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Extramedullary T lymphoid blast crisis representing an additional translocation, t(6;8)(q25;q22) in a patient with Ph-positive CML after allogeneic bone marrow transplantation.

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Key words: chronic myelogenous leukemia, T-lymphoblastic crisis, extramedullary crisis, chromosome translocation, bone marrow transplantation.

Abstract A patient with extramedullary crisis from CML after allogeneic BMT is reported. A pathological neck lymph node observed after transplantation revealed pre-T lymphoblastic phenotype, and the FISH analysis showed recipient type sex chromosomes and bcr/abl fusion gene. The cells represented an additional translocation, t(6;8)(q25;q22). No rearrangements of the TCR β, γ or δ chain genes were observed. The absence of TCR rearrangement indicated the clonogenic involvement of pluripotent hematopoietic stem cells by Ph chromosome. Bone marrow specimens at that time showed donor type sex chromosomes and no bcr/abl-positive cells by FISH.

List of abbreviations: CML, chronic myelogenous leukemia; CP, chronic phase; BC, blast crisis; Ph, Philadelphia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; BMT, bone marrow transplantation; EBV, Epstein-Barr virus; TCR, T cell receptor; TdT, terminal deoxynucleotidyl transferase; FISH, fluorescence in situ hybridization; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

Introduction

CML is a clonal myeloproliferative disease characterized by the proliferation and accumulation of myeloid lineage cells in the bone marrow and the peripheral blood. The disease has a triphasic clinical course, consisting of chronic, accelerated and blastic phases. The blastic phase morphology in CML resembles acute leukemia, and lymphoid crisis is observed in approximately 30% of these patients. Most cases of lymphoid crisis show a B cell immunophenotype, and only scattered cases of T lineage crisis have been reported. Studies of such rare cases with T lymphoid crisis may help clarify the clonal involvement of hematopoietic precursors by Ph chromosome, in addition to the sequential differentiation and hierarchy of pluripotent hematopoietic stem cells.

One patient with CML showed T lineage crisis after allogeneic bone marrow transplantation. We intensively studied the characteristics of the blasts using immunological and biological examinations.

Materials and Methods

Clinical features of the patient

A 45-yr-old man was admitted for leukocytosis (150.0 x10^3 /μl) in June, 1996. Karyotype analysis was performed on the bone marrow which showed a simple Ph abnormality: 46, XY, t(9;22)(q34;q11). He was diagnosed with CML in the chronic phase. He was treated daily with oral hydroxyurea (1,500 mg/day) and a hypodermic injection of natural interferon-alpha (αIFN, 3 x 10^6 U/day).

He was admitted again for lymph node swelling in the left neck in October, 1999. A lymph node biopsy revealed diffuse lymphoma of medium to large cell type. The specimen showed $MT1^+$, $UCHL1^-$, $CD3^+$ (possibly cytoplasmic CD3) and $CD20^-$ T-lymphoblasts by immunohistochemistry. The karyotype analysis of the specimen showed a simple Ph abnormality in all 4 metaphases studied. He was treated with 5 courses of conventional CHOP (intravenous cyclophosphamide, adriamycin, vincristine and

oral prednisolone), followed by 30 Gy of radiotherapy. The neck lymph node swelling disappeared after radiotherapy.

In June, 2000, he received a BMT from an unrelated male donor who had a congenital abnormal karyotype: 47, XY, +Y. The conditioning treatment for the BMT included intravenous cyclophosphamide (60/kg, x2 days), oral busulfan (1 mg/kg, x16 times) and 6.0 Gy of total body irradiation. The bone marrow hematopoiesis recovered within one month after the transplantation, which showed the donor karyotype in all 20 metaphases studied.

Lymph node swelling appeared again in the right neck at day 80 after the transplantation. The whole body computed tomograph showed no other tumors. The bone marrow specimen, at that time, showed normal features and the donor karyotype: 47, XYY in all 20 metaphases studied. A biopsy of the right neck lymph node was performed, and the sample was morphologically, immunologically, and biologically studied as follows. He was treated with 60 Gy of radiotherapy, and the lymph node swelling disappeared.

Lymph node swelling appeared in the right axilla in December, 2000. The whole pathological lymph node in right axilla was surgically removed. He was then treated with further radiotherapy on the right axilla and hypodermic injections of αIFN in February, 2001. The bone marrow specimens at each time of nodal relapses showed normal features.

Monoclonal antibodies

FITC-conjugated anti-LeuM1 (CD15), CALLA (CD10), anti-HLe1 (CD45), Leu4 (CD3), Leu5b (CD2), Leu9 (CD7), Leu1 (CD5), Leu7 (CD57), Leu3a (CD4), TCR-α/β, TCR-γ/δ, control IgG1, and PE-conjugated anti-HPCA2 (CD34), LeuM9 (CD33), IL2R (CD25), Leu2a (CD8), Leu14 (CD22), Leu12 (CD19), LeuM3 (CD14), Leu16 (CD20), Leu19 (CD56), Leu20 (CD23), Leu15 (CD11b), Leu54 (CD54), HLA-DR, control IgG1 were purchased from Becton Dickinson Immunocytometry Systems, Mountain View, CA. FITC-conjugated anti-MCS2 (CD13), c-kit (CD117) and Mik-β1 (CD122) were purchased from Nichirei, Tokyo, Japan. FITC-conjugated anti-CD36 (FA6.152), CD38 (T16), CD41 (P2), FMC7, and PE-conjugated anti-Glycophorin A (KC16), CD24, CD16 were purchased from Immunotech, Marseille, France. FITC-conjugated anti-CD1 was purchased from DAKO, Denmark. PE-conjugated anti-CD21 (B2) was purchased from Coulter Immunology, Hialear, FL. FITC-conjugated anti-CD40, CD70, and PE-conjugated anti-CD27, CD154 were purchased from Pharmingen, San Diego, CA. PE-conjugated anti-AC133 was purchased from Miltenyi Biotec, Bergisch Gladbach, Germany. FITC-conjugated anti-MPO, control IgG1, PE-conjugated anti-cytoplasmic-CD3, cytoplasmic-CD22 and control IgG1 were purchased from Caltag Laboratories, Burlingame, CA.

Flow cytometry

For phenotype analysis, a single cell suspension was obtained from a pathological lymph node of the patient by mincing using Medimachine™ (DAKO), and aliquots of the sample were stained simultaneously with FITC- and PE-conjugated monoclonal antibodies. For cytoplasmic marker analysis, aliquots of the sample were stained with antibodies against cytoplasmic antigens and Fix & Perm™ cell permeabilization kits (Caltag). Cell fluorescence was analyzed with a FACScan™ Flow Cytometer using CELLQuest™ software (Becton Dickinson).

Southern blotting analysis

The rearrangements of the TCR β, γ and δ-chain genes, the immunoglobulin heavy chain gene, bcr gene, and the integrated EBV TR-region gene were evaluated according to the standard method. In brief, 5 μg of DNA from the sample was extracted according to the standard methods, digested with *Ech*RI, *Bam*HI, *Hind*III, *Kpn*I, *Bgl*II, or *Bam*HI and *Hind*III, electrophoresed through a 0.6% agarose gel and transferred onto a nylon membrane filter. The filter was hybridized with a ^{32}P-labeled TCR/Cβ1 and Cβ2 probes, TCR/Jγ1.3 and Jγ2.3 probes, TCR/Jδ1 probe, Ig/JH probe, bcr 3'-end's probe, bcr 5'-end's probe, or EBV/TR-region probe, and washed under appropriate stringency conditions, then bands were visualized by autoradiography.

FISH

The lymph node and bone marrow samples were also analyzed by means of FISH designed for detection of X- and Y-chromosomes or bcr/abl fusion gene. The Translocation DNA Probe kits were purchased from Vysis, Dawners Grove, IL. Cells were placed on the slide in the same manner as that for chromosomal analysis. The denatured slide was hybridized with the probe labeled with

SpectrumOrange™ (DYZ3 sequence of Y-chromosome or abl gene) or SpectrumGreen™ (DXZ1 sequence of X-chromosome or bcr gene) in the Translocation DNA Probe kits. Following hybridization, the slide was washed and counter stained with DAPI. Signal numbers were counted in 1,000 cells.

Results

Morphology and karyotype

The biopsy specimen of the right neck lymph node showed extensive infiltration of small to medium-sized lymphoid cells with a starry sky background and a low mitotic rate (Fig. 1A). The cells had scanty cytoplasm and obvious nucleoli (Fig. 1B). Some of the cells presented TdT stained by a mouse monoclonal antibody (Fig. 1C).

The karyotype analysis of the lymph node cells showed a complex abnormality: 46, XY, t(6;8)(q25;q22), der(9)del(9)(p22)t(9;22)(q34;q11), der(22)t(9;22) in all 20 metaphases studied (Fig. 2). The karyotype analysis of the bone marrow, at that time, showed donor karyotype: 47, XY, +Y in all 20 metaphases studied (data not shown).

Phenotype of the lymph node cells

The surface and cytoplasmic phenotypes are shown in Fig. 3. The surface phenotype of the cells was found to be CD2, CD5, CD7, CD38, CD45-positive, CD8, CD11b, CD33, CD34, CD54, HLA-DR-partially positive, and CD1, CD3, CD4, TCRαβ, TCRγδ, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD13, CD14, CD15, CD36, CD41, GPA, CD16, CD56, CD57, CD122, CD27, CD40, CD70, CD154, FMC7-negative. The cells also expressed cytoplasmic CD3ε and did not expressed myeloperoxidase or cytoplasmic CD22.

Southern blotting and FISH analysis

The sample obtained from the pathological lymph node did not show rearranged bands for JH, TCR/Cβ, TCR/Jγ or TCR/Jδ1 genes (Fig. 4). The sample showed rearranged fusion bands for bcr/abl by both 3'-end's and 5'-end's bcr probes. No EBV-DNA was detected by means of Southern blotting analysis.

The incidence of 46, XY (recipient) and 47, XYY (donor) cells by FISH analysis of the lymph node sample was 97.5% and 2.5%, and bone marrow sample was 5.4% and 94.6%, respectively. The incidence of bcr/abl-positive and negative cells in the lymph node sample was 97.3% and 2.7%, and bone marrow 5.9% and 94.1%, respectively (Fig. 1). False positive rate of bcr/abl signal in normal human blood lymphocytes was 2.8 $\pm$ 1.3 % (n=20).

Discussion

A patient with T cell lineage lymphoid crisis from CML (T-BC) is reported. Most cases with extramedullary lymphoid blast crisis of CML have been reportedly treated with chemotherapy even when the bone marrow was free from crisis, and the tumors in those patients were resistant to chemotherapy which resulted in death of acute transformation. The case reported here was the first treated with chemotherapy, and the tumor was shown to be resistant. Then the tumor disappeared after radiotherapy before the BMT. The relapsed T lymphoblastic tumor on the opposite side of his neck after BMT was also treated by radiotherapy, and the treatment was successful. Therefore, it is possible that the extramedullary blast cell tumor, which appears as an acute transformation of CML, is sensitive to radiotherapy, not to chemotherapy in some cases, when the bone marrow is free from the crisis.

The lymph node cells of this patient showed Ph chromosome and an additional translocation, t(6;8)(q25;q22). There have been no reports of hematopoietic malignancy representing this karyotype of translocation. No noteworthy genes have been reported closely located at these breakpoints on chromosomes 6 and 8.

The patient was transplanted with bone marrow from a donor who had a unique genetic marker of sex chromosomes. Therefore, the relapsed disease of CML and a secondary disease after bone marrow transplantation arose from the residual recipient's clones and transplanted donor's clones were clearly recognized by utilizing these two genetic markers, the bcr/abl and sex chromosomes.

There have been approximately 30 cases with CML in T-BC reported [1 – 28], and approximately half were intensively studied for immunophenotype and the molecular events of TCR genes. The characteristics of these pathological T lymphoblasts appeared to be separated into 2 groups, i.e. surface CD3-negative, cytoplasmic CD3-positive early thymocytes and surface CD3-positive common thymocytes. The primary involved tissues by the transformed blasts were mostly superficial lymph nodes [23], although bone marrow was involved in some

cases. Hence, the blastic transformation of the Ph-positive pluripotent precursors to T lymphoblasts occurred extramedullary in such cases. T cell type LBL, another type of hematopoietic malignancy of thymocytes, generally expresses TCRαβ [29]. However, TCRγδ was more frequently expressed than TCRαβ in T cell type ALL [29]. In contrast, more than half of all cases with T-BC did not show TCR rearrangement [23 , 29], and the rest of such cases showed a rearranged TCRδ or TCRβ gene. The reason for the difference in TCR genotypes in the 3 groups of thymocyte malignancies, T-BC, T-LBL and T-ALL, is not yet known. A reasonable suggestion to explain this difference may be that the transformation of the 3 diseases arises during different stages of T lineage maturation, i.e. T-BC in pluripotent stem cells, T-ALL in bone marrow T lymphoid precursors, and T-LBL in intrathymic T lymphocytes according to their TCR gene maturation.

T-ALL and T-LBL often involve the thymus as a reflection of the homing nature of their normal counterparts. In contrast, the transformation of T-BC occurs in superficial lymph nodes [23, 29], although the reason is also not known. Bone marrow samples show the CP in most cases with T-BC, therefore, a clone of the Ph-positive pluripotent stem cells in such cases were transformed as a result of the unknown environmental conditions in a lymph node that had allowed growth of the blasts, whereas similar conditions were not present in the marrow.

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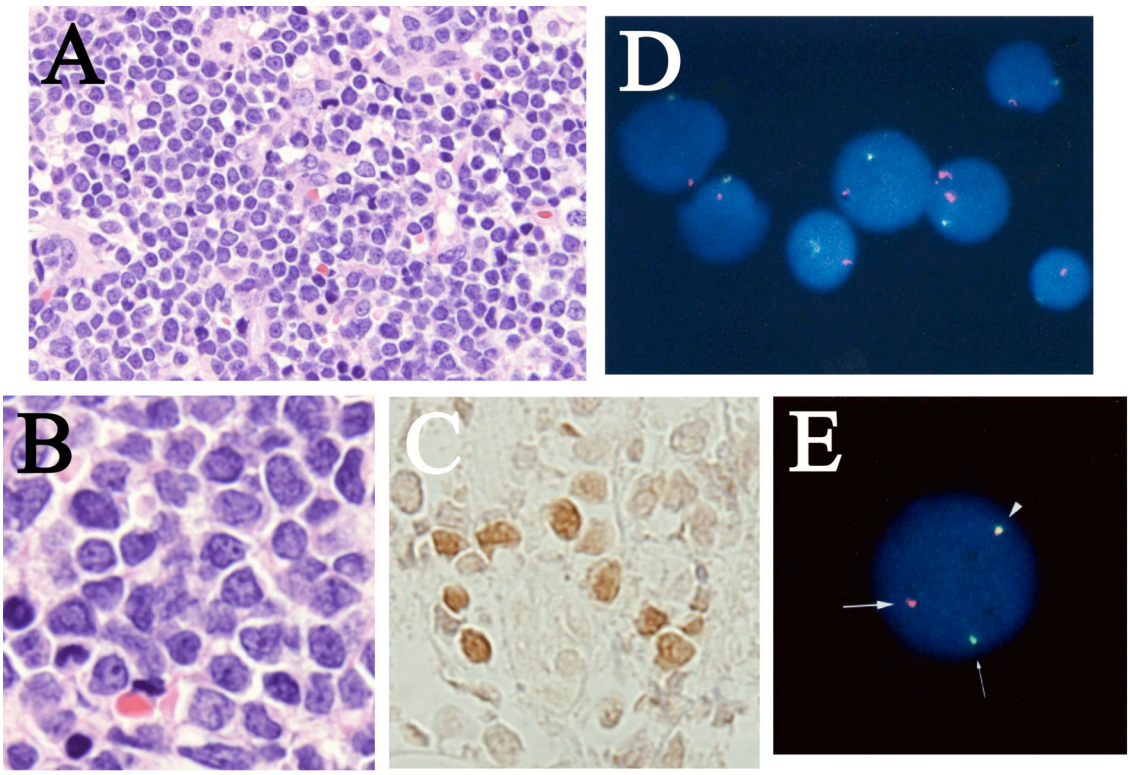

Fig. 1 Histology and FISH analysis of the pathological lymph node. Panels A and B, HE stain (Original magnification x400 and x1,000, respectively). There are small to medium sized lymphoid cells with scanty cytoplasm and clearly visible nucleoli. Panel C, TdT (horseradish peroxidase, x1,000). Panel D, an image of FISH (X- and Y-chromosomes): green spot, X-chromosome; red spot, Y-chromosome; the patient, 46, XY; the donor for bone marrow transplantation, 47, XYY. Panel E, an image of FISH (bcr/abl fusion gene): green spot, bcr (22q11); red spot, abl (9q34); yellow spot, bcr/abl fusion gene.

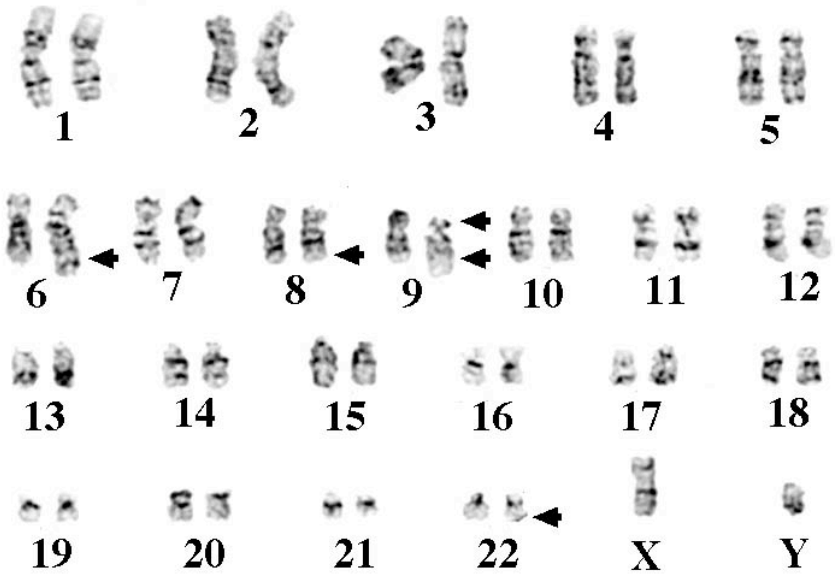

Fig 2. Karyotype of the lymph node. The karyotype analysis showed 46, XY, t(6;8)(q25;q22), der(9)del(9)(p22)t(9;22)(q34;q11), der(22)t(9;22) in all 20 metaphases studied. The arrows indicate breakpoints of the chromosomes.

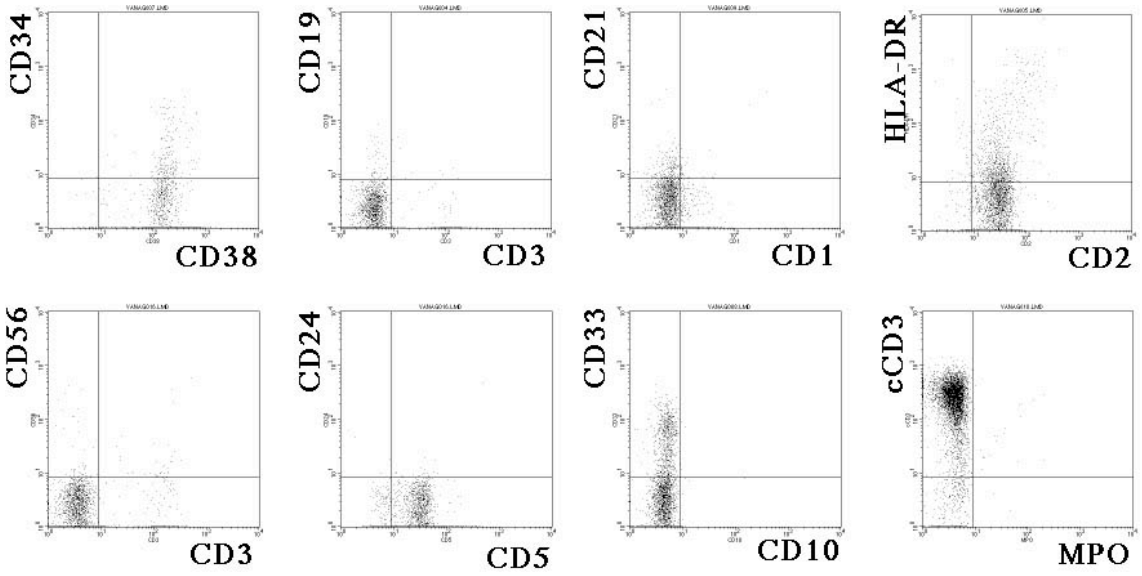

Fig 3. Phenotype analysis. Single cell suspension was obtained from the pathological lymph node of the patient, and simultaneously stained with FITC- and PE-conjugated monoclonal antibodies. Horizontal and vertical axis, FITC and PE fluorescence of the cells, respectively. MPO, myeloperoxidase; cCD3, cytoplasmic CD3ε.

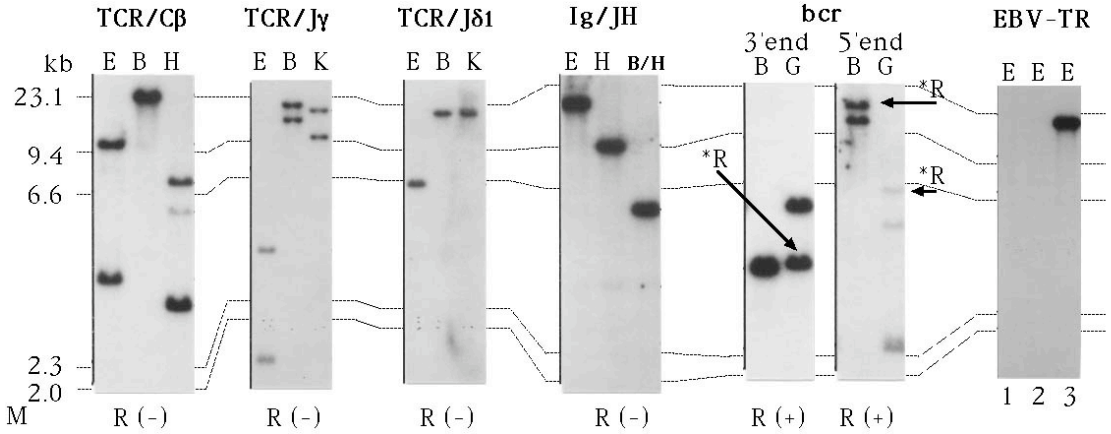

Fig 4. Southern blot analysis for the genes JH, TCR/Cβ, TCR/Jγ, TCR/Jδ1, bcr and TR-region of Epstein-Barr virus. E, B, H, K and G, digestion using *Eco* RI, *Bam* HI, *Hind* III, *Kpn* I and *Bgl* II, respectively. B/H, digestion using *Bam* HI and *Hind* III. *R, rearranged bands, otherwise germline bands. M, λ phage DNA *Hind* III digestion marker. Lanes 1, 2 and 3, normal controls, the pathological lymph node of the patient, and positive controls (Raji cell), respectively. Rearranged bands were not observed for JH, TCR/Cβ, TCR/Jγ, or TCR/Jδ1 genes. Rearranged fusion bands of bcr/abl were observed by both 3'- and 5'-end's bcr probes. No EBV-DNA was detected in the sample.