Acquired Immunodeficiency Syndrome-Associated T-Cell Lymphoma: Evidence for Human Immunodeficiency Virus Type 1-Associated T-Cell Transformation

By Brian G. Herndier, Bruce T. Shiramizu, Nancy E. Jewett, Kenneth D. Aldape, Gregory R. Reyes, and Michael S. McGrath

The majority of lymphomas in the setting of acquired, iatrogenic, or congenital immunodeficiencies are B-cell lymphoproliferations. We describe a rare T-cell lymphoma in a fulminantly ill patient infected with human immunodeficiency virus type 1 (HIV-1). The T-cell nature of the process was defined genotypically (monoclonal T-cell receptor \( \beta \)-chain \([C_\beta]\) rearrangement) and phenotypically (CD45RO\(^+\), CD4\(^+\), CD5\(^+\), CD25\(^+\), CD8\(^-\), CD3\(^-\) and negative for a variety of B-cell and monocyte markers). The CD4\(^+\), CD25\(^+\) (interleukin-2 receptor [IL-2R]) phenotype with production of IL-2 and IL-2R RNA is analogous to human T-lymphotropic virus type 1 (HTLV-I)-associated adult T-cell leukemia/lymphoma (ATLL); however, no HTLV-1 could be detected. Southern blot analysis did demonstrate monoclonally integrated HIV-1 within the tumor genome. Furthermore, the tumor cells were producing HIV p24 antigen as shown by immunohistochemistry. This is the first case of acquired immunodeficiency syndrome (AIDS)-associated non-Hodgkin’s lymphoma in which HIV-1 infection may have played a central role in the lymphocyte transformation process.

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N ON-HODGKIN’S lymphomas are one of the most common classes of malignancies in individuals infected with the human immunodeficiency virus type 1 (HIV-1). These lymphomas are predominantly of B-cell origin; however, rare T-cell lymphomas have been observed. The pathologic mechanisms by which these lymphomas occur have not yet been completely delineated, although subsets of the B-cell lymphomas with 8;14 chromosomal translocations or infected with Epstein-Barr virus (EBV) have been identified.

The role of HIV-1 in the pathogenesis of acquired immunodeficiency syndrome (AIDS)-associated lymphomas is currently unclear. In animals, the development of non-Hodgkin’s lymphoma is highly associated with oncogenic retrovirus infection. As the lymphoma develops, clonally integrated forms of animal retrovirus can be found within the lymphoma DNA. In animal species that develop non-Hodgkin’s lymphoma after viral infection, the lymphomas typically are not detected until approximately one sixth of the animal’s normal life span. Lentiviruses such as HIV-1, as opposed to leukemia viruses, are not thought to play direct roles in cellular transformation. However, lentivirus-infected animals, such as sheep infected with visna virus, typically die of lentivirus-associated disease earlier than lymphomas might develop in leukemia virus-infected animals. To date, HIV genomes have not been found integrated in DNA isolated from AIDS-associated lymphoma tissue, suggesting that the AIDS-associated lymphomas that have occurred over this past decade have not arisen because of direct HIV-1 involvement in cellular immortalization events. We report the first case of an AIDS-associated non-Hodgkin’s lymphoma that appears to have arisen from a T lymphocyte containing integrated HIV-1. This case suggests that similar to the animal leukemia/lymphoma retroviruses, HIV-1 in certain circumstances may be associated with malignant transformation.

CASE HISTORY

The patient was a 45-year-old black man whose chief complaints at the time of admission were cough, shortness of breath, sweats, and chills of a 1-week duration. The patient also noted concomitant fever and a weight loss of 10 kg. Physical examination revealed a 75-kg man in significant respiratory distress with fluctuating consciousness with a temperature of 38.5°C and blood pressure of 92/50 mm Hg. Pertinent physical findings included cervical, inguinal, and axillary adenopathy. Lungs were dull to percussion. Other conditions included seborrheic dermatitis, a 7-month history of increasing alopecia, and oral thrush. The laboratory workup was highlighted by a severe lymphopenia with an absolute CD4\(^+\) cell count of 45/\mu L and CD8\(^-\) cell count of 193/\mu L. The patient was normocytic.

The patient was admitted with the presumptive diagnosis of an opportunistic pulmonary infection: the leading infectious candidate was Pneumocystis carinii. Early in his hospital course, the patient’s condition rapidly deteriorated, requiring intubation and admission into the intensive care unit. Cytologic examination of pleural fluids consistently showed highly atypical lymphocytes. Examination of a fine-needle aspiration of lymph node and a lymph node biopsy were read as high-grade lymphoma. High-dose steroids were initiated. A positive purified protein derivative (PPD) and chest x-ray (Fig 1) featuring a diffuse nodular pattern, worsening upper lobe consolidation, and pericardial effusion led to pleural fluids shifted the focus of treatment to multiple pulmonary infections. The antibiotic course included several cephalosporins, triple-drug antituberculous therapy, amphotericin B,
trimetrexate, and high-dose intravenous trimethaprim-sulfamethoxazole. On the sixth day after admission, the patient's physicians decided not to aggressively treat the lymphoma because of the patient's deteriorating status and 20% cardiac ejection fraction. The patient died on the seventh hospital day. At autopsy, extensive lymphomatous involvement of lungs, tumor nodules in the heart,

Table 1. Antibody Specificities

<table>
<thead>
<tr>
<th>Cluster Designation</th>
<th>Major Specificities</th>
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<tbody>
<tr>
<td>CD45 (LCA)</td>
<td>Leukocyte common antigen</td>
</tr>
<tr>
<td>Anti-human IgM</td>
<td>B cells</td>
</tr>
<tr>
<td>CD20 (L26)</td>
<td>B cells</td>
</tr>
<tr>
<td>CD19</td>
<td>B cells</td>
</tr>
<tr>
<td>CD45RO (UCHL-1)</td>
<td>T cells</td>
</tr>
<tr>
<td>CD5</td>
<td>T cells, autoimmune B cells</td>
</tr>
<tr>
<td>CD3</td>
<td>T-cell receptor (α-chain)</td>
</tr>
<tr>
<td>CD4</td>
<td>T-helper</td>
</tr>
<tr>
<td>CD8</td>
<td>T-suppressor/cytotoxic</td>
</tr>
<tr>
<td>CD25</td>
<td>IL-2R</td>
</tr>
<tr>
<td>CD14 (Leu-M3)</td>
<td>Monocyte, follicular dendritic cells</td>
</tr>
</tbody>
</table>

Table 2. Immunohistology of the Patient's Lung Tumor

<table>
<thead>
<tr>
<th></th>
<th>Lung Tumor</th>
<th>Perihepatic Node</th>
<th>HIV + Follicular Hyperplasia Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CD45RO</td>
<td>+++</td>
<td>++</td>
<td>+ Paracortex</td>
</tr>
<tr>
<td>CD3</td>
<td>—</td>
<td>++</td>
<td>+ Paracortex</td>
</tr>
<tr>
<td>CD4</td>
<td>++</td>
<td>+</td>
<td>+ Paracortex</td>
</tr>
<tr>
<td>CD8</td>
<td>—</td>
<td>+</td>
<td>+ Paracortex</td>
</tr>
<tr>
<td>CD25</td>
<td>++</td>
<td>R</td>
<td>—</td>
</tr>
<tr>
<td>CD5</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>—</td>
<td>+</td>
<td>+ GC</td>
</tr>
<tr>
<td>CD20</td>
<td>—</td>
<td>+</td>
<td>+ GC</td>
</tr>
<tr>
<td>CD19</td>
<td>—</td>
<td>+</td>
<td>+ GC</td>
</tr>
<tr>
<td>CD14</td>
<td>—</td>
<td>—</td>
<td>+ GC, DP</td>
</tr>
<tr>
<td>p24</td>
<td>++</td>
<td>—</td>
<td>+ GC, DP</td>
</tr>
</tbody>
</table>

Abbreviations: —, no cells stained; +, 5% to 30% of cells stained; ++, 30% to 80% of cells stained; ++++, >80% of cells stained; GC, germinal center pattern of stain; DP, dendritic pattern; R, rare.
liver, left adrenal, and an enlarged periaortic lymph node were discovered. There was widespread lymphadenopathy not associated with tumor. Special stains for Pneumocystis carinii, acid-fast bacilli, and fungi were negative. The cerebrum featured microglial nodules and subpial gliosis with no evidence of lymphoma.

**MATERIALS AND METHODS**

Patient specimens. Samples were obtained at autopsy, quick-frozen, and stored at −70°C. Specimens were carefully aliquoted to ensure that representative samples were submitted for formaldehyde fixation/paraffin embedding and subsequent hematoxylin and eosin staining (H&E), as well as cryostat sections for immunohistochemistry and also for nucleic acid isolation.

Immunohistochemistry. Tissue stored at −70°C in the optimal cutting temperature (OCT) cryopreservative medium was thinsctioned using standard methodology. The two-stage immunoperoxidase technique of Wood and Warnke was used with minor modifications. Antibody sources included Dako Patts, Carpinteria, CA (anti-human-IgM, CD19, CD45, CD20, CD45RO, CD4, CD8, CD3, CD25, CD14), Becton-Dickinson, San Jose, CA (CD5), and Dr J.R. Carlson, University of California Davis (anti-HIV p24).
Second-stage biotinylated antibodies were from Zymed Laboratories (South San Francisco, CA) and avidin horseradish peroxidase from Vector Laboratories (Burlingame, CA). Table 1 summarizes cluster designations and major specificities of the antibodies used in this study.

**DNA studies.** Genomic DNA was isolated from each specimen and Southern blots prepared as previously described. Ten micrograms of high molecular weight DNA were restriction endonuclease-digested and separated by electrophoresis on a 0.8% agarose gel. The gel was denatured and neutralized before transfer to a nitrocellulose membrane. After baking (in vacuo) at 80°C for 2 hours, the membranes were prehybridized and then hybridized 16 to 24 hours. Following washes to a final stringency of 0.1X SSC (1X SSC = 0.15 mol/L NaCl + 0.015 mol/L sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) at 60°C for 30 minutes to 1 hour, the blots were exposed to x-ray film.

Various probes were used. To analyze rearrangements of the T-cell receptor β-chain (Cβ), HindIII- and BamHI-digested DNA was hybridized with a constant region probe for Cβ. DNA from peripheral blood lymphocytes (PBL) was digested and used as a Cβ germ line control. A clonal T-cell population would be expected to give a hybridization with the Cβ probe different from that of germ line bands present in PBL DNA. A human immunoglobulin heavy-chain J probe was used to assess immunoglobulin gene rearrangements. The J probe was hybridized to genomic DNA digested with HindIII or EcoRI. To detect the presence of human T-lymphotropic virus type I (HTLV-I) genes, the 9-kb HTLV-I insert (gift from Dr F. Wong-Staal, National Cancer Institute) was used with EcoRI-digested DNA. DNA extracted from the HTLV-I-infected T-cell line MT-2 was used as a positive control for HTLV-I hybridization. To detect the presence of the HIV genomes, a 3.5-kb end fragment (SacI-SacI) of the HIV-1 strain HXB-2 gene clone was used with BamHI-digested DNA (gift from Dr R. Gallo, National Cancer Institute, National Institutes of Health). DNA was extracted from the HTLV-IIB HXB-2 gene clone—transfected H9 T-cell line and used as a positive control for hybridization. An EBV terminal repeat gene probe and a human herpes virus-6 (HHV-6) specific oligonucleotide probe were used to test lymphoma DNA for the presence of these viral genes.

**Amplification of RNA.** RNA was isolated from the tumor tissue, as well as the negative control, PBL. The RNA was reverse transcribed into cDNA as previously described, with minor changes. RNA (3 μg) was denatured for 5 minutes and cooled. A mixture of 10% reverse transcriptase (RT) buffer, oligo-dT primer, dNTP, spermidine-HCl, RNAsin, and H2O to 50 μL was added to the denatured RNA with Moloney murine leukemia virus (MoMuLV-RT). The mixture was incubated at 37°C for 60 minutes. Following the addition of NaCl and phenol extraction, the cDNA was ethanol-precipitated.

The polymerase chain reaction (PCR) was performed essentially as previously described. Primers for interleukin-2 (IL-2) and IL-2 receptor (IL-2R) were derived from the Genebank library (IntelliGenetics, Mountain View, CA), in collaboration with Dr E. Engleman (Stanford University, Department of Pathology, Palo Alto, CA): IL-2 5' primer: 5'-GGGAATTCTATGTTATGGATGCAGAC-3'; IL-2 3' primer: 5'-GGGAGCAATTTGTCAGT-3'; IL-2R 5' primer: 5'-CTCGAGACACTGCTAGG-3'; IL-2R 3' primer: 5'-AGTGAACAAAGAAACAGAAG-3'. The primers were synthesized on a PCR Mate (Applied Biosystems, Foster City, CA).

A small portion of the RT product was subjected to PCR with each set of primers, as well as using both sets of primers in one reaction with the tumor cDNA. Equal aliquots of the PCR reactions were loaded with dye mix and electrophoresed in an 80-V constant voltage field agarose gel.

**RESULTS**

Table 2 summarizes the immunophenotype data on two autopsy tissue samples from the patient. The phenotypic results of the perihepatic lymph node are representative of other three “nonlymphomatous” lymph node sites (periaor-
tic, peripancreatic, and axillary). Histologically, these four sites did not meet surgical pathological criteria for malignant lymphoma and were best categorized as within the spectrum of benign HIV-related lymphadenopathy.23

In contrast to the previously described sites, the lung/hilar node mass was definitively malignant lymphoma. Although there was a small subset of benign-appearing lymphocytes, the vast majority of the cellular population were large cells with hyperchromatic nuclei featuring prominent nucleoli, often opposed to nuclear membranes—an appearance consistent with large-cell lymphoma as described in the Working Formulation for the classification of lymphomas24 (Fig 2). A subset of cells showed extreme atypia characterized by hyperchromatic nuclei with multiloculated forms, not unlike that previously described in high-grade T-cell lymphoma such as the HTLV-I-associated adult T-cell leukemia/lymphoma (ATLL).

The immunophenotype of the lung tumor was best described as T-lymphocytic: CD45+, CD45RO+, CD4+, CD25+, CD8+, CD3+. Expression of CD5 was noted in the tumor, but with weak immunostaining. The tumor was virtually devoid of B-cell markers (Table 2). The immunophenotype of the perihilar lymph node was that of a heterogeneous lymphocytic mixture. Both B cells (CD20, CD19 coexpression) and CD3+ T cells (CD4+ and CD8+) were clearly present. Of note was the rare appearance of CD25+ (“activated” T cells, IL-2R-positive) T cells (Table 2).

The positive control for the patient’s immunophenotyping was another HIV-positive patient, with classic follicular hyperplasia of the lymph node.25 This node showed strong B-cell immunostaining in germinal centers (CD19 and CD20), and a reactive mixture of T cells (CD4+ and CD8+) in the paracortex. The follicular dendritic/monocyte marker (CD14) was prominent in the germinal centers (Table 2).

The anti-HIV p24 monoclonal antibody was applied to two tissue sites from the patient and from the follicular hyperplasia control (Fig 3). The control showed faint anti-p24 staining in a follicular dendritic pattern identical to the pattern of CD14 staining. The immunostaining of the pulmonary tumor, unlike the faint staining of the control, featured dense anti-p24 antibody staining of tumor cells. The perihilar node was not positive for p24 at equivalent titrations of primary antibody.

**MOLECULAR ANALYSIS**

The predominant cell present in the tumor specimen was a CD4+ T cell. To test whether these T cells were monoclonal, lymphoma and control tissue DNA were analyzed by Southern blot for clonal rearrangement of the Cβ T-cell receptor gene. The tumor DNA had a rearranged Cβ gene (Fig 4, lane 1) when analyzed by two different enzymatic digests (HindIII, BamHI) as compared with the germ line Cβ configuration (Fig 4, lane C). Note that the non-tumor-involved lymph node DNA showed only the germ line configuration of Cβ genes (lane 2), which is characteristic of a polyclonal lymphocytic population. Immunoglobulin gene rearrangement studies using the J5 gene probe failed to detect monoclonal B-cell involvement at any site analyzed (data not shown).

DNA from the pulmonary tumor and uninvolved lymph node were analyzed for HIV integration (Fig 5). When DNA from the pulmonary tumor was digested with BamHI, two distinct bands at approximately 6 kb and 3 kb were found when probed with the 3.5-kb 3’-HIV fragment (Fig 5, lane 1). When the same DNA was digested with XbaI and hybridized with the same probe, a single band of approximately 9.6 kb was noted (lane 2). HIV DNA hybridization to high molecular weight DNA in an undigested tumor DNA specimen shows that the hybridizing band is larger than the HIV-1 genome, demonstrating the clonal integration of the HIV-1 provirus (lane 3). The uninvolved lymph node was completely negative for HIV (lane 4). The presence of two distinct bands in the BamHI digest, but a single fragment when digested with XbaI, suggested that the integrated HIV contained an internal BamHI site with closely flanking XbaI sites or XbaI sites within the long terminal repeats. The intensity of HIV gene probe hybridization to the tumor was similar to the control HXB-2 transfected H9 cell hybridization intensity (lane C), suggesting that the majority of tumor cells contained an integrated copy of HIV.

To determine if RNA encoding IL-2 and IL-2R was expressed in the tumor, products of RT RNA (cDNA) were amplified with specific primers using the PCR. DNA product (500 bp) was visualized for the IL-2 reaction (Fig 6, lane 1) and the IL-2R (lane 2) reaction (200 bp). Control unstimulated peripheral blood mononuclear cells (PBMC) did not produce discernable signal in an analogous RT, PCR experiment (lane 3). Simultaneously amplified IL-2 and IL-2R gene fragments showed that the IL-2 RNA was
HIV-ASSOCIATED T-CELL LYMPHOMA

Fig 5. Analysis of the patient’s lung tumor with the HIV gene probe. The control HXB-2 transfected T-cell line, H9/HXB (lane C), the patient’s lung tumor (lane 1), and control uninvolved patient perihepatic lymph node (lane 4) were all digested with BamHI. The lung tumor DNA was also digested with XbaI (lane 2), or run undigested (lane 3). The Southern blot was probed with the 3.5-kb 3’ end (SacI-SacI) of the HXB gene clone.

Fig 6. Ethidium bromide-stained agarose gel containing PCR products resulting from amplification of cDNA products. Lane 1 is from tumor cDNA using IL-2 primers (500 bp); lane 2, from tumor cDNA using IL-2R primers (200 bp); lane 3, negative control consisting of cDNA from PBMC; lane 4, tumor cDNA using both sets of primers.

DISCUSSION

We have described the rapid and fatal development of an acute T-cell lymphoma in a patient infected with HIV-1. The lymphoma was composed almost exclusively of CD4+ T lymphoblasts that also expressed HIV p24 antigens. Molecular studies confirmed this to be a monoclonal T-cell process with tumor cells containing integrated HIV-1. This appears to be the first case of AIDS-associated non-Hodgkin’s lymphoma in which the tumor arose from an HIV-infected progenitor cell.

This tumor was defined as an acute T-cell lymphoma based on its histologic and immunophenotypic similarity to another class of acute T-cell lymphoma (ATLL) that occurs in individuals infected with HTLV-I. The tumor was histologically similar to ATLL, as it was made up of pleomorphic, activated T lymphocytes with occasional multilobed and multinucleated cellular forms. Immunophenotypically, these were activated T cells that expressed the CD25, IL-2R. Although the tumor cells expressed the CD4 molecule, they did not express the CD3 antigen. Unlike ATLL, there was no evidence for HTLV-I infection of this tumor as assessed by Southern blot analysis. Similarly, although EBV and HHV-6 have been found in several human T lymphomas, no EBV or HHV-6 genes were found in this tumor’s DNA.

Although we cannot definitively establish the role of HIV-1 in lymphomagenesis, there are several specific HIV-1-related mechanisms that could have contributed to T-cell transformation. The HIV-1 may have integrated near a cellular oncogene such as c-myc, thereby causing constitutive c-myc expression and cellular transformation. Because the tumor expresses the IL-2R, the HIV-1 tat gene product could also have upregulated IL-2 production, causing the tumor to proliferate by an autocrine process. In PCR amplification of tumor IL-2 cDNA, the presence of IL-2 RNA was demonstrated in apparent levels at least as high as IL-2R RNA and substantially greater than in PBMC. Because high levels of IL-2R protein (CD25) were detected immunohistochemically, it is likely that a similarly
high level of IL-2 protein was being made by the tumor. Finally, the tumor-associated HIV may have undergone mutation or picked up a cellular oncogene and become modified into a truly oncogenic retrovirus. Molecular cloning of the integrated provirus is in progress to test this final hypothesis.

The finding of an HIV-1-positive lymphoma may be thought of as unusual, since lentiviruses have not been associated with the development of malignancies. However, as the second decade of the AIDS epidemic progresses, more HIV-1-infected individuals are living into their own second decade of infection. This time of infection (10 to 15 years) is approximately one sixth the normal human life span and by analogy with animal models of non-Hodgkin’s lymphoma, might be the time that virus-transformed lymphomas would begin to appear if HIV were behaving like a classic leukemia virus. A careful molecular analysis of the integrated HIV-1 in this tumor is in progress. These molecular studies will hopefully delineate the role, if any, of HIV-1 as a transforming agent in infected individuals.

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Acquired immunodeficiency syndrome-associated T-cell lymphoma: evidence for human immunodeficiency virus type 1-associated T-cell transformation

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