Infection with Dual-Tropic Human Immunodeficiency Virus Type 1 Variants Associated with Rapid Total T Cell Decline and Disease Progression in Injection Drug Users

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The characteristics of sequential human immunodeficiency virus type 1 (HIV-1) isolates from 12 seroconverters among injection drug users selected for either rapid or slow disease progression were evaluated. All 6 patients who developed AIDS within 5 years were initially infected with syncytium-inducing (SI) variants or showed a transition from non-SI–inducing (NSI) to SI variants. Detection of SI variants was associated with rapid decline of both CD8+ and CD4+ T cells. In contrast, the 6 slow progressors carried only NSI variants and maintained stable or increasing CD8+ T cell levels. The SI variants that were associated with initial infection were dual tropic, with efficient replication in primary macrophages and T cell lines. These results suggest that the ability to replicate in macrophages, rather than the SI or NSI phenotype per se, may be an important determinant of HIV-1 transmission and that dual-tropic viruses, when transmitted, may be associated with rapid progression to AIDS.

Epidemiologic studies have shown that the median time from human immunodeficiency virus type 1 (HIV-1) infection to the development of AIDS is 8–12 years [1–5], with ~20% of infected people developing AIDS within 5 years and another 5% showing minimal disease progression after ≥10 years [6–10]. The factors that influence this variable rate of disease progression are poorly understood. It is clear that HIV-1 replication is active during all stages of the disease, as monitored in both the peripheral blood [11–15] and lymphoid tissues in vivo [16–17].

Phenotypic properties of the various HIV-1 variants, such as replication rate, cell tropism, and syncytium-inducing (SI) ability, correlate with the stage and rate of disease progression [18–28]. The occurrence of a cluster of slowly progressing patients who had been exposed to one source of attenuated HIV-1 demonstrated that the phenotype of the infecting virus can determine subsequent disease progression [29]. Infection with apparently attenuated HIV-1 variants may have also contributed to the slow disease progression in other patients [30, 31].

Disease progression may also be affected by host factors. For example, the appearance of virus-specific CD8+ cytotoxic T lymphocyte activity has been associated with control of viremia during primary HIV-1 infection [32, 33], and a correlation between the degree of CD8+ T lymphocyte anti-HIV suppressive activity and clinical status has also been reported [34]. Recovery of replication-competent HIV-1 from some long-term nonprogressors suggests that host factors are responsible for the control of HIV-1 replication in vivo [30, 35]. People who are homozygous for a deletion in the CCR-5 gene are relatively resistant to HIV-1 infection [36–38], and heterozygosity for this deletion may also provide some degree of protection against disease progression [36, 37, 39].

HIV-1 isolates have been grouped phenotypically into two classes: the macrophage-tropic (M-tropic), non-SI–inducing (NSI) class and the T cell line–tropic (T-tropic), SI class (for a recent review see [40]). M-tropic, NSI viruses use primarily CCR-5 as the coreceptor for entry [41–45], while the T-tropic, SI viruses use CXCR-4 [46]. The M-tropic, NSI viruses predominate during HIV-1 transmission and can be detected at all stages of HIV-1 infection [40]. In contrast, the T-tropic, SI viruses are more often isolated from persons with late-stage disease [40]. An association of SI viruses with rapid disease progression has been demonstrated, but a causal relationship has not been established [40].

AIDS among injection drug users (IDUs) remains a major and growing public health problem. IDUs account for more than one-third of the total AIDS cases in the United States and represent one of the fastest growing areas of the HIV-1 epidemic in this country. IDUs have shown less reduction in risk behaviors and HIV-1 incidence than other segments of the population. Information regarding the characteristics of HIV-1 isolates and the rate of disease progression among IDUs in the United States is scarce. As part of the ongoing natural history study of a cohort of IDUs in Baltimore (ALIVE) [47], we have been studying characteristics of HIV-1 variants from...
documented seroconverters who had either unusually rapid (progression to AIDS within 5 years after estimated time of infection) or more typical, slower courses of disease.

Patients, Materials, and Methods

Study population. The ALIVE study recruited 2960 IDUs (95% are African Americans) in Baltimore as a result of extensive community outreach efforts in 1988–1989. HIV serostatus was determined, and then the subjects were followed semiannually with interviews, physical exams, and venipuncture to obtain cells and serum for further analysis. The ALIVE study had identified ~200 HIV-1 seroconverters by the time this study was initiated; those seroconverters are the population from which the samples for this study were obtained.

We limited subject selection to those participants with an interval of <9 months between the last seronegative and the first seropositive visits. The mid-point between these visits was taken as the estimated time of infection. Of the ALIVE subjects, 135 were considered eligible, and we further required that they have at least three sequential visits on which T cell counts were done. We identified 105 qualified seroconverters, of whom 6 had developed clinical AIDS within 5 years (rapid progressors). To have a total of 12 subjects, we also identified randomly 6 control subjects who were eligible on the basis of the following criteria: They had more than three sequential T cell counts, they had no signs or symptoms of clinical AIDS for at least 5 years, and they had initial CD4+ T cell counts similar to those of the rapid progressors.

Protease inhibitor or combination therapy was not used by these subjects. Although zidovudine was used by some individuals at certain time points, no subject received zidovudine for the first year after seroconversion. Also, the rapid progressors tended to receive zidovudine earlier than slow progressors because zidovudine was given to individuals who had low CD4 cell counts.

Virus isolation and characterization. Viable peripheral blood mononuclear cells (PBMC) (2 × 10⁶) from cryopreserved PBMC were cocultured with 2 × 10⁶ phytohemagglutinin-stimulated PBMC from HIV-seronegative donors for virus isolation as previously described [48]. Culture media were monitored for p24 antigen (DuPont NEN, Wilmington, DE) twice a week for up to 4 weeks after coculture. A positive culture was defined by two consecutive HIV-1 p24 antigen values >200 pg/mL. Ninety-five percent of HIV-1–infected patients, who were not on effective antiviral therapy or were not long-term nonprogressors with stable, high CD4 cell counts, were culture positive using this procedure.

The ability of HIV-1 isolates to replicate in MT-2 cells [49] or primary macrophages [50] was evaluated as previously described. Cell-free viruses recovered from infected PBMC were passed twice through MT-2 cells to enrich for SI variants. For comparison of viral replication in MT-2 cells and primary macrophages, cell-free viruses were standardized by the level of p24 (100 pg) and used to infect ~2 × 10⁶ MT-2 cells or macrophages from HIV-negative donors. Virus replication was monitored by the presence of p24 in the culture supernatants. Syncytium formation was examined daily by use of a light microscope.

DNA preparation, polymerase chain reaction (PCR) amplification, and DNA sequencing of C2-V3 region of HIV-1 env. Cryopreserved PBMC (1 × 10⁶) were washed once with RPMI 1640 and resuspended in 250 mL of lysis buffer (10 mM Tris hydrochloride [pH 8.3], 50 mM KCl, 2.5 mM MgCl₂, 1% NP-40, 1% Tween 20, 100 mg of protease K/mL). Samples were then incubated at 65°C for 2 h, followed by heating at 95°C for 10 min. Cell lysates corresponding to 2 × 10⁵ PBMC were subjected to PCR, using external env primers as previously described [49]. The first PCR (10 mL) was subjected to a second PCR using nested primers containing BamHI or EcoRI restriction enzyme site as previously described [49]. Both first- and second-round PCRs were run at 95°C for 2 min, followed by 30 cycles of denaturing for 30 s at 95°C, annealing for 30 s at 60°C, and synthesis for 45 s at 72°C. At the end of the 30 cycles, the samples were held at 72°C for 10 min and then stored at 4°C for further analysis. The nested PCR products were digested with BamHI and EcoRI and cloned into pGEM7Z(+) Recombinant plasmids that contained PCR products were sequenced by the Sanger method using T7 and SP6 primers from both directions. HIV-1 env sequences were also determined from infected MT-2 cells and macrophages as described above.

Results

Relationship between characteristics of sequential primary HIV-1 isolates from IDUs and disease progression. Demographic data for the 12 study subjects are presented in table 1. All subjects seroconverted between 1988 and 1991. Ages ranged from 20 to 36 years old, 8 of 12 subjects were men, all were African American, and 11 had injected drugs during the seroconversion interval. Of the 6 subjects who progressed rapidly to AIDS (R1–R3 and SW1–SW3), 3 (R1–R3) had a rapid CD4+ T cell decline immediately after infection, which was followed by a diagnosis of AIDS within 4 years of the

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years), sex</th>
<th>Year of seroconversion</th>
<th>Clinical AIDS, illness</th>
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<tr>
<td>R1</td>
<td>30, M</td>
<td>1989</td>
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</tr>
<tr>
<td>R2</td>
<td>34, M</td>
<td>1989</td>
<td>Yes, PCP</td>
</tr>
<tr>
<td>R3</td>
<td>39, M</td>
<td>1991</td>
<td>Yes, MAI</td>
</tr>
<tr>
<td>SW1</td>
<td>27, F</td>
<td>1989</td>
<td>Yes, PCP</td>
</tr>
<tr>
<td>SW2</td>
<td>33, F</td>
<td>1990</td>
<td>Yes, Pneu</td>
</tr>
<tr>
<td>SW3</td>
<td>24, F</td>
<td>1989</td>
<td>Yes, PCP</td>
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<tr>
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<td>28, M</td>
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<tr>
<td>S6</td>
<td>32, M</td>
<td>1989</td>
<td>No</td>
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</tbody>
</table>

Note. All subjects were African American, and all subjects except R2 used injection drugs during seroconversion interval. Clinical AIDS was defined by 1993 CDC criteria [77]. CMV, cytomegalovirus infection; PCP, Pneumocystis carinii pneumonia; MAI, Mycobacterium avium-intracellulare complex; Pneu, recurrent pneumonia.
estimated time of infection (figure 1). Three others (SW1–SW3) also developed AIDS within 4 years of the estimated infection date (figure 1); however, they had stable CD4 cell counts for the first 20–40 months after infection before having a rapid decline in CD4$^+$ T cells and progressing to AIDS. Another feature of the 6 rapidly progressing subjects was that they had no compensatory increase in CD8$^+$ T cell counts during the period of rapid CD4$^+$ T cell decline. Consequently, all 6 had a rapid total T cell (CD3$^+$) decline prior to progressing to AIDS (figure 1).

In contrast to the subjects with rapid disease progression, 6 control subjects (S1–S6) were asymptomatic and maintained relatively stable total T cell counts (figure 1) over a comparable period of time. One control subject (S1) had a significant decline in CD4$^+$ T cells but also had a compensatory increase in CD8$^+$ T cells (figure 1).

To investigate the possible relationship between disease progression and the characteristics of HIV-1 variants, sequential primary HIV-1 isolates were obtained from all subjects except 1 slow progressor (S6), who maintained stable and very high CD4 cell counts (figure 1); the primary virus isolate from the first seropositive visit was the only isolate obtained from this subject. All primary HIV-1 isolates were replication competent in phytohemagglutinin-stimulated PBMC from HIV-negative donors. The peak production of HIV-1 p24 antigen in the culture supernatants from infected PBMC was routinely >100 ng/mL (data not

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**Figure 1.** Absolute nos. of circulating T cell subsets (CD3$^+$, CD4$^+$, and CD8$^+$ lymphocytes) and phenotypes of primary HIV-1 isolates in 12 seroconverters. Dotted vertical lines indicate time points at which clinical AIDS [50] was diagnosed. All virus isolates were replication competent in phytohemagglutinin-stimulated peripheral blood mononuclear cells from HIV-negative donors. Syncytium-inducing (S) or nonsyncytium-inducing (N) phenotype of viruses was determined in MT-2 cells; mid-point between last seronegative visit and first seropositive visit was used as estimated time of infection. Time points (x axis) after HIV-1 infection are indicated in months.
The viral phenotype (NSI or SI) was determined using MT-2 cells as previously described [49]. The NSI phenotype was characterized by a p24 level of <5 pg/mL in the supernatant from infected MT-2 cells and no observed syncytium formation during the first 3 weeks after infection. The SI phenotype was characterized by prominent syncytium formation in MT-2 cells, accompanied by p24 levels of ≥100 ng/mL.

SI isolates were detected in the 3 rapid progressors (R1–R3) at the first seropositive visit and at subsequent visits (figure 1). The remaining 9 subjects had NSI variants at this visit (figure 1). A transition from NSI to SI was detected in all 3 subjects (SW1–SW3) who progressed from stable CD4+ T cell counts to rapid declines in CD4+ T cell levels and development of AIDS (figure 1). For subjects SW1 and SW2, SI variants were first detected before the period of rapid CD4+ T cell and total T cell decline (figure 1). A more precise time at which the NSI-to-SI transition occurred in subject SW3 could not be determined.

NSI variants were consistently detected in the 5 control subjects (S1–S5) who maintained relatively stable total T cell counts and did not progress to AIDS (figure 1). For subject S6, the viral phenotype was only available for the first seropositive visit (figure 1). We obtained sequential viral env V3 sequences from uncultured PBMC samples that were obtained from subject S6 during subsequent visits (up to 5 years after infection). All of these sequences predicted the NSI phenotype (data not shown).

Genotypic analysis of HIV-1 env sequences using the first seropositive PBMC samples from subjects R1–R3 confirmed the presence of SI variants. It has been previously reported that M-tropic, NSI variants are the predominant viruses in the period immediately following sexual [52, 53] or parenteral [54] transmission of HIV-1. Therefore, the detection of SI variants in 3 early seroconverters (R1–R3) raised the possibility that these subjects had been coinfected with both NSI and SI variants and that the SI variants may have constituted only a minor population in these subjects.
To address this question, HIV-1 env V3 loop sequences were determined from uncultured PBMC samples collected from these subjects at their first seropositive visits. Ten to 16 sequences were determined for each subject as previously described [49]. For subjects R2 and R3, homogenous viral sequences were obtained (figure 2), consistent with the interpretation that they were infected with a relatively restricted viral variant. At the same time, viral sequences from infected MT-2 cells were also analyzed to confirm the SI genotype. Viral sequences obtained from infected MT-2 cells representing SI sequences were also homogenous. The viral V3 loop sequences from uncultured PBMC samples of subjects R2 and R3 were identical to the sequences obtained from MT-2 cells infected with these isolates (figure 2). Previous studies have suggested that proviral sequences in PBMC samples obtained within 6 months after infection represent the virus strains that established the initial infection [52, 53]. Therefore, it is likely that the SI variants were the major viruses that established infection in subjects R2 and R3. However, the possibility that NSI variants established infection in subject R2 and R3 and rapidly underwent transition from NSI to SI strains cannot be excluded.

On the basis of V3 sequences, 2 major virus populations were detected in subject R1 (figure 2). The viral sequences, clone numbers 5–9, from the uncultured PBMC samples matched the viral sequences from infected MT-2 cells and therefore represent SI variants. The existence of viral sequences in the uncultured PBMC samples that were not detected in the infected MT-2 cells suggests that NSI variants were also present in this subject at the time of sampling. These putative NSI variants have the genotype of NSI HIV-1 in the V3 loop. Although it is unusual to find very heterogeneous HIV-1 sequences in recent seroconverters, it is also unlikely that this was due to PCR contamination for several reasons. First, sequences identified from R1 were not found in the samples from the other 11 seroconverters and are distinct from sequences of HIV-1 clones used in the laboratory (data not shown). Second, phylogenetic analysis of env sequences from all 12 subjects indicated that the sequences from R1 clustered together but not with sequences from other unrelated subjects (data not shown).

Third, samples from subsequent visits of R1 were also analyzed. In this case, HIV-1 sequences obtained from subsequent visits of R1 were closely related to the original sequences from R1, but not to the sequences from the other unrelated 11 seroconverters (data not shown). These results strongly suggest that PCR contamination did not occur. It is not clear whether coinfection with both NSI and SI variants or rapid switching from one phenotype to another is responsible for the findings for subject R1. Previous studies have shown that coinfection with different variants of HIV-1 can occur but does so infrequently [55, 56].

The SI variants from subjects R1–R3 were dual tropic. It has been speculated that the ability to infect macrophages is
The replication of HIV-1 isolates in MT-2 cells and macrophages is shown in Table 2. The table indicates that the dual-tropic clone 89.6 replicates efficiently in both MT-2 cells and macrophages (table 2). Primary NSI isolates from the first seropositive visit of 3 slow disease progressors also replicated efficiently in macrophages (data not shown).

To further exclude the possibility that the efficient replication of these 3 primary isolates in macrophages could be attributed to the presence of NSI variants, viral env sequences were determined from both infected MT-2 cells and macrophages (figure 3). Homogenous V3 loop sequences (5 clones) were identified from each virus isolate infected—MT-2 cells for all 3 subjects. More important, viral V3 loop sequences from the infected macrophages matched those from infected MT-2 cells in all cases. These results indicate that efficient replication in both MT-2 cells and primary macrophages by these virus isolates was not due to the coexistence of different variants. Therefore, the SI variants from subjects R1–R3 were dual tropic.

**Discussion**

In this study, the relationship between the characteristics of primary HIV-1 isolates and a combination of rapid total T cell decline (CD4⁺ and CD8⁺ T cells) and development of AIDS were systematically evaluated in 12 documented seroconverting IDUs. Previous studies of this type in IDUs are scarce. Several interesting patterns of disease progression were noted. First, SI variants were detected in all of the 6 subjects who had the most rapid disease progression. This high incidence of SI variants in patients who developed AIDS is in contrast to the lower prevalence of SI variants among IDUs who developed AIDS in the Amsterdam cohort [61]. Second, the SI variants that were responsible for initial infection in 3 of the rapid progressors were apparently dual tropic. Third, detection of SI variants was associated not only with rapid CD4⁺ T cell decline but also with rapid CD8⁺ T cell decline.

It has been reported that SI variants can be detected in AIDS patients [24, 28]. Although this is apparently the case for homosexual men in both the United States [24] and Europe [28], the Amsterdam study reported a lower proportion (21%) of SI variants in IDUs who developed AIDS [61]. This difference raises the possibility that the route of virus transmission or that illicit drug use itself might influence the selection of HIV-1 variants. In this study, SI variants were detected in all 6 IDUs who developed AIDS within 5 years of seroconversion from the Baltimore ALIVE cohort. It is possible that this high rate of SI variants results from the fact that we examined the most rapid disease progressors, while in the Amsterdam study the prevalence of SI was investigated for all patients who developed AIDS, including rapid, intermediate, and slow progressors.
As previously mentioned, viruses recovered from recently infected persons are predominantly M-tropic, NSI variants [40]. The ability to enter and productively infect macrophages both at the mucosal surface during sexual transmission and in the lymphoid tissues during parenteral transmission may account for this selection. In vivo, CD4+ T lymphocytes and macrophages are the primary targets of HIV-1 infection [40]. PBMC from healthy donors are largely quiescent, as judged by the lack of cell proliferation [62, 63] and the absence of surface activation markers, such as HLA-DR and CD25 [64, 65]. Although a small fraction of T lymphocytes does express these markers in resting PBMC, these cells cannot support productive HIV-1 infection without exogenous stimulation by mitogen and cytokines [62–65]. On the other hand, tissue macrophages are more susceptible to HIV-1 replication [59, 66]. At the time of HIV-1 transmission, more macrophages than activated CD4+ T cells may be available for initial virus infection, and therefore the M-tropic variants are favored. Detection in early seroconverters of primary SI variants that can also efficiently replicate in macrophages is consistent with the interpretation that the ability to infect macrophages, rather than SI or NSI phenotype per se, may be critical for HIV-1 transmission.

A link between the detection of SI variants and a rapid CD4+ T cell decline in vivo has been previously established [40]. In this study, a rapid decline not only in CD4+ T cells but also in total T cells was observed in 3 rapid disease progressors who were initially infected with SI variants. A rapid decline in total T cell count followed by AIDS was also observed in 3 subjects who showed viral phenotype switching from NSI to SI during the course of the disease. On the other hand, total T cell counts were stable in the 6 subjects who were infected with and continued to harbor NSI variants whether they had or did not have a significant decline in CD4+ T cells. These results raise the interesting possibility that SI variants of HIV-1 might have a more detrimental effect on CD8+ T cells than do NSI variants.

It has been suggested that indirect killing of CD8+ T cells by HIV-1 infection results from apoptosis [67] or shortening of the telomeres [68]. A possibility of direct CD8+ T cell infection by certain HIV-1 variants has also been raised [69, 70]. If these are true mechanisms for killing of CD8+ T cells in vivo, it is possible that SI variants may be more efficient in inducing these mechanisms than are NSI variants. Alternatively, efficient infection and killing of CD4+CD8+ thymocytes by HIV-1 may contribute to a total T cell decline in vivo [71, 72]. Since these thymocytes or similar precursor cells in other parts of the body may contribute to regeneration of CD4+ and CD8+ T cells, infection and killing of these cells by HIV-1 may influence total T cell counts in vivo. To this end, it is worth noting that SI variants appear to kill CD4+CD8+ cells more efficiently than do NSI variants [71–74], and a recent study has suggested that CD4+CD8+ cells express high levels of CXCR-4, the coreceptor used by SI variants [75]. A rapid decline in the total T cell count beginning ~2 years before the development of AIDS has been observed in several cohort studies, suggesting failure of T cell homeostasis [76, 77]. These observations suggest that the possible association between the emergence of particular strains of HIV-1, such as SI variants, and a rapid decline in total T cell count deserve further investigation.

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References


