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Case Study

T-Cell Prolymphocytic Leukemia, Small Cell Variant, Possibly at the Stage of Intracytoplasmic Expression of CD3 in T-Cell Ontogenesis

Yuriko Yoshioka,[1)] Miho Nagao,[1)] Toshiharu Saitoh,[1)] Satoshi Yoshioka,[2)] Hiroko Tsunemine,[2)] Kiminari Itoh,[1,2)] Taiichi Kodaka,[2)] and Takayuki Takahashi[2)]

T-cell prolymphocytic leukemia, small cell variant (T-PLL-s), is a rare lymphoid neoplasm associated with a poor prognosis. We encountered a case of T-PLL-s with a characteristic phenotype. A 67-year-old female was referred to our hospital because of lymphocytosis in August 2013. Hepatosplenomegaly, lymphadenopathy, and skin lesions were absent. Hematologic examination revealed a white blood cell count of 17.9 × 10^9/L with 81.2% mature lymphocytes, which were small with a high nuclear/cytoplasmic ratio, lacking a nucleolus and cytoplasmic granules. Anemia and thrombocytopenia were not observed. Flow cytometric analysis showed that these lymphocytes were positive for CD2, cyCD3, CD4, CD5, CD7, CD21, and CD38 (partially), but negative for smCD3, smTCR-$\alpha\beta$ and -$\gamma\delta$, cyTCR-β, CD1a, CD8, CD25, HLA-DR, and terminal deoxynucleotidyl transferase. Polymerase-chain reaction analysis of cells from both the peripheral blood and the bone marrow demonstrated monoclonal rearrangement of TCR-γ. A possible rearranged band of the TCR-β gene was observed by Southern blot analysis. The karyotype of the marrow cells was 46, XX. A diagnosis of T-PLL-s, possibly at the stage of cytoplasmic CD3 expression in the ontogenesis of T-cells, was made. The patient has been asymptomatic, and the white blood cell count has gradually increased during one-year observation, being 69.0 × 10^9/L with 89.7% lymphocytes in August 2014. 〔*J Clin Exp Hematop 55 (1) : 17-21, 2015*〕

Keywords: T-cell prolymphocytic leukemia, small cell variant, smCD3, cyCD3, smTCR

INTRODUCTION

T-cell prolymphocytic leukemia (T-PLL), small cell variant, had been designated as T-cell chronic lymphocytic leukemia (T-CLL) ;[1,2] however, it was classified as a subtype of T-PLL, that is, small variant of T-PLL (T-PLL-s), in the Classification of the World Health Organization, 2008.[1] T-PLL-s is very rare; T-PLL itself comprises 2% of mature adult lymphocytic leukemia, and 20% of this is the small cell variant.[2] T-PLL-s has been reported to share its phenotype, cytogenetic background, and clinical picture with T-PLL.[3] Morphologically, T-PLL cells are medium-sized with a high nuclear/cytoplasmic ratio and basophilic and agranular cytoplasm. The nucleus of T-PLL cells is round, elliptical, or irregular in shape, and contains a few conspicuous nucleoli regardless of its condensed chromatin network. On the other hand, the cells of T-PLL-s are small and the nucleolus is unremarkable.[1,3]

As for the surface expression of T-lineage-related antigen in T-PLL, 60% of them are solely CD4-positive, 25% are both CD4- and CD8-positive, and 15% are solely CD8-positive.[1,3,4] Furthermore, T-PLL cells are typically positive for CD2, CD3, CD5, and CD7, but negative for CD1a and terminal deoxynucleotidyl transferase. The subtype of the T-cell receptor (TCR) is mostly the $\alpha\beta$ type, while the $\gamma\delta$ type is rare. We encountered a patient with T-PLL-s, possibly at the stage of cytoplasmic CD3 expression in the ontogenesis of T-cells.

CASE REPORT

A 67-year-old asymptomatic female was referred to our hospital because of lymphocytosis in August 2013. In terms of her past history, she had undergone ocular decompression for glaucoma of the right eye, cholecystectomy, and treatment for a duodenal ulcer. Physical examination at presentation revealed a soft lymph node with a diameter of 7 mm at the left

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[1)]Departments of Cell Therapy and [2)]Hematology, Shinko Hospital, Kobe, Japan
Corresponding author: Dr. Takayuki Takahashi, Department of Hematology, Shinko Hospital, 4-47 Wakihama-cho, Chuo-ku, Kobe 651-0072, Japan
E-mail: takahashi.takayuki@shinkohp.or.jp

Table 1. Laboratory data of this case

Hematology		Chemistry		Serology	
White blood cell	17.9 × 10^9/L	Total protein	7.1 g/dL	HTLV-1	(−)
Neutrophil	14.0%	Albumin	4.7 g/dL	**Bone marrow**	
Eosinophil	1.6%	T-bilirubin	0.7 mg/dL	NCC	194.3 × 10^9/L
Basophil	0.4%	AST	20 IU/L	G/E ratio	2.23
Monocyte	2.4%	ALT	17 IU/L	Total-EBL	19.3%
Lymphocyte	81.2%	LDH	235 IU/L	Total-granul.	43.1%
Atyp. lym.	0.4%	ALP	179 IU/L	Lymphocyte	37.3%
Red blood cell	4,860 × 10^9/L	γ-GTP	25 IU/L	**Karyotype**	46, XY
Hemoglobin	14.2 g/dL	BUN	11.5 mg/dL		
Platelet	15.1 × 10^9/L	Creatinine	0.57 mg/dL		
		CRP	0.0 mg/dL		

Atyp. lym., atypical lymphocytes; AST, aspartate aminotransferase; ALT, alanine transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; CRP, C-reactive protein; CK, HTLV-1, serum antibody for T-lymphotropic virus type-1; NCC, nucleated cell count; G/E ratio, granulocytic/erythroid ratio, Ebl, erythroblasts; granul., granulocytes

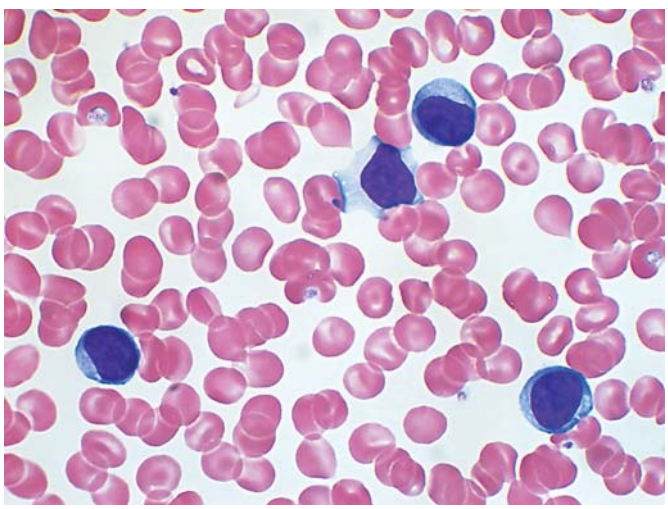

Fig. 1. Circulating abnormal lymphocytes. They are small with a high nuclear/cytoplasmic ratio, lacking a nucleolus and cytoplasmic granules. Wright-Giemsa staining, ×1,000.

axilla. Hepatosplenomegaly and skin lesions were absent.

The results of laboratory examinations are shown in Table 1. Hematologic examination revealed a white blood cell count of 17.9 × 10^9/L with 81.2% mature lymphocytes, which were small with a high nuclear/cytoplasmic ratio, lacking a nucleolus and cytoplasmic granules (Fig. 1), a hemoglobin concentration of 14.2 g/dL, and a platelet count of 151 × 10^9 /L. Biochemical and serological examinations showed that the serum concentration of C-reactive protein was within normal limits (normally below 0.3 mg/dL), while that of lactate dehydrogenase was slightly elevated to 235 IU/L (normally, 150 to 230 IU/L). A serologic test for human T-lymphotropic virus type-1 yielded a negative result. Serological analysis of Epstein-Barr virus (EBV) revealed past EBV infection.

Flow cytometric analysis of the circulating lymphocytes showed that these cells were positive for CD2, cyCD3, CD4, CD5, CD7, CD21, and CD38 (partially), but negative for smCD3, smTCR-αβ and -γδ, CD1a, CD8, CD25, HLA-DR, and terminal deoxynucleotidyl transferase (Fig. 2). To confirm the negativity of smCD3, we stained the circulating lymphocytes of the patient with mouse IgG1 as a negative control (Fig. 3A). We then showed the pattern of CD3-staining of the patient's lymphocytes (Fig. 3D) and mouse IgG1 staining (Fig. 3C) as histograms. These histograms clearly showed the negativity of smCD3 of these lymphocytes. In addition, we attempted to detect cyTCR-β protein in these leukemic cells using a monoclonal antibody that had been engineered to detect cyTCR-β protein (GeneTex, Los Angeles, USA), with a negative result. Bone marrow aspirate showed many similar lymphocytes (37.3% of marrow cells), which were phenotypically identical to those in the peripheral blood. Polymerase-chain reaction (PCR) analysis of cells from both the peripheral blood and the bone marrow demonstrated the monoclonal rearrangement of TCR-γ but not that of the immunoglobulin heavy chain. To examine the rearrangement of TCR genes further, we performed Southern blot analysis of TCR-β and TCR-δ genes. As shown in Fig. 4, a single rearranged band was detected in the analysis of the TCR-β chain Jβ1 region, but not in the TCR-β chain Jβ2 and Cβ regions, and the TCR-δ chain Jδ1 region (data not shown). However, the rearranged band in the analysis of the TCR-β chain Jβ1 region was detected only in *Hind* III digestion but not in *Eco*R I and *Bam*H I digestions. Therefore, the nonspecific nature of this band cannot be ruled out. Chromosomal analysis of the marrow cells showed a normal karyotype of 46, XX. From these results, a diagnosis of T-PLL-s was made.

Computed tomography scanning of the chest and abdomen showed mild splenomegaly but not lymphadenopathy.

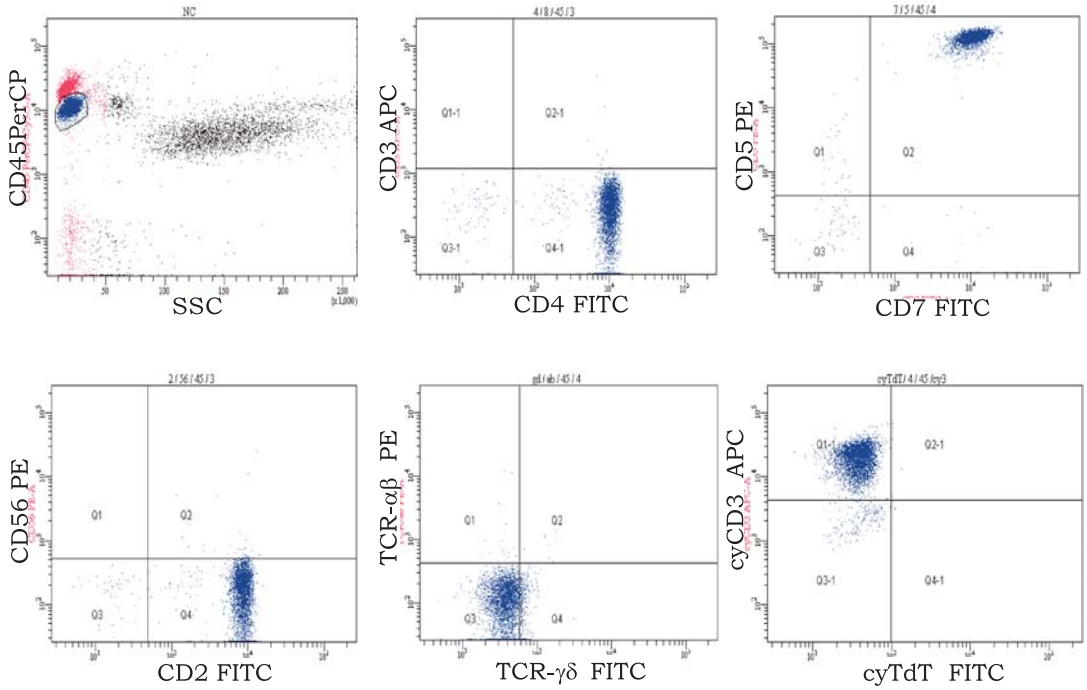

Fig. 2. Flow cytometric analysis of the circulating abnormal lymphocytes. Low CD45-gated analysis demonstrates that they are positive for CD2, CD4, CD5, CD7, and cyCD3 but negative for smCD3, CD56, T-cell receptor (TCR)-γδ, TCR-αβ, and cyTdT. cyTdT, cytoplasmic terminal deoxynucleotidyl transferase.

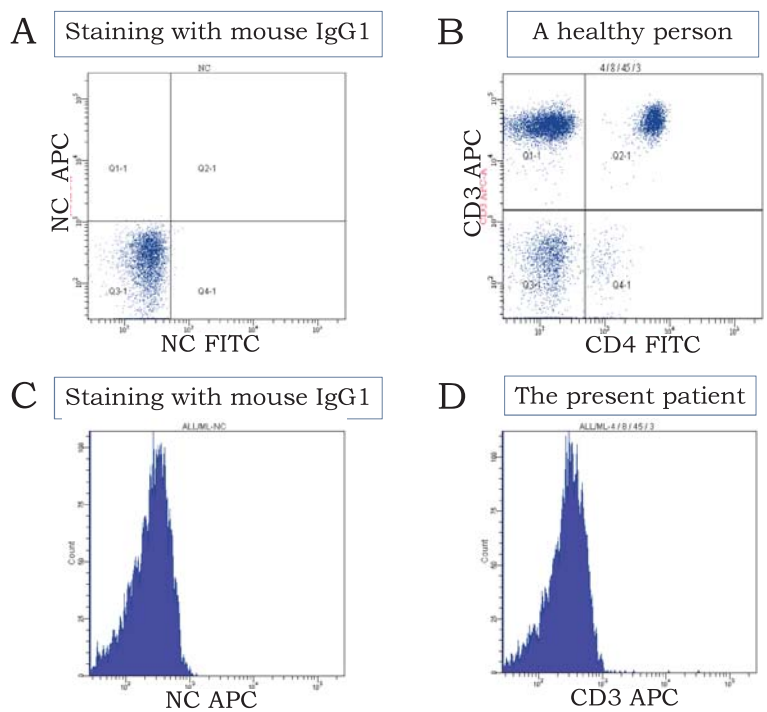

Fig. 3. Confirmation of smCD3 negativity of leukemic cells in the present patient. Staining of the leukemic cells with mouse IgG1 (negative control) (*3A*), confirmation of CD3 and CD4 staining using circulating mononuclear cells from a healthy person (*3B*), demonstration of *3A* staining as a histogram (*3C*), and demonstration of smCD3 staining of the patient's leukemic cells (Fig. 2, upper panel) as a histogram (*3D*).

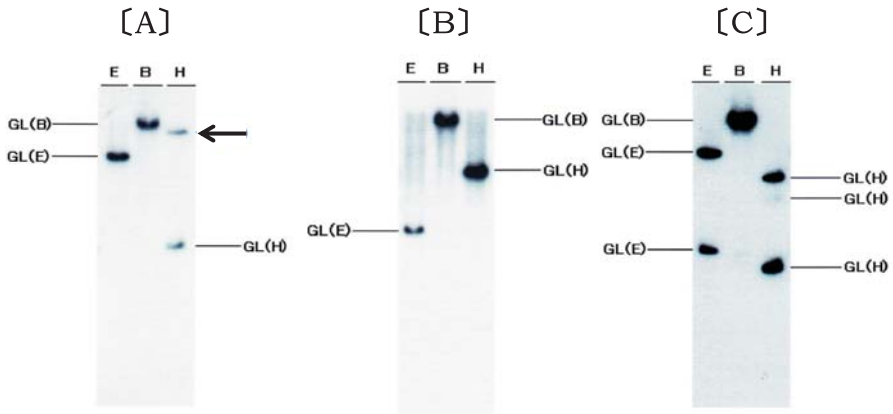

Fig. 4. Southern blot analysis of the patient's leukemic cells regarding T-cell receptor (TCR) gene rearrangement (performed by LSI Medience Corporation, Tokyo, Japan). (***4A***) Analysis of the TCR-*β* chain Jβ1 region. A single rearranged band is seen in *Hind* III digestion (*arrow*). (***4B***) Analysis of the TCR-*β* chain Jβ2 region with no rearranged band. (***4C***) Analysis of the TCR-*β* chain Cβ region with no rearranged band.

Although the pattern of serum antibodies for EBV showed past infection, abnormal lymphocytes in the present patient expressed CD21; therefore, we performed multiplex virus PCR analysis, which is available at the Department of Cell Therapy, Shinko Hospital.[5] PCR analysis of whole blood revealed low-level EBV viremia of 5.0×10^2 copies/mL, but the absence of EBV in the plasma.

The patient has been asymptomatic since the first visit to our hospital in August 2013. The white blood cell count, however, gradually increased to as high as 69.5×10^9/L in August 2014, without anemia, hepatosplenomegaly, lymphadenopathy, or skin lesions, although the platelet count decreased to 70×10^9/L. We are conducting allogeneic hematopoietic stem cell transplantation after appropriate chemotherapy.

DISCUSSION

The majority of T-PLL patients show progressive disease, which is associated with a poor prognosis and a median survival of less than one year,[1] while a small number of patients are asymptomatic with a slow-growing disease. Currently, the present patient may belong to the latter. Chemotherapies for T-PLL include purine analogs such as fludarabine or pentostatin; however, the response of T-PLL cells to these chemotherapeutic agents is generally poor.[1,3] Currently, immunotherapy with alemtuzumab (monoclonal antibody against CD52 antigen) followed by allogeneic hematopoietic stem cell transplantation appears to be the optimal therapeutic strategy.[6]

In the present patient, abnormal lymphocytes expressed CD2, CD4, CD5, and cyCD3, but not smCD3, smTCR-*αβ*, or smTCR-*γδ*, as T-lineage-related antigens. This phenotype may correspond to pre-T-cells at the thymus stage in the ontogenesis of the T-cell lineage. In addition, about 20% of T-PLL cases lack smCD3 and, instead, express cyCD3.[7] However, it has been reported that neoplastic cells from adult T-cell leukemia express cyCD3 when the expression of smCD3 is diminished due to its excess activation.[3] The single-positive T-cell stage of leukemic cells in the present patient, therefore, cannot be ruled out because these leukemic cells expressed CD4 but not CD8. As for the reduced expression of smCD3, CD3 and TCR are usually present on the cell surface, forming a complex together;[7,8] therefore, it may be likely that cyTCR is expressed when the expression of smCD3 is diminished. From this point of view, either cyTCR-*αβ* or cyTCR-*γδ* may be expressed in leukemic cells in the present patient, as shown by a possible rearranged band in the analysis of the TCR-*β* chain Jβ1 region as examined by Southern blotting (Fig. 4). To verify this possibility, we attempted to detect cyTCR-*β* protein in these leukemic cells, obtaining a negative result. In addition, no antibody that is able to detect cyTCR-*δ* protein is currently available. Regarding CD7 expression, T-PLL cells show characteristically intense CD7 expression, as observed in the present patient, while its expression is weak or absent in adult T-cell leukemia, T-cell large granular leukemia, or other neoplasms such as Sezary syndrome.[3]

In T-PLL, chromosomal abnormality involving the No. 14 chromosome is observed in about 75% of cases. Recurrent chromosomal abnormalities include inv14q(q11:q32) and t(14;14)(q11;q32), and, less frequently, t(X;14)(q28;q11). The leukemic cells in the present patient, however, showed a normal 46, XX karyotype.

In conclusion, we have reported a case of characteristic T-PLL-s, possibly at the stage of intracytoplasmic expression of CD3 in T-cell ontogenesis. The accumulation of cases with a similar phenotype will clarify the clinical, phenotypic, and molecular picture of such T-PLL-s.

DISCLOSURE

The authors declare that there are no conflicts of interest with any individuals or companies.

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