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Co-Existence of Cutaneous T-Cell Lymphoma and B Hairy Cell Leukemia

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A primary cutaneous form of peripheral T-cell lymphoma (PTCL) and a low grade B-cell non-Hodgkin's lymphoma that was classified as a variant of hairy cell leukemia (HCL) were simultaneously diagnosed in a 79-year-old woman by both phenotypic and genotypic analyses. The coexistence of a T- and B-cell lymphoma in the same patient is rare, and, to our knowledge, this particular association has not been previously described. The patient was referred to our Department for evaluation of multiple cutaneous itchy, reddish plaques; laboratory analyses disclosed a lymphocytosis, that presented 6 years earlier. A bone marrow aspirate showed a 50% B-cell interstitial infiltrate, while a skin biopsy surprisingly revealed a PTCL. Clonality of both neoplastic processes was assessed by Southern blot analysis. The indolent clinical course of the cutaneous disease, and the low and stable number of circulating neoplastic T cells supported the diagnosis of a mycosis fungoides (MF)-like PTCL. Possible oncogenic events and/or putative underlying viral infections which could have played a role in the occurrence of B- and T-cell non-Hodgkin's lymphomas in the same patient are discussed. Am. J. Hematol. 64:197–202, 2000.

Key words: hairy cell leukemia; peripheral T-cell lymphoma; semi-nested PCR; Southern blot analysis

INTRODUCTION

Hairy cell leukemia (HCL) is a rare lymphoproliferative malignancy accounting for approximately 2% of all leukemias. This chronic B-cell neoplasm is characterized by pancytopenia, splenomegaly, immunological abnormalities, and the presence of morphologically distinct "hairy cells" with irregular cytoplasmatic projections in the blood, bone marrow, liver, spleen, and other tissues. Hairy cells express the usual markers of B-cell lineage, namely CD19, -20, -22, -45RO, and -79a, while they are negative for CD5, -10, and -23. Moreover they are CD11c, -25, and -103 positive; this last molecule, usually present on mucosal T lymphocytes, is an important diagnostic marker for HCL [1] and its atypical form, as in the present case. Before effective therapy was introduced, the median survival time of HCL patients was approximately 4 years. However, HCL occasionally has an indolent course, and some patients have lived longer than 25 years without systemic treatment. A small number of patients, mainly those with smaller spleens, normal blood counts, and few hairy cells in the blood and bone marrow, may survive untreated for long periods. In our patient, the clinically silent HCL dated back at least 6 years and was discovered during evaluation of the patient for multiple cutaneous reddish and itchy plaques, later proven to be a mycosis fungoides (MF)-like peripheral T-cell lymphoma (PTCL).

Cutaneous PTCL belongs to the group of non-Hodgkin's lymphomas. The main representatives of

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PTCL, MF and Sezary syndrome (SS), are non-Hodgkin's lymphomas with low malignant potential. The coexistence of T and B non-Hodgkin's lymphomas (NHL) in the same patient is rare. Cutaneous T-cell lymphomas, such as MF and SS, have occasionally been described in association with myeloma [2], B-CLL [3], or other low-grade B-cell NHL [4]. Unusual cases associated with intermediate grade (centroblastic–centrocytic) lymphoma have also been reported [4]. We describe a rare association between a primary cutaneous form of PTCL and a low-grade B-cell NHL diagnosed as a variant of HCL.

CASE REPORT

A 79-year-old woman was referred to our Department in January 1998 to evaluate multiple itchy reddish plaques (Fig. 1A) that had appeared 6 months earlier on the trunk and submammary folds and then extended to the abdomen, pelvis, and proximal regions of the upper and lower limbs (Fig. 1B). The patient had a 25-year history of diabetes mellitus, which was well controlled by diet and tolbutamide, and had bilateral cataracts. She was otherwise well and in good general condition; neither hepatosplenomegaly nor lymph node enlargement was present. Ultrasound examination of the abdomen showed normal findings; laboratory evaluation showed lymphocytosis (WBC 11,800/μL, Ly 6,608/μL) with a normal hemoglobin concentration and platelet count. A retrospective search disclosed the presence of lymphocytosis since 1992 (WBC 10,600/μL, Ly 7,420/μL). Apparently, this finding was ignored until 1998, when morphologic and immune phenotipic evaluation of a peripheral blood sample was performed and found consistent with a B-cell variant of HCL. Serum immunoglobulin concentration and electrophoresis were normal; antiglobulin test was negative and cryoglobulins were absent. The serum LDH level was in the normal range; tartrate-resistant acid phosphatase (TRAP) was negative. An abundant sternal bone marrow aspirate showed a 50% B-cell interstitial infiltrate. Skin involvement by the leukemic cells was strongly suspected but, surprisingly, a PTCL was diagnosed. A serological test for HTLV-I/II was negative.

In March, therapy with α-IFN (6×10^6 U three times a week) was attempted due to increasing cutaneous involvement by the itchy plaques but was discontinued after 6 months because of poor tolerance with no appreciable clinical improvement, and no effect on the lymphocyte count (WBC 12,200/μL, Ly 6,100/μL). Symptomatic treatment with an antihistaminic drug plus prednisone was then administered; this afforded the patient better relief but has left the clinical pattern unchanged up to the present.

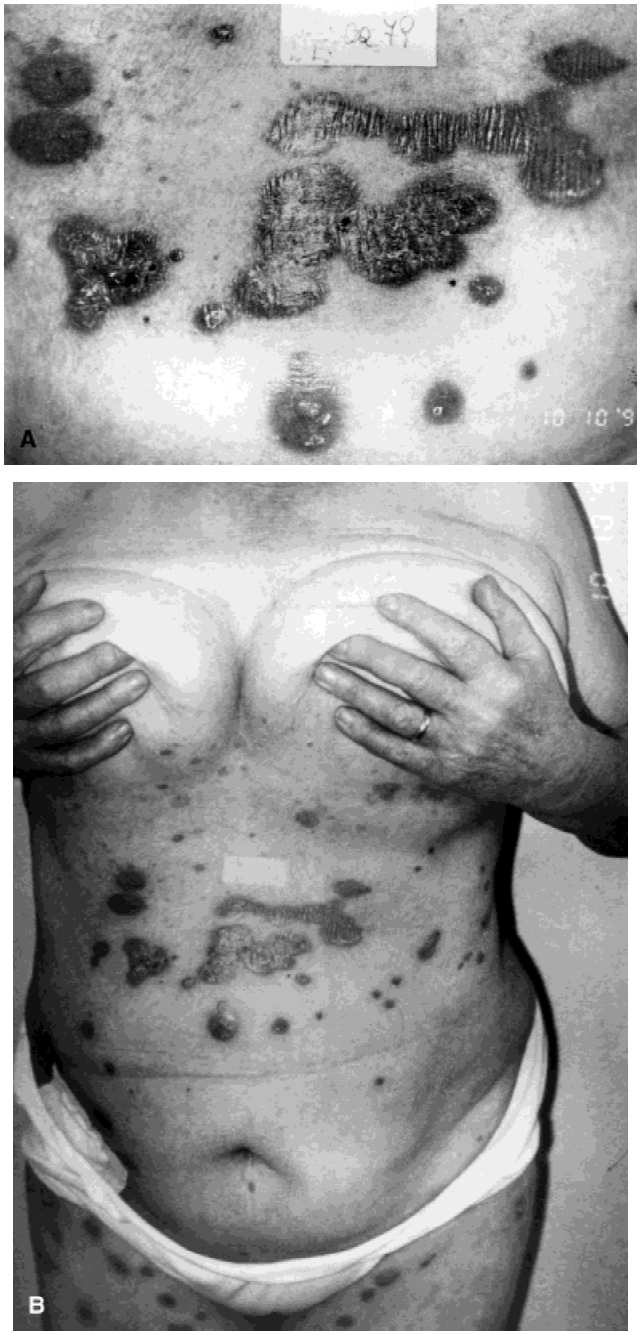

Fig. 1. Skin lesions of the patient. (A) Macroscopic aspect of the abdominal lesions. (B) Multiple plaques on submammary folds, abdomen, and proximal lower limb region.

MATERIALS AND METHODS

Morphologic and Immunophenotypic Studies

Formalin-fixed, paraffin-embedded sections were stained with hematoxylin-eosin and Giemsa. Immunohistochemistry (IHC) was performed on Zenker-fixed decalcified and formalin-fixed paraffin-embedded tissues (bone and skin biopsies, respectively), as previously reported [5].

Molecular Studies

Tumor tissues were also analysed by Southern blot with human immune receptor and Epstein-Barr virus specific probes, as described [6]. The semi-nested polymerase chain reaction (PCR) approach for the detection of clonal V_H-D_H-J_H junctional rearrangements was applied to tumor sample DNA; amplified fragments were then separated by denaturing polyacrylamide gel electrophoresis. EBNA 2 specific PCR on tumor sample DNA was performed as previously described [7].

RESULTS

Immune Phenotyping and Histopathology

At the light microscope, the peripheral lymphocytes appeared as small round cells with a bean-shaped nucleus, and at times an abundant cytoplasm with "hairy" projections (Fig. 2A). A few lymphocytes had a cerebriform nucleus, similar to "atypical" monocytoid cells (Fig. 2B). Immune phenotyping of the peripheral lymphocytes disclosed a dominant population of monoclonal ($CD19^+$ and $CD103^+$) B cells expressing high-density surface immunoglobulins restricted to the λ light chain; CD5 and CD25 were negative, thus suggesting a variant of HCL. On the other hand, a T-cell phenotype for the "atypical" monocytoid cells was confirmed by immune phenotyping of the peripheral smear; these neoplastic T-cells represented less than 1% of the circulating lymphocytes.

A bone marrow aspirate and biopsy showed a hypercellular pattern, with a 50% lymphoid interstitial infiltrate; a slight and focal increase in reticulin was also observed. The neoplastic cells expressed CD45 and CD20, while only few cells were DBA 44 positive, at variance with the classical form of HCL.

A skin biopsy showed the presence of a rich lymphoid infiltrate prevalently located in the superficial and reticular derma (Fig. 2C); however, at high power, small intradermal groups of atypical lymphocytes with convolute or cerebroid nuclei, inconspicuous nucleoli, and pale cytoplasm were also observed (Fig. 2D). Phenotypic characterization demonstrated a peripheral T-cell phenotype (CD45, CD3, CD4, CD43, CD45RO positive; CD8, TIA1, Granzyme-B, Perforin, CD20, CD30 negative). As an example, specific staining for CD45RO is presented in Figures 2E and F.

Molecular Characterization

Southern blot analysis of the DNA from two blood samples collected at 3 month intervals (PBL-1 and PBL-2) disclosed superimposable patterns of IgH gene rearrangement, thus suggesting the presence of the same clonal B-cell subpopulation in both samples, while DNA from the skin biopsy was found in the germline configuration (Fig. 3A). The opposite result was obtained using the TCRβ specific probe because a rearranged band was detected only in the skin biopsy, while DNA from both blood samples presented the same germline pattern as the DNA of control K562 cells (Fig. 3B).

To better ascertain the persistence of the same clonal B-cell population in peripheral blood, tumor samples were analyzed by a semi-nested PCR approach. As shown in Fig. 4A, comigrating bands could be detected in both blood samples. Surprisingly, identical bands were amplified from skin DNA, suggesting the presence of rare clonal B cells, that could not be detected by Southern blot analysis; this latter finding could be due to the very high sensitivity of the PCR approach.

Finally, the presence of EBV specific sequences was analyzed by both Southern blot and PCR methods: using a terminal repeat specific probe, neither linear (replicative), nor episomal (latent) EBV genomes could be detected in any of the patient's samples (data not shown), while an amplified EBV type A EBNA 2A fragment was observed only in the skin (Fig. 4B).

DISCUSSION

Our patient presented with multiple, reddish, itchy plaques on an otherwise normal skin; no extracutaneous involvement was apparent in accordance with the diagnosis of primary cutaneous T-cell lymphoma (PTCL). The presence of sporadic T lymphocytes in the peripheral blood smear, together with the atypical hairy cells, was first suspected on morphological grounds and then confirmed by immune phenotyping. The low number of circulating neoplastic T cells (less than 1%) explains the absence of clonal TCRβ gene rearrangements in the peripheral blood cells. The indolent clinical behavior without lymph node involvement in the absence of any important treatment supports the diagnosis of PTCL, MF-like, in agreement with tumor histopathology and immune phenotype. However, a close follow-up of the circulating T-cell neoplastic component in this patient is mandatory in order to detect a potential systemic involvement as early as possible. We could document that peripheral lymphocytosis was present 6 years prior to our observation, thus suggesting that the B-cell neoplasm preceded the cutaneous T-cell lymphoma. Morphological and immune phenotypic patterns defined the B-cell proliferation as a variant of HCL; the absence of splenomegaly, the lack of a "dry tap" and the low expression of CD25 and DBA44 were consistent with this diagnosis.

The occurrence of both B- and T-cell lymphomas in one patient may merely be coincidental. Nevertheless, possible explanations for such a condition can be advanced, such as genetic predisposition for lymphoproliferative diseases, underlying viral infections, and possible mutagenic effects of cytostatic drugs. While oncogenic

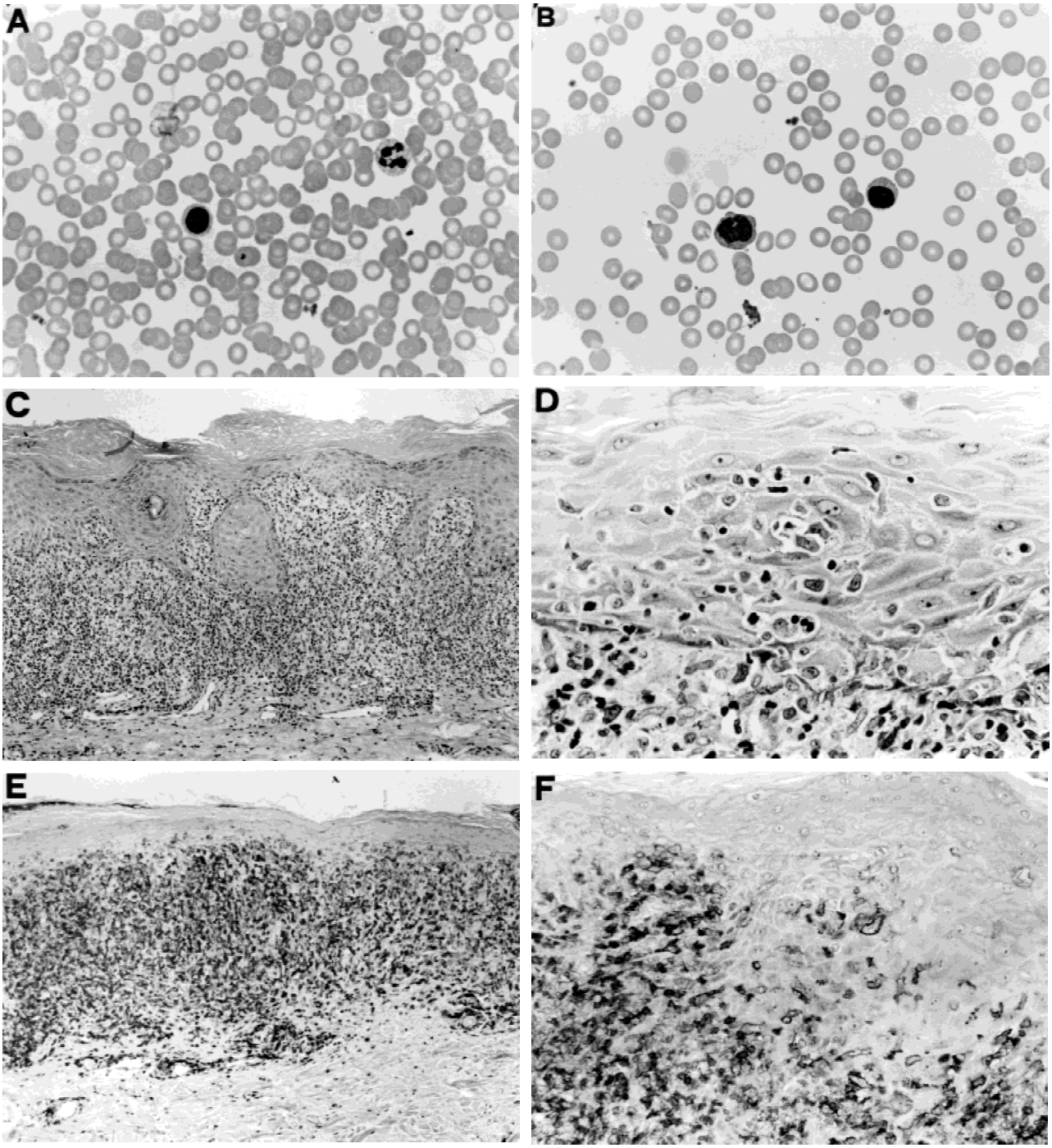

Fig. 2. Morphology and immune phenotype of tumor samples. (A) Peripheral blood. Hairy cell with small cytoplasmic projections and a neutrophilic granulocyte (May Grünwald-Giemsa, original magnification ×120). (B) Peripheral blood. Atypical, cerebroid cell (left) near a small mature lymphocyte (May Grünwald-Giemsa, original magnification ×120). (C) Panoramic view of skin biopsy with heavy dermal infiltration by lymphoid cells (H.E. original magnification ×20). (D) At higher power, the intradermal infiltrating atypical lymphoid cells resemble Pautrier's microabscesses (H.E. original magnification ×80). (E) Immunoperoxidase stain with CD45RO antibody. The majority of the dermal lymphoid cells are positive (ABC indirect method, original magnification ×20). (F) Immunoperoxidase stain with CD45RO antibody. Intraepithelial atypical lymphoid cells are also strongly positive (ABC indirect method, original magnification ×80).

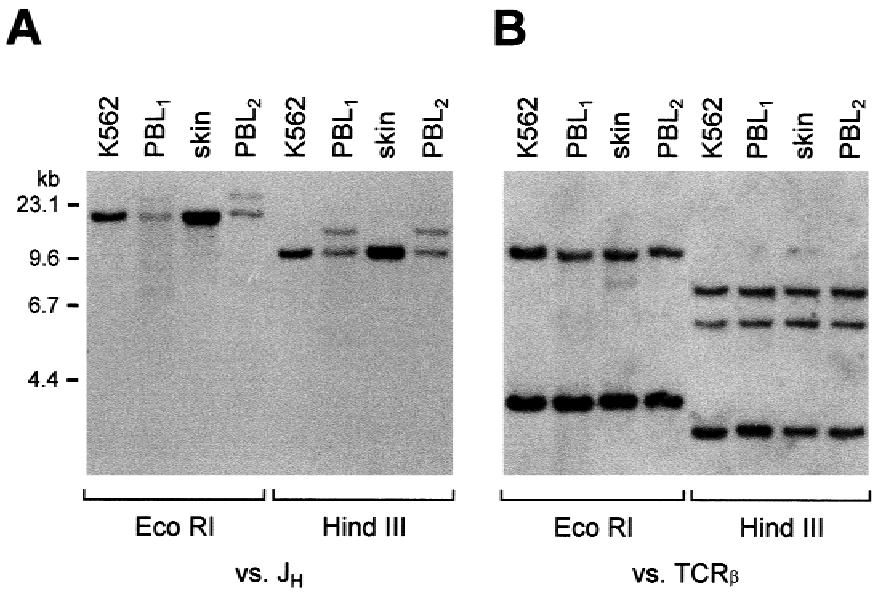

Fig. 3. Southern blot analysis of immune receptor gene rearrangements in tumor samples. High molecular weight DNA from two separate blood samples (PBL1 and PBL2) and a skin biopsy (skin) was digested with the indicated restriction enzymes, blotted, and hybridized with IgH (A) and TCRβ (B) specific probes. K562 cell line DNA served as germline control, while lambda/*Hin*dIII DNA fragments were used as molecular weight markers.

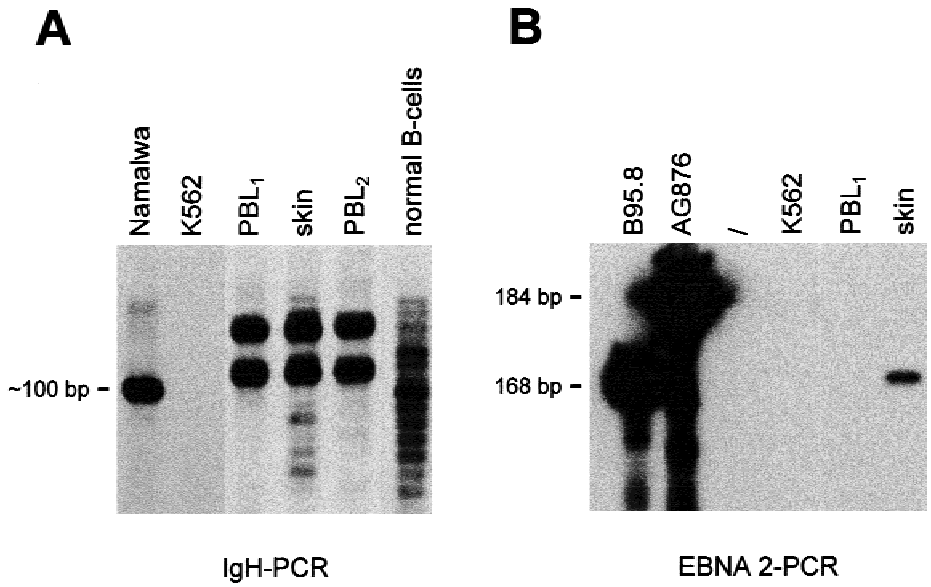

Fig. 4. PCR analysis of B-cell clonality and EBV infection in tumor samples. (A) Semi-nested PCR analysis of IgH gene rearrangements. An aliquot of the denatured amplified product was electrophoresed on a denaturing gel, and the dry gel was processed for autoradiography. Namalwa and K562 cell line DNA were used as positive and negative controls, respectively. DNA from normal peripheral blood cells was also run in parallel as a reference pattern for a polyclonal sample. (B) EBV-specific EBNA 2 PCR products were electrophoresed on an agarose gel, blotted, hybridized with a specific oligonucleotide, and processed for autoradiography. DNAs from B95.8 and AG876 cells were used as type A and B positive controls, respectively, while DNA from K562 cells served as negative control.

events related to the advanced age of the patient might have played a role, we found no evidence of HTLV-I/II and EBV infection in tumor cells; the EBV positive result in the skin sample obtained by PCR was not confirmed by either Southern blot analysis or in situ hybridization (data not shown), and is likely explained by the presence of latently EBV infected bystander B cells in the skin.

There is also evidence of the existence of a pluripotent stem cell that generates both mature B and T cells [8]. Indeed, composite lymphomas with multiple clones of different lineages coexisting in the same lesion [9], and cases of lymphomas containing a single clone with both immunoglobulin and T-cell receptor gene rearrangements [10] have been documented. In our case, however, no lineage infidelity or promiscuous rearrangements could be demonstrated by Southern blot analysis. A helper activity of neoplastic T lymphocytes, which could be specific for a B-cell clone, has been proposed as the triggering mechanism for B lymphoma development by workers who claim that T-cell neoplasms more often precede than follow B-cell lymphoma [2]. Nevertheless, in the majority of the reported cases [4], as well as in ours, the B-cell neoplasm preceded the T-cell lymphoma.

Small T-cell clones were identified in 3 of 13 patients with B-CLL [11]. Since the malignant B-cells of B-CLL are, as in HCL, well differentiated, they may secrete cytokines or other factors that could chronically stimulate T-cells, thus increasing the risk for the emergence of a T-cell clone. These clonal T-cells in turn could undergo additional genetic alterations, that might lead to malignant transformation with development of a T-cell lymphoma. Similar mechanisms could explain the exceptional development of a high grade T-cell lymphoma in patients with B-cell CLL [12], and the development of PTCL in this patient with HCL. An impaired T-cell functioning, namely a decrease number of CD4+ CD45RO+ cells, can occur in the course of HCL, and it might be related to the frequent occurrence of opportunistic infections in these patients [13,14]. Since this T-cell subset plays an important role in tumor invasion [15], its depletion could further explain the emergence of a second malignancy; interestingly, in our HCL patient, clonal T-cells express precisely this phenotype.

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REFERENCES

1. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PJ, Knowles DM, Mason DY, Muller-Hermelink H-K, Pileri SA, Piris MA, Ralfkiaer E, Warnke RA. A revised European-American classification of lymphoid neoplasms: a proposal from the international study group. Blood 1994;84:1361–1392.
2. Bryant E, Ronan SG, Iossifedes IA. Plasma cell myeloma in a patient with cutaneous T-cell lymphoma. Cancer 1982;50:2122–2125.
3. Sheibani K, Forman SJ, Winberg CD, Rappaport H. Coincidence of B-cell chronic lymphocytic leukemia and cutaneous T-cell lymphoma (mycosis fungoides): immunologic characterization by monoclonal antibodies. Blood 1983;62:1176–1181.
4. Grange F, Avril MF, Esteve E, Joly P, Bosq J, De Murets A, Thomine E, Ortoli J-C, Duvillard P, Vaillant L, Bagot M, Wechsler J, and the French Study Group on Cutaneous Lymphomas. Coexistent cutaneous T-cell lymphoma and B-cell malignancy. J Am Acad Dermatol 1994; 31:724–731.
5. Poletti A, Giacon C, Pennelli N. Simultaneous visualization of immunodetected antigens and tissue components revealed by non-enzymatic histochemical stains. J Histochem Cytochem 1992;40:1965–1970.
6. Strazzabosco M, Corneo B, Iemmolo RM, Menin C, Gerunda G, Bonaldi L, Merenda R, Neri D, Poletti A, Montagna M, Del Mistro A, Maffei-Faccioli A, D'Andrea E. Epstein–Barr virus-associated post-transplant lympho-proliferative disease of donor origin in liver transplant recipients. J Hepatol 1997;26:926–934.
7. Menin C, Ometto L, Veronesi A, Montagna M, Coppola V, Veronese ML, Indraccolo S, Bruni L, Corneo B, Amadori A, De Rossi A, Chieco-Bianchi L, D'Andrea E. Dominance of a single Epstein–Barr virus strain in SCID-mouse tumors induced by injection of peripheral blood mononuclear cells from healthy human donors. Virus Res 1995; 36:215–231.
8. Greaves MF, Chan LC, Furley AJW, Watt SM, Molgaard HV. Lineage promiscuity in hemopoietic differentiation and leukemia. Blood 1986; 67:1–11.
9. York JC, Cousar JB, Glick AD, Flexner JM, Stein R, Collins RD. Morphologic and immunologic evidence of composite B- and T-cell lymphomas: a report of three cases developing in follicular center cell lymphomas. Am J Clin Pathol 1985;84:35–43.
10. Biondi A, Di Celle PF, Rossi V, Casorati G, Matullo G, Giudici G, Foa R, Migone N. High prevalence of T-cell receptor Vd2-(D)-Dd3 or Dd 1/2-Dd3 rearrangements in B-precursor acute lymphoblastic leukemias. Blood 1990;75:1834–1840.
11. Wen T, Mellstedt H, Jondal M. Presence of clonal T-cell populations in chronic B lymphocytic leukemia and smoldering myeloma. J Exp Med 1990;171:659–666.
12. Lee A, Skelly ME, Kingma DW, Medeiros LJ. B-cell chronic lymphocytic leukemia followed by high grade T-cell lymphoma. Am J Clin Pathol 1995;103:348–352.
13. van der Host FAL, van der Marel A, den Ottolander GJ, Kluin-Nelemans HC. Decrease of memory T helper cells ($CD4^+$ $CD45RO^+$) in hairy cell leukemia. Leukemia 1993;7:46–50.
14. Raspadori D, Rondelli D, Birtolo S, Lenoci M, Nardi G, Scalia G, Sestigiani C, Tozzi M, Marotta G, Lauria F. Long-lasting decrease of $CD4^+$/$CD45RA^+$ T cells in HCL patients after 2-chlorodeoxyadenosine (2-CdA) treatment. Leukemia 1999;13:1254–1257.
15. Jacob MC, Favre M, Lemarc'Hadour F, Sotto MF, Bonnefoix T, Sotto JJ, Bensa JC. CD45RA expression by CD4 T lymphocytes in tumors invaded by B-cell non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD). Am J Hematol 1992;39:45–51.