Transfusion-Associated AIDS: Donor–Recipient Human Immunodeficiency Virus Exhibits Genetic Heterogeneity


The genetic diversity of the human immunodeficiency virus (HIV) isolated from transfusion-associated AIDS patients has been examined. Restriction enzyme mapping studies of integrated proviral DNA of donor and recipient origin demonstrated genomic variation between isolates. Analysis of the molecularly cloned viral genomes of one donor-recipient pair showed that virus from the recipient had restriction enzyme site differences from the donor, notice-

ably clustered in the env and orf-2 regions, and also had a greater number of restriction sites in common with the donor as well. These results suggest that HIV may undergo genomic variation in vivo. Comparison of donor-recipient viruses may further the understanding of the molecular basis for AIDS pathogenesis.

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A CQUIRED immunodeficiency syndrome (AIDS) and related immunologic disorders have been etiologically linked to human immunodeficiency virus (HIV), which is also known as human T-lymphotropic virus type III (HTLV-III).1 lymphadenopathy-associated virus (LAV),2 or AIDS-associated retrovirus (ARV).3 HIV is cytopathic and has tropism for the T4 helper lymphocyte subset.1,4 Molecular cloning of different HIV isolates led to the characterization of the genome by restriction enzyme mapping.5,7 and comparison of the primary nucleotide sequences further revealed a variation of 1.8% between HTLV-III and LAV and up to 9.3% between LAV and ARV-2.8,11 Restriction enzyme polymorphism studies carried out with a number of HIV isolates from persons not epidemiologically linked to each other showed an altered restriction pattern from one isolate to another.7,12,14

The genetic variation observed with HIV isolates from the natural population could result from high mutability of the genome. To examine the extent of genetic changes, we have focused our attention on HIV isolated from transfusion-associated AIDS patients between 18 and 31 months after they had received blood and the blood donors who were the source of their infection. Here we report that virus isolated from blood recipients had restriction enzyme patterns that were different from the patterns of virus from their blood donors.

MATERIALS AND METHODS

Cells and viruses. HIV were isolated from samples obtained from transfusion-associated AIDS patients and their blood donors as described.13,18 Briefly, peripheral blood collected was subjected to a Ficol-Hypaque gradient for collection of mononuclear cells. The cells were grown in RPMI 1640 (GIBCO, Grand Island, NY) containing 10% fetal calf serum and 10 μg/mL phytohemagglutinin (PHA, GIBCO) for three days and were further propagated in RPMI medium containing T cell growth factor and 1:5000-diluted goat antibody to human interferon (Miles Scientific, Naperville, IL). Periodically the culture was tested for virus production by cocultivation with 3-day-old PHA-stimulated normal adult T cells. Cell-free supernatants from the cocultivated cultures were analyzed for retrovirus by reverse transcriptase assay,1 and virus stock was frozen after two passages in PHA-stimulated peripheral blood lymphocytes (PBLs).

Restriction enzyme analysis. High–molecular weight DNA was extracted from 7-day-old virus-infected adult T cells.17 DNA (10 μg) was digested with different restriction enzymes and subjected to electrophoresis on a 0.8% agarose gel. The DNA fragments were transferred to nitrocellulose paper and hybridized to a nick-translated HIV full-length genomic probe.18 The hybridization was performed at 42°C for 18 hours in a buffer containing 50% formamide, 5× SSC, (1× SSC = 0.15 mol/L NaCl and 0.015 mol/L Na2-citrate, pH 7), 5× Denhardt’s solution (1 mg/mL bovine serum albumin, polyvinylpyrrolidone, and Ficoll), 20 mmol/L NaPO4 (pH 6.5), 100 μg/mL salmon sperm DNA, and 10% sodium dextran sulfate as described.19 The filters were washed at 65°C with 0.1× SSC, 0.1% sodium dodecyl sulfate, and exposed to Kodak XAR-5 film for one to two days.

Construction and screening of recombinant phage library. Hirt DNA,19 extracted from adult T cells 72 hours after virus infection, was digested with ScaI and fractionated on 10% to 40% sucrose density gradients.14 Fractions containing human immunodeficiency virus sequences were pooled and ligated to ScaI arms of λgt WES.AB. The DNA was packaged by using the extract and protocol from Promega Biotech (Madison, WI). Plaques containing recombinant phage were located by the plaque filter hybridization.20

Restriction endonuclease mapping. Recombinant phage DNA was prepared as described.13 DNA insert containing viral sequences were separated from λgt WES.AB arms by ScaI digestion and preparative agarose gel electrophoresis. Restriction endonuclease mapping was carried out with the partial digestion method of Smith and Birnstiel.21

RESULTS

Transfusion-associated AIDS patients and their blood donors. Previous studies in our laboratory revealed the presence of HIV in the blood of patients with transfusion-associated AIDS and their blood donors. All donor-recipient pairs had antibodies to HIV as determined by enzyme-linked immunosorbent assay and Western blot analysis.14,15 Viruses isolated from four donor-recipient pairs were propagated in PHA-stimulated normal PBLs. The clinical conditions of the donor-recipient individuals is given in Table 1. In all cases, only one HIV-infected donor was found to be the source of
Table 1. Findings in Investigations of Patients With Transfusion-Associated AIDS and Their Blood Donors

<table>
<thead>
<tr>
<th>ID No. of Donor-Recipient Pair From Whom HTLV-III/LAV Was Isolated</th>
<th>Total No. of Donors</th>
<th>No. Donors With AIDS Risk Factor* / No. Interviewed</th>
<th>No. Donors With HIV Antibody/ No. Tested</th>
<th>Interval From Transfusion to AIDS Onset in Recipient (mo)</th>
<th>Donor Health at Time of Viral Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1/3</td>
<td>1/3</td>
<td>18</td>
<td>Healthy</td>
</tr>
<tr>
<td>2</td>
<td>9†</td>
<td>1/4</td>
<td>1/4</td>
<td>31</td>
<td>Healthy</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>1/30</td>
<td>1/26</td>
<td>18</td>
<td>Healthy‡</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1/5</td>
<td>1/3</td>
<td>18</td>
<td>Healthy‡</td>
</tr>
</tbody>
</table>

Virus from the recipient was isolated at the time of AIDS onset.

*All donors with AIDS risk factor were homosexual men.
†Five donors were collected in Europe.
‡Subsequently developed AIDS.

the recipient’s infection with virus. Interestingly, all the blood recipients had AIDS at the time of virus isolation, whereas all donors were asymptomatic during the time between blood donation and virus isolation.

Southern hybridization analysis of donor-recipient HIV proviral DNAs. High-molecular weight DNA isolated from virus-infected cells was digested with restriction enzymes and analyzed by Southern hybridization to a molecularly cloned full-length HIV-Zr6 DNA. HIV-Zr6, isolated from an African AIDS patient, was cloned by using Hirt DNA extracted from the virus-infected cells. SacI enzyme-digested DNA was used for cloning because this enzyme has cleavage sites only in the LTR sequences of viral DNA. From our analyses involving a number of isolates from North America and Zaire (Anand et al, unpublished data) the 9-kb full-length probe hybridizes intensely to all isolates despite the restriction enzyme site differences. No detectable hybridization was found in uninfected cultures of the same PBL.

Figure 1 shows the hybridization patterns observed with donor-recipient pair 1 proviral DNA cleaved with 14 different restriction enzymes. Donor DNA, cleaved with SacI restriction enzyme, showed a faint hybridization at the 9-kb range in addition to ones at 5.5 kb and 3.5 kb. Further experiments by taking lesser amounts of high-molecular weight DNA in the restriction digests indicated that the 9-kb band was due to partial digestion and not to the presence of a second virus in the cells (data not shown). Also, restriction enzyme patterns show that only one predominant form of the virus was detected in a single donor or recipient.

Four donor-recipient pairs of viruses analyzed in our experiments exhibited varying degrees of restriction enzyme site polymorphism (Table 2). SacI enzyme, which cleaves the viral DNA in long terminal repeat sequences of HIV, generated different restriction fragments in three donor-recipient pairs: 1, 3, and 4. A characteristic feature of HIV-infected lymphocytes is the presence of considerable

![Fig 1](image-url). Southern blot analysis of HIV proviral DNA from donor-recipient pair 1. PBLs, separated by Ficoll-Hypeque technique and stimulated with PHA (10 µg/mL), were infected with HIV isolated from the respective donor-recipient pair as described. Six days after infection, high-molecular weight DNA was extracted and digested with different restriction enzymes. Hybridization was carried out under standard conditions. O, blood donor; R, blood recipient; 1, SacI; 2, KpnI; 3, HindIII; 4, HindII; 5, EcoRI; 6, BglII; 7, BamHI; 8, PstI; 9, PvuII; 10, SacII; 11, HhaI; 12, HphI; 13, HaeII; 14, EcoRV. Full-length HIV-Zr6 fragment cloned in pUC18 plasmid was purified twice on low-melting agarose gel and used for nick-translation.
unintegrated linear and circular DNA in addition to the integrated proviral DNA.5-7 To avoid the restriction site differences due to the different forms of DNA and to compare the internal restriction fragments, we have also tested with enzymes BgIII, HindIII, Kpnl, PvuII and ScaI, which cleave the genome at multiple sites. Table 2 lists the differences in the four pairs of viruses with regard to ten different restriction enzymes. Donor-recipient pairs 1 and 4 showed six restriction site differences, and donor-recipient pair 2 showed only two restriction site differences. Restriction analyses failed to reveal a specific genotypic pattern that distinguished isolates obtained from the AIDS patients and isolates obtained from the asymptotically infected blood donors.

Molecular cloning of donor-recipient human immunodeficiency viral DNA. To determine more precisely the extent of genomic variation between donor-recipient viruses, we have molecularly cloned the viral genomes of donor-recipient pair 1 (Table 1). PHA-stimulated lymphocytes were infected at a high multiplicity with viruses derived from the donor-recipient pair. Unintegrated viral DNA extracted 72 hours after infection19 was used as a substrate for constructing the recombinant DNA library, and positive clones were identified with the HIV-Zr6 probe. Representative clones containing donor and recipient viral genome were selected for detailed analysis. The physical maps of the cloned genomes are shown in Fig 2. Donor viral DNA had 17 unique restriction sites, and the recipient virus showed seven new restriction sites. Restriction enzymes Neol and Scal, which cleave viral DNA only once, exhibited no alteration. On the other hand, changes were evident with restriction enzymes such as HindIII, PvuII, BgIII, and Scal, which cleave at multiple sites in the viral DNA. The restriction enzyme site differences between the donor and recipient viruses are noticeably clustered in the env and downstream orf-2 gene. Comparison of the physical maps of the donor-recipient viral DNA with that of HTLV-III and ARV indicated the presence of common restriction enzyme sites. In addition, donor-recipient viruses exhibited a number of differences from HTLV-III and ARV, especially with respect to the enzymes that cleave viral DNA less frequently. A primary nucleotide sequence comparison of donor-recipient viruses would be required to define these changes more precisely.

**DISCUSSION**

HIV has been shown to exhibit considerable genetic heterogeneity as determined by restriction enzyme cleavage patterns. Comparison of 18 different viral isolates by Wong-Staal et al14 revealed that variation between isolates ranged from very minimal (one of 23 restriction enzyme sites) to considerable (16 of 31 enzyme sites different). Benn et al15 have noted variation in ten isolates derived from different geographic locations. These differences in restriction enzyme

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**Table 2. Summary of Restriction Fragment Differences in Donor-Recipient Pairs of HIV**

<table>
<thead>
<tr>
<th>Virus Isolate</th>
<th>Sacl</th>
<th>Kpnl</th>
<th>HindIII</th>
<th>HindII</th>
<th>EcoRI</th>
<th>BgIII</th>
<th>BamHI</th>
<th>PstI</th>
<th>PvuII</th>
<th>Scal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor-recipient pair 1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Donor-recipient pair 2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Donor-recipient pair 3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Donor-recipient pair 4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: +, change in the restriction fragment length generated by the indicated enzyme between donor-recipient viral DNA; -, no change in restriction fragment length.

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![Fig 2. Restriction endonuclease maps of HIV from donor-recipient pair 1. The location of genes are indicated below the physical map.](image-url)
patterns could result from many kinds of genotypic differences including: (a) one or more individual bases could differ, resulting in loss of the cleavage site or formation of a new one, and (b) insertion or deletion of blocks of sequences could also alter its size. Donor-recipient viruses not only share a number of common restriction enzyme sites but they also possess sites unique to each isolate. Our analysis of donor-integrated viral DNA suggests that there was only one kind of virus present in the donor’s blood, which may be the predominant form at the time of collection. By using different HLA-unrelated donor lymphocytes, viruses with similar restriction pattern were repeatedly recovered, indicating a lack of genetically distinct viruses at the detectable level. However, we cannot rule out the possibility that blood donors harbor multiple viruses and that one may be able to grow better upon transmission into the recipient’s body. In vitro lymphocyte cultures used for propagating the virus may have selected one form over the other. This possibility is less likely because PBLs seem to have less selection bias for virus growth in comparison with the established human T cells in culture.

Whether the changes in the genomic sequences result in the changes of the translational products encoded by the virus is a very important question. Neel et al suggested that electrophoresis should detect about one third of all nucleotide substitutions in the coding sequences because of altered molecular charge of polypeptides due to amino acid substitutions. There are changes in the gag and env gene proteins encoded by donor-recipient viral genomes (data not shown) similar to the data reported for nine independent HIV isolates. The differences in the apparent molecular weight of proteins may be due to substitutions in amino acid sequence and deletions as well. The changes in env gene protein may have significant implications because of its role in recognition of receptor and tropism.

Molecular cloning of AIDS viruses from donor-recipient individuals enabled us to analyze the similarities and differences in detail. The differences between donor-recipient viruses are most prevalent in the env and orf-2 gene. It should be mentioned that a span of 18 to 31 months separate donor-recipient viruses. Noticeably, the extent of changes in the recipient virus does not correlate with the time elapsed from transfusion in the recipient because donor-recipient pair 2 showed minimal changes. Since the spontaneous mutation rate is much lower in humans, a special hypermutation mechanism may have to be invoked for the differences seen with donor-recipient viruses. Sequence analyses of different HIV isolates indicate that point mutation, presumably due to misincorporation of bases and rearrangements (reduplication, inversions, deletions, etc) contribute to the observed genetic variation between isolates. Also, nucleotide variation is not consistent over the whole genome. The genes encoding the internal virion proteins gag and pol are considerably more conserved than env. Such a picture also emerges from the restriction enzyme data of the donor-recipient isolates and other independent isolates. The underlying mechanism of such variation is not known. The error-prone nature of virus-encoded reverse transcriptase enzyme may partly be responsible for such variation. Studies concerning the retrovirus point mutation rate with Rous sarcoma virus indicate a frequency on the order of $3 \times 10^{-4}$ bases per passage of virus. Hahn et al reported a higher mutation rate for HIV. Studies on viruses recovered from molecularly cloned HIV proviral DNA using an animal model system would be useful to quantitate the mutation rate in HIV.

Wong-Staal and coworkers reported that AIDS virus does not accumulate genetic changes in cell culture. The changes observed with different AIDS viruses, including the donor-recipient pair, may reflect the selective pressure of the immunologic mechanisms existing in different hosts. The changes in HIV may be similar to changes observed with visna virus and equine infectious anemia virus (EIAV), members of the lentivirus family. Both visna virus and EIAV undergo changes in their envelope glycoproteins during progression of infection. It has been suggested that such changes allow these viruses to evade host immune surveillance mechanisms. Whether the emergence of antigenic variants plays any role in the pathogenesis of these diseases or in the pathogenesis of AIDS and related human diseases remains to be proved. In all four of the donor-recipient pairs studied, the recipients developed AIDS before their donors, and in three cases, the donors have so far remained asymptomatic carriers. The viral genotype, host immune surveillance mechanisms, and/or other factors may contribute to the development of disease in the recipients. Recently, Hahn et al reported genetic changes in HIV isolated sequentially from persistently infected individuals. The data generated on transfusion-associated AIDS viruses further attest the changing nature of HIV in vivo. It will be interesting to know whether HIV does change more rapidly when passing from one person to another than when multiplying in a single person. The elucidation of this should await further studies.

ACKNOWLEDGMENT

We gratefully acknowledge Mead Morgan and Richard Green for help with the figures and Cindy Whitehead and Susan Shaw for the excellent preparation of the manuscript. We also thank the following physicians for their assistance in obtaining blood samples from donors and recipients: W. James Alexander, Robert Hamm, Rand Stoneburner, and Robert Weinstein.

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Transfusion-associated AIDS: donor-recipient human immunodeficiency virus exhibits genetic heterogeneity

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