Splenic Lymphoma with Villous Lymphocytes with CD5+, CD11c+ B-Cell Phenotype

Shuichi Miyawaki, Takashi Machii*, Hisami HiraBayashi, Toru Sakura, Hiroko Shiozaki, Kunihiro Yashiro, Hirokazu Murakami**, Jun Tsuchiya***, Kenji Kashiwabara**** and Teruo Kitani*

A 64-year-old male suffered from splenomegaly without lymphadenopathy. His WBC count on admission was 6.1×10^9/l with 55% abnormal lymphocytes. No monoclonal gammopathy was detected. Abnormal cells shown in films usually had relatively abundant cytoplasm with serrated edges. Under phase-contrast microscopy, the cells displayed short, needle-like processes. The immunophenotype of peripheral blood mononuclear cells were CD19+, CD20+, CD11c+, FMC7+, CD5+, CD10−, CD25− and SIg+. The spleen histology showed a distinctive pattern of white pulp infiltration by abnormal lymphocytes with features of plasma cell differentiation. These findings were compatible with the features of splenic lymphoma with villous lymphocytes.

(Key words: B-cell malignancy, hairy cell, splenomegaly, immunophenotype)

Introduction

Hairy cell leukemia (HCL) is defined as a distinct clinico-pathologic entity on the basis of several distinguishing features (1), but similar findings are also observed in a certain non-Hodgkin’s lymphoma. Such a lymphoma has been described under a variety of names (2–8). The disease is characterized by splenomegaly, inconspicuous adenopathy, frequent occurrence of a small amount of monoclonal paraprotein in the serum, and the presence of lymphocytes with cytoplasmic projections (villous lymphocytes) in the peripheral blood. Sprino et al (6) considered the disease as a distinct variant of an immunocytoma on the basis of histological and hematological examinations and named it, splenomegalic immunocytoma with circulating hairy cells. Melo et al (7, 8) evaluated the immunophenotype and morphology of villous lymphocytes, which revealed the cytological differences between the villous lymphocytes in this disease and the hairy cells in HCL; they proposed the name, splenic lymphoma with villous lymphocytes (SLVL). Although SLVL is recognized as a distinct B-cell disorder classified among the chronic lymphoid leukemias as proposed by the French-American-British (FAB) group, only a few cases have been reported in Japan. This may be because the features of SLVL are inadequately defined or are not clearly understood. We present here the clinical, cytological, immunological and pathological features that were observed in a patient with SLVL.

Case Report

A 64-year-old male was admitted to Saiseikai Maebashi Hospital for an evaluation of splenomegaly on March 13, 1991. His evaluation revealed the presence of splenomegaly (15 cm below the left costal margin), but there was no evidence of lymphadenopathy. His blood analyses were as follows: Hb 126 g/L, RBC 3.82×10^12/l, PCV 31.3, platelets 66×10^9/l, WBC 6.1×10^9/l with 26% segmented neutrophils, 1% monocytes, 18% lymphocytes and 55% abnormal lymphocytes. Bone marrow aspirations resulted in dry taps. The bone marrow biopsy was normal and revealed no characteristic pattern for HCL. Total serum protein was 7.4 g/dl with 61% albumin and 16.5% γ-globulin. No monoclonal gammopathy was detected. Abnormal lymphocytes had a round nucleus with well-condensed chromatin and a distinct nucleolus, and relatively abundant cytoplasm that stained moderately basophilic. The abnormal lymphocytes preferentially displayed serrated or villous edges in the thickly smeared areas of the film (Fig. 1a), but polar distribution of the villi was not apparent. On the other hand,
Cytoplasmic projections were hardly seen in the cells placed in the thinly smeared areas (Fig. 1b). These cells resembled the cells of hairy cell leukemia variants (1, 8). Under phase-contrast microscopy, most of the abnormal lymphocytes revealed surface villi consisting mainly of short, needle-like projections (Fig. 2a). Viewed under electron microscopy (Fig. 3), the abnormal cells possessed short, thin and randomly distributed villi. A round nucleus, with slight indentations, possessed a prominent nucleolus and scattered heterochromatin. No ribosome-lamella complex was found. The cytochemically tartrate-resistant acid phosphatase (TRAP) reaction was invariably negative.

The immunophenotypes of peripheral blood mononuclear cells were analyzed by fluorescence activated cell sorting (FACS) with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAb).

![Fig. 1. Peripheral blood film (Wright's stain). Lymphoid cells showing relatively abundant cytoplasms and round nuclei with a distinct medium-sized nucleolus. In thickly smeared areas the cytoplasm appears serrated (a) but does not in thinly smeared areas (b).](image1)

![Fig. 2. Phase-contrast micrograph of peripheral blood mononuclear cells. Villous lymphocytes showing short needle-like cytoplasmic processes (a). Hairy cells display ruffles associated with long microvilli (b).](image2)

![Fig. 3. Electron micrograph. Cells showing a slightly indented nucleus with a prominent nucleus. The cytoplasm shows many short villi. Bar: 1 μm.](image3)
to human Igk, Igλ, CD5, CD10, CD11c, CD19, CD20 and CD25 (Becton Dickinson, Tokyo, Japan). The reaction with FMC7 MoAb (Sera-Lab, Crawley Down, U.K.) was evaluated using FITC-conjugated anti-mouse IgM antibodies (TAGO Inc., Burlingame, CA, U.S.A.) by indirect immunofluorescence. The abnormal lymphocytes reacted with anti-Igλ, CD19 and CD20. They expressed CD11c and FMC7, as do hairy cells in HCL, but they did not react with anti-CD25 MoAb. The double immunofluorescent staining with FITC-conjugated anti-CD5 and PE-conjugated anti-CD19 MoAb revealed that 17% of the CD19 positive cells expressed CD5 antigen.

On April 10, a splenectomy was performed to relieve the patient's stomach fullness. The spleen histology showed a distinctive pattern of white pulp infiltration of abnormal lymphocytes (Fig. 4a). The abnormal lymphocytes displayed morphological features of plasma cell differentiation. No blood lakes or pseudosinuses, as described in HCL cases (9), were seen. A diagnosis of SLVL was made based on these stated results. The patient has progressed satisfactorily since the operation and, as of March 1993, he is doing quite well and seems free of any clinical problems (Fig. 5).

Discussion

Our patient had marked splenomegaly with the absence of lymphadenopathy. The circulating lymphocytes had relatively abundant cytoplasm with serrated edges on the blood film and they showed the phenotype of SIg+, CD19+, CD20+, CD11c+, FMC7+ as well. These clinical, cytological and immunophenotypical findings were very similar to those described for HCL cases (1). Under phase-contrast microscopy, most abnormal lymphocytes displayed short, needle-like villi (Fig. 2a). The morphological features of the surface villi, however, appeared to differ from the thin, membranous cytoplasmic projections (ruffles) seen in HCL cells (Fig. 2b). In addition, other

Fig. 4. Spleen section showing white pulp infiltration by lymphocytes with features of plasmacytic differentiation (HE stain, ×10 (a), ×50 (b)).

Fig. 5. Clinical course.
features including the negative TRAP reaction, the absence of ribosome-lamella complex, and the expression of CD5 antigen are unusual for HCL. From these findings, a B-cell lymphoproliferative disorder simulating HCL was suspected. The spleen histology showed predominant involvement in the white pulp by abnormal cells with morphological features of plasmocytic differentiation.

Although the abnormal cells expressed CD5 antigen, they were found to have a low nuclear/cytoplasmic ratio and surface villi, unlike chronic lymphocytic leukemia (CLL) lymphocytes. The present patient is histologically differentiated from non-Hodgkin’s lymphoma in the leukemic phase, including intermediate lymphocytic lymphoma and follicular lymphoma. Although the present findings are fundamentally in accordance with the features described for SLVL by Melo et al, some differences were noted between the present results and theirs. For example the CD5 antigen was positive in our case, but was negative in all the cases reported by Melo. The CD11c antigen was also positive in the present case, but Bennett et al (1) reported that SLVL cells did not react to Leu-M5 (CD11c). Melo described a high incidence of a monoclonal gammopathy and, although the presence of monoclonal-protein (M-protein) is helpful in the differentiation of HCL, it was not detected in the present case. Most pertinent to the present case is the data of Valensi et al (10), who reported that the monoclonal band was positive in only 2 of 8 cases, and that in 7 of these 8 cases, the CD5 antigen and the CD11c antigen were expressed in 3 and 5 cases, respectively. These findings suggest that SLVL is heterogenous in the expression of CD5, CD11c, and also in the existence of monoclonal gammopathy. Melo et al stressed an uneven, often polar distribution of surface villi in the blood films as a characteristic feature of SLVL cells, which differs from the HCL cells. Such a difference in cellular outlines of the cells in the present patient and that of the HCL cells was not apparent on the blood films. In our experience, the shape of cellular outlines on blood films depends on the method of making smears and also on the examination areas of the film. Villous lymphocytes in the present patient were shown to have distinguishing morphological features under phase-contrast microscopy. This finding may be useful for the differential diagnosis of SLVL. However, more research on this point is necessary. Except for CD19 and CD20, the expression of CD5, CD10, CD11c, CD25 and CD38 in SLVL varies from case to case (1, 8, 10), and a specific surface marker for SLVL has not yet been discovered (1, 8, 10). Perhaps a reliable marker for SLVL will be reported at a later time.

SLVL is a low grade non-Hodgkin’s lymphoma, and it follows either a stable or a slowly progressive clinical course. Thus, reports that focus on the therapy of this disease are rare. Recently, Mulligan and associates (11) reported on the natural history of 50 cases of SLVL as well as on their response to therapy; they reported a survival rate for all patients of 82% at 3 years and 78% at 5 years, and they recommend splenectomy as the first choice of treatment for SLVL. Although villous lymphocytes are still detected in the present patient’s peripheral blood, his clinical status is good after undergoing splenectomy.

References


Table 1. Surface Marker of Peripheral Blood Mononuclear Cells

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<thead>
<tr>
<th>Immunologic markers</th>
<th>Reactivity observed (%)</th>
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<tbody>
<tr>
<td>CD5</td>
<td>20.2</td>
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<tr>
<td>CD10</td>
<td>1.4</td>
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<tr>
<td>CD11c</td>
<td>67.5</td>
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<td>CD19</td>
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