Findings that shed new light on the possible pathogenesis of a disease or an adverse effect

Are increased Foxp3+ regulatory T cells responsible for immunosuppression during HTLV-1 infection? Case reports and review of the literature

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Summary

Research of human T lymphotropic virus type I (HTLV-1)-associated diseases is mostly focused on inflammatory and lymphoproliferative disorders. However, the immunosuppressive consequences of HTLV-1 infection are frequently ignored. In developing countries where exposure to parasitic and other tropical diseases is frequent, the burden of disease is significantly increased by opportunistic infections. Regulatory T cells (Tregs) are a CD4 T-cell subset capable of suppressing effector responses. During HTLV-1 infection, CD4+Foxp3+ cells are increased in HTLV-1-associated leukaemia/lymphoma (ATLL) as well as in non-leukaemic presentations. However, controversy exists regarding the actual regulatory function of these cells. In this report, we present two cases of HTLV-1 ATLL complicated by parasitic organisms and we provide a brief review of the literature regarding FoxP3+ regulatory T cells and their role as a possible mechanism for the immunosuppressive manifestations that take place during HTLV-1 infection.

BACKGROUND

Human T lymphotropic virus type I (HTLV-1) was the first retrovirus known to cause human infection and it was first isolated from a patient with cutaneous T-cell lymphoma. Although most of the infected individuals remain asymptomatic throughout their lives, around 3–5% will develop adult T-cell leukaemia/lymphoma (ATLL).¹

This severe and progressive lymphoproliferative disorder is characterised by generalised lymphadenopathy, hepatosplenomegaly, lytic bone lesions, systemic infiltration and disseminated cutaneous lesions. Disease progression may be rapid and has a poor prognosis. Although ATLL presents varied clinical manifestations along a continuous spectrum of disease, it has been classically categorised in four types: acute, chronic, lymphomatous and smouldering.² The diagnosis and subtype classification constitute the Shimoyama criteria (table 1).³ Although there is no standard recommended treatment, several chemotherapy schemes are being studied. Vincristine, cyclophosphamide, doxorubicin and prednisone (VCAP); doxorubicin, ranimustine and prednisone (AMP); and vindesine, etoposide, carboplatin and prednisone (VECP) regimens may result in better outcomes than traditionally used biweekly cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP).⁴ Other trials have suggested that Zidovudine in association with high doses of interferon- α may lead to high remission rates.⁵ However, each patient's risk should be evaluated individually and should be included in clinical trials whenever possible.²

The expression of the viral protein Tax seems to play a pivotal role in the initial neoplastic transformation and

expression of HBZ for the maintenance of the transformed T-cell lines.⁶ ATLL cells are CD4 T lymphocytes that usually coexpress high levels of the IL-2R α (CD25) and chemokine receptor 4 (CCR4).⁷ In addition, the expression of Forkhead box p3 (Foxp3), a transcription factor critical to the development and function of regulatory T cells (Tregs), has been found to be increased in ATLL.⁸

HTLV-1 patients are at risk of recognised opportunistic infections including parasites, fungal pathogens and tuberculosis.^{2–9} We recently reported highly increased regulatory T-cell numbers (CD4+CD25+Foxp3+) in strongyloidiasis/HTLV-1 co-infection patients without ATLL. In addition, an increase in Tregs is correlated with a poor Th2 response against *Strongyloides* antigen, low eosinophil numbers and an increased parasite load in stools.¹⁰ Increased Treg numbers have also been found in HTLV-1

Table 1 Clinical characteristics of adult T-cell leukaemia/lymphoma subtypes (24)

	Acute	Chronic	Lymphomatous	Smouldering
Lymph	Present	Present	Absent	Absent
LDH	High	$\leq 2N$	Variable	$\leq 1.5N$
Hypercalcaemia	Usually present	Absent	Maybe present	Absent
Organs involved	Any organ	Any organ except bone, GIT and CNS	Lymph node and any other organ*	Only skin and/or lungs and/or blood
Body effusion	Present	Absent	Maybe present	Absent

*Histology proven lymphadenopathy. CNS, central nervous system; GIT, gastrointestinal tract; LDH, lactic dehydrogenase; N, normal upper limit.

asymptomatic carriers, although in lower numbers when compared with strongyloidiasis co-infected patients.¹¹

Some have speculated that ATLL cells represent a clonal expansion of infected regulatory T cells, which may have a suppressive capacity.¹² It is possible that a similar expansion of regulatory T cells may be responsible for the immunosuppression associated with HTLV-1 patients without ATLL. Here, we present two cases of ATLL complicated by parasitic infections and discuss the role of Foxp3 expression during HTLV-1 infection, immunosuppression and ATLL.

CASE PRESENTATION

Case 1

A 70-year-old woman born in Cajatambo (Peruvian highlands) was admitted to the Internal Medicine ward at Cayetano Heredia Hospital in Lima, Peru with a 20-year history of desquamative plaques on her left lower limb. They had been treated as a recurrent tinea corporis infection. These lesions slowly progressed compromising her right lower limb, chest, upper extremities, back and abdomen. The patient stated that approximately 2 months prior to admission, these chronic lesions became raised and very pruritic, especially on pressure zones (back, elbows and knees, also scalp and ears). She noted weight loss of approximately 11 kg, fever and facial erythroderma. Two weeks prior to admission, she developed multiple enlarged inguinal, axillary and cervical lymph nodes.

Upon physical examination, the patient showed multiple desquamative macular lesions with raised erythematous borders located on her back, upper and lower limbs, scalp and ears (figure 1A); she also presented crusted-like lesions mainly on her back and lower limbs (figure 1B) and facial and upper thorax erythroderma (figure 1C).

Blood cell count showed mild anaemia (haemoglobin: 12.4 g/dl) and marked lymphocytosis (86 330 cells/mm³) with the presence of characteristic flower cells in the

peripheral blood smear. Flow cytometry of peripheral blood was performed showing that 94% of CD4 lymphocytes presented a Treg phenotype (CD25+Foxp3+).

A skin biopsy was taken from the erythematous lesions, showing a hyperplastic epidermis with band infiltrate and multiple intraepithelial lymphoid collections, known as Pautrier's abscesses and epidermotropism of the lymphocytes. All of this is suggestive of cutaneous T-cell lymphoma. Immunohistochemistry was performed and the results indicated that the abnormal lymphocytes that were infiltrating the epidermis were CD3 and Foxp3+ (figure 2).

HTLV-1 EIA was reported as positive. Multiple skin scrapings were performed at the time of admission. The sample from the desquamative lesions showed multiple hyphae when stained with KOH, confirming a ringworm infection and the samples from the crusted lesions were positive for *Sarcoptes scabiei*. As part of the protocol for evaluating possible opportunistic infections in HTLV-1-infected individuals, three stool samples were sent for evaluation of gastrointestinal parasitic infections. The patient was found to be infected with *Strongyloides stercoralis*. She was admitted with the following diagnosis: HTLV-1-associated ATLL of acute type, ringworm infection, crusted scabies and *S stercoralis* infection. She received oral ivermectin for both *S scabiei* and *S stercoralis* infections. In addition, biweekly CHOP was started. Despite clinical care, the patient rapidly deteriorated and died.

Case 2

A 62-year-old woman born and raised in Lima but currently living in Iquitos (Peruvian jungle) was seen at the Tropical Medicine Institute outpatient clinic with a 2-month history of recurrent episodes of watery diarrhoea up to 4–5 times a day and associated with epigastric pain. She had no contributing history of previous medical problems. Initial physical examination was positive for

Figure 1 Skin manifestations. (A) Enlarging raised erythematous ring lesions in the back. These lesions were desquamative and highly pruriginous. (B) Crusted-like lesions at the foot. Scraping from the lesions was positive for *Sarcoptes scabiei*. (C) Several raised macular ring lesions within an erythematous skin.

Figure 2 Immunohistochemistry of skin lesions. (A) Immunohistochemistry for FoxP3+ was performed from a paraffin-embedded tissue using the Streptavidin-Horseradish peroxidase technique. (B) Immunohistochemistry for CD3 was performed from a paraffin-embedded tissue using the Streptavidin-Horseradish peroxidase technique.

multiple small non-tender adenopathies in neck and axillae, and no hepatomegaly or splenomegaly was found. The remaining portion of the exam was unremarkable.

Whole blood count showed microcytic anaemia (Hb 8.8 g/dl) with mild leukocytosis (12 700 cells/mm³) with 20% lymphocytes and 2% eosinophils. A stool examination was performed and reported as positive for *S stercoralis* larvae on two consecutive stool samples; no other intestinal parasites were reported. During this period, an HTLV-1 EIA was performed and reported as positive.

Flow cytometry analysis of peripheral blood showed an increased proportion of Tregs (CD4+Foxp3+) (figure 3). Peripheral blood smear revealed the presence of characteristic flower cells. The patient was hospitalised and treated with oral ivermectin (200 µg/kg/day for 2 days repeated 14 days later). During hospitalisation, she also developed further diarrheal episodes. At this point, new stool examination revealed *Entamoeba histolytica* cysts. Baermann examinations were negative for *S stercoralis*. Peripheral lymph node biopsies showed inflammatory changes and a bone marrow biopsy showed evidence of lymphoma. The patient's condition was classified as adult T-cell leukaemia/lymphoma of the lymphomatous type. She was started on a combination of antiretrovirals (tenofovir and lamivudine) and interferon-α. During hospitalisation, the patient developed septic shock secondary to hospital-associated pneumonia. Despite appropriate support measures, the patient died.

DISCUSSION

Regulatory T cells are a CD4 T-lymphocyte subset that actively suppress immune activation and control the expansion of self-reactive lymphocytes. Thus, they play an important role in peripheral tolerance. Although Tregs express high levels of IL-2Rα (CD25) and glucocorticoid induced tumor necrosis factor receptor (GITR), these markers are also upregulated in recently activated T cells.¹³ In addition, HTLV-1 infection of CD4 T cells yields the expression of CD25 in 80% of the cases.¹⁴ HTLV-1 induces upregulation of CD25, making CD4+CD25+ T cells the major reservoir of HTLV-1 infection.¹⁵ As a consequence, CD25 cannot be used as a reliable regulatory T-cell marker in HTLV-1 infection.

The expression of Foxp3 confers T cells with a suppressive capacity, making it the single most reliable marker of

regulatory T cells.¹⁶ Foxp3 is highly expressed in CD4+CD25+ T cells during ATLL and confers the suppressive capacity through a cell-to-cell mechanism, mainly cytotoxic T lymphocyte antigen 4 (CTLA-4) and in some cases by TGF-β. In addition, patients with Foxp3 negative tumours had better survival.¹⁷ The increment of Foxp3 expression and TGF-β production after infection of CD4 T cells suggests that the acquisition of a Treg phenotype is a potential mechanism to explain the broad immunosuppression observed in ATLL patients.¹⁴

It has been postulated that ATLL cells could result after the expansion of cells with a newly acquired Foxp3 phenotype or the preferential infection of Foxp3+ cells. Although higher expression of Foxp3 was found in HTLV-1-infected T-cell lines, Foxp3 expression in tumour cells is detected in only two-thirds of ATLL patients.¹⁸ Furthermore, CD4+Foxp3+ cells did not correlate with CD4+CD25+ T cells.

Figure 3 Flow cytometry of peripheral blood mononuclear cells. Peripheral blood mononuclear cells were isolated and stained with antihuman CD4, CD25 and FoxP3+ monoclonal antibodies. Flow cytometry analysis showed an increased proportion of CD4+Foxp3+ cells.

In addition, ATLL cells from only a subset of patients were hyporesponsive to TCR-mediated activation and suppress the proliferation of autologous CD4+ non-ATLL cells.¹⁹

Recently, it was demonstrated that unlike CD4+Foxp3+ T cells, expanded CD4+TCRVc cells are unable to inhibit heterologous CD4 T cells. In addition, HTLV-1 is preferentially found in tax-expressing lymphocytes. However, only Foxp3+Tax- T cells are able to correlate with the percentage of inhibition. Furthermore, the expanded ATLL clones express very low frequencies of Foxp3, suggesting that the Foxp3+ regulatory T cells are distinct from ATLL cells.¹⁹

Although it was first reported that the Treg proportion decreased and correlated with inflammatory diseases during HTLV-1 infection, more recent reports indicate the opposite.²⁰ This was suggested to be the result of using CD25 as a marker of Tregs during HTLV-1 infection in the early reports. The increase of CD4+Foxp3+Tax- cells is inversely correlated with the HTLV-1-specific CTL response. Foxp3+ cells are also correlated with tax expression and proviral load.²¹ Only Foxp3 protein, but not mRNA, are able to discriminate Tregs from CD4+CD25+ T cells.²²

In summary, immune suppression due to HTLV-1 infection might be due to an increased proportion of CD4+CD25+Foxp3+ regulatory T cells; whether these cells are responsible for blunting the immune response to invading pathogens is a question with an elusive answer. Also, it has been postulated that ATLL cells represent a clonal expansion of regulatory T cells, but the underlying mechanism for this process remains uncertain, ATLL will be developed in 3–5% of HTLV-1 infected individuals. Although the ATLL subtypes differ slightly in their clinical presentation and prognosis, all are at increased risk of infection by opportunistic organisms. The presented cases support the possibility of an increased FoxP3+ Tregs phenotype as a possible mechanism for the severe immunosuppression observed in HTLV-1 associated ATLL.

Learning points

- ▶ Adult T-cell leukaemia/lymphoma (ATLL) develops in 3–5% of human T lymphotropic virus type I (HTLV-1) infected individuals. ATLL can be further classified as acute, chronic, lymphomatous or smouldering, according to established criteria.
- ▶ Chemotherapy should be started after evaluating the individual's risks. Regimens including vincristine, cyclophosphamide, doxorubicin and prednisone, AMP and vindesine, etoposide, carboplatin, and prednisone are available and may be superior to cyclophosphamide, doxorubicin, vincristine, and prednisone.
- ▶ ATLL individuals have a greater risk of contracting opportunistic infections, including *Strongyloides stercoralis* hyperinfection.
- ▶ Patients with *S stercoralis* hyperinfection, with no underlying known risk factor for immunosuppression, should be tested for HTLV-1. Individuals residing or migrating from HTLV-1 endemic areas who are infected with *S stercoralis* should be tested for HTLV-1.
- ▶ Whether increased regulatory T cells are responsible for blunting specific effector responses to opportunistic pathogens in ATLL remains a critical question to answer.

Competing interests None.

Patient consent Obtained.

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