

## Case Report

# Splenic gamma/delta T-cell Lymphoma Report of a Case

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### Abstract

We described a case of splenic CD4 positive  $\gamma/\delta$  T-cell lymphoma with bone marrow involvement, and leukemic change in the terminal stage. A 23-year-old male had high fever and hepatosplenomegaly with pancytopenia and histiocytic hemophagocytosis. In order to make an accurate diagnosis and to improve symptom, splenectomy was performed. Histological section of the spleen showed marked congestion and diffuse proliferation of atypical lymphocytes having oval nuclei with or without nuclear indentation in the red pulp, indicating diffuse, large cell lymphoma.

Immunohistochemically, the atypical cells were positive for CD2, CD3, CD4, CD15 and CD25. The lymphoma cells were also positive for  $\delta$  TCS1 and negative for TCR  $\beta$  F1. Despite the chemotherapy, his lymphoma did not improve and developed into leukemic change in the critical state. When his bone marrow was largely involved by blastic cells about 90%, phenotype examination by flow cytometry revealed CD3-positive and TCR  $\gamma/\delta$ -1-positive, but CD 4-negative.

This case is thought to be an unusual splenic  $\gamma/\delta$  T-cell lymphoma with onset of histiocytic hemophagocytosis and CD4 expression in the initial stage.

There are two forms of the T-cell receptor; TCR  $\alpha/\beta$  and TCR  $\gamma/\delta$ . The former is expressed on the majority of peripheral T-cells, whereas the latter is present on the minority, reported about 9% of the peripheral blood lymphocytes<sup>1)</sup>. These TCR  $\gamma/\delta$  positive lymphocytes in the peripheral blood are small to medium-sized cells in normal donors. The spleen is

the organ with highest population of TCR  $\gamma/\delta$  lymphocytes with preferential location in the sinusoids of red pulp while TCR  $\alpha/\beta$  lymphocytes occupies the periarteriolar sheaths of penicillary arteries. The marker expression of peripheral  $\gamma/\delta$  lymphocytes are usually double negative, but there are only a few single positive lymphocytes for CD4 or CD8.

A similar distribution has been demonstrated in patients with T-cell lymphoma (T-ML); i.e., most peripheral T-ML cells express TCR  $\alpha/\beta$ , whereas TCR  $\gamma/\delta$  expression is very rare. Since the first report by Farcet et al<sup>2)</sup> on 1990, only a few more than ten cases with  $\gamma/\delta$  T-ML have been reported. The majority of these cases shows hepatosplenic and bone marrow involvement with sinusal/sinusoidal pattern by infiltration of medium sized abnormal lymphocytes without azurophilic granules, male predominance, marker expression of CD3,  $\delta$  TCS1, TCR  $\delta$  1, but negative for CD4,5,8, and TCR  $\beta$  F1<sup>2-6)</sup>. These tumor cells were also characterized by the genotypic expression of monoclonal rearrangement of TCR  $\gamma$  and  $\delta$ . We describe an unusual case of T-ML with TCR  $\gamma/\delta$  expression arising in the spleen.

**Key words:** Splenic lymphoma, T-cell lymphoma, gamma-delta T-cell receptor

### Case report

A 23-year-old man was admitted to a hospital on December 14, 1992, with a high fever and headache. He had been well until 7 days before admission, when he developed some common cold symptoms. Physical examination showed severe hepatosplenomegaly. Neither lymphadenopathy nor skin rash was observed. Peripheral blood examination revealed pancytopenia, and bone marrow examination showed histiocytic hemophagocytosis and a few atypical lymphoid cells.

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On June 7, 1993, a diagnostic and therapeutic splenectomy was performed. Under the diagnosis of splenic lymphoma by the pathological examination of the spleen, chemotherapy regimen of MACOP-B was applied, and his symptoms were reduced for a short period, though bone marrow involvement of abnormal lymphoid cells was mildly observed. On October, 1993, he had persistent high fevers, increase in liver size and mild liver dysfunction, and progressive pancytopenia. Since January 1994, marked increase of abnormal lymphoid cells had been observed in the peripheral blood and bone marrow. On March 1994, many large blastic cells were observed in the bone marrow and peripheral blood. The patient died on June 29, 1994, about 19 months after the onset of pancytopenia and hemophagocytosis. An autopsy was not granted.

### Materials and Methods

The splenectomy specimen was fixed in 20% phosphate buffered formalin before conventional tissue processing for paraffin embedding. A part of spleen was stored  $-80^{\circ}\text{C}$  until required for immunohistochemical studies.

Immunohistochemical examination of cryostat sections from the spleen was carried out using alkaline phosphatase-labeled avidin technique. The monoclonal antibodies used in this study were: CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD13, CD14, CD15, CD19, CD20, CD21, CD25, CD30, CD56, CD68, HLA-DR, TCR  $\beta$  F1 and  $\delta$  TCSI.

Eight bone marrow biopsy examinations were performed during the course.

### Results

#### *Cytological features*

Light microscopic examination of the bone marrow aspirate specimens revealed a proliferation of abnormal lymphoid cells from the initial biopsy to the later stage. The cells in the early stage were relatively uniform-sized abnormal lymphoid cells involving up to 5% in the bone marrow. The cells had mildly irregularly shaped nuclei with moderately condensed chromatin and inconspicuous nucleoli, and abundant cytoplasm with small vacuoles (Figure 1). There were no azurophilic granules in the cytoplasm. The histiocytic hemophagocytosis was observed in the bone marrow of initial stage. The terminal phase of this patient's disease was accompanied by a significant change in morphology of abnormal lymphoid cells. The neoplastic cells became increasingly large and anaplastic, with fine chromatin and marked nuclear irregularities, involving up to 93% in the bone marrow (Figure 2).

#### *Pathological and Immunohistological findings*

The resected spleen was enlarged, weighing 720 grams with soft consistency. The surface was stretched and

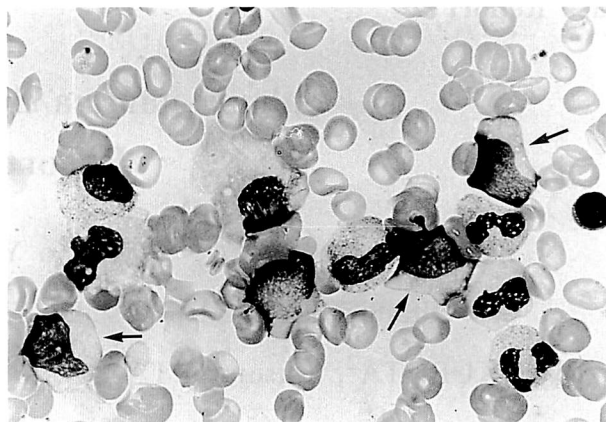


Fig. 1. Bone marrow smear. Abnormal lymphoid cells (arrows) have irregular nuclei with moderately condensed chromatin, and inconspicuous nucleoli and abundant cytoplasm with small vacuoles. (May-Gruenwald-Giemsa stain,  $\times 250$ )

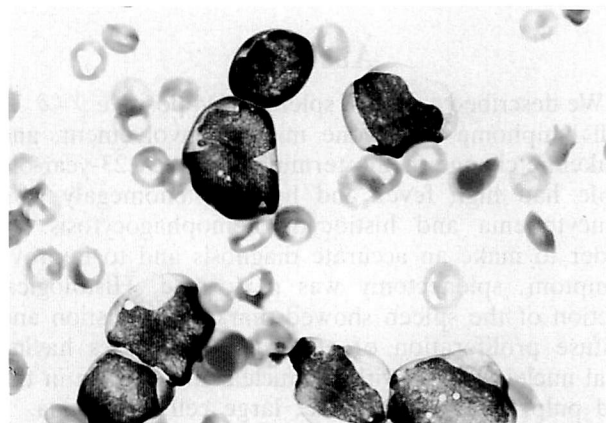


Fig. 2. Bone marrow smear in critical state. Neoplastic cells are large and anaplastic with nuclear irregularities and fine chromatin, and markedly different from those shown in Figure 1. (May-Gruenwald-Giemsa stain,  $\times 330$ )

the cut surface was dark red, soft and swollen. There was no nodular lesion. The histological section of spleen showed congestion and diffuse proliferation of atypical cells having oval nuclei with or without irregular indentation, distinct nuclear margin and somewhat fine chromatin pattern. The cytoplasm was abundant and pale eosinophilic. These atypical cells were diffusely infiltrating the cords and sinuses in the red pulp with marked atrophy of the white pulp (Figure 3). Mitotic figures are also observed. These histological findings are indicative of malignant lymphoma, diffuse, large cell type (Figure 4).

Bone marrow examinations showed hypercellularity in the early stage, and hypocellularity in the later stage. The lymphoid cells in the bone marrow were positive for CD3 by immunohistochemistry using paraffin sections. Infiltrating CD3-positive T cells had mostly small to medium-sized round nuclei with mild indenta-

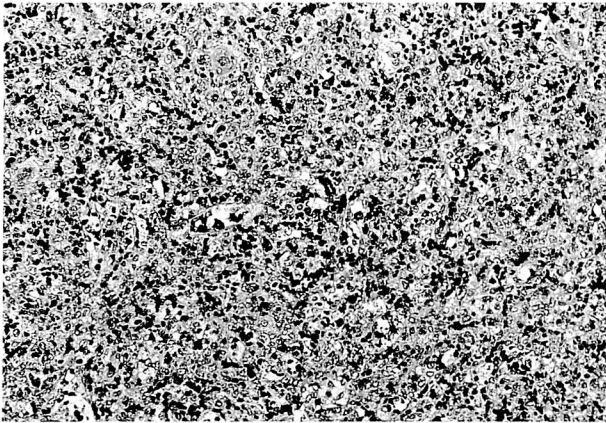


Fig. 3. Histology of the spleen. Diffuse proliferation of atypical cells is observed in the red pulp. (hematoxylin and eosin stain,  $\times 50$ )

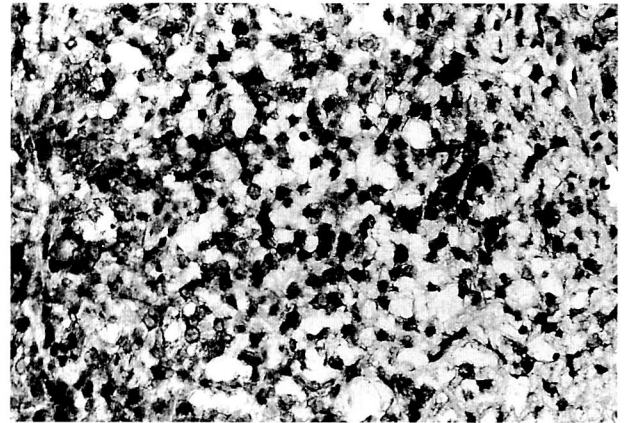


Fig. 6. Immunohistologic study on frozen section of the spleen with anti-CD4 monoclonal antibody. Atypical lymphocytes are positive for CD4 antibody. (alkaline phosphatase-labeled avidin technique,  $\times 100$ )

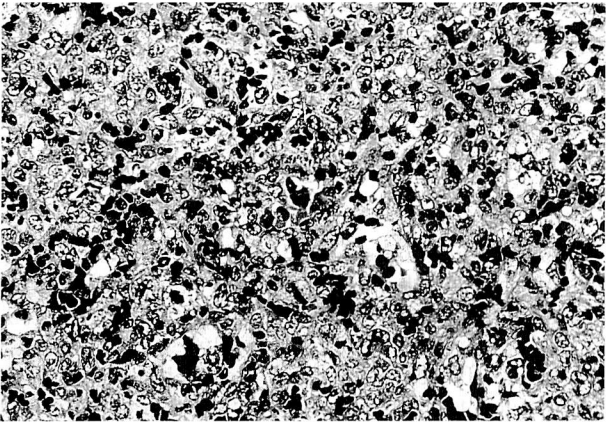


Fig. 4. Histology of the spleen. Proliferating atypical cells have oval nuclei with or without irregular indentation. (hematoxylin and eosin stain,  $\times 100$ )

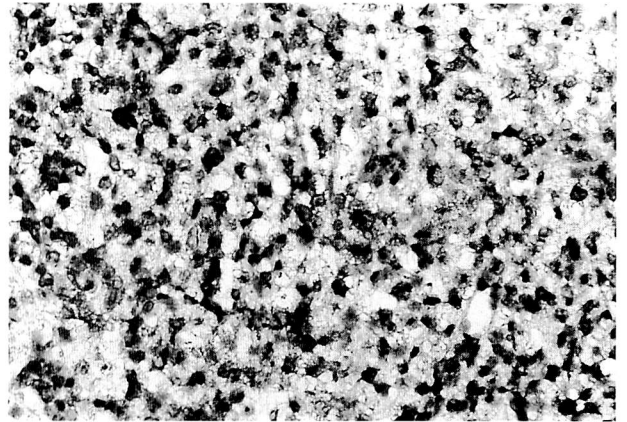


Fig. 7. Immunohistologic study on frozen section of the spleen with  $\delta$  TCS1. Atypical lymphocytes express TCR  $\delta$ . (alkaline phosphatase-labeled avidin technique,  $\times 100$ )

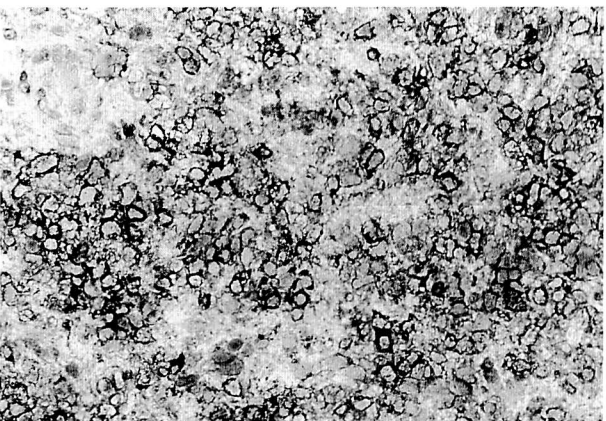


Fig. 5. Immunohistologic study on frozen section of the spleen with anti-CD3 monoclonal antibody. Most atypical lymphocytes strongly express CD3. (alkaline phosphatase-labeled avidin technique,  $\times 100$ )

tion from the early to the later stage with increasing trend in number, and sinus involvement. In the critical state, hypoplastic bone marrow was replaced by CD3-positive T cells with large round or oval nuclei. All bone marrow specimens showed increased number of CD68 positive histiocytes which have erythrophagocytic activity and siderophagic change in the terminal stage.

The lymphoma cells of the spleen were positive for CD2,3,4,15 and 25 (Figure 5,6), and negative for CD1,5,7,8,13,14,19,20,21,30,56, and HLA-DR. These cells expressed also the TCR  $\delta$  chain detected by  $\delta$  TCS1 (Figure 7), but not the TCR  $\beta$  chain detected by TCR  $\beta$  F1 (Table 1).

On March 1994, when his bone marrow was largely involved by blastic cells about 90%, phenotype examination by flow cytometry was performed (Figure 8). These cells were positive for CD3 and TCR  $\gamma/\delta$  —

Table 1. Phenotype of neoplastic cells

Tissue	Monoclonal antibodies							
	CD2	CD3	CD4	CD8	TCR $\beta$ F1	TCR-1 $\alpha/\beta$	$\delta$ TCS1	TCR $\gamma$ $\delta$ -1
Spleen*	+	+	+	-	-	nd	+	nd
Bone marrow**	nd	+	-	-	nd	-	nd	+

nd: not done

\* immunohistochemistry

\*\* analysis by flow cytometry

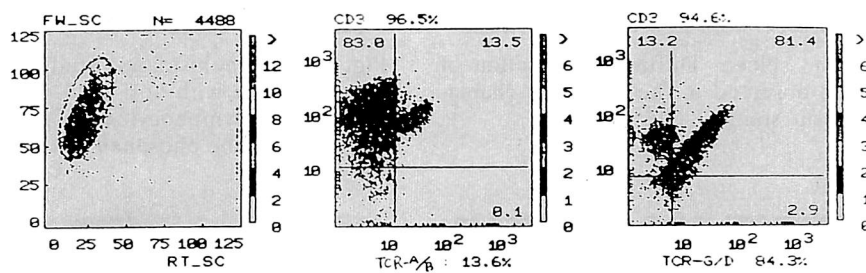


Fig. 8. Two color analysis by flow cytometry. left: FW-SC-RT-SC, center: CD3-TCR-A/B, right: CD3-TCR-G/D.

1, but negative for CD4 (Table 1).

## Discussion

We have reported a case of splenic  $\gamma/\delta$  T-ML with onset of pancytopenia and histiocytic hemophagocytosis, and leukemic change with blast transformation in the terminal stage. This case is thought to be best fit of the findings reported as hepatosplenic T-ML with TCR  $\gamma/\delta$  expression, although we could not examine the histology of enlarged liver.

Various  $\gamma/\delta$  T-cell neoplasms have been reported previously, namely, acute lymphocytic leukemia, lymphoblastic lymphoma, large granular lymphocyte disorders, and peripheral T-cell lymphoma<sup>2-6)</sup>. The common clinical and histopathological features of peripheral T-cell lymphoma (PTCL) with TCR  $\gamma/\delta$  expression are marked hepatosplenomegaly, no lymphadenopathy, and sinusoidal/sinusoidal infiltration by neoplastic cells in the spleen, liver and bone marrow occurring in young male patients. The majority of these cases shows a pattern of hepatosplenic lymphoma, but there are a few cases of nasal lymphoma and cutaneous lymphoma<sup>3)</sup>.

The diagnosis of PTCL of TCR  $\gamma/\delta$  type is based on the histologic evaluation of tissue biopsies and on the demonstration of CD3+, CD4-, CD5-, CD7-, CD8-, TCR $\beta$ F1-, and  $\delta$  TCSI+. The tumor cells of this patient had CD3+, CD4+, CD5-, CD7-, CD8-,  $\delta$  TCSI+ phenotype of the spleen, and in the

terminal stage, CD3+, CD4-, CD8-, TCR  $\gamma$   $\delta$ -1 + phenotype of the bone marrow tumor cells. CD4 expression in two of three cases with hepatosplenic lymphoma is reported previously<sup>7)</sup>. In this case, therefore, CD4 expression of the splenic lymphoma has changed to CD4 negative in the bone marrow of terminal stage with similar TCR  $\gamma$   $\delta$  expression of the tumor cells. Phenotypic changes during the course from CD3+,  $\gamma/\delta$  + to CD3-,  $\gamma/\delta$  - of hepatosplenic lymphoma has been reported by Farcet et al<sup>2)</sup>. The abnormal lymphoid cells of bone marrow in the initial stage and later stage are similar in the nuclear pattern, but different by the number of cells. The cells changed from a small number of medium-sized cells in the bone marrow of initial stage to the large blastic cells which were thought to be leukemic in the terminal stage. These findings suggest that same tumor cells have changed in size and terminated by the blast crisis in the critical state with loss of CD4 expression. It is likely that a second genetic event occurred to produce these changes, though there is no direct evidences in these changes of the same tumor cells. Similar change of the clinical status from chronic lymphoproliferative disorder with small lymphocytes to acute terminal course with excess large blastic cells in a case of hepatosplenic lymphoma has also been reported<sup>6)</sup>. The genotype of the lymphoma cells in this patient could not be fully evaluated by Southern blot analysis, because of the small amount of sample, but clonal rearrangement of TCR  $\gamma$  and  $\delta$  genes are suspected from the phenotypic

expression of the spleen and bone marrow.

This patient had onset of pancytopenia and histiocytic hemophagocytosis with findings of infection, suggested at first virus associated hemophagocytic syndrome. But known virus infection could not be detected. Erythrophagocytic histiocytes in this case observed in all bone marrow specimens are thought to be possibly induced by lymphokines which are produced by tumor cells<sup>8,9)</sup>.

In conclusion, we present an unusual case of splenic  $\gamma/\delta$  T-cell lymphoma with initial findings of infection which shows histiocytic hemophagocytosis, typical histology of splenic  $\gamma/\delta$  T-cell lymphoma with sinus pattern, CD3 and CD4 expression, and loss of CD4 expression and acute leukemic change in the terminal stage. Although there are only ten or more cases of hepatosplenic  $\gamma/\delta$  T-cell lymphoma previously reported, there is a variety in the clinical findings, phenotypic expression, and morphology of the tumor cells. Further case collection of this type of lymphoma with clinical, morphological, phenotypic and genotypic informations is recommended for the knowledge of the disease entity.

### References

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