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Akinori Hara, MD, PhD[1], Takashi Wada, MD, PhD[2], Shinji Kitajima, MD[1], Tadashi Toyama, MD[1], Toshiya Okumura, MD[1], Kiyoki Kitagawa, MD, PhD[1], Yasunori Iwata, MD, PhD[1], Norihiko Sakai, MD, PhD[1], Kengo Furuichi, MD, PhD[1], Masato Higuchi, PhD[3], Shuichi Kaneko, MD, PhD[1]

Departments of [1]Disease Control and Homeostasis, and [2]Laboratory Medicine, Graduated School of Medical Science, Kanazawa University, Kanazawa, Japan

[3]Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd, Shizuoka, Japan

Correspondence: Takashi Wada

Department of Laboratory Medicine, Graduate School of Medical Science, Kanazawa University

13-1 Takara-machi, Kanazawa 920-8641, Japan

phone +81-76-265-2000(ext 7270)

fax +81-76-234-4250

e-mail twada@m-kanazawa.jp

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Abstract

A 42-year-old woman with SLE was admitted to our hospital because of severe anemia. Her bone marrow was almost normocellular and erythroblasts were nearly absent. Laboratory data showed elevated levels of lactate dehydrogenase and positive findings on Coombs' tests. Based on these findings, her anemia was diagnosed as the overlap of PRCA with AIHA. Radioimmunoprecipitation assay revealed that her serum was positive for anti-erythropoietin antibodies before therapy. Furthermore, the autoantibodies inhibited proliferation of an EPO-dependent cell line in a dose-dependent manner. Immunosuppressive treatment improved the anemia accompanied with disappearance of the autoantibodies.

Introduction

Systemic lupus erythematosus (SLE) is a multiorgan disorder that commonly involves the hematological system [1]. Among hematological complications, anemia is common under such mechanisms of induction as anemia of chronic disease (ACD), iron deficiency anemia (IDA), autoimmune hemolytic anemia (AIHA), anemia of chronic renal insufficiency and drug-induced myelotoxicity [1, 2]. In addition, anemia in SLE may be accompanied by various syndromes of hematopoietic failure, such as aplastic anemia, hemophagocytic syndrome and rare pure red cell aplasia (PRCA) [1, 2].

We encountered a case of SLE accompanied with combined PRCA and AIHA, which is extremely rare. While searching for a mechanism responsible for the anemia in this case, anti-erythropoietin (EPO) autoantibodies were detected in the patient's serum. In 1997, Casadevall *et al.* reported the presence of autoantibody to EPO in a patient with transient PRCA who had anemia with erythroid hypoplasia [3]. In their patient, anti-EPO antibodies functionally blocked the interaction between EPO and EPO receptor, and were thus responsible for the impaired erythropoiesis [3]. The serum from our patient also inhibited EPO-dependent cell proliferation, suggesting that the anemia in this case may also be mediated by anti-EPO antibodies as one of its immunological mechanisms. This is the first case report of SLE with PRCA and AIHA associated with

functional anti-EPO autoantibodies.

Case report

A 42-year-old woman was admitted to hospital in November 1992 because of fatigue with severe anemia. She was first described as having thrombocytopenia in 1991. Thereafter, a diagnosis of SLE was made, based on the presence of bicytopenia and anti-nuclear antibody, elevated anti-DNA antibody titer and proteinuria in accordance with the American College of Rheumatology criteria for SLE [4]. There was no history of fever, rash or exposure to drugs or toxic agents thought to interact with erythrocytes or erythroblasts. Physical examination showed that her palpebral conjunctiva was anemic. Systemic computed tomographic scans showed no evidence of solid tumor, thymoma, or lymphadenopahty. The laboratory examination revealed marked anemia (red-cell count $2.67\times10^{6}/mm^{3}$, hemoglobin level 7.8 g/dl, reticulocyte count $1.3\times10^{4}/mm^{3}$) and thrombocytopenia (platelet count $9.2\times10^{4}/mm^{3}$). The erythrocyte sedimentation rate was 80 mm/h. Electrophoresis of serum proteins showed polyclonal gammopathy with a total gamma globulin concentration of 2.4 g/dl. The serum iron and ferritin levels were normal and elevated (157.8 ng/ml), respectively. C3 and C4 complement components were reduced. Direct and indirect Coombs' tests gave positive results with an elevation

of lactate dehydrogenase (540 IU/l). The serum EPO level was elevated at 585.0 MIU/ml. Anti-nuclear, anti-ds DNA and anti-platelet antibodies were positive. Anti-U1 RNP, anti-Sm and anti-phospholipid antibodies were all negative. Serology for human immunodeficiency virus, hepatitis, cytomegalovirus, Epstein-Barr virus, and parvovirus B19 was negative. The prothrombin time, activated partial thromboplastin time and fibrinogen level were all normal. Although renal function was normal, 24-h urinary protein excretion was 0.5 g. Bone marrow aspirate on admission showed almost normal representation of myeloid and megakaryocyte precursors, but erythroid precursors were nearly absent. Cytogenetic analysis of the marrow showed a normal female karyotype of 46 XX. Taken together, a diagnosis of SLE accompanied with PRCA and AIHA was made. On the 5th hospital day, the patient was treated with oral prednisolone at a dose of 40 mg/d. In addition, 3-day intravenous pulse therapy with 500 mg of methylprednisolone was added on the 11th, 18th and 25th hospital days. The anemia as well as the thrombocytopenia responded well to therapy, resulting in an increase in the hemoglobin level of 11.5 g/dl (Figure 1A). Concomitantly, an indirect Coombs' test became negative and, subsequently, a direct Coombs' test gave a negative result. Follow-up bone marrow aspiration revealed the appearance of erythroblasts (46.0% of 18.5×10^4 nucleated cells).

As it has been reported that autoantibodies to erythropoietin can be detected in

patients with SLE and/or PRCA [3, 5] and that the frequency of these antibodies is significantly higher in patients with severe anemia [5], the mechanisms underlying the anemia were examined in our patient using radioimmunoprecipitation assay for serum autoantibodies against erythropoietin as described previously [6]. The patient was positive for serum anti-erythropoietin autoantibodies before treatment, which disappeared 6 months after treatment.

To further examine the pathogenic role of anti-EPO antibodies, the patient's serum at the time of diagnosis was examined for its ability to inhibit EPO-induced cell proliferation. The EPO-dependent human erythroid cell line, AS-E2, was cultured in the presence of human EPO with the patient's serum or those from healthy controls [6, 7]. The serum from the patient inhibited proliferation of an EPO-dependent cell line in a dose-dependent manner, indicating the presence of EPO-neutralizing activity (Figure 1B). To evaluate the possibility of antierythrocyte antibody action against the differentiation and proliferation of erythroid progenitor cells, the patient's sera positive or negative for indirect Coombs' test, both of which were negative for anti-EPO antibodies, were examined for its ability to inhibit the EPO-induced proliferation of AS-E2. Unexpectedly, the patient's sera, positive or negative for the Coombs' test, similarly inhibited EPO-dependent cell proliferation (Figure 1C), suggesting the

presence of putative inhibitor(s) other than anti-EPO antibodies and antierythrocyte antibodies.

Discussion

This is the first report of the presence of autoantibodies to EPO and functional evidence in an SLE patient with combined PRCA and AIHA. According to the revised American College of Rheumatology criteria for SLE incorporating hemocytopenia [4], hematological abnormalities are common features of this disease [1]. Among these manifestations, anemia is found in about 50% of patients, with chronic anemia being the most common form [2]. While AIHA occurs in 7–15% of the patients, iron deficiency anemia, drug-induced myelotoxicity and anemia of chronic renal failure have also been reported [1]. On the other hand, PRCA associated with SLE is rare [2]. The combination of PRCA with AIHA in a patient with SLE was first described by Meyer *et al.* in 1978 [8]. Since then, there has been only 1 case in which anti-EPO antibodies were detected [5, 9]. Therefore, we have summarized these patients in Table 1.

One of the causes of EPO resistance is the presence of antibodies against EPO, which inhibit binding of EPO to its receptors and interfere with the differentiation of erythroid progenitor cells [3]. In a recent study, anti-EPO antibodies were detected in SLE

patients mainly with severe anemia and active disease [5]. In addition, it has been reported that the frequency of anti-EPO antibodies in patients with SLE is about 15% [5] and that in SLE patients with anemia is 21% [10]. On the other hand, another study reported that 46% of patients with SLE have anti-EPO antibodies but at least a significant proportion of these antibodies in SLE were not functional, since there was no relation to the degree of anemia [11]. Thus, its functional role in inducing anemia in SLE has not been elucidated. In the present study, we demonstrated that the patient's serum contained anti-EPO antibodies blocked the proliferation of an EPO-dependent cell line in a dose-dependent manner, and that interferon-γ and tumor necrosis factor-α, proinflammatory cytokines which promoted an apotosis of erythroid progenitor cells (data not shown). In addition, antierythrocyte antibodies which could interfere with the differentiation and proliferation of erythroid progenitor cells [8] were not detected in the serum. Interestingly, existence of putative inhibitor(s) against the erythroid progenitor cells in the patient's serum other than anti-EPO antibodies was suggested. These results suggest that anti-EPO antibodies in the present patient have a biological role in neutralizing erythropoiesis.

A blunted EPO response in patients with SLE has been considered to be a leading factor in anemia in SLE [11]. Our patient presented with elevated levels of EPO on

admission. The presence of anti-EPO antibodies in the patient's serum was studied by the competitive radioimmunoassay (RIA) in the present study. It has been known that serum EPO determination by competitive RIA results in the rather high levels of EPO due to the existence of antibodies that will compete for tracer binding with antiserum used in RIA [6]. On the other hand, Schett *et al*. reported the EPO levels measured by ELISA and the interference by anti-EPO antibodies in the measurement of EPO [11]. Taken together, it may be difficult to measure real serum EPO level in anti-EPO antibody-positive patients, and a careful interpretation of the EPO level in these patients may be required.

Our patient responded to immunosuppressive therapy including corticosteroids. As a result, the anemia improved gradually accompanied with the disappearance of the anti-EPO and antierythrocyte autoantibodies. It has been reported that the presence of inhibitory autoantibodies is typically related to SLE activity and can be suppressed by successful treatment [2]. Taken together, it is suggested that the coexistence of at least two different autoantibodies to the erythroid series contributed to the anemia in our patient, as reported previously [12, 13], and that these antibodies were effectively regulated by treatment with immunosuppressive drugs.

In summary, we described functional evidence of autoantibodies to EPO in an SLE

patient with PRCA and AIHA. This case may provide a basis for the pathogenesis of the immune-mediated anemia in SLE and for further investigation of the regulation of erythropoiesis in hematological disorders.

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Figure legends

Figure 1

A. Clinical course of the patient and response to treatment. Reticulocyte percentage and hemoglobin levels are reported in the graph. Abbreviations: PSL, prednisolone; mPSL, methylprednisolone; MZB, mizoribine.

B. Neutralization of erythropoietin activity by the anti-erythropoietin antibodies in the patient' serum studied by inhibition of proliferation of the erythropoietin-dependent cell line, AS-E2. Normal control (○), patient's serum before treatment (■), patient's serum after treatment (□).

C. Effect of the patient's serum positive or negative for the indirect Coombs' test on the proliferation of AS-E2 cells. patient's serum negative for Coombs' test (■), patient's serum positive for Coombs' test (◆)