Circulating Abnormal Cells Detected in a Patient with Immunoblastic Lymphadenopathy

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An unusual case of immunoblastic lymphadenopathy (IBL) with circulating CD3+CD4+ cells is reported. A lymph node biopsy specimen showed the characteristic features of IBL. Two-color analyses demonstrated that the circulating abnormal cells were CD3~, CD4+, HLA-DR+, and CD25+. Chromosomal analysis revealed unrelated clones in the lymph node. Though 48% of the peripheral blood lymphocytes were abnormal, no clonal rearrangement of the TcR β chain gene was detected in the peripheral blood. This case might point out the possibility that some cases of IBL truly had no TcR gene rearrangement and were dysplasias.

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Key words: AILD, circulating abnormal cells, CD3+CD4+ abnormal cells, prednisolone

Introduction

Immunoblastic lymphadenopathy (IBL) is a systemic lymphoproliferative disease characterized by generalized lymphadenopathy, hepatosplenomegaly, skin rash, and immunological abnormalities (1-4). As a result of many studies, it has become clear that many cases of IBL are identified as T-cell lymphoma (IBL-like T-cell lymphoma) (5-9). However, there remains a problem that there is no clonal rearrangement of the TcR β gene in 30% of IBL (10). It has been thought that this problem is due to the inability to find clonal rearrangements for a slight underlying monoclonal proliferation below the threshold of detection.

In this paper we describe many circulating abnormal T cells detected in a patient with IBL by two color analyses. But neither cytogenetic nor immunogenotypic analysis revealed clonality despite the presence of many immunophenotypically abnormal T cells. Our data show dysplastic features of IBL.

Case Report

A 54-year-old Japanese woman was admitted to our hospital in June 1991 because of skin rash. She had had recurrent episodes of systemic lymphadenopathy, fever and skin rash after taking some analgesic agents (ibuprofen, etc.) during the preceding two years. The skin rash had disappeared spontaneously on the first occasion, and after taking prednisolone on the second occasion. On physical examination, generalized lymphadenopathy, fever, skin rash and hepatosplenomegaly were found. A biopsied right cervical lymph node exhibited the typical features of IBL. The cervical lymph node biopsy specimen showed diffuse effacement of the nodal architecture with complete absence of germinal center and prominently arborizing postcapillary venules (Fig. 1a). Proliferating cells consisted of small lymphocytes, plasma cells, large immunoblasts with pale cytoplasm, and eosinophils (Fig. 1b). The pale cells were stained with UCHL-1. The chromosomal analysis of the lymph node revealed a clone with 3 trisomy (4/24 mitotic cells) and another clone with 21 trisomy (9/24 mitotic cells) (Table 1). These findings were believed to indicate the presence of karyotypically unrelated abnormal clones. Laboratory findings (Table 1) showed the WBC to be 4,900/µl, and decreased gamma-globulin level in the serum was found. The number of CD4+ plus CD8+ cells exceeded that of CD3+ cells. Serum antibody against human T-cell leukemia virus (HTLV)-I and HTLV-I proviral DNA of the peripheral blood mononuclear cells (PBMC) were negative. She was diagnosed as IBL. The patient received prednisolone therapy. Soon after beginning the therapy, the lymph node swelling, hepatosplenomegaly and skin rash subsided. She was discharged on August 1991, but was soon readmitted because of pneumonia. She died of pneumonia in November 1991.

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Materials and Methods

Lymphocyte subset identification

For two-color immunofluorescence analyses, PBMNC were incubated for 30 min on ice with monoclonal antibody (MoAb) conjugated to fluorescence isothiocyanate (FITC) and biotin. After washing, flow cytometry was carried out on a FACScan (Becton Dickinson, Mountain View, Ca). For each monoclonal antibody (MoAb) used, 5,000–10,000 cells were analyzed and all subsequent analyses were performed on the samples of the cell population within the lymphoid window. We used MoAb to the following antigens [cluster designations (CD) follow in parentheses]: CD3 (Leu4); CD4 (Leu3a); CD25 (anti-IL-2 receptor); HLA-DR (anti-HLA-DR); 1/10 dilution (all antibodies were purchased from Becton Dickinson, Mountain View, Ca).

Sorting of CD3⁻CD4⁺ lymphocytes

PBMNC were incubated with conjugated anti-Leu 4 (CD3) and Leu 3a (CD4) and sorted in CD3⁻CD4⁺ fractions on a

Table 1. Laboratory Findings

<table>
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<th>Peripheral blood</th>
<th>Surface phenotype of lymphocytes</th>
<th>Coombs test ID</th>
<th>ANF</th>
<th>anti-DNA</th>
<th>Thyroid test</th>
<th>Microsome test</th>
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</table>

| Bone marrow     |                                 |               |     |         |              |                |      |
| NCC             |                                 |               |     |         |              |                |      |
| MGK             |                                 |               |     |         |              |                |      |
| Erythroid series|                                 |               |     |         |              |                |      |
| Granuloid series|                                 |               |     |         |              |                |      |
| Monocyte        |                                 |               |     |         |              |                |      |
| Lymph           |                                 |               |     |         |              |                |      |

Circulating Abnormal Cells in IBL

FACStar cell sorter (Becton Dickinson). The purity of the sorted cells consistently exceeded 95%.

Rearrangement of TcR and Ig genes

Southern blot hybridization of the mononuclear cells (MNC) of the peripheral blood (PB) and lymph node (LN) revealed

Southern blot analysis

Five microgram portions of DNA were digested with the restriction enzymes, Eco RI, Bam HI, Hind III. After electrophoresis, the DNA fragments were transferred to nylon membranes by the method of Southern (11) and were hybridized with C8, Jy, and Jh as probes.

Results

Phenotypic findings of circulating abnormal cells before and after treatment

The number of CD4+ plus CD8+ lymphocytes exceeded that of CD3+ lymphocytes in the peripheral blood (PB) and lymph node (LN) (Table 1). Two-color analyses showed circulating abnormal cells expressed as CD4+ and HLA-DR+ but not CD3 or CD25 (Fig. 2). After prednisolone therapy, the circulating abnormal cells decreased remarkably.

Morphological findings of sorted circulating abnormal cells

Circulating abnormal cells showed a slightly convoluted nucleus with a pale cytoplasm.

Fig. 2. Two-color analyses of circulating abnormal cells.

Fig. 3. Sorted CD4+CD3− circulating abnormal cells (May-Giemsa stain, ×1,000).
negative TcR and Ig rearrangement (Table 1).

Discussion

The clinical characteristics of this patient were compatible with those of typical IBL except for the reduced serum γ-globulin, in contrast to the hypergammaglobulinemia seen in the most cases, and a definite diagnosis was made on the basis of the histologically characteristic features of the lymph node. The present case was shown to be clonal by immunophenotypic analysis, but not by cytotogenetic or immunogenotypic analysis.

Malignant T-lineage populations often manifest pan-T antigen loss (12). This phenomenon has not been observed in any non-neoplastic disorder (13). The results suggest that the loss of one or more pan-T markers is a useful phenotypic finding supporting the diagnosis of T-cell lymphoma. In a series of 88 T-cell lymphomas (13), the CD3 was lost in 17/88 (19%). No loss of pan-T antigens is reported in IBL or IBL-like T-cell lymphoma (14). We showed that half of the circulating lymphocytes in this patient were abnormal cells with CD3-CD4+ surface markers. Sorted circulating abnormal cells showed slightly convoluted nuclei with pale cytoplasm. Although the circulating abnormal cells constituted half of the peripheral mononuclear cells, they did not show TcR rearrangement. As a result of the finding of gene rearrangement in IBL cases, it became clear that many cases of IBL were identified as IBL-like T-cell lymphoma. However, the problem is that several different frequencies of monoclonal TcR B gene rearrangement are reported to occur in IBL (10), and there is no evidence of T cell clonality in 30% of the IBL.

There are two explanations for this anomaly. First, there was no evidence of T cell clonality because the underlying monoclonal T cell proliferation was below the level of detection in IBL. Previous studies have shown that gene rearrangement analysis cannot accurately detect a monoclonal population of B or T cells when they account for less than 5% of the cells present in a tissue sample (15). However, in the present case there was no evidence of T cell clonality despite the high percentage (48%) of abnormal PB lymphocytes. Secondly, there is no clonal T cell proliferation of IBL cases in nature. This is precisely why we understand IBL to be a dysplasia. Nanba and Sasaki (16) proposed that IBL is a lymphodysplastic syndrome, based on the low rate of TcR gene rearrangement (1/11). Frizzera et al. (10) emphasized that in about 30% of IBL cases, as determined by histopathology, no evidence of clonality is found by immunogenotypic analysis. These authors proposed that the designation “IBL” should be limited to such cases, whereas the term “IBL-like lymphoma” should be reserved for cases shown to be clonal by immunogenotypic analysis. In the present case we could not detect TcR gene rearrangement despite the numerous abnormal T cells, so this would be a non-neoplastic disease.

Some abnormal lymphocytes have been observed in the PB of patients with IBL, but it is not clear if the abnormal lymphocytes are neoplastic or non-neoplastic cells. There have been few studies of the peripheral lymphocytes of IBL. Activated T cells are demonstrated in the peripheral blood of the IBL patient, whereas most of the T cells express HLA-DR antigens (17). It was shown that prednisolone alone decreased the circulating abnormal cells. The good response to prednisolone therapy is an important feature in IBL-like T-cell lymphoma (9).

In conclusion, we found many circulating abnormal cells in IBL but these circulating abnormal cells did not have TCR B gene rearrangement. These data may prove the dysplastic nature of IBL.

References