Peripheral T-cell Lymphoma With a Regulatory T-cell Phenotype: Report of a Nodal and an Extranodal Case From Peru

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Abstract: T-cell regulatory lymphocytes (T_{reg}) are identified by their reactivity with CD4, CD25, and FOXP3, and are variably present in the background of various neoplasms including hematopoietic tumors, and function modulating the immune response, including control of autoimmunity. Adult T-cell leukemia/lymphoma is an aggressive lymphoma associated with human T-lymphotrophic virus 1 infection characterized by the presence of neoplastic lymphocytes with a T_{reg} phenotype; however, this phenotype is not characteristically found in other lymphomas. Here, we report 2 apparently immunocompetent human T-lymphotrophic virus 1-negative patients with nodal and extranodal peripheral T-cell lymphoma, not otherwise specified with a T_{reg} immunophenotype, based on the strong CD25 and FOXP3 positivity of the neoplastic cells. One patient was a 48-year-old woman with an early stage tumor in the cavum, who despite of chemotherapy subsequently developed systemic disease and died of tumor progression 46 months from diagnosis. The second patient was a 65-year-old male with generalized adenopathy and B symptoms who received chemotherapy achieving a complete remission but had recurrence and died 36 months from diagnosis. The histopathology revealed a diffuse infiltrate with an interfollicular distribution in the second case, with nodal involvement, consisted of large cells with clear cytoplasm associated with vascular proliferation and abundant mitoses. Neoplastic cells of first case showed typical T_{reg} phenotype, whereas the second case had a CD4/CD8 double negative T_{reg} variant. Only a single similar case was found in a review of the literature. We conclude that peripheral T-cell lymphoma, not otherwise specified with a T_{reg} phenotype may represent a distinct category of T-cell lymphoma with an aggressive clinical course and poor prognosis.

Key Words: PTCL, T-cell regulatory, lymphoma, peripheral T-cell lymphoma, immunohistochemistry

(*Appl Immunohistochem Mol Morphol* 2012;20:196–200)

T cells include subpopulations of effector T cells (TH1, TH2, and germinal center B-helper T cells), memory cells (central and effector memory), and cytotoxic T cells, usually defined by the secretion of certain cytokines.[1] In addition, some T cells that inhibit autoimmune reactions have been designated as regulatory T cells (T_{regs}).[2] T_{regs} can occur naturally or be inducible, suppress effector T cells and prevent reactivity to self-antigens and tumor immunity.[3] T_{regs} are identified by their expression of αβ T-cell receptor, CD4, CD25, and FOXP3.[4] T_{regs} non-specifically suppress proliferation and activation of other T cells, such as CD4 and CD8.[5,6] In solid tumors, such as pancreatic and breast adenocarcinomas, T_{regs} suppress lymphocyte activity against tumor antigens that may result in neoplastic progression.[7] Gene transfer of FOXP3 in mice can turn $CD4^+$ T cells into $CD4^+/CD25^+/FOXP3^+$ cells with a regulatory phenotype; hence, expression of FOXP3 constitutes a reliable marker of T_{reg} lymphocytes.[8,9]

After the current paradigm in which lymphoma cells have a normal cell counterpart, adult T-cell leukemia/lymphoma (ATLL), an aggressive T-cell malignancy associated with the human T-lymphotrophic virus 1 (HTLV-1) is the only lymphoma subtype, which proposed cell of origin has a T_{reg} phenotype.[10,11] The objective of this study is to report 2 apparently immunocompetent HTLV-1-negative patients with nodal and extranodal peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS) with a T_{reg} immunophenotype, based on the strong CD25 and FOXP3 positivity of the neoplastic cells.

CASE REPORTS

Case 1

A 48-year-old woman from south coastal Peru without a significant past medical history and an excellent performance status presented with a 6-month course characterized by epistaxis and nasal congestion, but no B symptoms. Initial evaluation revealed a 2 × 3-cm lesion in the cavum, where multiple biopsies were taken. Physical examination did not show lymphadenopathy

Received for publication March 28, 2011; accepted May 18, 2011.
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or hepatosplenomegaly. Laboratory testing showed leukocytes, 6×10^9/L, with a normal differential; hemoglobin, 9.6 g/dL; and a platelet count of 300×10^9/L. The liver enzymes were within normal limits, lactate dehydrogenase level was 214 IU/mL (upper range: 400 IU/mL) and β2-microglobulin was within normal range. Serologic studies for HTLV-1, hepatitis B, hepatitis C, and human immunodeficiency virus were negative. A computed tomographic (CT) scan revealed that tumor was confined to the cavum; and no cervical lymphadenopathy was noted. Biopsy diagnosis was PTCL, NOS, with a T_{reg} phenotype. Patient was categorized as stage IA with a low-risk International Prognostic Index[12] and received standard doses of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) for 6 cycles, administered every 3 weeks. Patient achieved a complete response based on restaging CT scans at the end of chemotherapy. Recurrence was noted in left tonsil and cervical nodes 2 years later. Patient received ICE (ifosfamide, carboplatin, and etoposide) for 4 cycles with progression of disease; subsequently she received GDP (gemcitabine, dexamethasone, and cisplatin) for 6 cycles with locoregional progression. Radiotherapy was then pursued to her right cervical area up to 4000 Gy with a good response. Six months later, patient had a duodenal recurrence that presented with perforation. Patient died at 46 months from diagnosis.

Case 2

A 65-year-old man who presented with an 8-month course of weight loss of approximately 30 pounds; cervical and inguinal lymphadenopathy. He had a performance status ECOG 0 and denied fevers or night sweats. A CT scan revealed cervical, axillary, and inguinal lymphadenopathy, but no extranodal involvement. Lactate dehydrogenase level was within normal limits and β2-microglobulin was 3.0 mg/L (normal range, 0.8 to 1.5 mg/L). Hemoglobin was 10.7 g/dL, leukocyte count was 5.6×10^9/L, and platelets were within normal range. HTLV-1 serology was negative. Cervical lymph node biopsy was diagnosed with PTCL, NOS. The patient was considered as a stage IIIB with a low-intermediate risk International Prognostic Index score. Patient received CHOP for 6 cycles given every 3 weeks, and achieved a complete response; however, he relapsed on cervical and axillary lymph nodes, 7 months later. Patient received 6 cycles of ICE with progressive disease, and then pixantrone in a clinical trial setting for 4 cycles with a partial response. Patient died from lymphoma progression, 36 months after his diagnosis.

PATHOLOGIC FINDINGS

Case 1

Histologic sections demonstrated a diffuse effacement of the nasopharyngeal architecture with a noninvolved subepithelium, where reactive small lymphocytes and histiocytes were noted (Fig. 1). The majority of the neoplastic infiltrate was deep into the specimen, and consisted of large, pleomorphic cells with abundant clear cytoplasm (Fig. 2). Nuclei were oval, vesicular with distinct central nucleoli, or hyperchromatic, irregular, elongated with dense chromatin. Numerous mitotic figures and scattered high endothelial venules, partially hyalinized were noted. Rare large Reed-Sternberg-like cells and scattered small to intermediate size cells with folded, hyperchromatic nuclei were also noted. Focal ulceration, necrosis, and karyorrhexis were noted. Immunohistochemical studies revealed that the neoplastic lymphocytes were positive for CD3 (Biocare, Concord, CA; dilution 1:200), CD25 (Novocastra, Buffalo Grove, IL, dilution 1:1600), and FOXP3 (Abcam, Cambridge, UK; dilution 1:50). The neoplastic cells were negative for CD4 (Novocastra, Buffalo Grove, IL, dilution 1:20). Polymerase chain reaction used to detect proviral HTLV-1 DNA in the tumor using described methods was negative.[13]

FIGURE 1. Case 1: Histologic section of cavum shows a diffuse effacement of the architecture with small lymphocytes and histiocytes under the mucosal surface. Hematoxylin and eosin, × 100.

Case 2

Histologic sections revealed subtotal effacement of the nodal architecture. The infiltrate was diffuse and interfollicular, surrounding residual lymphoid follicles with germinal centers (Fig. 3). The infiltrate consisted of large, pleomorphic cells with abundant clear cytoplasm. Numerous mitotic figures and scattered small-to-intermediate size hyperchromatic lymphocytes were admixed (Fig. 4). Neoplastic cells were positive for CD3 (Fig. 5), CD4, CD25, and FOXP3 (Fig. 6).

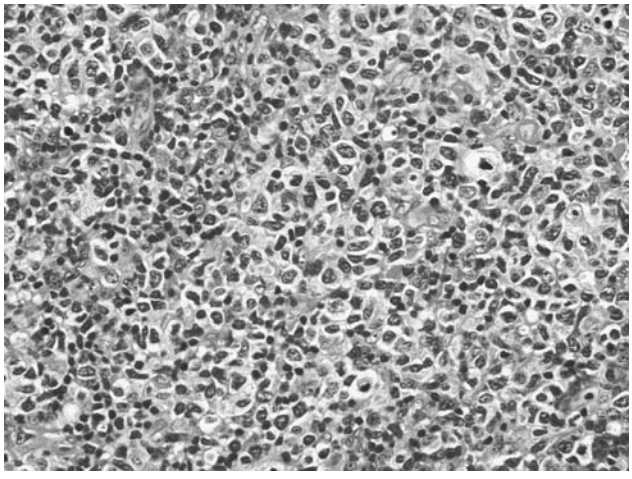

FIGURE 2. Case 1: The neoplastic infiltrate consisted of large, pleomorphic cells with abundant clear cytoplasm. Cells display oval vesicular nuclei with distinct central nucleoli. There are numerous mitotic figures and scattered high endothelial venules, partially hyalinized. Hematoxylin and eosin, × 400.

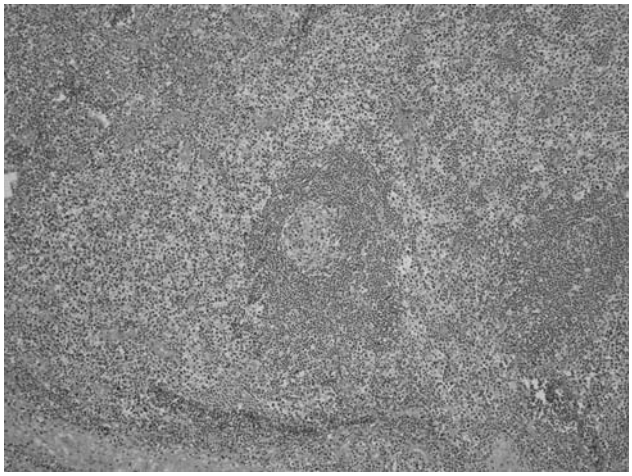

FIGURE 3. Case 2: Section of lymph node shows malignant lymphoma with a diffuse and interfollicular pattern. Hematoxylin and eosin, × 100.

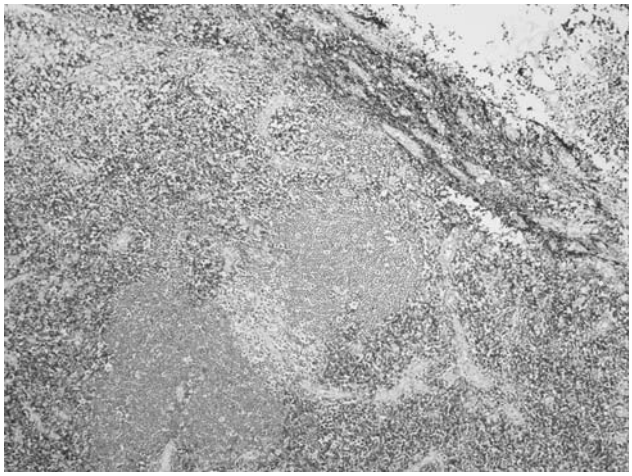

FIGURE 5. Case 2: CD3 immunohistochemistry highlights the neoplastic cells growing in an interfollicular/paracortical pattern. × 100.

Both cases had scattered $CD20^+$ aggregates of small-to-intermediate size lymphocytes, including lymphoid follicles with a rare germinal center in case 2. Interfollicular lymphocytes included large $CD20^+$ cells, some of which were positive for Epstein-Barr virus encoded RNA (EBER). EBER reacted mainly with large cells, but not with small background lymphocytes. Epstein-Barr Virus (EBV) latent membrane protein-1 was also positive in case 2. CD30 highlighted scattered intermediate and rare large cells. No reactivity of neoplastic cells with CD56 and T-cell intracellular antigen-1 was noted. CD21 highlighted scattered disrupted follicular dendritic cell (FDC) meshworks, which focally were perivascular in case 2. Bone marrow was negative for lymphoma in both cases. Biopsies of recurrence were performed in case 1, but not in case 2. Biopsies of recurrence of case 1 in left tonsil and cervical lymph node showed similar histopathology and phenotype as original biopsy.

DISCUSSION

In this study, we describe 2 cases of nodal and extranodal HTLV-1-negative, PTCL, NOS with a T_{reg} immunophenotype, based on the coexpression of CD25 and FOXP3 by the malignant cells; only 1 case was positive for CD4. T_{reg} lymphocytes are identified by their reactivity with FOXP3, and represent 5% to 10% of peripheral T cells; T_{regs} are responsible for the control of autoimmunity.[9] ATLL is the only lymphoma that expresses FOXP3, and is considered derived from T_{regs}.[11,14] Roncador et al[11] reported a more aggressive clinical course in patients with FOXP3-positive ATLL, although the survival difference did not reach significance. Neoplastic ATLL cells could act as T_{reg}-like cells and induce immunosuppression, especially in the FOXP3-positive group.[14] More recently, this finding has been challenged, suggesting that the FOXP3-positive cells are mainly a component of the reactive lymphocytes in the neoplastic microenvironment.[15]

A review of the literature disclosed that only ATLL is characterized by a T_{reg} phenotype.[11,14,16–18] In addition, Bonzheim et al[19] reported the only case of PTCL, NOS

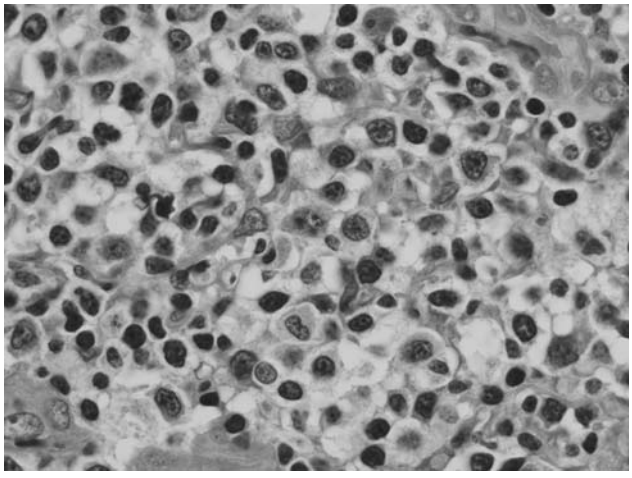

FIGURE 4. Case 2: Neoplastic cells are large, pleomorphic with abundant clear cytoplasm. Hematoxylin and eosin, × 1000.

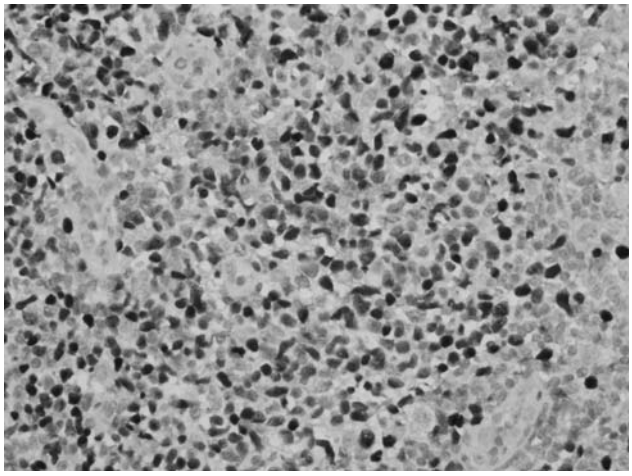

FIGURE 6. Case 2: FOXP3 immunohistochemistry highlights the majority of the neoplastic cells. × 400.

with an immunohistochemical profile similar to our cases. In this study on 83 cases including PTCL, NOS, angioimmunoblastic T-cell lymphoma (AITL), and anaplastic large cell lymphoma, only 4 of 6 cases of anaplastic lymphoma kinase-positive anaplastic large cell lymphoma showed weak and/or focal expression, consistent with the notion that T_{reg} PTCL is rare, if it at all exists, in the Western hemisphere. The only patient with PTCL, NOS with a T_{reg} phenotype was a 59-year-old man who presented with stage IV disease, had several complications during chemotherapy and died 1 month after diagnosis. Our first case presented with stage I PTCL, NOS, who had an initial response to CHOP and radiotherapy; however, had a locoregional relapse followed by progression of disease, and chemoresistance. It is interesting to note that, our case showed positivity for EBV latent membrane protein-1 and EBER within the Reed-Sternberg-like cells. It is considered that EBV infection induces transformation of lymphocytes into Reed-Sternberg-like cells in patients with PTCL.[20,21] It could be hypothesized that T_{regs} could have contributed to local immunosuppression and EBV activation, favoring a more resistant lymphoma.

From our review of 2 cases with HTLV-1-negative PTCL with a T_{reg} phenotype, the histopathology is characterized by a marked predominance of large cells, some of which display distinct central nucleoli, reminiscent of immunoblasts. The neoplastic cells show a clear cytoplasm and abundant mitoses. Few reactive small lymphocytes, increased vascularity and occasional eosinophils are found in the background. The immunophenotype that supports a T_{reg} phenotype is $CD3^+$, $CD4^+$, $CD8^-$, $CD25^+$, and $FOXP3^+$, however, rare subsets of T_{reg} lymphocytes are described as $CD4^-$, similar to the phenotype of our second case, constituting so-called double negative T_{regs}.[22] The differential diagnosis includes the lymphomatous form of ATLL, where large cells are predominant. Diagnosis of ATLL requires positive serology and confirmatory testing for viral integration.[23] Affected patients proceed from endemic areas such as Japan, the Caribbean basin or South America, including Peru. Our cases had negative serology and polymerase chain reaction testing for DNA provirus was negative. Otherwise, many of the described features of the 2 cases we describe with a T_{reg} phenotype may overlap with the usual findings of ATLL cases. T_{reg} PTCL may mimic AITL because of the presence of extensive interfollicular growth, neoplastic cells with clear cytoplasm and admixed large B cells, which frequently express EBV antigens or encoded RNA.[24] However, the neoplastic cells are not as abundant as in our cases. In addition, there usually is a predominance of small clusters of intermediate size atypical lymphocytes in AITL, and is not easy to determine cellular atypia. The neoplastic cells of AITL usually express CD4, CXCL13, CD10, and BCL-6, consistent with a germinal center helper T-cell. The increased vascularity observed in our cases of PTCL with a T_{reg} phenotype may also mimic the arborescent vascular proliferation of high endothelial vessels associated with FDCs of AITL. Only 1 of the 3 cases had a mild increase of FDC meshworks around a small vessel, whereas 2 cases did not have detectable FDC meshworks. The distinction between AITL and PTCL with a T_{reg} phenotype and increased FDC meshworks would rely on the neoplastic cell phenotype.

From our perspective, a strong expression of FOXP3 and a T_{reg} phenotype may have caused antitumor immunosuppression, leading to an aggressive behavior in these patients with PTCL, NOS, and a T_{reg} phenotype. As PTCL is heterogeneous, a T_{reg} phenotype may help define a distinct entity for those HTLV-1 negative cases, and the need to establish new therapeutic approaches for FOXP3-positive PTCL. Comparable with ATLL, immunodeficiency would be expected in this type of lymphoma with a T_{reg} phenotype. In fact, both of our cases had EBV^+ Reed-Sternberg-like cells admixed with the T-cell neoplastic infiltrate. However, the role of immunosuppression is rather unclear given the lack of associated immunodeficiency by laboratory or clinical parameters.

In conclusion, FOXP3 expression can be found in PTCL, NOS of nodal, and extranodal sites, and it may potentially constitute a target for molecular targeted therapies. Prospective studies should validate the potential prognostic role of the T_{reg} phenotype and the potential therapeutic value of targeting FOXP3 in PTCL.

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