Pure Red Cell Aplasia and Autoimmune Hemolytic Anemia Sequentially Occurring in a Patient with Large Granular T-lymphocytic Leukemia

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Abstract

Pure red cell aplasia (PRCA), a type of anemia, occurred in a 50-year-old man six weeks after a splenectomy. It was successfully controlled by ciclosporin A (CsA) treatment. However, the onset of acute autoimmune hemolytic anemia (AIHA) developed one week after the CsA had been tapered off. Fortunately, the AIHA responded well to a high-dose methylprednisolone and immunoglobulin treatment. Unexpectedly, the patient suffered from severe pulmonary infection three months after the AIHA therapy. Four months later, he completely stopped the methylprednisolone. The disease was diagnosed as large granular T-lymphocytic leukemia by T cell receptor gene rearrangement, a surface marker examination and immunohistochemical staining. To our knowledge, no similar cases have previously been reported in the literature.

Key words: pure red cell aplasia, autoimmune hemolytic anemia, splenectomy, large granular T-lymphocytic leukemia

Case Report

The patient was a 50-year-old man who had a five-year history of chronic hepatitis B and was treated with oral adeefovir dipivoxil. A splenectomy and cardia gastric devascularization had been performed two years previously based on the diagnosis of hepatitis B with compensated liver cirrhosis, portal hypertension and hypersplenism by CT scanning (Fig. 1). The baseline preoperative complete blood count (CBC) was as follows: white blood cells (WBC), 1.17×10^9/L (neutrophils, 0.23×10^9/L; lymphocytes, 0.80×10^9/L); hemoglobin (Hb) level, 80 g/L; red blood cells (RBC), 2.56×10^12/L; and platelets (PLT), 38×10^9/L. All other parameters, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were within their normal ranges. Bone marrow cytology suggested hypersplenism. The postoperative pathology of the spleen was in accordance with the histology of congestive splenomegaly. CBC determined one week after surgery revealed that all parameters had returned to their normal ranges (WBC, 6.87×10^9/L; neutrophil count, 4.33×10^9/L; lymphocyte count, 1.53×10^9/L; Hb, 123 g/L; and PLT, 300×10^9/L).

The patient developed symptoms of fatigue, pallor, palpitations and shortness of breath on exertion at six weeks after surgery. His Hb level gradually decreased from 130 g/L to 45 g/L within 10 weeks after surgery, with RBC, 1.36×10^12/L; hematocrit (HCT), 17%; MCV, 101fL; and MCH, 36 pg. The proportion of reticulocytes was only 0.78%, with an absolute count of 1.06×10^9/L. The WBC count was 9.06×10^9/L, with neutrophils constituting 43% and lymphocytes 48.6%. The PLT counts were within the normal range. The tests for rheumatoid factors including anti-streptolysin-O (ASO), rheumatoid factor (RF), antinuclear antibodies (ANA), extractable nuclear antigen (ENA), anti-double-stranded DNA antibodies and anti-phospholipid antibodies were negative. A human parvovirus B19 examination was negative. Hepatitis B virus (HBV)-DNA quantitation was within the normal range and liver and kidney functions were normal, with normal concentrations of serum bilirubin. The Coombs test was also negative. T-lymphocyte subsets ana-
Liver and renal function tests were normal and the serum total and indirect bilirubin levels were 177.7 μmol/L and 165.4 μmol/L, respectively. The concentration of lactate dehydrogenase was 1,375 U/L; serum lipids, myocardial enzymes and troponin were within their normal ranges, and the blood coagulation profile was also normal. The rheumatological tests were still negative, including ASO, RF, ANA, ENA and anti-double-stranded DNA antibodies. The human parvovirus B19 test was still negative. Both direct and indirect Coombs tests were positive (type IgG-C3d), and a high titer of warm auto-antibodies was detected in the serum. The expression of CD55 and CD59 on erythrocytes and neutrophils was normal. An analysis of T-lymphocyte subsets showed that CD3-, CD4- and CD8-positive cells represented 39.24%, 17% and 21.29% of the total lymphocytes, respectively, and the ratio of CD4/CD8-positive cells was 0.8. T cell receptor (TCR) and IgH gene rearrangements of peripheral blood (PB) tested by a polymerase chain reaction (PCR) were negative. Immunoglobulin quantitation was as follows: IgG, 8.62 g/L (normal range, 7-16); IgA, 0.51 g/L (normal range, 0.7-4); IgM, <0.17 g/L (normal range, 0.4-2.3); complement C3, 0.75 g/L (normal range, 0.9-1.8); and complement C4, <0.067 g/L (normal range 0.1-0.4). The bone marrow cytology showed a cellular marrow with an actively proliferating erythroid lineage. The erythroblasts at different stages of development were increased in numbers and had an approximately normal morphology. Granulocytes and megakaryocytes were normal. The diagnosis of a tumor was ruled out by a systemic CT examination. The patient was diagnosed with acute autoimmune hemolytic anemia (AIHA). Washed red blood cell infusion was attempted, but could not be administered because repeated blood cross-matching tests failed to confirm the compatibility between the donor and the recipient. The patient’s Hb level rapidly declined to 27 g/L, and the administration of methylprednisolone (500 mg/d) and intravenous immunoglobulins (25 g/d) was applied for three days. The patient was then treated with a red blood cell transfusion after a successful cross-matching test. The patient’s anemia and symptoms gradually improved and the methylprednisolone was reduced to an oral dose of 60 mg/day. The patient was discharged and the steroid dose was completely tapered off in three months.

Unexpectedly, the patient was admitted again because of severe pulmonary infection and respiratory failure three months after AIHA diagnosis and therapy. He was given anti-fungal, anti-bacterial and anti-viral treatments for one month and eventually recovered from the infection. During this time, lymphocytes increased gradually in PB after withdrawal of the steroid. An analysis of T-lymphocyte subsets showed that CD3-, CD4- and CD8-positive cells represented 94.71%, 14.65% and 78.27% of the total lymphocytes, respectively. Large granular lymphocytes constituted 15% of total lymphocytes in peripheral blood. However, tests re-checking the presence of TCR and IgH gene rearrangements were still negative. Because the disease did not fulfill the diagnostic criteria of large granular lymphocytic leukemia.

The patient had no signs of gastrointestinal hemorrhage and was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia and was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination. The patient was diagnosed with acquired pure red cell aplasia (PRCA) supported by both the clinical pictures and the laboratory tests. Tumors possibly relevant to PRCA, such as thymoma and other solid tumors, were ruled out by a systemic CT examination.
reactive lymphocytosis, TCR gene rearrangement, including in a blood smear (Fig. 2). In order to distinguish whether granular lymphocytes constituted 55% of total lymphocytes, CBC was as follows: WBC, 16.5×10^9/L; neutrophils, 21%; lymphocytes 76%; Hb, 144 g/L; and PLT, 196×10^9/L. Large granular lymphocytes constituted 55% of total lymphocytes in a blood smear (Fig. 2). In order to distinguish whether the lymphocyte proliferation involved a neoplastic disease or reactive lymphocytosis, TCR gene rearrangement, including TCR-β, TCR-γ and IgH gene, was analyzed by PCR for a third time. Finally, we found a gene rearrangement in a region of 188.06bp of TCR-γ, involving Vy9-JyL3/2.3. TCR-α, TCR-β and IgH gene rearrangements were negative. Flow cytometry immunophenotyping of PB cells indicated the following expression: TCRα/β+, CD2+, CD3+, CD4+, CD5+, CD7+, CD8+, CD16+, CD56+-/-, CD57+ and CD25-. In order to determine whether the tissue of the spleen subjected to a splenectomy two years previously indicated leukemic involvement or congestive spleen, immunohistochemical staining of a formalin-fixed spleen specimen was undertaken. We found the invasion of abnormal large granular T lymphocytes with the following expression: CD2+, CD3+, CD7+, CD5+, CD4+, CD8+, CD57+ and TIA-1+. (Fig. 3). The final diagnosis of the disease was PRCA and AIHA sequentially occurring in a patient with T-LGLL.

Surprisingly, five months after the patient had stopped taking the immunosuppressive medicine, he again suffered from acute AIHA. The clinical signs were the same as those at the first occurrence of AIHA. A test for plasma haptoglobin at the onset of AIHA was normal and the level of plasma free hemoglobin was 55.7 mg/L (0-50 mg/L). Direct and indirect Coombs tests were still positive (type IgG-C3d). Methylprednisolone (500 mg/d) and intravenous immunoglobulins (25 g/d) were applied for five days. The patient’s anemia and symptoms gradually improved and the methylprednisolone was gradually reduced. The patient is currently taking CsA for the treatment of T-LGLL.

Discussion

PRCA is a heterogeneous syndrome characterized by the failure of bone marrow erythropoiesis or disorders affecting only the precursors of red blood cells. It is classified as congenital or acquired PRCA. In the current patient, gradual exacerbation of anemia occurred over the six weeks after a splenectomy due to compensated liver cirrhosis and portal hypertension, with reductions in the absolute reticulocyte number and bone marrow erythropoiesis, as well as a significant decrease of normoblasts, all of which led to the diagnosis of PRCA. There are multiple causes of acquired PRCA, including infection with human parvovirus B19 or hepatitis virus, lymphoproliferative disorders, thymoma and other tumors, and it can also be induced by certain drugs (1). The acute arrest of hematopoiesis caused by human parvovirus B19 usually resolves without intervention after a few weeks. Viral hepatitis-induced PRCA is relatively rare. Although a few cases of PRCA caused by hepatitis A (2-5) or C (6) have been reported, cases caused by hepatitis B have not. In the current case, the patient had taken oral adefovir dipivoxil tablets for five years to treat hepatitis B; however, to date, adefovir dipivoxil tablet-induced PRCA has not been reported. The final diagnosis of the patient was T-LGLL, so we think PRCA maybe one of the manifestations of T-LGLL in this patient.

However, acute AIHA first occurred one week after CsA had been tapered off. The second occurrence of acute AIHA followed five months after steroid had been completely stopped. AIHA is an acquired type of anemia caused by immune dysregulation, resulting in the generation of auto-antibodies directed against the patient’s own RBC. RBC lysis is triggered when these auto-antibodies bind to cell surface antigens and activate the complement system. To date, no more than 20 reports have been published that address PRCA concurrent with AIHA. In these cases, the causes were also multiple and related to lymphoma (7-11), thymoma (12), parvovirus B19 infection (13), hepatitis A virus infection (4, 5) and bone marrow transplantation (BMT) (14).

We attempted to identify the pathogenesis of PRCA and AIHA sequentially occurring in this patient. However, TCR and IgH gene rearrangements were negative in the first two examinations. PB smear did not find a sufficient number of large granular lymphocytes. A splenectomy is one option for the treatment of PRCA refractory to treatment with immune inhibitors and a thymectomy (15-19). A splenectomy is also a widely recognized and effective therapy for AIHA. However, to date, no reports have been published describing the development of PRCA following a splenectomy or the progression to AIHA after the successful treatment of PRCA. Both of these autoimmune diseases are the result of a series of immune dysfunctions.

Since the disease was complicated and its progression was changeable, the patient was closely followed up for a long
time. After the steroid was completely stopped for four months because of a severe infection, the lymphocytes clearly increased and large granular lymphocytes constituted 55% of the total lymphocytes in a PB smear. Further examination by immunophenotyping and molecular analyses confirmed the diagnosis of T-LGLL. By immunohistochemical staining of a formalin-fixed spleen specimen, the invasion of abnormal large granular T lymphocytes was found, which indicated that T-LGLL was one of the causes of the splenomegaly.

Large granular lymphocyte (LGL) disorders include a spectrum of conditions, ranging from polyclonal to clonal indolent and/or overt leukemic LGL proliferation. The disease usually affects older people (mean 60 years). It is asymptomatic in nearly 30% of cases, with lymphocytosis representing the only observed hematological abnormality. Various diseases and viral infections may be associated with T-LGLL. However, there were no reports about HBV hepatitis accompanied by T-LGLL. Most cases involve clonal expansions of TCRα/β+ LGL displaying a CD8+ phenotype with the expression of cytotoxic T-cell antigens (CD57, CD16, TIA-1, perforin and granzyme B) (20). Evidence of granular lymphocytosis greater than 2,000/L lasting for more than six months has been generally accepted as the most relevant criterion for the diagnosis of LGLL (21). However, the absolute number of LGL is not critical for the diagnosis of LGLL. We emphasize that a multiparameter analysis including clinical, hematologic, immunologic and molecular data should be used for the diagnosis of LGLL. The literature also indicates that a Southern blot analysis can be used for the diagnosis (21). Considering that PCR is accurate enough for diagnosis and is also much more robust for clinical applications, we used PCR as the diagnostic standard.

Chronic LGL proliferations can arise from either cytotoxic T-cell lymphocytes or NK-cell lymphocytes. Individuals with either type of cell proliferation have a good prognosis and respond quite well to currently available immunosuppressive therapies. Because the current patient had received immunosuppressive therapy for PRCA and AIHA for at least 15 months, which is also the right regimen of LGLL therapy, the T-cell clone is no longer detectable by a PCR analysis during the immunosuppressive therapies.

Intriguingly, the disease progression in the current case was complicated and changeable (Fig. 4, Table). First, there was the diagnosis of hepatitis B with compensated liver cirrhosis, portal hypertension, hypersplenism and pancytopenia. Second, PRCA occurred six weeks post-splenectomy and an effective therapeutic outcome was achieved by CsA treatment. Third, the sudden onset of AIHA occurred twice after withdrawal of the immunosuppressive medicines, which was treated effectively by glucocorticoid and immunoglobulin. Fourth, there were severe pulmonary infection and respira-
Figure 4. The time course of WBC, Lymphocytes and HB.

Table. The Course and Progression of the Disease in the Patient.

|                          | WBC (*10^9/L) | Hb (g/L) | PLT (*10^9/L) | Surface marker | PCR (TCR and IgH) | Bone Marrow Or Blood Smear | Other Exam | Treatment                      |
|--------------------------|---------------|----------|---------------|----------------|------------------|---------------------------|------------|--------------------------------|
| before splenectomy       | 1.17 (N:0.23, L: 0.80) | 80       | 38            | Not acquired   | Not acquired     | Hypersplenism             | Liver cirrhosis, Portal hypertension | Splenectomy |
| after splenectomy         | 6.67 (N:4.33, L:1.53) | 123      | 300           |                |                  |                           | Hypersplenism, Esophageal varices, Spleen pathology was congestive splenomegaly |            |
| PRCA                     | 9.06 (N:3.89, L:4.40) | 45       | 226           | CD3 52.50%     | Not acquired     | The erythroblasts at different stages of development were increased in numbers in bone marrow | PET-CT was normal Bone marrow ECT was normal |            |
| First AIHA               | 36.80 (N:28.30, L:4.05) | 40       | 140           | CD3 39.24%     | TCR gene rearrangement (-) | The erythroblasts at different stages of development were increased in numbers in bone marrow | Coombs’ test (+) CD55,CD59 (-) Rheumatological tests(-) | Methylprednisolone was tapered off in 3 months |
| Pulmonary infection       |               |          |               | CD4 17.00%     | TCR gene rearrangement (-) | LGL occupied 15% of total lymphocytes in PB | Fungal spine in sputum | Anti-infection treatment for 1 month |
| Follow-up                | 16.90 (N:3.30, L:12.70) | 144      | 196           | CD1 94.71%     | TCR gene rearrangement (-) | LGL occupied 55% of total lymphocytes in PB |   | No treatment for 4 months |
| Second AIHA and T-LGLL   | 17.00 (N:7.50, L:9.70) | 54       | 210           | CD2+, CD4+, CD7+, CD8+, CD56+/-, CD57+, TCRαβ+ | TCR-γ gene rearrangement (+) | LGL occupied 50% of total lymphocytes in PB | Coombs’ test (+) plasma haptoglobin (+) plasma free hemoglobin was 55.7mg/L CD55,CD59 (-) | Methylprednisolone |

The patient was at risk of secondary infections because of LGLL and immunosuppressive therapies. The course and progression of the disease in the current patient suggest that LGLL has a chronic indolent clinical course. The disease may remain asymptomatic for many years in the majority of patients. A splenectomy may be considered as an adjuvant in LGLL patients with relevant splenomegaly and refractory cytopenia. Cytopenia (either neutropenia or anemia) is quite common. The leukocyte count may be normal or slightly elevated, but in most patients, there is an increase in circulating LGL, even without having absolute lymphocytosis; some cases are lymphopenic. In discrete patients, lymphocytosis develops after splenectomy (22).

In summary, to date, this is the first report of PRCA and AIHA sequentially occurring in a patient with T-LGLL. Once patients with LGLL begin treatment, the regimen should not be altered for a period of four months, and they must be closely observed by careful observation of complete...
blood counts (23).

The authors state that they have no Conflict of Interest (COI).

Xuemei Qin and Yuan Yu contributed equally to this work.

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