Human T-lymphotropic virus type 1 (HTLV-1) was the first human oncovirus isolated by Gallo et al in 1980 and established as an etiological agent for adult T-cell leukemia/lymphoma (ATL). Although more than 15 million individuals are infected by HTLV-1 through the world, the spread of the virus is highly endemic. The HTLV-1 infection is prevailing in southwestern Japan, inter-tropical Africa, Central and South America. In Kyushu district, Japan, the seroprevalence reaches >30% in the adult population. In the US, Europe and the Middle East the HTLV-1 infection is very rare, and cases of ATL have been reported sporadically. We describe here acute ATL in two patients of Jewish-Romanian origin. The epidemiological anamnesis and screening indicate that both patients acquired the HTLV-1 from their mothers leaving in Romania.

Case reports

Case 1. A 49-year-old woman who was born in Romania and immigrated to Israel at age of 9 years, presented in April 2001 with a 2-months history of fever, weight loss, night sweats and back pain. She denied intravenous drug abuse, blood transfusion or travel outside Israel. Physical examination revealed marked splenomegaly and mild cervical and axillary lymphadenopathy. Blood tests showed leukocytosis 15×10^9/L with 60% lymphocytes. Peripheral blood smear revealed numerous lymphocytes, part of them showing irregular, convoluted and polylobated nuclei, some resembling flower shape. The lymphocytes demonstrated the phenotype: CD3 60%, CD4 83%, CD25 54%. Bone marrow biopsy disclosed interstitial and focal infiltrate composed by medium-sized lymphocytes with irregular nuclei. Karyotype was 46,XX. Cervical lymph node biopsy demonstrated diffuse infiltrate by lymphoma cells positive for CD3 and CD4. Serum calcium was markedly elevated 19.4 mg/dL (normal 10.5-12.0), lactic dehydrogenase (LDH) was 521 u/L (normal 120-380), phosphorus, liver transaminases and parathyroid hormone were normal. Radiographic survey revealed multiple ostolytic lesions of the skull. Antibodies against HTLV-1 were detected by enzyme linked immunosorbent assay (ELISA). Western blot (INNO-LIA HTLV I/II, Innogenetics, Ghent, Belgium) showed reactivity to two gag (p19-I, p24) and two env (gp46-I, gp21) antigens of HTLV-1 corresponding to the Cosmopolitan type. Diagnosis of ATL associated with HTLV-1, was established. In addition, antibodies reactive to hepatitis C virus (HCV) were detected by serological screening, and the presence of viral RNA was confirmed by polymerase chain reaction (PCR).

Serological testing of family members revealed that her mother was seropositive for HTLV-1 but seronegative for HCV. One of her two sons was seronegative for both HTLV-1 and HCV, and her second son and husband refused to undergo testing. The chemotherapy according to the ProMACE-CytaBOM regimen (prednisone, adriamycin, cyclophosphamide, etoposide, cytarabine, bleomycin, oncovin, methotrexate) was started with addition of lamivudine and pamidronate. Partial remission was achieved following two cycles with resolution of symptoms and normalization of laboratory tests. Treatment course was complicated by recurrent episodes of chemotherapy-induced neutropenia and infection, including Corynebacterium sepsis, which were successfully treated with colony stimulating factor (G-CSF) and antibiotics.

In July 2001, three months following her initial presentation, the patient developed fever, recurrent hypercalcemia 16 mg/dl and generalized maculopapular rash. A skin biopsy revealed infiltration of the dermis by atypical lymphocytes with the same profile (CD2, CD3, CD4). Sixty percent of the cells were also positive for Ki-67, a marker of high proliferative index. The patient did not respond to chemotherapy with cytarabine, cyclophosphamide, high-dose methotrexate and fludarabine and died.

Case 2. A 56-year-old female was born in Bucharest, Romania and immigrated to Israel 16 years ago. She was hospitalized in July 2003 because of progressive weakness started two weeks ago, and hypercalcemia 15.1 mg/dL. She had no history of intravenous drug abuse or recent travel. The physical examination disclosed mild generalized lymphadenopathy and splenomegaly. Blood count showed Hb 12.5 g/dL, WBC 18.5×10^9/L with 69% lymphocytes, PLT 720×10^9/L. Peripheral blood and bone marrow contained >70% medium-sized lymphocytes showing pleomorphic multilobuled nuclei, some of them with flower-shaped occurrence. The lymphoid markers were CD3 64%, CD4 90%, CD25 85%. The cytogenetic analysis showed complex alterations: 48,XX,+3,der(10),t(10;15(q23;q22),+mar[11]. Antibodies to HTLV-1 were found by ELISA and confirmed by Western blot (INNO-LIA, Innogenetics, Belgium). The diagnosis of ATL was established. The patient was treated by the chemotherapy LSG15 regimen, suggested by Japan Clinical Oncology Group for treatment of acute ATL. Hematological and cytogenetic remission was achieved and lasted for 4 months. Then prompt relapse occurred, accompanied by refractory hypercalcemia, and the patient died. Her family serological testing revealed that mother, sister and brother (leaving in Romania) are carriers of HTLV-1.

Additional studies

Rearrangement of TCR gamma chains, tested by PCR method using primers for the corresponding gene (5’ GCT TCT AGC TT TCT GTC TC-3’) (Genset corp, Paris, France) and Jurkat cell control had detected monoclonality of T-cells (Figure 1). DNA extracted from peripheral blood of the patient was used to amplify TCR gamma sequences. RT-PCR was performed on 5 μL of the reverse transcription product using 1 μL of each primer 5’ GTC TCT AGC TTT CCT GTC TC-3’ (Genset corp, Paris, France) and 5’ GCT TCT AGC TTT CCT GTC GC-3’. The PCR products were resolved on a 2% agarose gel and subsequently stained with ethidium bromide.

1. Normal lymphocytes
2. Jurkat cell TCR-gamma-Vγ positive control
3. HTLV-1 patient
4. Negative control
5. Marker

Figure 1. Detection of TCR gamma rearrangement by PCR in HTLV-1 patient.
eral lymphocytes in case 1 was analyzed by quadrupli-
cate inverse PCR as previously described\(^7\) (kindly per-
formed by Dr. E. Wattel, Claude Bernard University,
Lyon, France) and revealed sequences of the HTLV-1
genome (Figure 2).

**Discussion**

We report two patients of Romanian origin who devel-
oped acute type T-cell leukemia/lymphoma associated
with the retrovirus HTLV-1. The transmission of the virus
occurs by transfer of infected CD4\(^+\) lymphocytes from
mother to child during breast-feeding, during sexual
intercourse or by blood transfusion.\(^5\) The virus is endem-
ic in Japan, West Africa and South America but is very
rare in the US, Europe and the Middle East including
Israel. In Israel, the antibodies to HTLV-1 were discov-
ered in 5 out of 276,000 blood donations (0.0018\%) [Israeli
Central Blood Bank, 2002], however, 1.3\% intraven-
ous drug abusers were found seropositive.\(^6\) A very
high rate of infection ~20\% has been identified among a
segregated community of Jews originated from the city of
Mashhad in the northern Iran.\(^7\) ATL is extremely rare in
Israel. The first Israeli patient was referred to the US
in 1981 because of unexplained hypercalcemia. He de-
veloped diffuse lymphadenopathy, lymphomatous lep-
tomeningitis and died.\(^8\) Another 49-year-old woman,
who presented with smoldering disease involving skin
only, turned to fulminant generalized leukemia and died
from Pneumocystis carinii pneumonia.\(^9\) Four other cases
were diagnosed in Mashhadi Jews, all of them demon-
strated a rapid and fatal disease course.\(^10\)

In Romania, antibodies to the HTLV-1 were found in
0.64\% of the blood donors indicated a 25-50-fold higher
seroprevalence rate compared to other areas of Europe
and the US.\(^11\) Only occasional ATL cases were reported in
Romania.\(^12-14\) So, our present report of 2 patients originat-
ed from Romania is important to assess the epidemiolo-
gy of the disease. The finding of seropositivity of moth-
ers in both patients supports the mother-to-child trans-
mision of the virus.

According to the clinical picture ATL has been clas-
sified into four types: smoldering, chronic, lymphomatous
and acute.\(^15\) Our patients are typical of the acute type and
manifested many of the clinical features of this disorder
including the abrupt onset of fever, night sweats, hyper-
calcemia, osteolytic bone lesions, lymphadenopathy,
splenomegaly and skin involvement. As determinative
feature, acute type of ATL is characterized by the appear-
ance of pleomorphic atypical lymphocytes on peripheral
blood smear showing markedly lobulated nuclei (includ-
ing so-called flower cells), which display membrane mark-
ers of mature peripheral T-cells: CD2, CD3, CD4, CD5
with the activated antigen CD25 and lack of CD7 and
CD8. Patients with acute type ATL have very poor prog-
nosis, only 17\% survive 5 years, and the median survival
is 10 months.\(^16\) Cause of death is often opportunistic infec-
tion. No treatment strategy has been found successful for
the management of acute ATL. The LSG15 regimen,
given to the second patient reported herein, has shown
reasonable improvement in overall survival.\(^17\) Novel ther-
apic strategies, more specific for T-line expansion,
such as purine analogs or monoclonal antibodies, have
been subject for clinical trials in ATL.\(^18\) The antiretroviral
therapy with combination of interferon alpha and
zidovudine had a beneficial effect even in patients refrac-
tory to chemotherapy.\(^19\) However, all of these regimens
induced very few long-term survivors.

In conclusion, we describe two ATL patients originated
from Romania and believe that this report has an impor-
tant epidemiological significance.

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**References**


