Effect of treatment of *Strongyloides* infection on HTLV-1 expression in a patient with adult T-cell leukemia

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Abstract

Human T-cell leukemia virus type 1 (HTLV-1) is associated with adult T-cell leukemia–lymphoma (ATLL) in about 5% of infected individuals. Coinfection by *Strongyloides stercoralis* has been suggested to be a cofactor for development of ATLL. We describe a patient who presented with HTLV-1-associated chronic ATLL and *Strongyloides* infection. Studies of this patient's viral RNA levels demonstrated stimulation of HTLV-1 replication by *Strongyloides,* which resolved with anti-helminthic therapy. This case provides support for the hypothesis that *Strongyloides* is a cofactor for ATLL via T-cell stimulation.

Introduction

Strongyloides stercoralis has been proposed as a cofactor for human T-cell leukemia Type 1 (HTLV-1)-associated adult T-cell leukemia–lymphoma (ATLL) [1]. This is based on epidemiologic association of *Strongyloides* and HTLV-1 infections in Brazil, the Caribbean Islands, and Southern Japan, and from small clinical series reporting more severe disease manifestations in the presence of both infections than with either one alone [2–4]. The mechanism for the synergism between these two infectious agents is unclear. The current report describes such a dual-infected patient, and the results of newly developed viral expression assays which were applied for the first time to assess the effects on HTLV-1 RNA expression of treatment of *S. stercoralis* infection.

Case Report

The patient is a 69-year-old Japanese-American woman from Okinawa, who was previously in excellent health. She presented with a 1-month history of abdominal discomfort, bloating, anorexia, vomiting, nonproductive cough, mild hoarseness, generalized weakness, and an 8 lb weight loss, representing 7% of her body weight. She had been breast-fed as an infant. She moved to U.S. from Okinawa 43 years ago. She had no exposure to illicit drugs. Her physical examination was remarkable for normal cardiopulmonary, neurologic, dermatologic, and

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lymph node examinations. She had moderate abdominal tenderness and distention, and pedal edema.

Laboratory data included a hemoglobin value of 12.3 g/dl, platelet count of 490,000/mm^3, and a total leukocyte count of 48,500/mm^3 with 26% lymphocytes. The absolute lymphocyte count (ALC) ranged from 14,800 to 22,700/mm^3 during the first week of hospitalization (Fig. 1). The peripheral smear showed many atypical lymphocytes with cerebriform nuclei, occasional "flower" cells, and rare cells with immature chromatin (Fig. 2a–c). Chemistries revealed a calcium of 7.8 mg/dl (lower limit of normal, LLN = 8.6) and an albumin of 2.4 g/dl, which declined to 1.6 g/dl (LLN = 2.6, Fig. 1). The lactate dehydrogenase (LDH) was 477 U/l (ULN = 250, Fig. 1).

Computerized tomographic scans of the chest, abdomen, pelvis, and brain showed thickening of the gastric, duodenal, and colonic walls, and a right lower lobe infiltrate. A skeletal survey showed no lytic lesions. Cerebrospinal fluid was normal. ELISA and Western blot assays for HTLV-1 were positive with detection of antibodies to Gag p19 and p24 and Env gp21 and gp46 proteins. Bone marrow biopsy showed a mature T-cell neoplasm. Flow cytometry studies showed that the T-cells expressed cytoplasmic CD3 and surface CD2, CD4, CD5, and CD25, but not surface CD3, CD7, CD8, CD10, or CD56, consistent with the diagnosis of ATLL. Stool examination and upper endoscopy and gastric and duodenal biopsies revealed a heavy infection with S. stercoralis adult worms and larvae (Fig. 2d–f).

The patient received two 5-day courses of ivermectin (100 µg/kg/d) 2 weeks apart. Repeat stool examinations performed 1, 2, and 3 months later showed that the treatment had cured the infection. Her abdominal symptoms and edema resolved within 1 month after ivermectin treatment; her appetite improved, she gained weight, and her albumin and LDH normalized (Fig. 1). The ALC gradually increased over the subsequent 9 months (Fig. 1). She developed mild peripheral adenopathy, but remained asymptomatic with no evidence of visceral disease or hypercalcemia. The patient received no chemotherapy or glucocorticoid treatment for her ATLL during the time period of this report.

Materials and Methods

HTLV-1 DNA assay

The HTLV-1 DNA assay, performed with peripheral blood mononuclear cells (PBMCs), measures the number of copies of viral genome. HTLV-I provirus load was measured using ABI PRISM 7700 sequence detector (Perkin Elmer/Applied Biosystems), as described elsewhere, using primers in pX [5]. The assay was standardized by measuring the number of copies of β-actin DNA, as described previously [5]. All samples were tested in triplicate. The amount of HTLV-I proviral DNA was calculated as copy number of HTLV-I (pX) per 100 PBMC = [(copy number of pX)/(copy number of β-*actin*/2)] × 100.

HTLV-1 RNA assay

The HTLV-1 RNA assay, performed with PBMCs, measured the number of copies of viral RNA that are expressed from the viral genome. RNA extraction, complementary DNA (cDNA) synthesis, and real time PCR were performed as described previously and summarized briefly below [6]. Primers were designed for the amplification of HTLV-1 *tax* cDNA, and the human housekeeping gene hypoxanthine ribosyl transferase (*hprt*) for internal calibration. Standard curves for the value of HTLV-1 *tax* mRNA and *hprt* mRNA were generated using cDNA from MT-2 cells. All standards and samples were assayed in duplicate. The thresh-old cycle (C_t) values were used to plot a standard curve in which C_t decreased in linear proportion to the log of the template copy number. The correlation values of standard curves were always more than

99%. The relative HTLV-1 *tax* mRNA load was calculated by the following formula: HTLV-1 tax mRNA load = (value of *tax*)/(value of *hprt*) × 10,000.

Xenotransplant studies

Xenotransplant studies were performed to assess the tumorigenicity of the patient's ATLL cells. A female NOD.Cg-Prkdc[SCID]IL2rg[tm1Wjl]/SzJ mouse (pathogen free), purchased from Jackson Laboratory, was inoculated intraperitoneally with 2×10^7 fresh PBMCs isolated from patient blood sample. Periodically, serum was collected via the anterior facial vein. Serum concentrations of the soluble released IL-2Rα chain (Tac) were measured using the R&D systems ELISA kit. At the time of necropsy, tissues were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin.

Results

Virus load assays were performed on samples obtained at presentation, and on samples collected 3 and 6 months later after she had completely recovered from *Strongyloides* infection. These included measurements of HTLV-1 DNA and RNA in peripheral blood mononuclear cells. The viral DNA level increased from 0.26 ± 0.68 copies/cell at presentation to 0.48 ± 0.68 and 0.38 ± 0.27 copies/cell, respectively, 3 and 6 months later (Fig. 1). These values reflected the levels of ALC. In contrast, the viral tax mRNA level at presentation was 1.05 copies/10^4 copies of control *hprt* mRNA, and exhibited marked decline to 0.05 and 0.18 copies/10^4 copies of *hprt* mRNA, respectively, 3 and 6 months later. These values correlated well with the levels of albumin and LDH.

To assess tumorigenicity of the patient's PBMCs, they were also injected into a severely immunocompromised NOD.SCID.IL2rg mouse. After four and a half months, the mouse manifested marked splenomegaly, hepatomegaly, and generalized lymphadenopathy as a result of infiltration by an aggressive T-cell lymphoma (Fig. 2g–j).

Discussion

ATLL is a mature CD4+ lymphoid malignancy that occurs in 5–10% of individuals infected by HTLV-1 by breast feeding [7]. It occurs in four different subtypes, smoldering, chronic, leukemic, or lymphomatous disease [8]. The leukemic and lymphomatous subtypes account for 60–90% of cases; these are characterized by an aggressive course, with frequent skin, bone, central nervous system, and other visceral involvement, hypercalcemia, and limited responses to chemotherapy [9–12]. In contrast, chronic ATLL is characterized by lymphocytosis and minimal adenopathy or skin involvement, as manifested by our patient. This disease is more prolonged, although conversion to acute ATLL may occur. Even though this patient's illness was consistent with chronic ATLL, her T-cells were capable of causing an aggressive lymphoma in a xenotransplant model system. This may be indicative of better immunological control of the malignancy in the patient than the immunocompromised mouse. *Strongyloides* infections have been reported in 5–20% of ATLL cases, and these infections are often more severe than in individuals who are not coinfected with HTLV-1 [13]. Okinawa, the birthplace of this patient, is endemic for both HTLV-1 and *Strongyloides* [4]. *S. stercoralis* is an intestinal nematode usually acquired transcutaneously. Adult worms reside in the small bowel and release larvae into the intestinal lumen that are excreted in the stool for years [14]. Autoin-fection is normally limited by the host immune system, but immunosuppressed patients can develop disseminated disease (hyperinfection syndrome). This patient had typical symptoms of a severe Strongyloidiasis, with bloating, vomiting, intestinal and peripheral edema, and weight loss, coinciding with the development of T-cell leukemia. Therapy for ATLL was not initiated because of the risk of exacerbating the parasitic infection. Ivermectin, used for

treatment of this patient, has been reported to cure 97–100% of patients with Strongyloidiasis, and is more effective than albendazole and better tolerated than thiabendazole [15].

The synergistic severity of disease caused by these infections has been suggested to be due to immunocompromise resulting from HTLV-1 as a result of interferon γ expression, and shift from Th2 to Th1 responses, resulting in decreased levels of IL4, IL5, IL13, and IgE [4,16–19]. Alternatively, a *Strongyloides* antigen has been postulated to induce a potent polyclonal T-cell mitogenic response, and reactivation of HTLV-1 expression [1,20]. Although the patient's ALC and viral DNA load did not decline after ivermectin treatment, the dramatic drop in viral RNA after treatment suggests that *Strongyloides* was a potent stimulus for virus expression, presumably through T-cell activation. Alternatively, *Strongyloides* may have caused general immunosuppression that permitted HTLV-1 replication and spread, which reversed after ivermectin treatment with associated weight gain and decreased intestinal inflammation. Expansion of infected T-cells may enhance genetic instability resulting in inactivation of DNA repair mechanisms, predisposing to cancer [3,21,22]. Thus, *Strongyloides* and HTLV-1 coinfection may lead to ATLL in a manner parallel to coinfection with malaria and Epstein–Barr virus which has been linked to Burkitt's lymphoma [23]. Thus, this case illustrates the complexities of pathogen and host interactions that may contribute to hematologic malignancy.

Acknowledgements

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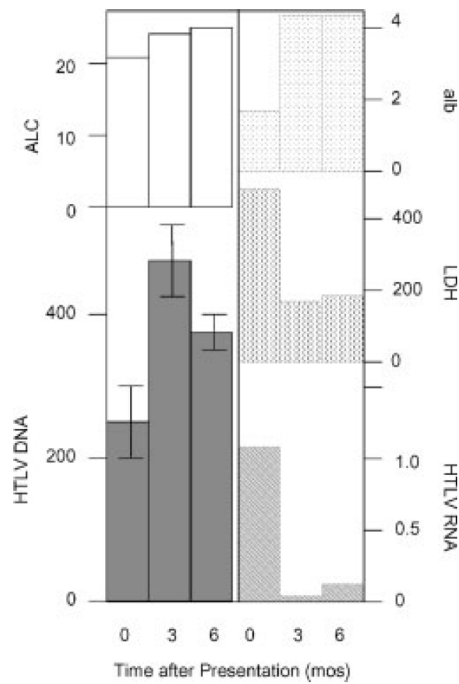
Figure 1.
Clinical course of patient. Values for absolute lymphocyte count (ALC, $\times 10^{-3}$), albumin (alb, g/dl), lactate dehydrogenase (LDH, U/l), HTLV DNA (copies/cell, $\times 10^{-3}$), HTLV RNA (copies/10^4 copies of hprt mRNA) are reported at presentation (0 months), and 3 and 6 months later. Bars on graph of HTLV DNA show standard errors.
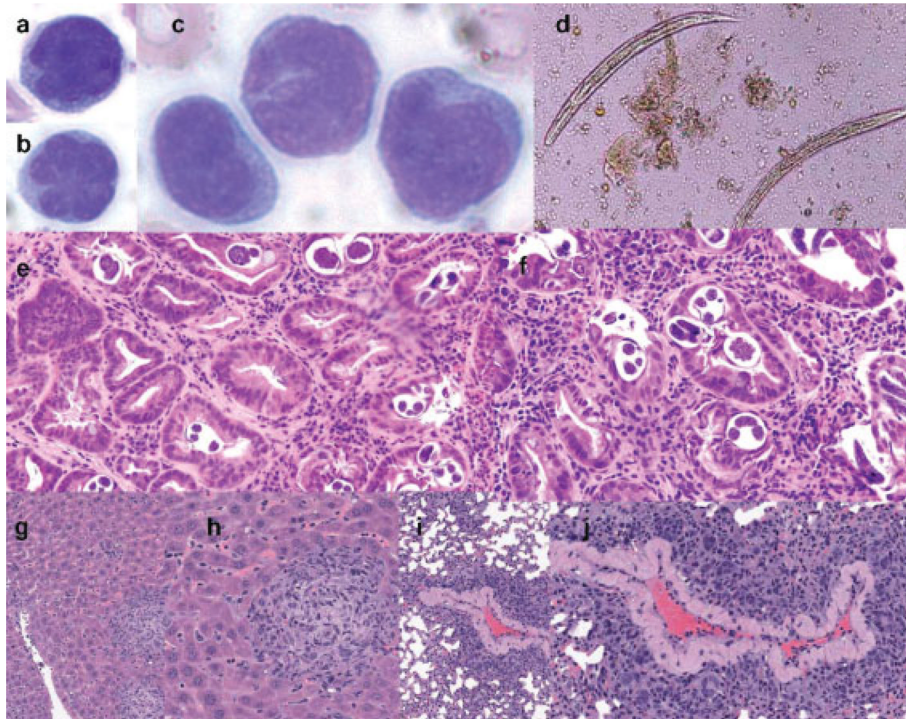
Figure 2.
Pathologic findings. (a–c) Atypical lymphocytes in peripheral blood, and (d) *Strongyloides stercoralis* in the stool and (e,f) gastric biopsy are shown. (g,h) Liver and (i,j) lung infiltration in NOD.Cg-Prkdc^{SCID}IL2rg^{tm1Wjl}/SzJ mouse inoculated with PBMCs from the patient. Routine hematoxylin and eosin staining is shown.