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LETTER TO THE EDITORS

Immune-mediated red cell aplasia after anti-CTLA-4 immunotherapy for metastatic melanoma

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A 55-year-old man presented in 2002 with a right lower extremity nodular melanoma, Breslow thickness 1.33 mm, without associated nevus or lymphocytic infiltrate. A sentinel lymph node biopsy was negative, and he received a wide excision and no further therapy. One year later, he presented with a subcutaneous in-transit metastasis which was resected. He began systemic adjuvant therapy on a melanoma vaccine trial containing twelve melanoma peptides in 2004, but 1 year later was found to have progressive disease with liver and subcutaneous metastases. In 2005, he participated in a clinical trial of the fibroblast activation protein (FAP) inhibitor PT100, but developed progressive disease after 6 months, with new subcutaneous and right inguinal lymph node lesions. He continued to have excellent performance status and in late 2005 participated in a phase III trial of carboplatin and paclitaxel along with Sorafenib or placebo, but developed progressive disease after 7 months. In 2006, he began on Ipilimumab (10 mg/kg once every 3 weeks), an anti-CTLA-4 monoclonal antibody, in a phase II clinical trial approved by the Institutional Review Board at the University of Chicago. He did not have a prior personal or family history of autoimmune disease. He developed vitiligo after 3 months of therapy and had radiographic evidence of response, with decreased size of the right inguinal lymph nodes and stable liver metastases. The following month, he developed hypothyroidism, which was successfully treated with levothyroxine. He had intermittent low grade diarrhea managed with kaopectate, and fatigue, but continued to have an active lifestyle with stable disease.

In 2007, after receiving four cycles of induction followed by three cycles of maintenance therapy over a total of 47 weeks, he had radiographic evidence of progressive disease of a subcutaneous mass and underwent re-induction with 10 mg/kg intravenous Ipilimumab, given once every 3 weeks. He continued to have vitiligo, hypothyroidism, and intermittent fatigue. After two cycles of re-induction, he became severely fatigued, pale and tachycardic. His hemoglobin was 7.4 g/dl and hematocrit was 20.4%, compared to a baseline of 14.4 g/dl and 42%, respectively. Ipilimumab dosing was held and he was transfused with packed red blood cells. However, he continued to have severe fatigue and persistent anemia. There was no melena or sign of active bleeding, and a stool Guaiac test was negative. Laboratory evaluation revealed hemoglobin 5.4 g/dl, hematocrit 14.9%, reticulocytes 0.1%, absolute reticulocyte count 1.72 K/μl, reticulocyte production index 0, elevated serum ferritin (839 ng/mL) and serum iron (206 mcg/dl), normal vitamin B12 and folate, and elevated serum erythropoietin (1,284 mIU/ml). Platelets were 173,000 μl^{-1} and white blood cells were mildly decreased at 2,900 μl^{-1}. Although a direct antiglobulin test was positive, the levels of lactate dehydrogenase, bilirubin, and haptoglobin were normal. He was admitted to the hospital for evaluation and treatment of an apparent underproduction anemia. He was transfused with packed red blood cells and underwent a bone marrow biopsy and aspirate with review of peripheral blood smears. The differential diagnosis at this time included red cell aplasia, aplastic anemia, parvovirus, and

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melanoma metastatic to bone marrow. The patient was initially treated with oral prednisone at 1 mg/kg day^{-1}, with little change in his transfusion requirement and no elevation of the reticulocyte count. After 4 weeks, he received intravenous immunoglobulin (IVIg) and rapidly showed a reticulocytosis and normalization of his hemoglobin.

The peripheral blood smear showed a marked anemia, moderate leukopenia and normal numbers of platelets. Red blood cells were normochromic and normocytic, but polychromatophilic cells were virtually absent, consistent with red cell aplasia. A marked erythroid hypoplasia accompanied by a granulocytic hyperplasia and adequate numbers of mature-appearing megakaryocytes was observed in the normocellular bone marrow biopsy. Only occasional singly dispersed immature-appearing erythroid precursors were found in the biopsy. The marrow aspirate essentially recapitulated the findings in the biopsy and revealed a myeloid to erythroid ratio of greater than 100–1. Moreover, there was no evidence of dysplasia in the marrow aspirate cells. Histologic, immunohistochemical, and polymerase chain reaction were negative for parvovirus. Also, there was no histologic evidence of metastatic melanoma in either the biopsy or aspirate, which was supported by negative immunohistochemical stains for Melan-A and HMB-45 carried out on the biopsy. Several intratrabecular lymphoid aggregates of varying sizes comprised of small mature lymphocytes, as well as two well separated large germinal centers were present in the biopsy (Fig. 1, bone core biopsy, H&E of one of the germinal centers). Small mature CD3$^+$ T lymphocytes far outnumbered small mature CD20$^+$ B cells in the interstitium and in the small lymphoid aggregates, with approximately equal proportions of CD4$^+$ and CD8$^+$ T cells. On the other hand, CD20$^+$ B cells comprised the majority of germinal center cells (Fig. 1, CD20, immunohistochemistry) and were surrounded by a thick rim of T cells, a higher proportion of which were CD4$^+$ compared to CD8$^+$ (Fig. 1, CD4 and CD8 immunohistochemistry). A background of reactive, singly scattered CD138$^+$ plasma cells was present. Only rare cells were highlighted by the NK marker, CD56. Flow cytometry analysis performed on the marrow aspirate cells demonstrated T:B and CD4:8 ratios of 17:1 and 2:1, respectively. T cells expressed the typical surface markers (CD2, CD3, and CD5), and no aberrant T cell surface marker expression was detected. The kappa:lambda ratio was concordant with the presence of a polyclonal B cell population. In summary, the bone marrow showed no evidence of metastatic melanoma, parvovirus, or lymphoproliferative disorder, but did show a pure red cell aplasia in the presence of lymphoid aggregates and prominent reactive germinal centers, which are not normally found in healthy bone marrow specimens. Therefore, the above described morphologic and immunophenotypic observations are supportive of an immune-mediated red cell aplasia [1].

Immune related adverse events (IRAEs) induced by anti-CTLA-4 therapy, including colitis, uveitis, hypophysitis,

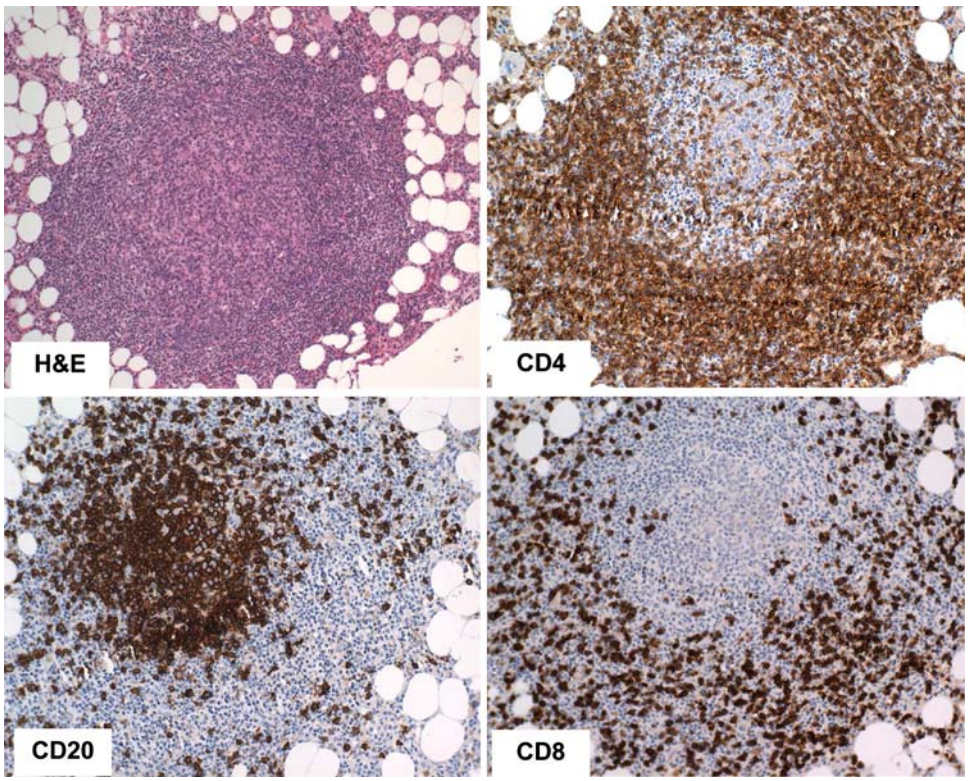

Fig. 1 Representative germinal center from the bone core biopsy. A thick rim of T cells, a higher proportion of which were CD4$^+$ compared to CD8$^+$, surround the germinal center which consists predominantly of CD20$^+$ B cells. Clockwise from upper left: H&E, CD4, CD8, and CD20 immunohistochemistry

and dermatitis, are well documented [2]. Indeed, the anti-tumor mechanism of CTLA-4 blockade relies on removing CTLA-4 inhibition of T cell responses, presumably resulting in augmented T cell recognition of tumor antigens and subsequent tumor rejection [3]. It is thought that increased T cell recognition of self antigens also occurs, resulting in a variety of autoimmune-like side effects [4]. In metastatic melanoma patients treated with anti-CTLA-4 therapy, IRAEs have been reported to correlate with tumor regression [5] and have been suggested to be prognostic for achieving a complete response [6].

This is the first report of pure red cell aplasia in a patient receiving anti-CTLA-4 immunotherapy for metastatic melanoma. Although the pathogenesis of acquired pure red cell aplasia is incompletely understood, the findings of cytotoxic serum antibodies to erythroid precursors or blocking antibodies against erythropoietin in some patients suggest an autoimmune etiology [7]. Therapy for pure red cell aplasia has classically consisted of immunosuppression with corticosteroids, as well as T cell directed agents, such as cyclosporine [8]. IVIg has also been shown to be an effective treatment option [9]. Our patient had a poor response to corticosteroids and rapid clinical benefit from IVIg, which along with the presence of lymphoid aggregates and germinal centers with abundant $CD4^+$ T cells and B cells, suggest a possible antibody-mediated etiology associated with anti-CTLA-4 therapy in this case. Pure red cell aplasia is a potentially life threatening complication of immune-stimulating cancer therapy, which should be considered in the differential diagnosis of anemia in patients being treated with such agents.

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