HTLV-I-Induced Lymphoma Mimicking Hodgkin’s Disease. Diagnosis by Polymerase Chain Reaction Amplification of Specific HTLV-I Sequences in Tumor DNA

By David B. Duggan, Garth D. Ehrlich, Frederick P. Davey, Shirley Kwok, John Sninsky, Jack Goldberg, Leon Baltrucki, and Bernard J. Poiesz

A patient with a localized HTLV-I-associated lymphoproliferative disease that was misdiagnosed as Hodgkin’s disease is presented. The patient’s serum was negative for HTLV-I antibodies by enzyme-linked immunosorbent assay (ELISA), Western blot, and radioimmunoprecipitation. Tumor tissue DNA was negative for HTLV-I by Southern blotting but was positive for distinct HTLV-I sequences when subjected to DNA amplification using the polymerase chain reaction. We conclude that the clinical and pathological diagnosis of HTLV-I-related lymphoma can be difficult and can be confused with Hodgkin’s disease. Extremely sensitive molecular biological techniques may be required to establish a diagnosis of HTLV-I-induced lymphoma.

MATERIALS AND METHODS

Antibody studies. ELISA and radioimmunoprecipitation were performed as described.

Western blots were performed using purified, detergent-solubilized HTLV-I proteins produced by the HUT 102 cell line according to standard methods.

Immunohistochemistry. Staining for HTLV-I in tissue sections was performed with an indirect immunoperoxidase method using the antibody described by Robert-Guroff.

DNA analysis. DNA from fresh tissue was extracted and analyzed by Southern blot hybridization with the HTLV-I gag-pol probe pATK 32 (gift of Dr. M. Yoshida) and by analysis of enzymatically amplified DNA. The latter process, also known as the polymerase chain reaction (PCR), has been described for detection of human immunodeficiency virus (HIV). PCR for HTLV-I involves the use of two primers (designated SKS4 and SK55) that are 20 bases in length and that correspond to the plus and minus strands, respectively, of target DNA (Fig 1). This primer pair recognizes sequences located 121 base pairs apart in the pol gene of HTLV-I. This region of the HTLV-I genome was chosen because it is highly conserved among HTLV-I isolates and because of its minimal homology with HIV and HTLV-II (Kwok et al, unpublished data).

Following heat denaturation of the target DNA (1 μg) and annealing of the primers, Klenow fragment of Escherichia coli DNA polymerase I is added, and synthesis of the complementary strands occurs. The DNA so synthesized can then serve as templates themselves, such that repeated cycles of denaturation, primer annealing, and extension results in an exponential increase of the DNA sequence flanked by the primers. Thirty cycles were used for all amplifications reported here.

Amplified sequences are detected by the oligomer restriction...
There is no history strictly heterosexual.

The patient was then seen at the Health Science Center at Syracuse in October 1985 with a retropharyngeal mass. A biopsy revealed many highly abnormal lymphoid cells thought to possibly represent a lymphoma. The biopsy was sent for review, a diagnosis most consistent with Wegener's granulomatosis was offered, and evaluation of the respiratory and genitourinary (GU) systems was recommended. The tissue was also reviewed at the SUNY Health Science Center in Syracuse where a diagnosis of lymphoma (either pleomorphic T cell lymphoma or Hodgkin’s lymphoma, mixed cellularity subtype) was made.

The patient was then seen at the Health Science Center at Syracuse. It was noted that there was no history of sinusitis, pulmonary symptoms, hematuria, or arthritis. Chest radiograph was normal, as was urine analysis and renal function tests. The patient’s physical examination was normal. There was no adenopathy or hepatosplenomegaly. Computed tomography (CT) scans of the chest, abdomen, and pelvis were remarkable for a single calcified granuloma in the lung. CT scan of the neck showed multiple, moderately enlarged lymph nodes within the jugular chains on both sides and a large left jugulodigastric node. There was also a superficial soft-tissue prominence in the posterior portion of the lower nasopharynx on the right. Biopsy of a left-neck lymph node showed effacement of the lymph node architecture by an infiltrate of lymphocytes with many eosinophils and multiple areas of necrosis, scattered plasma cells, atypical histiocytes in clusters or sheets, and small focal areas with diffuse fibrosis (Fig 2A).

Mummified cells, atypical mitoses, numerous multilobulated cells, and atypical mononuclear cells were scattered throughout the tissue. Reed-Sternberg cells were present with variable frequency. Some sections revealed residual, uninvolved node with hyperplastic follicles. Immunoperoxidase studies revealed a polyclonal staining of the large lymphoid cells with kappa and lambda light chains. Leu-M1 was positive in some atypical large cells, including those of the Reed-Sternberg morphology (Fig 2B). Immunophenotyping of cells in suspension revealed that CD5 antigen was positive in 83% of the cells, and other T cell antigens (CD1, CD2, CD4, CD8) were positive in 40% to 50%. Fifty-three percent of the cells stained with polyclonal anti-Ig, 45% with anti-IgM, 41% kappa, and 12% lambda. Approximately 40% of cells labeled with anti-B1 (CD20), B2 (CD21), and B4 (CD19) MoAbs, and 5% stained with anti-TAC MoAb.

Although the possibility of a pleomorphic T cell lymphoma was considered, a diagnosis of Hodgkin’s disease, mixed cellularity, was made because of the polymorphous cellular infiltrate, presence of Reed-Sternberg cells, and the positive Leu-M1 staining of Hodgkin’s-like cells.

After the diagnosis of Hodgkin’s disease was issued, this tissue was stained with monoclonal anti HTLV-I p19 and was positive (Fig 2C). A portion of this tissue was also submitted for HTLV-I nucleic acid amplification (vide infra) and Southern blot analysis, as were peripheral blood and bone marrow. The patient was seronegative for HTLV-I antibodies by ELISA, Western blot, and radioimmunoprecipitation. He subsequently had a staging laparotomy that was negative, and he received radiation therapy to the nasopharynx and a mantle port. Southern blots of all samples were negative for HTLV-I. PCR-OR was positive on the neoplastic lymph node but negative on the uninvolved bone marrow specimen (Fig 3). There is no evidence of disease at this time.

**Table 1. Summary of Patient Characteristics**

<table>
<thead>
<tr>
<th>Initial clinical manifestations</th>
<th>Nasopharyngeal mass, cervical adenopathy</th>
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<tr>
<td>Pathologic diagnosis</td>
<td>1. Wegener’s granulomatosis</td>
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<td></td>
<td>2. Hodgkin’s disease mixed cellularity</td>
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<td>3. Pleomorphic T cell lymphoma</td>
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<tr>
<td>HTLV-I ELISA</td>
<td>Negative</td>
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<tr>
<td>HTLV-I Western blot</td>
<td>Negative</td>
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<td>HTLV-I radioimmunoprecipitation</td>
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<td>Southern blot</td>
<td>Negative</td>
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<tr>
<td>Polymerase chain reaction</td>
<td>Positive</td>
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**CASE HISTORY**

The patient is a 38-year-old white man who is a lifetime resident of upstate New York (Table 1). He is married and is strictly heterosexual. There is no history of IV drug use or blood transfusion. He presented at another institution in October 1985 with a retropharyngeal mass. A biopsy revealed many highly abnormal lymphoid cells thought to possibly represent a lymphoma. The biopsy was sent for review, a diagnosis most consistent with Wegener’s granulomatosis was offered, and evaluation of the respiratory and genitourinary (GU) systems was recommended. The tissue was also reviewed at the SUNY Health Science Center in Syracuse where a diagnosis of lymphoma (either pleomorphic T cell lymphoma or Hodgkin’s lymphoma, mixed cellularity subtype) was made.

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**DISCUSSION**

The clinicopathologic expression of HTLV-I infection is diverse. Most seropositive people are asymptomatic and have no evidence of disease. The commonest presentation in endemic areas is that of peripheral blood involvement, with or without adenopathy or skin lesions. HTLV-I infection can also present in an indolent leukemic form, which may persist for some time before progressing to the better known acute ATL. ATL is the most frequently recognized form in the United States and is characterized by lymphadenopathy,

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**Fig 1.** Schematic diagram of primer pairs used to amplify HTLV-I. The two primers SK54 and SK55 are homologous to nucleotides 3385 to 3484 and 3465 to 3483, respectively, of the HTLV-I pol gene. The detection probe SK66 is homologous to nucleotides 3426 to 3460.

(OR) technique, wherein an end-labeled oligonucleotide hybridizes in solution to a region of the amplified sequence and reconstitutes a specific endonuclease cleavage site.26 Cleavage with the specific endonuclease produces a labeled oligomer of defined size that is then separated electrophoretically and identified by autoradiography. Figure 1 shows a schematic diagram of the primer pairs and detection probe used for these studies. The sensitivity of this procedure is estimated to be 25-fold greater than Southern blot (Kwok et al, unpublished data).
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Fig 2. (A) Biopsy of retropharyngeal mass; note the infiltrate of eosinophils and lymphocytes along with a large binucleated cell (arrow). Magnification × 200; hematoxylin and eosin. Insert shows a Reed-Sternberg-like cell; Magnification × 400; hematoxylin and eosin. (B) Biopsy of retropharyngeal mass stained with MoAb Leu-M1 (avidin-biotin peroxidase, counter stained with hematoxylin, magnification × 400). Note membrane and Golgi staining. (C) Biopsy of retropharyngeal mass stained with monoclonal anti-pi 9 (avidin-biotin peroxidase, counter stained with hematoxylin). See staining of large abnormal mononuclear and lymphoid cells, magnification × 400.

Peripheral blood and marrow involvement, hypercalcemia, elevated bilirubin, and, often, interstitial pneumonitis due to leukemic cell infiltration.10

Our patient presented with a localized tumor in which the diagnoses of Wegener’s granulomatosis and Hodgkin’s disease were suggested. When referred to our institution, the possibilities of a pleomorphic T cell lymphoma and of HTLV-1 infection were entertained. In addition, by polymerase chain reaction gene amplification, specific HTLV-1 sequences could be detected in the tumor tissues. It is important to note that the tumor DNA was negative for HTLV-1 on Southern blot, suggesting that a minority of cells (<10%) contained HTLV-1 sequences. This patient is unique in that he had a limited (stage 1) HTLV-1 lymphoma and because he represents the first seronegative patient in the United States demonstrated to have an HTLV-1-associated lymphoma.

The histopathologic and clinical confusion about this patient is noteworthy in many respects. HTLV-1 is a relatively recently identified pathogen that causes a wide variety of clinical manifestations and pathologic alterations. It is an uncommon pathogen in the United States but is being recognized much more frequently, and the incidence of HTLV-1 seropositivity is rising in the United States.

Kawano et al19 have defined patients with a variable clinical course of HTLV-1-induced lymphoproliferative disease. They include in their initial study five patients with indolent disease who had no evidence of leukemia and whose disease was limited to skin, skin and lymph nodes, or skin and liver. Bunn et al10 have described the usual course of HTLV-1-associated lymphoma in the United States, and all of the patients had an acute clinical course with skin lesions or hypercalcemia. Ten of eleven also had lymphadenopathy, six had organomegaly, six had bone marrow involvement, and five had pulmonary infiltrates. Pandolfi et al27 have identified HTLV-1 seropositivity and viral integration in three of 16 patients with T-CLL studied in Italy. Sohn has described an HTLV-1-positive patient with a chronic course who had massive hepatosplenomegaly and neutropenia and whose white count rose following splenectomy.28 These studies...
suggest that there are less common clinical expressions of HTLV-I distinct from the commonly recognized ATL.

Many authors have emphasized the diverse pathologic expression of HTLV-I. The clinicopathologic manifestations of HTLV-I infection have been described in detail by Japanese authors who, because of the endemic nature of HTLV-I, have had the greatest experience. In 1979 Hanaoka et al described the pathologic findings of 59 patients from HTLV-I-endemic Kyushu with a pleomorphic T cell leukemia who had acute clinical illnesses consistent with HTLV-I-induced ATL, although specific HTLV-I testing was not done. They commented on the variation in size and the convoluted, lobulated, distorted, nuclear forms of the neoplastic cells and noted that binuclear giant cells resembling Reed-Sternberg cells were common, as was eosinophilia. They also stated that the histologic features resembled those of various types of lymphoma, including Hodgkin's disease. They described a "mildly pleomorphic" subtype comprised primarily of medium-sized neoplastic cells with infrequent giant neoplastic cells. One patient in this subgroup was thought to have Hodgkin's disease because of a node biopsy revealing small neoplastic cells with marked eosinophilia, a proliferation of lacunar cells, and occasional Reed-Sternberg cells. When a leukemic phase ensued after three years the diagnosis was reclassified.

The peculiar problems relating to the histopathologic diagnosis of non-Hodgkin's lymphoma in Japan, now known to be due to widespread HTLV-I infection, were discussed by Suchi et al in a 1979 proposal of a new subclassification for Japanese lymphomas. They proposed a new classification, "pleomorphic," to distinguish rapidly growing lymphomas of peripheral T cells from the central T cell lymphoblastic type. It was suggested that the presence of such pleomorphism identified a unique type of lymphoma that could not be reproducibly classified according to the Rappaport classification. This subgroup of 13 tumors, when subjected to repeat classification by 16 Japanese pathologists and Drs Dorfman and Berard from the United States, was often misdiagnosed as Hodgkin's disease due to the presence of a diffuse proliferation of various sized cells with Reed-Sternberg cells and mononuclear variants, as well as reactive histocytes, eosinophils, and plasma cells. A high proportion of these patients were from the HTLV-I-endemic Kyushu district, but specific HTLV-I tests were not performed at the time of publication. Subsequent testing has determined that \( \approx 95\% \) of such patients are HTLV-I seropositive. Moreover, retrospective studies of Hodgkin's disease in Japan have revealed that 20% to 40% of cases initially diagnosed as Hodgkin's disease were non-Hodgkin's lymphoma. A retrospective evaluation of the high mortality due to Hodgkin's disease in Kyushu revealed that 12 of 36 cases diagnosed as Hodgkin's disease during 1969 to 1972 were reclassified as ATL in 1980, as were 4 of 92 cases seen from elsewhere in Japan. Studies of ATL in Taiwan have also commented on the frequent confusion with Hodgkin's disease.

The Japanese studies cited above reveal the diagnostic difficulty that Japanese pathologists encountered when attempting to classify a newly recognized type of non-Hodgkin's lymphoma and its not infrequent confusion with Hodgkin's disease. The issue became clearer in later publications when patients with ATL were found to be infected with HTLV-I; subsequent investigations established a causative role for this virus. In these patients the diagnostic confusion between Hodgkin's disease and non-Hodgkin's pleomorphic T cell lymphoma could be clarified by performing specific serologic tests. It is not unreasonable to expect similar confusion among pathologists in the United States who are just now beginning to see similar cases as the prevalence of HTLV-I appears to be rising. A recent report from the Mayo Clinic points out this diagnostic difficulty.

The crossreactivity of the monoclonal Leu-M1 antibody with the multinucleate cells of HTLV-I-induced lymphoma as well as with the Reed-Sternberg cells of Hodgkin's disease should be emphasized. Hsu and Jaffe reported Leu-M1 to stain positively for Reed-Steinberg cells but to stain negatively in 12 cases of peripheral T cell lymphomas. This finding was later questioned by Hyder and Schnitzer, who reported staining of some "Reed-Sternberg-like" cells in several peripheral T cell lymphomas. Our findings support the opinion that Leu-M1 is not specific for the Reed-Sternberg cells of Hodgkin's disease and that it may be positive in HTLV-I-induced lymphomas.

To our knowledge, confusion between Wegener's granulomatosis and HTLV-I has not been widespread. Our patient had an upper aerodigestive tract lesion but no other manifestations of Wegener's granulomatosis, and the histopathologic confusion was readily clarified by clinical criteria. We have performed ELISA assays for HTLV-I antibodies in 35 patients with Wegener's granulomatosis and related diseases and none were positive, suggesting that HTLV-I is not frequently associated with Wegener's (Poiesz B, Rosenwasser L, unpublished data, April 1986).

The detection of specific HTLV-I viral sequences by PCR in tumor tissue from our patient, who is seronegative for HTLV-I antibodies by ELISA and radioimmunoprecipitation and whose tumor tissue DNA is negative by Southern blot, is worthy of mention. PCR is capable of amplifying target DNA by several hundred thousand-fold and is a much more sensitive test than Southern blotting for detection of specific DNA sequences. This has been demonstrated for sickle cell disease and in HIV infection. Moreover, the comparisons of Southern blotting and PCR done on postmortem tissues from a patient with typical ATL clearly show PCR to be more sensitive (Kwok et al, unpublished data).

How, then, can the seronegativity of our patient be explained? It has been suggested that up to 10% of patients with tumors due to HTLV-I are seronegative. A substantial number of patients from highly endemic areas with typical clinopathologic syndromes of ATL have been seronegative and Southern blot negative for HTLV-I. Some of their families have also been seronegative. HTLV-I has been isolated from the peripheral blood of a seronegative person whose sibling had ATL. We feel it is possible that there are individuals who mount a limited or transient immune response to HTLV-I, perhaps related to HLA determinants of immune response. Seronegativity may also be due to acquisition of latent viral infection in early life, as has been suggested. Only further evaluation of seronegative patients...
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with suspicious clinical syndromes by the sensitive technique of PCR will answer that question.

We have described a patient with HTLV-I–associated lymphoma in whom the diagnoses of Hodgkin’s disease and Wegener’s granulomatosis were suggested. We recognize the clinical difficulty in establishing the diagnosis of HTLV-I–induced lymphomas and suggest that all patients with atypical lymphoproliferative disorders with identifiable T cell components or with atypical Hodgkin’s disease be studied further to rule out the possibility of HTLV-I infection.

Just as the tissues from the affected individuals are designated as pleomorphic, so are the clinical manifestations protean, and a high index of suspicion must be maintained.

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