CASE REPORT

T-γδ Large Granular Lymphocyte Leukemia Preceded by Pure Red Cell Aplasia and Complicated with Hemophagocytic Syndrome Caused by Epstein-Barr Virus Infection

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Abstract

A 51-year-old man developed anemia, and was diagnosed with pure red cell aplasia through the absence of erythroid progenitors. Initially, he was treated with cyclosporine and prednisolone for 6 months but they were ineffective. Large granular lymphocyte (LGL) leukemia with the T-cell γδ phenotype evolved after 6 months showing CD2+, CD3+, CD8- and CD56- with the T-cell receptor β gene rearrangement, clonalities of γ and δ genes and complex chromosome abnormality simultaneously with hemophagocytic syndrome (HPS). Epstein-Barr virus (EBV) genomic DNA was detected in the bone marrow cells. Administration of bolus methylprednisolone was ineffective, and the patient died one month later. In the present patient, it seemed that lymphoproliferative disease of large granular lymphocytes (LDGL) manifested initially as PRCA, γδ LGL leukemia evolved, and finally fatal HPS become complicated, presumably caused by the EBV reactivation in the immunodeficiency state with the administration of immunosuppressants.

Key words: pure red cell aplasia, large granular lymphocyte leukemia hemophagocytic syndrome, Epstein-Barr virus

(DOI: 10.2169/internalmedicine.45.1594)

Introduction

Granular lymphocytes have been characterized as lymphocytes with azurophilic granules in the cytoplasm. The disease of increased numbers of granular lymphocytes has been termed lymphoproliferative disease of granular lymphocytes (LDGL) or large granular lymphocyte (LGL) leukemia (1-3). LDGLs are divided into CD3+ T-cell lineage and CD3-CD16+ natural killer (NK) cell-lineage, and are heterogeneous disorders, ranging from cases with a prolonged clinical course and relatively favorable prognosis to rarely progressive or aggressive malignancy (1-3).

Immune-mediated cytopenia is recognized in association with LDGL or LGL leukemia, pure red cell aplasia (PRCA), aplastic anemia, autoimmune hemolytic anemia, neutropenia and thrombocytopenia (4-10). Granular lymphocytes of these patients have been shown to selectively inhibit erythroid progenitors but not myeloid progenitors (4-10).

Hemophagocytic syndrome (HPS) is associated with viral infection and T/NK lymphoproliferative disorders (11-15). Data on Epstein-Barr virus (EBV)-related peripheral T cell lymphoma (PTCL) has accumulated, and LDGL has been reported to be linked to EBV infection (15-19).

We observed a case initially presenting with PRCA, evolving LGL leukemia 6 months later and complicated with fulminant and fatal HPS caused by reactivated EBV infection in the immunodeficiency state by the administration of immunosuppressants.

Materials and Methods

Genomic DNA was obtained from the bone marrow aspirate cells. The rearrangement of T-cell receptor Cβ1 gene

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Received for publication October 4, 2005; Accepted for publication January 18, 2006
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Case Report

A 51-year old male was diagnosed with anemia during a health check examination; Hb 9.5 g/dl, in June 2004. He was examined by the Southern blot method as previously reported (20). The clonality of T-cell γ and δ genes was examined by gel electrophoresis using polymerase chain reaction (PCR) amplified product, since the amount of genomic DNA was insufficient for the analysis of the rearrangement of T-cell receptor gene. Epstein-Barr virus genomic DNA was examined using PCR amplified product.
was followed-up at another hospital, but his anemia progressed and thus he was referred to our hospital in September. Past history did not reveal any special diseases or medication. Physical examination revealed anemia, jaundice was not observed, and the lymph nodes, liver and spleen were not palpable. The hematological examination revealed the following: RBC 158×10^4/μl, Hb 6.6 g/dl, Ht 19.5%, MCV 123.4fl, MCHC 33.8%, Ret 1.4%, Platelet 25.4×10^4/μl and WBC 5000/μl (NSeg 53%, Eo 3%, Ba 1%, Lympho 29%, Mono 14%). The lymphocyte subset was not measured at that time. The bone marrow aspiration smear revealed slightly hypocellular marrow, decreased erythroid cells of M/E 9.0, basophilic erythroblast 0.6% and orthochromatc erythroblast 8.2%. Chromosome analysis of bone marrow cells showed normal karyotypes. Coombs tests, both direct and indirect, were negative. Parvovirus B19 DNA was negative.

Blood chemistry was as follows: total protein 6.2 g/dl, albumin 4.1 g/dl, CRP 0.2 mg/dl, total bilirubin 0.5 mg/dl, AST 38 IU/L, ALT 67 IU/L, LDH 200 IU/L, BUN 9.6 mg/dl, creatinine 0.74 mg/dl, uric acid 3.6 mg/dl, ferritin 994 ng/ml, vitamin B12 330 pg/ml, folic acid 3.3 mg/ml, antinuclear factor negative, anti-DNA antibody negative, RF<9.6 IU/ml, C3 78 mg/dl and C4 31 mg/dl. Computed tomography of the chest did not reveal thymoma.

The diagnosis of PRCA was made. The clinical course is shown in Fig. 1. He was initially treated by the administration of cyclosporine 200 mg/day orally, but improvement of anemia was not observed. Prednisolone, 30 mg/day orally, was added, and a slight improvement in anemia was observed: Hb 6.6 g/dl, Ret 3.0%. The patient was discharged in November and was followed-up at the outpatient clinic. The patient was admitted in December for acute renal failure accompanied with the disturbed control of diabetes mellitus. He was administered insulin thereafter, and his condition improved. Pancytopenia, Hb 5.2 g/dl, platelets 3.0×10^10/μl, and WBC 2500/μl with atypical lymphocytes of 6.0%, appeared on April 5, 2005. Pancytopenia progressed and large granular lymphocytes increased to 50-99%. The bone marrow aspiration smear revealed the appearance of large granular lymphocytes at 72.6% and hemophagocytic cells (Fig. 2). Flow cytometric analysis of bone marrow cells revealed the following: CD2 88%, CD3 33%, CD4 7.3%, CD5 10.4%, CD7 12.6%, CD8 8.1%, CD56 2.6%, HLA-DR 73.9%, TCR-γ δ 37.2% and α β 2.3%. The chromosome analysis of bone marrow cells revealed: 44, X, -Y, +1, der (1; 8) (q10; q10), -13, add (16) (q12), -22, +mar1 of 17/20 cells, and 44, idem [der (1; 8), +8, der (18) t (1; 18) (q12; q 23)] of 3/20 cells. The level of soluble IL-2 receptor was 25,800 U/ml and ferritin was 21,573 ng/ml. Rearrangement of the T-cell receptor (TCR) β gene, Cβ1, was observed by Southern blot analysis (Fig. 3). Monoclonally amplified bands of TCRγ and TCRδ were observed by polyacrylamide gel electrophoresis using polymerase chain reaction (PCR)
amplified products (Fig. 4). The patient was diagnosed with T-γδ LGL leukemia although the absolute number of granular lymphocytes was less than 2,000/μl (3), and it was complicated with HPS. The serological study for the EB virus revealed: EBEA IgG 08 (<1.0), EBVCA IgM 0.1 (<1.0), EBVCA IgG 9.0 (<1.0), EBVEBNA IgG 9.0 (<1.0), EBEBNA G 3.2 (<1.0), and cytomegalovirus (CMV IgM 0.32 (<0.8), CMV IgG 97.6 (<2.0)). Genomic EB virus DNA extracted from bone marrow cells was positive using PCR product. He was treated with bolus methylprednisolone starting on the end of April. However, it was ineffective and the patient died of sepsis one week later. Autopsy findings revealed the massive infiltration of immature lymphocytes into the Glisson’s sheath of liver and red pulp of the spleen. Bone marrow was hypocellular with increased immature lymphocytes and histiocytes.

Discussion

In patients with LDGL, cytopenia is usually manifested as anemia or neutropenia, and much less frequently, thrombocytopenia and pancytopenia (4-10). These are diagnosed as PRCA, myelodysplastic syndrome or aplastic anemia (4-10). The present case of PRCA may represent the initial manifestation of LDGL (10), however, LGL were not detected and the cytogenetic study was normal initially. Thus, LDGL is frequently underdiagnosed, and it is likely that a significant proportion of idiopathic or primary PRCA is secondary to T-LDGL (10).

T-LGL cells (LGLs) express CD3 and a TCR of αβ-type in the majority of cases, or γδ-type in a minority of cases (7). In contrast, NK-LGLs are CD3- and, consequently, do not express a TCR at the cell surface (7). In the present patient, the T-cell surface phenotype was CD2+, CD3+, CD4-, CD8- and γδ+, and showed rearrangement of the TCR Cβ1 gene and clonality of γ and δ genes. According to the analysis among 21 patients with CD3+ T-LDGL, 17 patients were CD4+, CD8+, 2 patients were CD4+, CD8-, and the remaining 2 patients were CD4-, CD8-, with the latter two exhibiting TCR-γ and δ gene clonality (1). These results supported the diagnosis of T γδ LGL leukemia in our patients, and this is found in only a minority of cases.

The possible mechanism of PRCA directly mediated by T- or NK LGLs has been demonstrated (6, 7). A case of PRCA with the clonal expansion of T-LGLs of γδ-type has been described in which the malignant LGLs were shown to carry functional inhibitory MHC class I receptors, killer cell inhibitory receptors (KIRs) (6). The cytotoxic T cells and NK cells express functional KIRs and destroy erythroid progenitors that show the downregulation of HLA class I expression, while myeloid cells are protected from lysis because they express higher levels of class I than the erythroid cells, leading to effective negative signaling via KIRs (6, 7). NK cells do not express TCR, but may be positively triggered by circulating antibodies against red-cell antigens activating NK cell receptors (6).

The proliferated LGLs are polyclonal or monoclonal, and the clinical course of PRCA complicated with LDGL usually takes the chronic, prolonged clinical course with a favorable prognosis (1-3, 21). LGL leukemia cases expressing CD56, whether of an NK (CD3-) or T-cell (CD3+) origin have acute leukemia like aggressive clinical features (1, 21). The LGL leukemia in the present patient evolved at the terminal of the clinical course, with the monoclonal proliferation as evidenced by the clonality from the cytogenetic study and the analysis of TCR genes.

Infection with EBV has been implicated in the development of a variety of malignancies, including Burkitt lymphoma, B-cell lymphoproliferative disorders associated with severe immunodeficiency, Hodgkin’s disease, certain types of T- and NK-cell lymphomas and nasopharyngeal carcinoma (10-15). In the present patient EBV genomic DNA was detected in the bone marrow cells, and suggested the link of EBV infection with the pathogenesis of the clonally evolved LGL leukemia. However, the precise pathogenetic significance of this finding is still unknown, since the localization of EBV in these cells was not examined by in situ hybridization for EBV RNA, immunoblotting for EBV genome products of EBV nuclear antigen (EBNA) and latent membrane protein (EBV-LMP) or clonal EBV episomal DNA.

The cause of PRCA is frequently immunological pathogenesis, and treatment for anemia in PRCA usually involves immunosuppressants, corticosteroids, cyclosporine and cyclophosphamide, and more than half of the patients respond to these treatments (1-7). The anemia in the present patient was refractory to the administration of cyclosporine and prednisolone. Indeed, PRCA patients with cytogenetic abnormalities as in the present patient, retrospectively considered, do not respond to immunosuppressive therapy (4).

Fulminant T/NK LDGL after acute/chronic EBV infection associated with HPS presents with fever and hepatosplenomegaly and leads to a poor prognosis (1, 18, 21). In the present patient, the clinical course after HPS appeared was rapid and aggressive, the administration of methylprednisolone was ineffective, and the patient died before other therapies, including chemotherapy and hematopoietic stem cell transplantation (13), could be started. In the present patient, it was considered that finally the fatal HPS complication was presumably caused by EBV reactivation in the immunodeficiency state with the administration of immunosuppressants of cyclosporine and prednisolone.

References

2. Dhodapkar MV, Li C-Y, Lust JA, Tefferi A, Phyliky R. Clinical


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