Effects of Immunosuppressive Therapy in a Patient with Aplastic Anemia-Paroxysmal Nocturnal Hemoglobinuria (AA-PNH) Syndrome during Ongoing Eculizumab Treatment

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Abstract

A 65-year-old woman experienced a hemolytic attack triggered by sepsis. She presented with markedly increased CD55−CD59− erythrocytes and the signs of bone marrow failure, which led to a diagnosis of aplastic anemia-paroxysmal nocturnal hemoglobinuria (AA-PNH) syndrome. There was a possibility of increasing hemolysis, as large PNH clones remained after immunosuppressive therapy (IST). Accordingly, eculizumab was first used to control the hemolytic attack followed by IST with antithymocyte globulin and cyclosporine A. The patient was successfully weaned from blood transfusions and has been followed up without any recurrence of hemolytic attacks.

Key words: paroxysmal nocturnal hemoglobinuria, aplastic anemia, eculizumab, antithymocyte globulin


Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells that are abnormally sensitive to attacks by the complement system. The clonal expansion of a hematopoietic stem cell with an acquired mutation in the PIG-A gene is the pathobiological mechanism underlying the development of PNH (1-3).

Hematopoietic stem cell transplantation is the only curative therapy for PNH. However, due to the risks associated with transplantation, the three major pathophysiological features of the disease, including hemolysis, bone marrow failure (BMF) and thrombosis, are symptomatically managed primarily with blood transfusions, steroids and warfarin, with little success (4, 5). In recent years, eculizumab, a humanized monoclonal antibody that binds specifically to human complement protein C5 and inhibits the formation of the terminal complement complex, has been clinically used to prevent the hemolysis associated with PNH (6-8).

Aplastic anemia (AA)-PNH syndrome was initially reported as a case of aplastic anemia presenting with symptoms characteristic of PNH during the course of the disease (9). There have also been reports of patients with pre-existing clinical symptoms of PNH at the onset of AA (10-14); in some cases, the size of the PNH clones may be significantly large. In these cases, there is a potential risk of inducing hemolysis during immunosuppressive therapy (IST) due to complement activation related to infection or xenoantibody-associated reactions (11, 12). These patients may benefit from eculizumab (13, 14). However, to date, no cases of AA-PNH syndrome treated with a combination of eculizumab and IST have been described in detail as a case report.

We herein report the case of a patient with AA-PNH who developed a hemolytic attack triggered by an infection. IST with antithymocyte globulin (ATG) and cyclosporine A (CsA) was initiated during ongoing eculizumab treatment, which allowed the patient to be successfully weaned from blood transfusions.
Case Report

A 65-year-old Japanese woman developed a fever, malaise, dorsal pain and chills four days before her first visit to our facility in October 2010. These symptoms gradually worsened, and she was admitted to our hospital. The physical examination findings revealed a body temperature of 38.9°C and an anemic and icteric conjunctiva. The patient’s urine was “port wine” in color. She also presented with petechiae and purpura on her extremities. A peripheral blood examination indicated pancytopenia: WBC count, 0.50×10⁹/L (neutrophils, 18.0%; lymphocytes, 70.0%); RBC count, 960×10⁹/L; Hb, 3.7 g/dL; Ht, 10.8%; platelet count, 9×10⁹/L; and reticulocytes, 2.3%. No morphological abnormalities were observed upon examination of a peripheral blood smear. A bone marrow aspiration showed hypocellular marrow with a predominance of plasma cells and reticulum cells, suggesting AA or hemophagocytic syndrome. The bone marrow cells displayed no morphological abnormalities, and a karyotypic analysis indicated normal 46,XX, which made a diagnosis of myelodysplastic syndrome unlikely. Other laboratory findings were as follows: C-reactive protein (CRP), 29.2 mg/dL; T-Bil, 5.9 mg/dL; D-Bil, 2.7 mg/dL; asparate aminotransferase (AST), 89 IU/L; alanine aminotransferase (ALT), 30 IU/L; lactate dehydrogenase (LDH), 1,118 IU/L (normal range: 120-230); blood urea nitrogen (BUN), 42 mg/dL; Cr, 1.5 mg/dL; and haptoglobin, <10 mg/dL, which indicated inflammation, hemolysis and renal dysfunction. Of the tests performed for hemolysis, the Coombs test was negative and the Ham test was positive. Consequently, a test for the surface markers of peripheral blood erythrocytes was performed, which revealed 27% CD55− CD59− erythrocytes (Fig. 1A). Based on these findings, the patient was diagnosed with PNH. As Escherichia coli was isolated from both urine and blood culture samples, we concluded that the elevated PNH-type blood cells had previously existed and that sepsis induced the PNH-associated hemolytic attack and hemophagocytic syndrome.

Since the patient was under septic shock and exhibited marked pancytopenia with hemolysis, systemic management with intravenous fluids and catecholamines was initiated accompanied by treatment with antibiotics, granulocyte colony-stimulating factor (G-CSF), erythrocyte transfusions, platelet transfusions, methylprednisolone (mPSL) pulse therapy and haptoglobin. The urinary tract infection and sepsis subsided on the 13th hospital day, and the patient’s general condition stabilized. A bone marrow aspiration and biopsy were performed due to prolonged pancytopenia. Hypoplastic marrow and the absence of atypical cells were observed. The patient was diagnosed with AA-PNH syndrome based on the clinical course and test findings.

After vaccination against Neisseria meningitidis, treatment with eculizumab was started on the 77th day (eculizumab at a dose of 600 mg via an intravenous infusion every seven days for four doses, followed by a maintenance dose of a 900 mg eculizumab infusion every 14 days). The LDH level decreased from 400 to 100 IU/L three weeks after the start of the treatment accompanied by an increase in the Hb level from 5-6 g/dL to 6-7 g/dL (Fig. 2). The neutrophil and platelet counts remained below 0.50×10⁹/L and 20×10⁹/L, respectively, even after the start of the eculizumab treatment. Since the BMF was not markedly alleviated (Fig. 2), IST using ATG and CsA for BMF was considered. Due to the persistent neutropenia, a subcutaneous injection of G-CSF (lenograstim 100 μg/day) was administered on the 124th day. Because the sixth administration of eculizumab was given five days before the start of the ATG treatment, we considered the possibility of weakened ATG activity. However, in practice, the patient’s lymphocyte count decreased sharply from 1.46×10⁹/L imme-

Figure 1. Immunophenotypic analysis of red blood cells using flow cytometry. A: The size of the PNH clone (CD55− CD59−) was 27% at the onset of the hemolysis attack. B: The size of the PNH clone was 90% 19 months after the eculizumab treatment. PNH: paroxysmal nocturnal hemoglobinuria
diately before the ATG treatment to 0.03×10^9/L on day 2 of the ATG treatment. On the other hand, the neutrophil count did not decrease markedly after the start of the ATG treatment, which may have been due to the influence of the G-CSF treatment. On day 8 of the ATG treatment (the 131st day), the administration of G-CSF was suspended because the neutrophil count increased (>3.0×10^9/L) (Fig. 3). Starting on the 156th day, CsA was administered at a dose that would maintain its trough level at 150 to 250 ng/mL. The patient subsequently followed an uneventful course without developing any severe infections. From the 183rd day, the Hb level was maintained over 6 g/dL and the platelet count was maintained over 30×10^9/L without blood transfusions. Prednisolone (PSL) therapy at a dose of 5 mg/day was started on the 196th day.

The patient was discharged from the hospital approximately seven months after being admitted. Since then, she has been managed as an outpatient and is receiving continuous treatment with eculizumab, CsA and PSL. Her peripheral blood neutrophil count remains low (0.3×10^9/L to 0.7×10^9/L), and G-CSF continues to be administered when the count falls below 0.5×10^9/L. The RBC and platelet counts have been gradually increasing; the Hb level has been maintained at approximately 10 g/dL and the platelet count has been maintained over 100×10^9/L since one year after the disease onset. The patient has been followed without any events to date (two years and two months after disease onset at the time of writing). When the levels of peripheral blood erythrocyte surface markers were analyzed 19 months after the start of the eculizumab treatment, the percentage of CD55 CD59 cells was 90%, which was higher than the percentage (27%) recorded during the first examination (Fig. 1B). Therefore, in the present case, normal hematopoi-esis was not sufficiently restored, even after the administra-

| Figure 2. The patient’s clinical course. A: Lactate dehydrogenase (LDH), B: Platelets (solid line) and hemoglobin (Hb, dotted line). |
| Figure 3. Changes in the neutrophil and lymphocyte counts with ATG treatment. The solid and dashed lines indicate neutrophils and lymphocytes, respectively. |

Discussion

Patients with AA-PNH syndrome are commonly treated using IST in a manner similar to that for AA, and there is no evidence that treatment with IST influences clonal expansion either positively or negatively (14). Most patients with AA-PNH syndrome have small PNH clones and require no specific PNH therapy prior to IST. In rare cases, significant amounts of the PNH clone remain even after IST and treatment, including eculizumab therapy, for the complications of PNH is required (11-17). Scheinberg et al. reported that seven of 14 patients with a PNH clone greater than 50% exhibited hemolysis or thrombosis after IST and required treatment using eculizumab (two patients) and/or oral anticoagulation (six patients) (13). Nakasone et al. administered ATG therapy in four PNH patients with cytopenia, and the anemia improved in all cases; however, three patients demonstrated hemolytic exacerbation and thrombocytopenia during ATG administration, which may have been due to xenoantibody-associated complement activation (11). In a conference presentation, five cases were reported in which IST was initiated during ongoing eculizumab treatment, and three of the five patients demonstrated a partial response to IST added to eculizumab (17).

In the present case, the results of a flow cytometry analysis (Fig. 1A), the high LDH level and the patient’s history of a severe hemolytic attack induced by infection suggested that large PNH clones may have remained and marked hemolysis may have recurred if IST for BMF had been applied without reducing the hemolysis. The potential risk of intravenous ATG in inducing xenoantibody-associated complement activation must be considered, especially in this patient (11, 12). As IST induces a severe decline in immune competence, infection-associated complement activation was also considered in this case. To prevent these problems, eculizumab was first administered, followed by ATG/CsA therapy after achieving a reduction in the LDH level and fre-

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quency of blood transfusions. In this manner, the BMF was alleviated without inducing a hemolytic attack.

Furthermore, since eculizumab is known to have immunosuppressive effects, such as reducing the immune function against *N. meningitidis*, we anticipated that eculizumab therapy with ATG and CsA might increase the risk of severe infection due to immunodeficiency. CsA treatment was then initiated approximately one month after the start of the ATG treatment, rather than starting both drugs simultaneously. This management may have allowed the patient to remain free of severe infection.

Eculizumab does not suppress PNH clone formation per se. In a phase III study of eculizumab, treatment of PNH with eculizumab resulted in a significant increase in the percentage of CD55-CD59 erythrocytes and escape from hemolysis (18). In another study, when patients with AA-PNH syndrome received ATG/CsA therapy without concomitant eculizumab treatment, most cases showed no increases in PNH clones (13). In the present case, the anemia decreased in response to preceding eculizumab treatment and the subsequent concomitant use of ATG/CsA; however, the percentage of CD55 CD59 erythrocytes in the peripheral blood markedly increased. Furthermore, CD55 CD59 granulocytes were also predominant (>90%, data not shown) after the eculizumab treatment. Therefore, the hemapoiesis appeared to greatly depend on the presence of PNH-type hematopoietic stem cells, even after the disease was alleviated in response to IST.

In summary, we herein reported a case of AA-PNH syndrome in which eculizumab treatment combined with IST was effective and well tolerated. The findings obtained in this case are valuable for clarifying the features of AA-PNH syndrome.

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

References