Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.4467-20
Intern Med 59: 2041-2045, 2020
http://internmed.jp

【 CASE REPORT 】

Pembrolizumab-induced Pure Red Cell Aplasia Successfully Treated with Intravenous Immunoglobulin

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Abstract:
We herein report a 64-year-old man who was treated with pembrolizumab for relapsed Hodgkin lymphoma. After the third administration of pembrolizumab, he showed acute anemia with a positive direct anti-globulin test. Because of the markedly erythroid hypoplasia, he was diagnosed with pure red cell aplasia (PRCA) caused by pembrolizumab. He was initially treated with prednisolone, but the reticulocytes decreased after tapering prednisolone. He then received high-dose intravenous immunoglobulin (IVIG) with prednisolone, and PRCA was successfully treated. Although the pathogenesis of PRCA caused by immune checkpoint inhibitors (CPIs) remains unclear, IVIG treatment may be effective for some steroid-refractory CPI-induced PRCA cases.

Key words: pure red cell aplasia, pembrolizumab, programed cell death 1, immune-related adverse events, direct anti-globulin test, intravenous immunoglobulin

(Intern Med 59: 2041-2045, 2020)
(DOI: 10.2169/internalmedicine.4467-20)

Introduction

Pure red cell aplasia (PRCA) is a hematological syndrome characterized by a normocytic normochromic anemia with severe reticulocytopenia and marked reduction in or absence of erythroid precursors from the bone marrow (1). With the increasing use of immune checkpoint inhibitors (CPIs) targeting cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and the programed cell death 1 (PD-1) pathway in a variety of malignancies, there is a growing number of reports of hematologic toxicities as immune-related adverse events (irAEs) (2, 3). However, PRCA as irAEs caused by CPIs has been rarely documented, and its management guidelines remain unclear.

We herein report a case of PRCA followed by the PD-1 antagonist pembrolizumab for refractory Hodgkin lymphoma, which was successfully treated with high-dose intravenous immunoglobulin (IVIG).

Case Report

A 64-year-old Japanese man was originally diagnosed with stage IIB classical Hodgkin lymphoma (mixed cellular type) and underwent chemotherapy with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) for 6 cycles. His disease relapsed two year later, but he refused to receive salvage chemotherapies or brentuximab vedotin because of the peripheral neuropathy. He was therefore started on immunotherapy using the PD-1 antagonist pembrolizumab (200 mg/m^2, every 3 weeks).

Shortly after the third administration of pembrolizumab, he developed acute normocytic normochromic anemia (hemoglobin 7.0 g/dL) with normal white blood cells and platelet counts. Although a direct anti-globulin test (DAT) converted to positive, his absolute reticulocyte counts were decreased (0.5×10^4/μL), and other laboratory data showed no apparent hemolysis (Table 1). A bone marrow examination revealed marked erythroid hypoplasia with no obvious morphological abnormalities, which was consistent with PRCA

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Received: January 11, 2020; Accepted: March 16, 2020; Advance Publication by J-STAGE: May 8, 2020
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Table 1. Laboratory Findings.

	Before pembrolizumab treatment	After 3rd pembrolizumab administration
WBC (/μL)	10,400	3,100
Neutrophil (%)	82.5	59.0
Lymphocyte (%)	5.5	36.0
Monocyte (%)	10.0	3.5
Eosinophil (%)	1.5	0.5
Basophil (%)	0.5	1.0
RBC ($\times 10^6$/μL)	3.89	2.45
Hemoglobin (g/dL)	11.5	7.0
Hematocrit (%)	34.5	20.5
Platelets ($\times 10^4$/μL)	26.1	31.4
Reticulocytes ($\times 10^4$/μL)	7.0	0.5
TP (g/dL)	6.6	7.0
T-Bil (mg/dL)	0.55	0.88
AST (IU/L)	19	18
ALT (U/L)	14	18
LDH (U/L)	271	162
ALP (U/L)	287	259
BUN (mg/dL)	14.0	14.9
CRE (mg/dL)	0.95	0.93
Na (mEq/L)	138	137
K (mEq/L)	3.9	4.2
Cl (mEq/L)	103	105
CRP (mg/dL)	4.21	0.47
DAT	negative	positive
Erythropoietin (mIU/mL)	30.0	65.9
SIL-2R (U/mL)	1,460	1,200

WBC: white blood cell, RBC: red blood cell, TP: total protein, T-Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, CRE: creatinine, CRP: C-reactive protein, DAT: direct anti-globulin test, SIL-2R: soluble interleukin-2 receptor

(Table 2, Fig. 1).

Computed tomography (CT) showed partial remission of Hodgkin lymphoma and no evidence of thymoma. Serological tests for parvovirus B19 and Epstein-Barr virus were negative, and parvovirus B19 DNA was not detected by polymerase chain reaction. No increase in large granular lymphocytes was found in the peripheral blood. Based on these findings, he was diagnosed with PRCA as irAEs due to pembrolizumab. He also exhibited severe blistering and ulceration on the oral and pharynx mucosa, which was diagnosed as an autoimmune bullous disease by dermatologists. The pembrolizumab treatment was therefore interrupted, and oral prednisone 60 mg daily was started (Fig. 2).

Four weeks later, his reticulocyte counts increased, and the oral mucosal lesions were transiently improved. However, after tapering prednisolone dosing to 35 mg daily, his reticulocytes decreased again, and the mucosal lesions worsened. Prednisone dosing was therefore returned to 60 mg daily, and 1 course of IVIG (400 mg/kg for 5 days) was added as second-line therapy for steroid-refractory autoimmune bullous disease.

One week after IVIG treatment, his reticulocyte counts promptly recovered, and the mucosal lesions disappeared again. Six weeks later, the DAT converted to negative, and his hemoglobin level reached the normal range despite tapering of prednisolone. Follow-up positron emission tomography (PET)/CT at 18 months after the last pembrolizumab infusion showed no recurrence of Hodgkin lymphoma, although no treatment for lymphoma was given during this period. PRCA and oral mucosal lesions have not recurred.

Discussion

Most acquired PRCA is considered to be caused by an immune mechanism that interrupts erythroid cell differentiation (1). In some primary and secondary PRCA associated with large granular lymphocytic leukemia or thymoma, cellular immunity has been regarded to be involved in the pathogenesis through the direct or indirect injury of erythroblasts by T-cells or natural killer cells (4-6). Therefore, immunosuppressants such as cyclosporine with or without concurrent corticosteroids are generally used as initial therapy for acquired PRCA (1). In contrast, a few case reports have shown that IVIG was effective for PRCA in patients with

Table 2. **Bone Marrow Examination.**

	Before pembrolizumab treatment	After 3rd pembrolizumab administration
Blast (%)	0.0	0.8
Promyelocyte (%)	3.6	1.8
Myelocyte (%)	24.6	11.6
Metamyelocyte (%)	13.2	9.4
Banded neutrophil (%)	16.0	7.0
Segmented neutrophil (%)	20.8	31.4
ProEBL (%)	0.0	0.2
BasoEBL (%)	0.2	0.4
PolyEBL (%)	14.6	1.6
OrthoEBL (%)	0.0	0.0
Lymphocyte (%)	3.2	24.8
Monocyte (%)	0.4	6.4
Eosinophil (%)	2.4	3.6
Basophil (%)	0.0	0.4
myelocyte/erythrocyte ratio	5.45	29.64

EBL: erythroblast

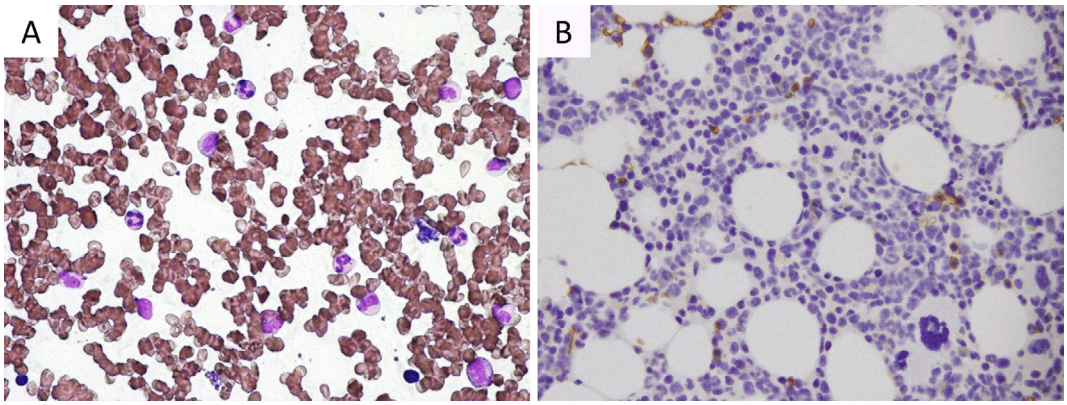

Figure 1. **Bone marrow pictures obtained at the diagnosis of PRCA. A Wright-Giemsa-stained slide of a bone marrow smear (A, 400×). A glycophorin A-stained slide of a bone marrow aspirate section (B, 100×). A bone marrow examination showed marked hypoplasia of erythroid cells.**

chronic lymphocytic leukemia (7) or persistent parvovirus B19 infection (8, 9).

In the present case, IVIG treatment was effective for pembrolizumab-induced PRCA that was refractory to corticosteroid therapy. Although PRCA as an irAE secondary to CPI is a rare finding, Gordon et al. reported a case of PRCA in a patient with metastatic melanoma receiving the anti-CTLA-4 antagonist ipilimumab (10). More recently, Nair et al. also described a case of PRCA following pembrolizumab therapy for relapsed malignant melanoma (11). Notably, these two previously reported CPI-induced PRCA cases were accompanied by DAT positivity and successfully treated with IVIG, as in our present case. The coexistence of PRCA and DAT positivity have been occasionally documented, especially in patients with lymphoid malignancies (12, 13) or autoimmune diseases (14, 15). Although the significance of DAT positivity in such cases is poorly understood, humoral mechanisms, including the production of autoantibodies against both erythrocytes and erythroid precursors, are suspected.

The mechanism of action of IVIG in most autoimmune diseases remains unclear; however, various mechanisms have been proposed, such as blockade of Fc receptors on macrophages and effector cells, neutralization of circulating autoantibodies, selective downregulation of autoreactive B-cells, regulation of the production of helper-T-cell cytokines, and decreased immune-complex-mediated inflammation (16). In pemphigus vulgaris, a type of autoimmune bullous disease, IVIG has been suggested to reduce the serum levels of pemphigus antibodies by increasing catabolism or manipulating the idiotype network (17). In contrast, the blockade of Fc receptors on macrophages, the immunomodulating effect on B-cell activation, and complement neutralization are thought to underlie the mechanisms of IVIG in idiopathic thrombocytopenic purpura and other autoantibody-mediated cytopenias (18). The pathogenesis of

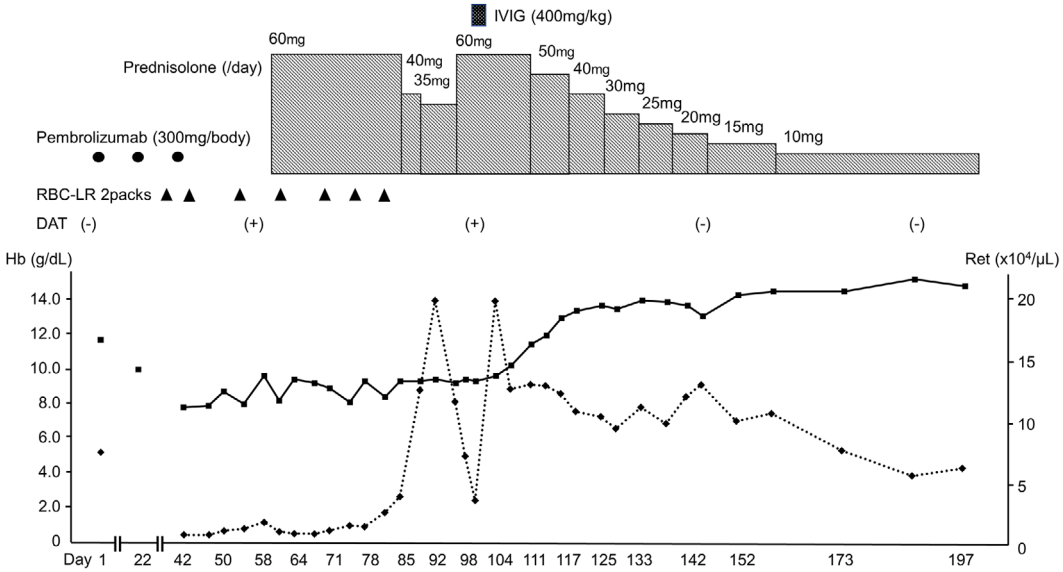

Figure 2. The clinical course of the patient. IVIG: intravenous immunoglobulin, RBC-LR: red blood cells-leukocytes reduced, DAT: direct anti-globulin test, Hb: hemoglobin, Ret: reticulocytes

PRCA induced by PD-1 antagonists has not been elucidated. PD-1 is expressed not only on CD8+ T-cells but also on CD4+ T-cells and activated B-cells, and the modulation of B-cells through both T-cell-dependent and T-cell-independent mechanisms has been shown in PD-1 pre-clinical models (19, 20). In addition, some clinical studies have shown that PD-1 antagonists can contribute to the production of pathogenic autoantibodies against thyroid (21) and muscle tissues (22). Furthermore, several case reports described cases in which rituximab, a humanized anti-CD20 monoclonal antibody, was effective for treating some irAEs caused by PD-1 antagonists, such as myasthenia gravis (23), cold agglutinin syndrome (24), autoimmune hemolytic anemia (25), and acquired hemophilia A (26). These observations suggest that humoral immunity may also play an important role in the etiology of various types of irAEs, including hematological toxicities.

In conclusion, we experienced a rare case of PRCA following pembrolizumab treatment in a patient with refractory Hodgkin lymphoma. Similar to the two previously reported CPI-induced PRCA cases (10, 11), our present case also had DAT positivity and was successfully treated with IVIG. Further studies are needed in order to clarify the specific mechanism underlying CPI-induced PRCA and its appropriate management.

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

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