Acquired Idiopathic Pure Red Cell Aplasia in a Hemodialyzed Patient with Inactive Systemic Lupus Erythematosus

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A male patient suffered chronic renal failure due to lupus nephritis and was undergoing hemodialysis. Six years after beginning hemodialysis, anemia developed, which improved by erythropoietin. Unresponsiveness to erythropoietin gradually appeared, and with a suspicion of pure red cell aplasia, he was treated with a high-dose corticosteroid but the unresponsiveness did not improve. Neither his serum nor lymphocytes inhibited erythropoiesis of either normal bone marrow stem cells or his own in vitro. These observations suggest an impaired hematopoietic microenvironment in his bone marrow.

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Key words: erythropoietin, corticosteroid, bone marrow stem cell

Introduction

We report a middle-aged male patient undergoing regular hemodialysis for 7 years due to lupus nephritis, and presenting with refractory anemia similar to pure red cell aplasia (PRCA). Clinical pictures and in vitro analysis of the bone marrow mononuclear cells indicated autoimmunity (1–4) or other known factors, i.e., thymoma, drugs, viral infections, or malignancy (1, 5, 6), and these factors did not seem to play a role in the pathogenesis of the present case of PRCA. Recently, human recombinant erythropoietin (EPO) has become available for patients who need regular dialysis, and almost all the anemia associated with chronic renal failure has been believed to be treatable with EPO (7–11). In spite of in vitro effectiveness of EPO, however, administration of high-dose EPO for 6 months was ineffective in the present case. To our knowledge, this is the first report of a hemodialyzed patient with inactive systemic lupus erythematosus who presented with idiopathic PRCA resistant to high-dose EPO and corticosteroid.

Case Report

A 43-year-old male hemodialyzed patient presented complaining of refractory anemia resistant to regular administration of EPO (9,000 U/week). He had been diagnosed with systemic lupus erythematosus (SLE) with polyarthritis, proteinuria, an-

tinuclear antibodies and LE cell 11 years before. He had been undergoing regular hemodialysis due to lupus nephritis for the 7 years since 1984. At the introduction of hemodialysis, his hemoglobin level was 7.5 g/dl.

Administration of 3,000 U/week EPO during regular hemodialysis was successfully introduced in this patient in February 1990 because of the diagnosis of normocytic and normochromic anemia. Since the level of hemoglobin began to decrease in January 1991 despite increases in the dose of EPO, blood transfusion was intermittently carried out. Discontinuing prescribed drugs, i.e., enalapril, ticlopidine, $\text{Al}(\text{OH})_3$, administration of iron, multiple vitamins, anabolic steroid, and increased doses of a corticosteroid were all ineffective. The number of peripheral reticulocytes had been below 1% of the peripheral total blood cell count, and the bone marrow aspiration and biopsy specimens revealed hypoplasia of immature erythroid cells with normal myeloid and megakaryocytic quantities (myeloblasts 2.0%, promyelocytes 0.8%, myelocytes-N 8.0%, metamyelocytes 12.6%, bands-N 21.0%, segs-N 31.0%, segs-E 4.2%, segs-B 0.4%, lymphocytes 14.0%, monocytes 4.6%, plasma cells 1.2%, reticulum cells 0.2%, pronormoblasts 0.2%, normoblasts-poly 0.2%, mitotic cells 0.2%, M/E ratio 200.0), indicating a possible diagnosis of PRCA (Fig. 1). No green coloration was detected in the bone marrow biopsy under polarized light microscopy after staining with Congo red. For further evaluation and treatment, he was admitted to our hospi-

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Fig. 1. Patients bone marrow biopsy specimen. General appearance is hypocellular. Immature erythroid cells are apparently hypoplastic, but the numbers and morphology of myeloid series as well as megakaryocytes are normal. Hematoxylin-eosin stain (HE stain, $\times 240$).

tal on August 2, 1991. On physical examination, he was a moderately developed, pale man. Conjunctivae were severely anemic, but not icteric. Neither thyromegaly nor cervical lymphadenopathy were present. Auscultation did not find any abnormalities in the lungs and the heart. Hepatosplenomegaly was absent, and no other abdominal masses were felt. An arteriovenous fistula was on his left arm. Neurological examinations revealed no abnormalities. Occult fecal blood was negative. The white cell count was 8,800 per microliter, red cell count was 2.48 million per microliter, hemoglobin level was 6.8 g/dl, the reticulocyte level was 1%, hematocrit was 21.2%. Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were 86 fl, 27.4 pg, and 32.1 g/dl respectively, and platelets were 530 thousands per microliter. Erythrocyte sedimentation rate was 82 mm/hr. The biochemical data were serum total protein 6.7 g/dl, serum albumin 2.6 g/dl, blood urea nitrogen 96.2 mg/dl, serum creatinine 14.5 mg/dl, glutamic oxaloacetic transaminase 12 IU/l, glutamic pyruvic transaminase 12 IU/l, lactic dehydrogenase 226 IU/l, alkaline phosphatase 165 IU/l, creatine phosphokinase 49 IU/l, amylase 478 IU/l, glucose 119 mg/dl, C-Reactive protein 0.18 mg/dl, sodium 137.1 mEq/l, potassium 5.3 mEq/l, chloride 104 mEq/l. Iron stores were plentiful (serum iron, total iron binding capacity, and ferritin were 244 μ g/dl, 254 mg/dl, and 1,700 ng/dl respectively). These biochemical data indicated the presence of chronic renal failure compensated skillfully by hemodialysis. Intravascular hemolysis was negligible because of the normal level of haptoglobin (564 mg/dl). The serum concentrations of inorganic phosphate (4.7 mg/dl), aluminium (3.5 μ g/dl) and parathyroid hormones (PTH-C 16.7 ng/ml, PTH intact 198 pg/ml, PTH-M 49,700 ng/ml) were above the normal range, but within the levels usually detected in hemodialyzed patients. A large dose of EPO was

administered just before each hemo-dialysis so the EPO serum level was high enough (936 mU/ml). Although diffuse and speckled type antinuclear antibodies were positive, the titer of anti-dsDNA antibodies was only 4.1 IU/ml.

Antiphospholipid antibody was 3.0 U/ml. LE test, RA test, anti Sm antibody, anti SS-A, and B antibody were all negative. Serum immunoglobulins G, A, and M were 1,259, 289, and 367 mg/dl respectively, and serum complement 3 and 4 were 50 and 40 mg/dl respectively. In spite of the repeated tests, anti-EPO antibodies were undetectable. Chest-radiographs, electrocardiogram, esophago-gastro-duodenography, chest- and abdominal-computed tomography were unremarkable. These findings and the normal serum levels of tumor markers suggested that malignancy was unlikely. Neither direct inhibitory factors in the serum nor suppression by T-lymphocytes were revealed by in vitro analysis of the bone marrow mononuclear cells, and the existence of responsiveness to EPO in the stem cells was suspected (see below). However, administration of high-dose EPO (9,000–27,000 U/week) for 6 months was ineffective.

Materials and Methods

Bone marrow mononuclear cells (MNC)

The bone marrow samples were obtained by posterior iliac crest aspiration from the patient and a healthy donor for bone marrow transplantation. Bone marrow MNC were isolated after Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation and suspended in alpha-MEM (GIBCO, Grand Island, NY, U.S.A.) supplemented with 1% penicillin and streptomycin. To separate T-lymphocytes from the MNC, sheep erythrocyte rosette formation method was used. Briefly, MNC were incubated with 10% (v/v) of sheep erythrocyte suspension in fetal calf serum (FCS) (GIBCO) for 60 minutes at room temperature, and then T-lymphocyte-depleted MNC were isolated from the interphase after Ficoll-Hypaque gradient centrifugation.

Burst forming units – erythroid (BFU-E) assay

Total MNC or T-lymphocyte-depleted MNC were plated at 2×10^5 cells/ml in 35 mm tissue culture dishes, each containing 1 ml mixture of alpha-minimum essential medium, 1.2% methyl cellulose (SIGMA, St. Louis, MO, U.S.A.), 30% FCS, 10% normal human serum, 1% deionized bovine serum albumin (SIGMA), 5×10^{-5} M 2-mercaptoethanol (SIGMA), 2 units of EPO, 10 ng of recombinant human granulocyte-colony stimulating factor (G-CSF), 10 ng of recombinant human interleukin-3 (IL-3). Each culture was done in four separate dishes. To investigate the influence of the patient's serum against MNC, 10% of the patient's stored serum was added instead of 10% normal human serum to the control mixture described above. Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO₂ in air. The number of BFU-E colonies (>40 cells/aggregate) were counted using an inverted microscope after 14 days of incubation.

Results

The colony assay for BFU-E were performed under various conditions. Although the data did not have statistical significance by Student's *t* test, the results seemed to be interpreted as below. With EPO, G-CSF and IL-3, a moderate number of erythroid bursts from the patient's total MNC was formed (Fig. 2), although fewer than that from the control total MNC. The number of colonies arising from the patient's T-lymphocyte-depleted MNC were fewer than that from the patient's total MNC. Otherwise, with the addition of the patient's serum, BFU-E formation of the patient's total MNC was apparently increased (Fig. 2), although the patient's serum did not affect BFU-E formation of the control total MNC.

Discussion

This report describes a rare case of a hemodialyzed patient with idiopathic PRCA. To our knowledge, the relationship between PRCA and chronic renal failure on regular dialysis has never been documented. Some patients with SLE presenting with PRCA were reported (12–17). Most patients with SLE show hematological disorders such as anemia, and the number of erythroid progenitor cells was often decreased in the bone marrow of anemic patients with SLE (18). Moreover, Otsuka et al. detected hematological abnormalities were also likely to occur at the multipotent stem cell level of such patients in vitro (19). As a cause of severe anemia, either circulating inhibitors of erythropoiesis including autoantibodies (20–22) or suppressor cellular autoimmunity (23–25) have been implicated, and clinical manifestations of such a hematological disorder usually appear with aggravation of SLE and is indicated by the increase in the titer of circulating anti-dsDNA antibodies.

Fig. 2. BFU-E colony assay of bone marrow MNC of the patient and a healthy control, with or without T-lymphocyte depletion or addition of the patient's serum. BFU-E colonies were moderately formed from the patient's total MNC, although fewer than those from the control total MNC. T-lymphocyte depletion similarly reduced the colony number of each MNC. Addition of the patient's serum increased the formation of BFU-E not from the control total MNC, but from the patient's total MNC. Data of the number of colonies are mean ± SE for assays in four separate dishes.

However, patients with end-stage renal failure undergoing regular dialysis due to lupus nephritis often show SLE decreased activity and are unlikely to manifest further disorders related to SLE (26, 27). Although there have been some exceptional cases with clinically active SLE receiving dialysis (26–29), they responded easily to administration of corticosteroids. Therefore, the PRCA that occurred in this patient treated with high-dose of corticosteroids did not seem to be dependent on autoimmunity associated with SLE. The activity of in vitro BFU-E formation of the patient's total MNC demonstrated that those cells could respond to hematopoietic factors supplemented in the culture medium in vitro. The difference in the erythropoietic activity of MNC of the present patient between in vitro and in vivo leads us to postulate that some suppressor factors and/or essential factors such as IL-3 existed, which were supplemented in the culture medium, and were insufficient in vivo. According to the observation of the in vitro study with T-lymphocyte depletion and the addition of the patient's serum, T-lymphocytes or circulating suppressor factors were unlikely to be associated with the present hematological disorder, which was incompatible with the usual findings of patients with PRCA and/or SLE (1–4, 12, 18–24). On the other hand, the activity of the patient's total MNC was lower than that of the control MNC (Fig. 2). This observation might be explained by abnormalities in the bone marrow stem cell itself, e.g., effects of uremic toxins (7), subclinical SLE-related disorders (18, 19) or persistent viral infection, especially by B19 parvovirus (1, 30), and the possibility that such abnormalities in the bone marrow stem cell itself played a role in the present case of PRCA cannot be ruled out. In addition, unknown hematopoietic factors other than EPO were also supposed to be in the patient's serum because the addition of the serum to the control medium which was fully supplemented with recombinant EPO induced marked BFU-E formation from the patient's total MNC. The control total MNC did not react with the patient's serum, leading to a speculation of enhanced reactivity to those factors in the patient's MNC that were masked in vivo. Marmont reviewed several treatments of PRCA (31), but some of them were ineffective and others involving immunosuppression were not applicable to this malnourished and immunocompromised patient. Meanwhile, Aoki and colleagues (32) reported in vitro responsiveness of bone marrow erythropoietic stem cells in aplastic anemia and myelodysplastic syndrome to EPO, and in vivo therapeutic potentials of EPO for these diseases were proposed. Although bone marrow cells of the present patient certainly responded to EPO in vitro, administration of high-dose EPO for 6 months failed to improve the anemic state. Anemia in patients with chronic renal failure occurs due to a number of causes (7, 33, 34), and Scigalla and colleagues (8) and Drueke (9, 10) reviewed several modulating factors involved in the hematopoietic response to EPO, but those factors could be eliminated and/or overcome by high-dose or long-term administration of EPO (11). Recently, it has been reported that systemic amyloidosis can reduce the hematopoietic response of hemodialyzed patients to EPO (35). Those modulating factors were negligible in the present patient, and we speculate that

some suppressor factors may work and/or some essential factors may be insufficient in the hematopoietic microenvironment, interrupting hematopoiesis in the bone marrow of this patient. Certainly, abnormalities in the hematopoietic microenvironment of patients with SLE were pointed out by Otsuka and colleagues (36), and they proposed that a diminished production of hematopoietic growth factors by bone marrow fibroblastoid cells as a result of a diminished activity of monocyte derived factors may be one cause of the hematocytopenia which occurs in patients with SLE. Their consideration was compatible with the present findings of the in vitro analysis, and it cannot be denied that similar pathogenetic mechanisms played a role in the present patient in spite of clinically inactive SLE.

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