



Attack of the clones: a T-cell population resembling adult T-cell leukemia/lymphoma, and large granular lymphocytosis, in an HTLV-2-infected patient

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Abstract

The HTLV-1 and HTLV-2 viruses, though closely genetically related, have divergent clinical impact: HTLV-1 has been causally linked with adult T-cell leukemia/lymphoma as well as neurologic disorders, whereas HTLV-2 shows no clear association with lymphoproliferative disorders. We report a patient who presented with two concomitant clonal T-cell lymphoproliferative disorders, who was found to have an HTLV-2, but not HTLV-1 infection. The patient's clonal T-cell proliferations were characterized and compared using multiparameter flow cytometry, fluorescence in situ hybridization, and TCR gene rearrangement analysis on flow-sorted cell samples from blood, bone marrow, and skin biopsy specimens. The patient had a CD4 + /CD25 + T-cell population, resembling adult T-cell leukemia/lymphoma both by clinical presentation and immunophenotype, as well as a separate clonal CD8 + population of large granular lymphocytes. Interestingly, she also had a *STAT3* mutation; large granular lymphocytic leukemia has been reported in association with HTLV-2 infection. This patient has had an indolent clinical course to date and is being managed expectantly. The unexpected finding of HTLV-2 infection is of uncertain significance with an unusual CD4 + /CD25 + clonal T-cell population resembling adult T-cell leukemia/lymphoma, but fits a reported association with clonal LGL-expansion and *STAT3* mutation.

Keywords Adult T-cell leukemia/lymphoma (ATLL) · Human T-cell leukemia virus type 1 (HTLV-1) · Human T-cell leukemia virus type 2 (HTLV-2) · T-cell lymphoproliferative disorder (T-LPD) · Large granular lymphocytes (LGLs) · T-cell large granular lymphocytic leukemia (T-LGLL)

Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm caused by the human retrovirus HTLV-1, with characteristic strong CD25 expression and a

poor clinical prognosis [1]. HTLV-1 infection is linked causally with ATLL, and the frequency is estimated to be 2.5% in HTLV-1 carriers and distributed in endemic regions (Caribbean basin, central Africa, southwestern Japan). In contrast, the HTLV-2 virus has not been associated with lymphoproliferative disorders, despite genetic similarity with HTLV-1. Interestingly, a minority of HTLV-2 infected patients have recently been found to have T-large granular lymphocytic leukemia (T-LGLL). We report a case of a patient with a clinically indolent T-cell proliferation resembling ATLL, found to have both HTLV-2 (but not HTLV-1) infection and a clonal large granular lymphocyte (LGL) expansion. This is the first description to our knowledge of clonal ATLL-like T-cells in HTLV-2 and adds to the spectrum of lymphoproliferative disorders associated with viral infection.

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Clinical history

A 68 year-old African-American woman presented with weakness, fatigue, and a 60-pound weight loss over several months. She is a Baltimore native with no history of international travel, blood transfusions, or drug use. She had been in her usual state of health until a recent hospitalization for septic thrombophlebitis affecting the bilateral lower extremities. Physical examination revealed mild splenomegaly and shotty inguinal lymphadenopathy, both confirmed by imaging and skin hyperpigmentation and thickening involving the lower extremities. Complete blood counts demonstrated a white blood cell count of 9.7 K/ μ L, hemoglobin of 10.9 g/dL, MCV of 85.5 fL, and platelet count of 66 K/ μ L, with a differential including 17% atypical lymphocytes which had the morphology of large granular lymphocytes (LGLs).

Materials and methods

Cytologic preparations were routinely made on peripheral blood and bone marrow aspirates. Histologic sections were routinely made on bone marrow trephine biopsy and skin biopsy samples in 10% neutral buffered formalin fixative. Flow cytometric studies were performed on peripheral blood and bone marrow aspirates using a 4-color assay with auto and internal controls for determination of antibody binding patterns. Standard cytogenetic studies were performed on bone marrow aspirates, and 20 G-banded metaphase cells were analyzed, karyotyped, and described according to official ISCN nomenclature. Fluorescence in situ hybridization (FISH) was performed on aspirate material using commercially available direct labeled probes, according to manufacturer recommendations. Three hundred interphase nuclei for each probe set were analyzed using standard fluorescence microscopy by 2 independent clinically licensed technologists. TCR gene rearrangement analysis was performed by polymerase chain reaction (PCR) technology, on the bone marrow and skin biopsy, and on flow-sorted material from the peripheral blood sample, using a multiplex primer method and capillary gel electrophoresis.

Results

Flow cytometry was performed on the peripheral blood sample and demonstrated an atypical T-cell population (13% of leukocytes) expressing CD4, CD5 (bright), CD7 (bright), CD25, and CD52, with variable cytoplasmic CD3 expression and negativity for surface CD3, CD2, CD8, CD10, CD30, CD56, CD1a, CD34,

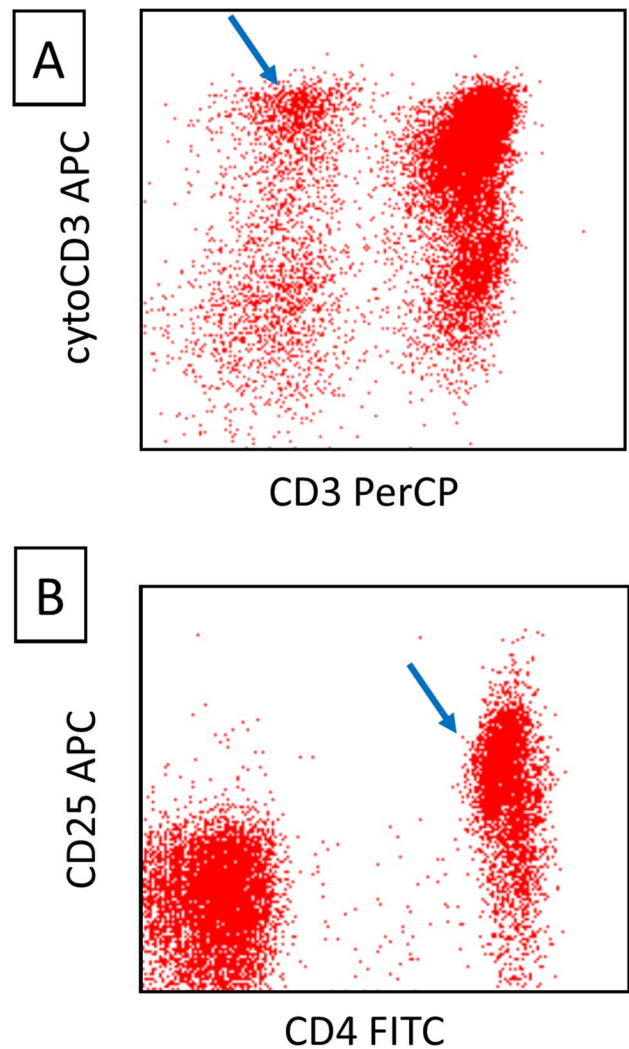


Fig. 1 Flow cytometry histograms, peripheral blood. The ATLL-like population (13%, blue arrows) is **A** cCD3+/ sCD3- and **B** CD4+/ CD25+

nuclear TdT, and TCRg/d (Fig. 1). Flow analysis additionally identified 25% CD8+ T-cells coexpressing CD3, CD2, and CD57, and negative for CD16, consistent with T-LGLs. A bone marrow biopsy was hypercellular for age (60%), with flow cytometric involvement by both the atypical CD4+, CD25+ T-cells (6%) and the LGL expansion (41%).

A skin punch biopsy from the left leg (Fig. 2) demonstrated a superficial dermal perivascular lymphocytic infiltrate, with scattered CD25+ T-cells and CD8+ T-cells by immunohistochemistry, consistent with low-level involvement by both T-cell populations. TCR gene rearrangement testing demonstrated a monoclonal pattern with identical peak sizes shared between the peripheral blood, bone marrow, and skin biopsy samples (Fig. 3).

Cytogenetic testing on the bone marrow demonstrated a normal female karyotype, though fluorescence in situ hybridization (FISH) testing demonstrated rearrangements of *TCRAD* (15%),

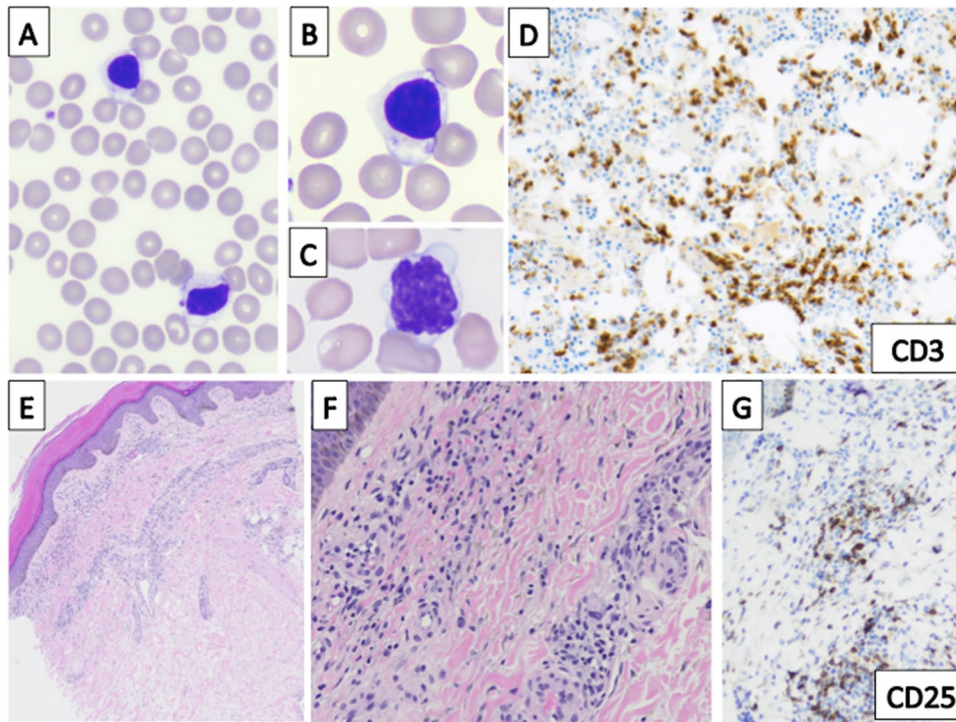


Fig. 2 Microscopic images. Peripheral blood smears demonstrated scattered lymphocytes with large granular lymphocytic morphology (A Wright Giemsa stain, 400×magnification and B Wright Giemsa stain, 1000×magnification), as well as few atypical lymphocytes with irregular nuclear contours, mature chromatin, and scant pale agranular cytoplasm (C Wright Giemsa stain, 1000×magnification). D CD3 immunohistochemical stain from the bone marrow core biopsy

demonstrates increased interstitially scattered CD3+T-cells, consistent with large granular lymphocytes (200×magnification). Histologic sections from the left leg skin punch biopsy demonstrated a superficial dermal perivascular lymphocytic infiltrate (E H&E stain, 20×magnification and F H&E stain, 100×magnification), with scattered CD25+ cells (G 100×magnification)

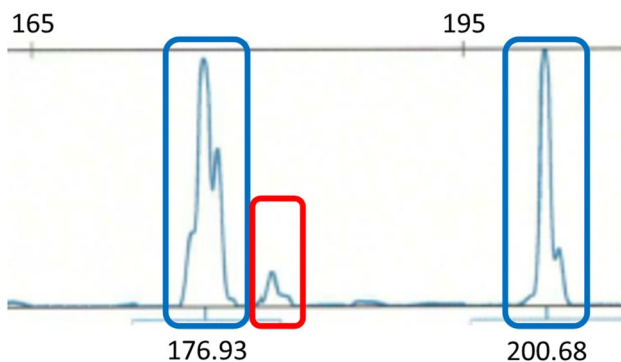


Fig. 3 TCR gamma gene rearrangement testing by polymerase chain reaction, performed after cell sorting, on peripheral blood sample. Blue boxes — clonal peaks in CD8+T-cells, red box — clonal peak in CD4+T-cells. Reactions showed identical peak sizes shared between peripheral blood, bone marrow, and skin biopsy samples

confirmed two separate T-cell clones: a CD3+/CD8+ population which harbored a 17p (*TP53*) deletion and a CD3-/CD4+ population with 14q *TRA/D* rearrangement and no del(17p) (see Fig. 2). Anti-HTLV antibody testing by enzyme-linked immunosorbent assay (ELISA) was repeatedly negative, and reflex HTLV-1/2 testing by Western blot confirmed positivity for HTLV-2.

Our final diagnosis was an atypical T-cell population resembling adult T-cell leukemia/lymphoma and concomitant clonal large granular lymphocytosis, both involving the skin, peripheral blood, and bone marrow. Given the unusual nature of the patient's T-cell lymphoproliferative disorders (LPDs), we elected for close monitoring without treatment; her cytopenias normalized, atypical T-cell populations decreased in size, and rash and lymphadenopathy all improved, and she was lost to follow-up 2 years after presentation.

loss of *P16* (13.5%), loss of *TP53* (35%), and three copies of *TCL1* (11%). Mutation analysis demonstrated an abnormality in exon 21 of *STAT3* (c.1919A>T; p.Y640F). Cell-sorted T-cell clonality and mutation analysis performed on peripheral blood

Discussion

The human T-cell leukemia virus types 1 and 2 (HTLV-1 and HTLV-2) are delta-retroviruses, and share roughly 70% nucleic acid sequences with similar genome structures [2].

Despite this, they have differing clinical impact: HTLV-1 has been associated with several diseases, including adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), whereas HTLV-2 shows no clear association with LPDs. While HTLV-2 was initially discovered in a patient with hairy cell leukemia [3], only rare infections are linked with a HAM/TSP-like or neurodegenerative disorder [4], and the relationship is difficult to evaluate, as the associations may be confounded by concomitant HIV-1 infection and/or intravenous drug use [5].

The viruses also display different *in vivo* tropism, with HTLV-1 being primarily detected in CD4+ T-cells and HTLV-2 in CD8+ T-cells [2, 6]. Both scenarios lead to clonal T-cell proliferation, though a study of 28 HTLV-2-infected individuals demonstrated significant differences from HTLV-1, including HTLV-2 proviral confinement to CD8+ T-cells, a small number of often highly expanded clones, and relative clone stability; these results suggest different influences on clonal expansion between the viruses, and that proliferation does not necessarily predispose to malignant T-cell transformation [7].

We are unsure of whether the HTLV-2 infection is in the CD4+ or CD8+ T-cells in our specific case, and the basis for calling the CD4+ clonal proliferation “ATLL-like” is the CD25+ phenotype and clinical presentation. It is not possible to show monoclonal integration of HTLV-2 in the neoplastic cells, and note that HTLV-2 infection could be an incidental finding in a patient with any T-cell LPD. Of further interest in this case is the potential link between HTLV-2 and T-cell large granular lymphocytic leukemia (T-LGLL). HTLV-2 infection was first detected in a T-LGLL patient in 1992 [8], and a subsequent study detected infection in 4 of 53 patients [9], suggesting pathogenetic participation in a minority of T-LGLL cases. Kim et al. recently reported *STAT3* mutations in CD8+ sorted T-cells from 4 of 30 HTLV-2-positive patients, suggesting that HTLV-2 may promote *STAT3* mutagenesis outside the context of T-LGLL [10]. Our patient’s *STAT3* mutation, clonal LGL expansion, and incidental HTLV-2 infection fit this description, though this is the first description to our knowledge of clonal ATLL-like T-cells in HTLV-2 infection, and the finding is of uncertain significance.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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