	AS	18	−	0.5	0	7	9 ± 1.89	−	−
16	AS	20	−	0.5	<0	6	18 ± 2.06	−	−
17	AS	15	+	0.8	<0	4	17 ± 2.0	−	−
18	AS	15	+	1.1	0	2	32	−	−
19	AS	30	−	0.6	7	2	19	−	−
20	AS	31	−	1.0	9	2	27	−	−

[a] All subjects were asymptomatic and HIV seropositive at the onset of the study. Eleven individuals remained asymptomatic, and nine developed ARC or AIDS at the last time point studied.
[b] OI, Opportunistic infections; KS, Kaposi's sarcoma.
[c] Expressed as the percentage of decrease of CD4+ cells per year.
[d] In antigen capture assay.
[e] NSI isolates.
[f] SI isolates.
[g] Conversion for serum HIV p24 in the course of the period of observation (see Table 2).
[h] Azidothymidine treatment was begun at the end of the period of observation.

phenotypic switch occurred, the properties of sequential isolates remained stable over time, we investigated the relation between the occurrence of specific variants of HIV and the clinical outcome of HIV infection. In this panel, again, SI isolates were obtained more frequently from individuals who progressed to ARC or AIDS (3 of 9) than in persons who remained asymptomatic (1 of 11). A significantly higher rate of CD4+ cell decrease ($P < 0.001$) was seen in individuals from whom SI isolates were recovered

TABLE 2. Absolute number of patient cells cocultivated with blood donor MNC does not significantly influence rapidity of detection of viral replication

No. of patient cells added to 2.5 × 10^6 blood donor MNC	Day of detection for patient sample:								
	A	B	C	D	E	F	G1	G2	G3
Expt 1									
3 × 10^6	7	11	11	7	4	7			
1 × 10^6	7	11	7	4	4	7			
3.3 × 10^5	7	11	11	7	4	7			
1.1 × 10^5	−[a]	−	−	7	7	7			
Expt 2									
1 × 10^6							7	4	7
2 × 10^5							7	7	7
4 × 10^4							−	7	7
8 × 10^3							−	−	−
1.6 × 10^3							−	−	−

[a] −, No virus detectable. Culture supernatants were tested for p24 twice a week, as described in Materials and Methods.

compared with that in individuals who yielded NSI isolates (Fig. 1B).

Comparison of the mean replication rate of a set of isolates from an individual with the clinical outcome revealed a significant difference between the replication rates of isolates from individuals who progressed to ARC or AIDS and individuals who remained asymptomatic ($P < 0.05$) (Fig. 1C). Moreover, a significant correlation was observed between the mean first day of detection in the antigen capture assay of a set of sequential isolates from an individual and both the outcome of CD4+ cell count and rate of CD4+ cell decrease observed in this individual ($\rho = 0.81$, $P < 0.001$, and $\rho = 0.85$, $P < 0.001$, respectively, in the Spearman rank order test) (Fig. 2A and B).

A similar, highly significant correlation was found between both the rate of CD4+ cell decrease and final CD4+ cell count and two other parameters of virus replication rate, i.e., mean first day of detection in the RT assay and mean day of peak RT activity ($P < 0.001$ for both parameters; data not shown). Since sequential isolates from an individual were generally found to have constant replication rates in primary isolation, in this group the rate of CD4+ cell decrease and the outcome of CD4+ cell count can apparently be related to the replication rate of HIV isolates recovered at the beginning of the study period.

Switch of biological properties of HIV isolates with subsequent development of disease. Sequential data from the four individuals from whom SI isolates were recovered are summarized in Table 3. These four individuals were studied for
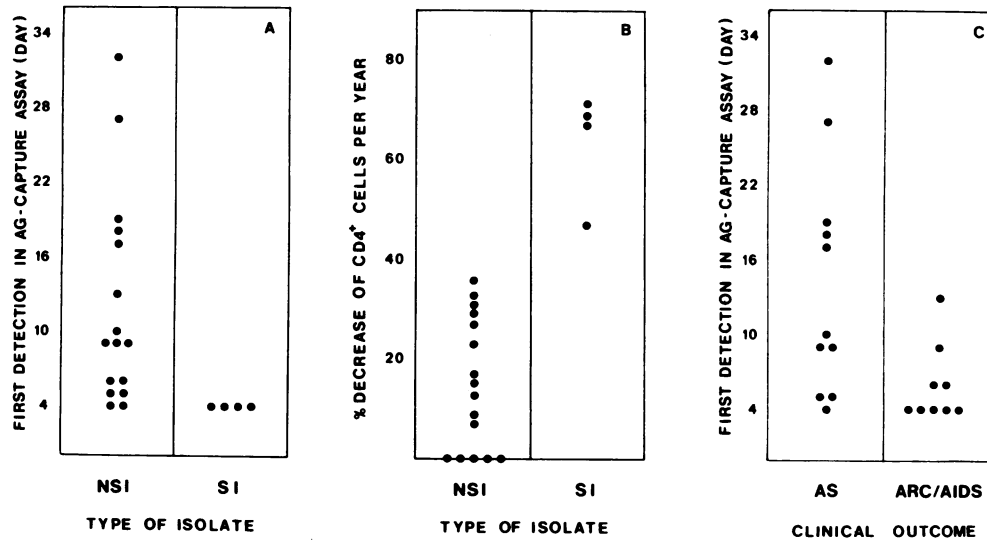
FIG. 1. (A) Comparison of the replication rates of NSI and SI isolates. The mean day of detection in the antigen capture assay of the sequential isolates obtained from a given person is used as a parameter for virus replication rate. (B) Comparison of the rate of decrease of the $CD4^+$ cell count in individuals from whom NSI or SI isolates were recovered. (C) Comparison of the mean replication rate of sequential HIV isolates (expressed as day of detection in the antigen capture assay) and the clinical outcome of the individual from whom the isolates were recovered. For statistical analysis (see text), the Wilcoxon-Whitney test was used.

a period of 18 to 27 months. In this period, five to eight isolates per individual were obtained. In two of these individuals (subjects 3 and 10), in whom virus isolation was successful in all but the first MNC sample studied, all isolates obtained were SI isolates. Interestingly, in the other two individuals (subjects 1 and 2), a transition from NSI to SI isolates was observed. In these subjects, the NSI isolates preceding the SI isolates had a slightly lower mean replication rate, which was only observed when judged by day of appearance of RT activity and peak RT activity (not shown). Concomitant with the development of SI capacity, a broadening of host range occurred (Table 3). A gradual transition was suggested by the finding that transmission of early SI

isolates from subjects 2 and 10 was relatively inefficient and required a prolonged time of culture before viral replication became detectable. Also, in preliminary experiments, SI isolates could be transmitted from the H9 cell line to the U937 and RC2A cell lines, but only SI isolates obtained at later time points could be transmitted to these cell lines directly from MNC culture (data not shown).

In all four individuals, recovery of SI isolates with a broad host range was followed by a rapid decline of $CD4^+$ cell numbers. ARC or AIDS ensued in three of these individuals. The one individual (subject 10) of these four who remained asymptomatic converted for serum HIV antigen during the period of observation (Table 3). During the last 3 months of

TABLE 3. Switch of biological properties of sequential HIV isolates in relation to clinical parameters and development of disease

Subject	Parameter	Properties of HIV isolates at 3-mo intervals[a]									
		1	2	3	4	5	6	7	8	9	10
1	SI capacity	−	NT	−	−	+	+	+	+	AIDS	
	Transmission to H9 cells	−	NT	−	−	+	+	+	+		
	$CD4^+$ cells (10^9)	0.4	0.4	0.4	0.3	0.3	0.3	0.2	0.1		
	Serum HIV antigen	−	−	−	−	−	−	−	−		
2	SI capacity	−	−	−	+	+	+	NT	NT	+	ARC
	Transmission to H9 cells	−	−	−	(+)	+	+	NT	NT	+	
	$CD4^+$ cells (10^9)	NT	NT	0.4	0.4	0.5	0.4	0.3	0.2	0.1	
	Serum HIV antigen	−	+	+	+	+	+	+	+	+	
3	SI capacity	0	+	NT	+	+	+	+	AIDS		
	Transmission to H9 cells	NT	+	NT	+	+	+	+			
	$CD4^+$ cells (10^9)	0.7	0.7	0.6	0.5	0.3	0.2	0.2			
	Serum HIV antigen	+	+	+	+	+	+	+			
10	SI capacity	0	+	+	+	+	+	+	+	NT*	+ (AS)
	Transmission to H9 cells	NT	(+)	(+)	+	+	+	+	+	NT	+
	$CD4^+$ cells (10^9)	NT	0.3	0.4	0.6	0.6	0.5	0.3	0.2	0.2	0.2
	Serum HIV antigen	−	+	+	+	+	+	+	+	+	+

[a] 0, No virus detectable; (+), inefficient transmission (see text); AS, asymptomatic; ARC, transition to ARC; AIDS, transition to AIDS; NT, not tested; *, zidovudine treatment initiated.
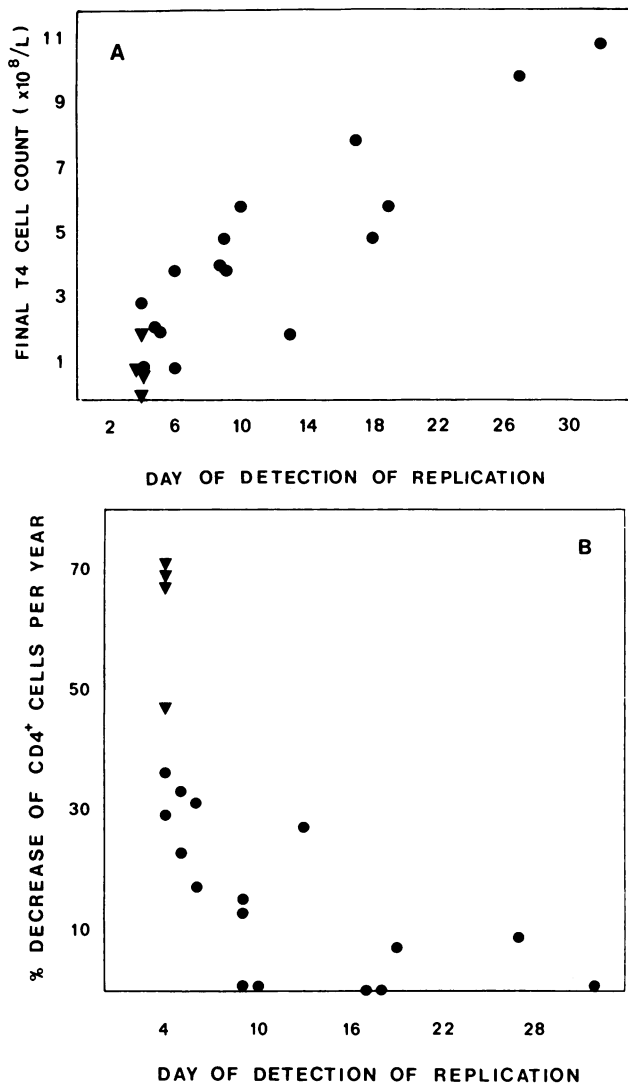
FIG. 2. (A) Relation between the outcome of CD4+ cell count at the end of the period of observation and the mean replication rates of sequential isolates of an individual (expressed on day of detection in the antigen capture assay). (B) Relation between the CD4+ cell decrease and the mean replication rate of sequential isolates from an individual. Statistical analysis (see text) was performed with the Spearman rank correlation test. ●, NSI isolates; ▼, SI isolates.

the observation period, this individual participated in a clinical trial on zidovudine, which may explain why progression to ARC or AIDS did not occur.

In most cases, recovery of SI isolates was accompanied by the detection of serum HIV antigen (subject 3) or conversion for serum HIV antigen during the observation period (subjects 2 and 4) (Table 3). In subject 1, serum HIV antigen was not detectable, but only low levels of anti-p24 antibodies (titers ≤1:8) were detected, possibly reflecting clearance of these antibodies by elevated production of HIV p24 (20).

Molecular analysis of the isolates obtained from subject 3 demonstrated a high degree of similarity among these isolates, although some differences could also be demonstrated (Fig. 3). All isolates from subject 3 possessed a characteristic KpnI site in their long terminal repeat, which is lacking in the human T-cell lymphotrophic virus type IIIB isolate. Moreover, they all lacked an EcoRI site present in human T-cell

lymphotrophic virus type IIIB. In three of the isolates, the presence of more than one clone could be demonstrated. These findings indicate that the similarity in biological properties between these isolates is not due to in vitro selection for a single dominant clone.

DISCUSSION

In this study, we investigated sequential HIV isolates from 20 individuals to obtain answers to the following questions. (i) What is the extent of biological variability of HIV isolates within one individual in the course of time, and (ii) in what way is the occurrence of syncytium-inducing and high-replicating HIV isolates related to clinical markers and the progression to disease? The same variability was found between isolates from different individuals, as observed previously for a transsectional panel (34), contrasting with the constancy generally observed among the isolates from the same individual.

Apparently, a spectrum from low- to high-replicating HIV viruses exists, with SI isolates having the highest replication rates. In contrast to the gradual differences in replication rate, there is a clear distinction between SI and NSI isolates with respect to their ability to replicate in the permanent cell lines tested. At present, it is not clear whether the infectivity block for NSI isolates in these cell lines is absolute, although our complete failure to transmit NSI isolates to these cell lines in numerous attempts both by infection with cell-free virus and cocultivation seems to indicate that this is the case.

Previously, we showed that the high replication rate of SI isolates observed in primary isolation reflects the rapid spread of these isolates in an MNC culture compared with that of low-replicating NSI isolates (34), presumably indicating a higher infectivity. Envelope variability seems to be the most likely explanation for both differences in infectivity and SI capacity. It has been shown that amino acid substitution (18) or modification of glycosylation (13) may substantially affect the SI capacity of HIV isolates. Mutational analysis of the 3' open reading frame region pointed out that variation in this gene might also be responsible for the differences in SI capacity and replication rate observed (11, 21). Possibly, due to less efficient binding and fusion capacity, NSI variants have a need for cellular factors other than CD4 for penetration and uncoating which are lacking in the cell lines tested. Alternatively, the difference in cellular tropism between SI and NSI variants could be due to differences in the long terminal repeat, resulting in a differential sensitivity to cellular transcriptional activators (17, 24). The finding that slow-replicating viruses were able to replicate in a T-cell line transfected with the tatIII gene (B. Åsjön, T. Barkhem, J. Albert, F. Chiodi, H. van Gegerfelt, P. Biberfeld, and E. M. Fenyö, IV Int. Congr. AIDS, abstr. no. 1569, 1988) may also indicate that NSI variants have a less competent tat gene product.

In a previous transsectional study, we showed that SI isolates are observed more often in patients with AIDS or ARC (34). In the present study, we demonstrate that in individuals with AIDS or ARC who yielded SI isolates, SI isolates were already present 9 to 15 months before development of the disease. Moreover, first isolation of an SI isolate was always followed by a rapid decline of CD4+ cells. All features of SI isolates can well be imagined to represent determinants of virulence in vivo. Therefore, it seems likely that appearance of these isolates is causally related to the subsequent CD4+ cell loss and development of the disease. The SI capacity and the apparent broader host range of these
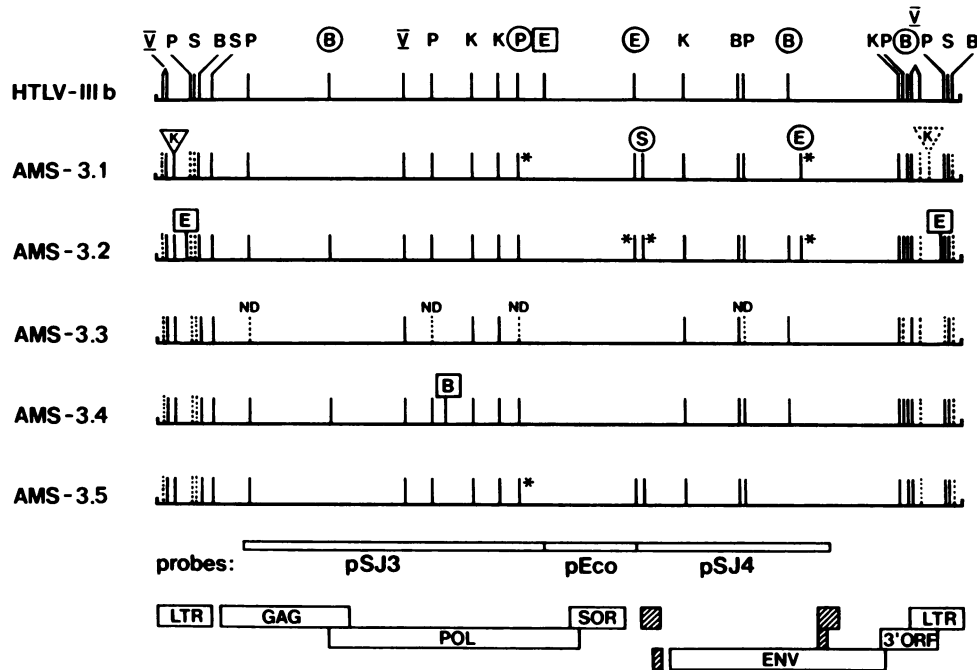
FIG. 3. Putative physical maps of HIV isolates HIV$_{AMS}$ 3.1 to 3.5 compared with that of HIV-HTLV/IIIB. Restriction sites are indicated as follows: V, EcoRV; P, PvuII; S, SacI; B, BglII; E, EcoRI; K, KpnI. Circles indicate restriction sites not present in all isolates; boxes indicate restriction sites present only in one isolate; triangles indicate restriction sites present only in HIV$_{AMS}$ 3.1 to 3.5. *, Partial cleavage; ND, not determined; LTR, long terminal repeat. The sites in the long terminal repeat indicated with dotted lines are restriction sites deduced from findings in the other long terminal repeat.

isolates seem to fit well with the hypothesis (30) according to which syncytium formation of long-living, low-CD4-expressing, HIV-infected cells with high-CD4-expressing cells may be an efficient mechanism to accomplish CD4$^+$ cell depletion. Nevertheless, SI isolates are not observed in all individuals who develop ARC or AIDS. Therefore, it is of particular interest that a significant correlation exists between both the CD4$^+$ cell decrease and outcome of the CD4$^+$ cell numbers of an individual at the end of the period of observation and the (mean) replication rate of the sequential isolates obtained from this individual. The most rapid decline in CD4$^+$ cell numbers was observed in the individuals from whom SI isolates were recovered, followed by those from whom high-replicating NSI isolates were obtained. In five clinically stable asymptomatic individuals from whom slow-replicating NSI isolates were obtained, no significant CD4$^+$ cell decrease was observed over a 15- to 31-month period. Thus, it seems that the replication rate of an HIV isolate is directly associated with the decrease in CD4$^+$ cell numbers in the subsequent period.

Next to decrease of CD4$^+$ cell counts, HIV p24 antigenemia has been used as a clinical marker for progression of disease (2, 8, 12). Although in this study antigenemia or low titers of antibodies to p24 coincided in most cases with high virus replication in vitro, two individuals, who remained asymptomatic in spite of existence of antigenemia for 15 months, did not demonstrate significant CD4$^+$ cell loss over this period and yielded consistently low-replicating HIV isolates. Therefore, the precise nature of the relationship between HIV p24 antigenemia and in vitro virus replication rates remains to be elucidated.

Recently, the degree of genomic variability of HIV clones obtained from one person at the same or different time points has been estimated (mean predicted nucleotide differences, 2

and 4%, respectively) (29). The result of the molecular analysis of the isolates of subject 3 are in agreement with these observations. This degree of genomic variability, however, does not appear to affect the stability of the biological phenotype of sequential isolates studied here. Also, the relation found between the occurrence of particular virus variants and clinical course in itself indicates that the in vitro biological properties of these isolates are relevant to the in vivo situation.

The results of this study are compatible with the hypothesis that differences in the rate of CD4$^+$ cell decrease observed between individuals, presumably one of the main determinants for the variation in the latency period of HIV infection, may be caused by differences in virulence between HIV variants. In this study, we could not find evidence for the gradual development of high-replicating NSI variants from low-replicating NSI variants. Nevertheless, such a development early in infection, possibly under the influence of host factors, cannot be excluded, because the individuals studied here were all seropositive at the beginning of the observation period. In two individuals, who eventually developed AIDS or ARC, a switch from high-replicating NSI isolates to SI isolates with still higher replication rates was observed 9 to 15 months before the onset of disease. Recently, a similar finding in two seropositive individuals has been reported (7). Theoretically, such a phenotypic switch could result from either mutational changes or superinfection with an SI isolate. The gradual changes occurring in the isolates from subjects 2 and 10 and the fact that superinfection with a second HIV isolate until now has not been demonstrated in humans (15) seem to argue in favor of a switch due to mutational changes. Interestingly, in two persons who yielded only SI isolates, it could not be ruled out that a switch in fact did occur as well. More extended

studies, preferably on individuals in whom the moment of seroconversion is well documented, will be needed to find out whether SI isolates can be recovered at all in the early stage of HIV infection and to establish to what extent in vivo mutagenesis of HIV is important as a mechanism in the pathogenesis of AIDS. Detailed molecular analysis of NSI variants, which apparently are predominant in early HIV infection, and comparison of these variants with SI isolates may help to localize and identify the viral structures important for HIV pathogenicity.

ACKNOWLEDGMENTS

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