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【 CASE REPORT 】

Pembrolizumab-induced Autoimmune Hemolytic Anemia and Hemophagocytic Lymphohistiocytosis in Non-small Cell Lung Cancer

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Abstract:
We herein report a 78-year old man with squamous cell carcinoma of the lungs treated with pembrolizumab. At 10 days after the administration of pembrolizumab, he showed progressive anemia and increased levels of bilirubin. Because the findings of a direct coombs test and cold hemagglutinin were positive, we diagnosed the patient with autoimmune hemolytic anemia and treated him with prednisolone. Subsequently, he was admitted to our hospital owing to fatigue, a high fever, and jaundice. His clinical findings met the diagnostic criteria of hemophagocytic lymphohistiocytosis, and he was rescued with a high dose of glucocorticoids. Marked tumor regression was obtained and has been maintained since then.

Key words: pembrolizumab, immune checkpoint inhibitors, non-small cell lung cancer, autoimmune hemolytic anemia, hemophagocytic lymphohistiocytosis

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Introduction

Pembrolizumab, an immune checkpoint protein inhibitor (ICI), exerts antitumor activity by targeting programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1). A recent clinical study showed that pembrolizumab was associated with a significantly longer progression-free and overall survival and with fewer adverse events than platinum-based chemotherapy (1). However, severe adverse events affecting the endocrine system or leading to an autoimmune disorder induced by PD-1 inhibitors including pembrolizumab have also been reported.

We herein report a unique case of non-small cell lung cancer in a patient who experienced autoimmune hemolytic anemia (AIHA) and subsequent hemophagocytic lymphohistiocytosis (HLH) induced by pembrolizumab.

Case Report

A 78-year-old man with a 20-pack-year smoking history was diagnosed with unresectable squamous cell carcinoma of the lung in his right lower lobe (cT2aN3M0, stage IIIB) without an oncogenic driver mutation. At first, his Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 0, and his hepatic and renal functions were normal on laboratory examinations (Table). He had slight macrocytic anemia and several episodes of agglutination of blood on blood tests. He had no history of stomach surgery, and his serum vitamin B12 and folic acid levels were normal. He had none of the typical physical findings suggesting connective tissue disease, and an antinuclear antibody test was negative. On immunohistochemistry, PD-L1 was expressed in more than 50% of the cancer cells in the specimen obtained by bronchoscopy. In addition, his PS was good, and he required treatment with pembrolizumab, a new commercially available anticancer drug. Therefore, he received 200 mg of pembrolizumab intravenously as first-line chemotherapy, according to the guidelines established by the Japan Lung Cancer Society.

On day 10 after receiving the first dose of pembrolizumab, rapidly progressive anemia and increased levels of to-

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Table. Laboratory Findings on Pre-treatment, Day 10, and Day 24 of the First Dose of Pembrolizumab.

		Pre-treatment	Day 10	Day 24
White blood cell	(/μL)	9,400	13,300	15,200
Hemoglobin	(g/dL)	11.7	6.0	8.2
Platelet	($\times10^4$/μL)	37.9	42.4	25.4
Reticulocyte	(%)	6.5	7.8	20.7
AST	(U/L)	19	25	98
ALT	(U/L)	8.0	12	14
CRE	(mg/dL)	0.8	0.81	1.1
Total bilirubin	(mg/dL)	1.0	4.2	3.8
Indirect bilirubin	(mg/dL)		1.4	1.6
LDH	(U/L)	277	492	1,533
Ferritin	(ng/mL)	244		35,400
FDP	(μg/mL)			59.3
D-dimer	(ng/mL)	<0.5		5.28
sIL-2R	(U/mL)		2,450	4,325

AST: asparatate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CRE: creatinine, FDP: fibrin degradation products, sIL-2R: soluble interleukin-2 receptor

tal bilirubin (4.2 mg/dL), indirect bilirubin (1.4 mg/dL), reticulocytes (7.8%) and lactate dehydrogenase (LDH) (492 U/L) were observed (Table). In addition, a direct coombs test and cold hemagglutinin showed positive results, suggesting that the patient might have AIHA. We were compelled to abandon further pembrolizumab treatment due to fears of other potentially unknown autoimmune adverse effects and instead treated him with 25 mg of prednisolone for AIHA.

On day 24 after treatment initiation, he was admitted to our hospital because of generalized fatigue, a high fever, and jaundice. A hematological examination showed leukocytosis and progressive anemia (white blood cell count, 15,200/μL; hemoglobin, 8.2 g/dL). On admission, thrombocytopenia was not observed (25.4×10^4/μL) (Table). His total bilirubin level was 3.5 mg/dL, and serum LDH and ferritin levels were elevated to 1,533 U/L and 35,400 ng/mL, respectively. The patient's blood cultures were all negative. Although Epstein-Barr virus (EBV) IgG and antibody to EBV nuclear antigen was positive, EBV IgM was negative. Serum soluble interleukin-2 receptor (IL-2R) was also elevated (up to 4,325 U/mL). In addition, a hypercoagulable state was observed [D-dimer 34.5 μg/mL; fibrin degradation products (FDP) 59.3 μg/mL], meeting the diagnostic criteria of acute disseminated intravascular coagulation. A bone marrow examination showed hypocellular marrow with histiocytic hyperplasia (macrophages, 1.6%) and hemophagocytic macrophage infiltration (Fig. 1). CT of the abdomen showed marked splenomegaly.

The patient had an elevated fever, splenomegaly, hemophagocytosis in his bone marrow, hyperferritinemia and elevated soluble IL-2R; these findings met five of the eight diagnostic criteria of HLH described in HLH-2004 (2). Therefore, we diagnosed the patient with HLH in addition to AIHA as complications of pembrolizumab treatment. Because his laboratory data worsened within 3 days, as shown in Fig. 2, we started steroid pulse therapy with antibiotics. After the steroid dosage was tapered and the patient was started on 20 mg of prednisolone, all of the laboratory findings gradually improved. On admission, the patient was suspected of potentially having had an allergic reaction to the radiocontrast agent, but a lymphocyte stimulation test and patch test were both negative. CT of his chest showed remarkable tumor shrinkage, which has been maintained without disease recurrence (Fig. 3).

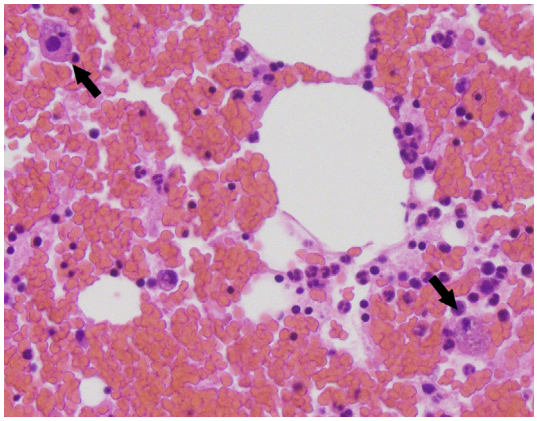

Figure 1. A bone marrow examination showed hypocellular marrow with histiocytic hyperplasia and hemophagocytic macrophage infiltration (indicated with the arrow).

Discussion

We encountered an interesting case of non-small cell lung cancer in a patient who seemed to develop AIHA and subsequent secondary HLH caused by pembrolizumab therapy. Many cases showing adverse events associated with the endocrine system or autoimmune disease due to PD-1 inhibitors have been reported. However, adverse autoimmune hematologic events related to these PD-1 antibodies have rarely been described in published studies. Pföhler et al. reported a case of immune thrombocytopenia as a rare side effect of nivolumab and pembrolizumab for metastatic melanoma (3). They suggested that immune hematology testing to diagnose or rule out immune thrombocytopenia is indispensable when patients are treated with ICIs and thrombocytopenia is observed.

The anti-PD-1 and anti-PD-L1 immune checkpoint inhibitors, including nivolumab and pembrolizumab, can trigger immune-related adverse events (4). When our patient was first diagnosed with lung cancer, he had mild anemia and a blood test showed agglutination. We suspect that the patient may have had AIHA that became clinically apparent when we started pembrolizumab treatment. AIHA related to immunotherapy is not well documented, and only a few cases have recently been reported. Palla et al. reported a case of AIHA after the use of nivolumab and summarized four cases of nivolumab-induced AIHA in patients with melanoma, cutaneous squamous cell carcinoma, Hodgkin's lymphoma and pulmonary adenocarcinoma (5). In addition, Nair

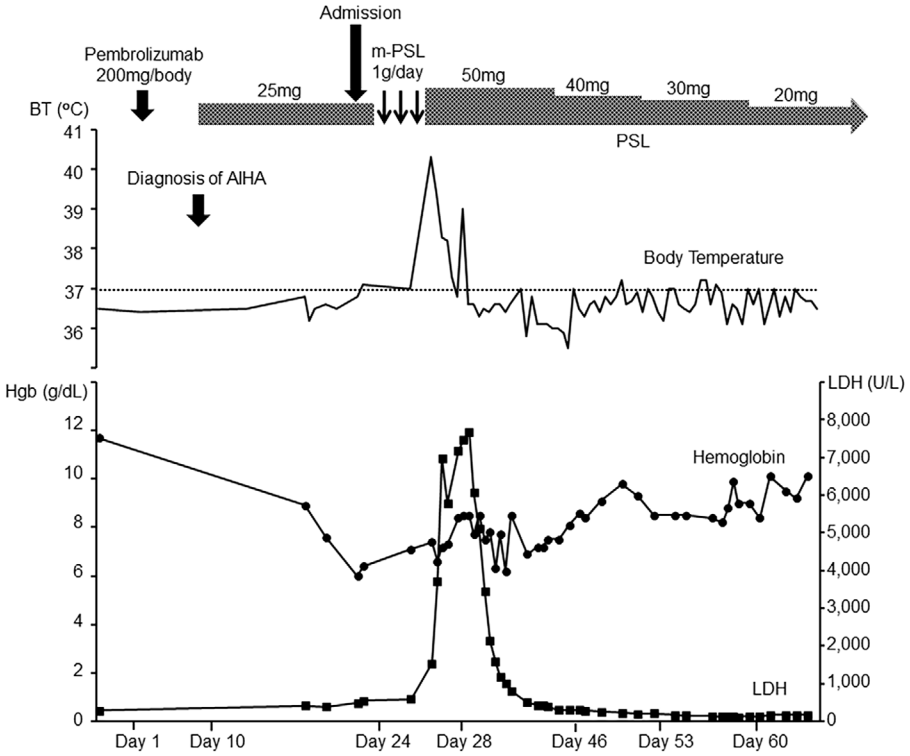

Figure 2. **The clinical course after the first dose of pembrolizumab showing the dose and duration of steroid therapy and its efficacy for hemophagocytic lymphohistiocytosis. The high fever immediately resolved, and the serum lactate dehydrogenase and hemoglobin levels remarkably improved.**

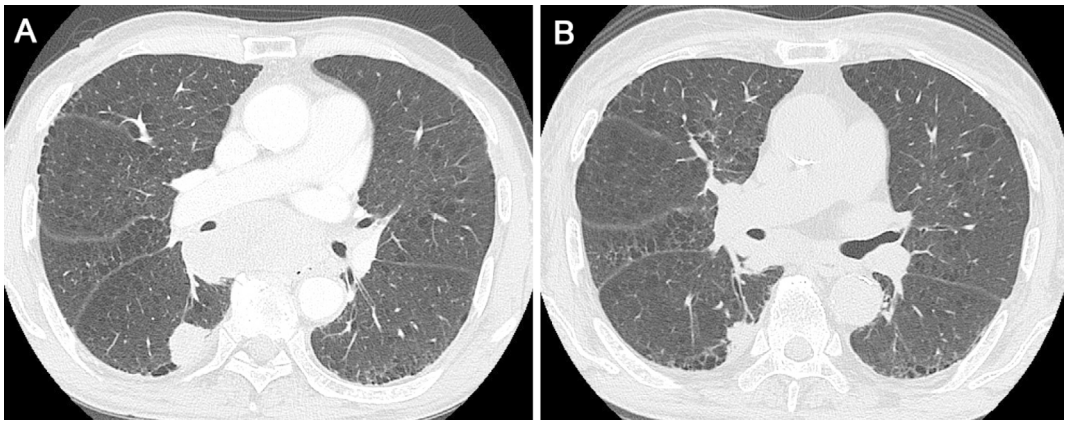

Figure 3. **Computed tomography findings of the chest before pembrolizumab treatment (A). A mass can be observed in the right lower lobe of the lung. Mediastinal lymphadenopathy markedly regressed after glucocorticoid treatment for autoimmune hemolytic anemia and hemophagocytic lymphohistiocytosis as complications of pembrolizumab on day 73 after the first dose (B).**

et al. reported a case of AIHA with pure red blood cell aplasia in malignant melanoma, suggesting a correlation between AIHA and pembrolizumab (6).

In contrast, there has been only one case report of immune checkpoint inhibitor-associated HLH. Shah et al. described a 76-year-old Caucasian man with metastatic bladder cancer treated with pembrolizumab, which may have facilitated an exaggerated immune response perpetuating HLH (7). HLH, which can be divided into primary and secondary types, is a hyperinflammatory syndrome defined by a defective cytotoxic function and hypercytokinemia leading to macrophage expansion and hemophagocytosis (8). Secondary HLH in particular has been reported in association with genetic mutations, infections, autoimmune disorders, and various malignancies (9). As Ammann et al. have already reported that T-cells, mainly CD8+ cytotoxic T-cells, are strongly activated in secondary HLH (10), we speculate that pembrolizumab activated T-cells and a subsequent immune response in our patient, resulting in HLH in addition to AIHA. To our knowledge, this is the first report to describe pembrolizumab-associated AIHA combined with subsequent rapidly progressive HLH.

Glucocorticoids are thought to be the mainstay of treatment for autoimmune adverse effects by immunotherapy. Ciclosporin and VP-16 are also used for initial therapy, according to HLH-2004 (2). Ramos-Casals et al. pointed out that using immunoglobulin improves the prognosis for HLH induced by infectious and autoimmune diseases, with an estimated survival rate of 59-75% (11). In the present case, the administration of glucocorticoids with pulse therapy was able to control the autoimmune complications. AIHA and HLH frequently induce life-threatening complications. Therefore, immediate detection and therapy using immunosuppressants for AIHA and/or human leukocyte antigen (HLA) are urged during treatment with immune checkpoint inhibitors.

Some researchers have suggested that the antitumor activity may be associated with the severity of the adverse events induced by immune checkpoint inhibitors (12). Osorio et al. indicated that thyroid dysfunction that can occur early in treatment is frequently preceded by transient hyperthyroidism associated with an improved outcome of pembrolizumab (13). In addition, a recent prospective cohort study by Teraoka et al. showed a significantly longer median progression-free survival in patients with immune-related adverse events induced by nivolumab (14). In the present case, the patient obtained favorable tumor regression and has maintained a long-term response, as shown in Fig. 3, which seems to be consistent with the findings of previous reports.

In conclusion, we experienced an interesting case of pembrolizumab-induced AIHA and HLH. The side effects of ICI are milder than those of any other cytotoxic anticancer drugs. However, uncommon adverse events can potentially be lethal, as in the present case. To avoid such an occurrence, a clear criterion for screening before using ICIs should be established. In addition, further investigations will be required to reveal the mechanisms underlying the development of ICI-related toxicity, and we need to enact immediate countermeasures to ensure such immune-related adverse effects do not occur in the future.

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

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