Relationship between Autoimmune Diseases and Imbalances in Helper/Suppressor T-cell Populations after Rituximab-containing Chemotherapy for Non-Hodgkin Lymphoma

Satoko Oka[1]*, Miho Hiramatsu[2], Tomoaki Kawano[2], Naruto Matsuoka[2] and Masaharu Nohgawa[1]

[1]Department of Hematology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Japan
[2]Department of Clinical Laboratories, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Japan

Abstract

Immunologic abnormalities including autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) have been described in patients with non-Hodgkin lymphoma. In some cases, anti-red cell or platelet antibodies are produced by lymphoma cells. However, cases of the occurrence of autoimmune diseases without recurrence of lymphomas have been also reported. The relationship between autoimmune diseases with post-bone marrow transplantation and Hodgkin disease may be attributed to immune dysfunctions, particularly those involving T cells. This autoimmune phenomenon may be related to imbalances in helper/suppressor T-cell populations. Imbalances in helper and suppressor T-cell populations have been reported after R-CHOP chemotherapy, and the recovery of serum IgG and CD4+ counts was observed more than 2 years after R-CHOP therapy in patients with B-cell lymphoma. In this report, we present a case of an 87-year-old man who was treated with rituximab-containing chemotherapy and maintained complete remission. However, the immunophenotyping of peripheral blood and bone mallow mononuclear cells revealed a reversed CD4/CD8 ratio. Two years later, he developed ITP. He was treated with intravenous immunoglobulin and eltrobopag, and thrombocytopenia improved. Five years later, he developed pneumonia and sudden Coombs-positive hemolytic anemia caused by autoantibodies against D antigen and thrombocytopenia without the recurrence of lymphoma. He was treated with prednisolone and a pulse dose of methylprednisolone; however, his response to therapy was poor and he subsequently died. We herein report a case who have been showed reversed imbalances in the helper/suppressor T cell populations over 2 years after R-CHOP therapy, developed AIHA and ITP without recurrence of lymphoma. The long-term monitoring of T-cell counts after rituximab containing chemotherapies is important, and careful attention to infection signs.

Introduction

The relationship between autoimmune diseases, such as autoimmune hemolytic anemia (AIHA), immune thrombocytopenic purpura (ITP) and Evans syndrome, and non-Hodgikin lymphomas (NHL) has been discussed in detail in the literature and has also been reviewed [1-4]. The underlying mechanisms may vary between entities and patients. These phenomenon is observed in primarily or the recurrence of lymphomas in some cases, and anti-red cell or platelet antibodies are produced by lymphoma cells [5]. Cases of the recurrence of autoimmune diseases without that of lymphomas have been also reported [6,7]. The relationship between autoimmune diseases with post-bone marrow transplantation and Hodgkin disease may be attributed to immune dysfunctions, particularly those involving T cells [8,9]. This autoimmune phenomenon may be related to imbalances in helper/suppressor T-cell populations [10]. Imbalances in helper and suppressor T-cell populations have been reported after R-CHOP chemotherapy, and the recovery of serum IgG and CD4+ counts was observed more than 2 years after R-CHOP therapy in patients with B-cell lymphoma [11,12]. Our patient, who had anti-red cell or platelet antibodies, showed evidence of a T-cell imbalance more than 5 years after R-CHOP chemotherapy. Although weaker immunity in older patients is a possible explanation, further investigations are needed in order to reach a definitive conclusion. We herein report a case that exhibited reversed imbalances in helper/suppressor T-cell populations more than 2 years after R-CHOP therapy, and developed AIHA and ITP without the recurrence of lymphoma.

Case Report

In November 2012, an 87-year-old man visited our hospital because a fluoro-deoxy-glucose (FDG)-positron emission tomography (PET)/computed tomography (CT) scan revealed intense FDG uptake in the pancreas. He had no symptoms. Laboratory tests showed a white blood cell (WBC) count of 4.7×10^9/L (normal range: 4.0×10^9 to 7.0×10^9/L), red blood cell count (RBC) of 415×10^{10}/L (normal range: 390×10^{10} to 520×10^{10}/L), hemoglobin (Hb) concentration of 12.1 g/dL (normal range: 12.0 to 14.0 g/dL), platelet count of 248×10^9/L (normal range: 150×10^9 to 400×10^9/L), and soluble interleukin 2 receptor (sIL2R) level of 1100 U/ml (normal range: 145 to 519 U/ml). The patient underwent fine needle biopsy from the pancreas, and the diagnosis of diffuse large B-cell lymphoma (DLBCL) was confirmed. No evidence of neoplastic infiltration was detected in bone marrow. We categorized the Ann Arbor staging of DLBCL as Stage IE. He received rituximab (375 mg/m², day 1) plus cyclophosphamide (750 mg/m², day

*Corresponding author: Satoko Oka, M.D, Department of Hematology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Japan, Tel: 81734224171; Fax: 81734218132; E-mail: okas@jasmine.ocn.ne.jp

2), doxorubicin (50 mg/m², day 2), vincristine (1.4 mg/m², day 2), and prednisolone (50 mg/m², days 2 to 6) (R-CHOP) chemotherapy, and his CT findings and sIL2R level (525 U/ml) markedly improved after 1 course of chemotherapy. After 3 courses of chemotherapy, the complete remission of lymphoma was confirmed in PET/CT scans. The patient remained completely free of lymphoma for five years; however, the immunophenotyping of peripheral blood mononuclear cells showed a reversed CD4/CD8 ratio (normal range: 0.7 to 2.8) (Figures 1a and 1b).

In February 2014, when he was 89-year-old, petechiae and ecchymosis developed on the extremities. He had neither "B symptoms" nor lymphadenopathy and hepatosplenomegaly. Laboratory tests showed a WBC count of 9.4×10^9/L, RBC count of 422×10^{10}/L, Hb concentration of 11.8 g/dL, and platelet count of 2×10^9/L (Figure 1). Bone marrow showed marked megakaryocytic hyperplasia without dysplasia and lymphoma cell infiltration. A cytogenetic study of bone marrow was normal, showing 46, XY. The immunophenotyping of bone marrow nuclear cells showed a reversed CD4:CD8 ratio (CD4 3.0%; CD8 10.8%) (Figure 2a). Tests for a series of autoantibodies related to collagen diseases were negative. A diagnosis of ITP was made. He was treated with intravenous immunoglobulin at 15 mg/kg and eltrobopag at 25 mg/day, which improved thrombocytopenia.

In April 2017, the 92-year-old patient was referred to a local hospital due to pneumonia. Ten days later, he was referred to our hospital with the rapid deterioration of his general condition and progression of anemia. A physical examination revealed a temperature of 38.6°C. He was pale and jaundiced, but did not have lymphadenopathy or hepatosplenomegaly. His laboratory results revealed severe anemia. Laboratory tests showed a WBC count of 6.4×10^9/L, RBC count of 176×10^{10}/L, Hb level of 46 g/L, and platelet count of 409×10^9/L (Figure 1). A peripheral smear showed spherocytes. His LDH level was 1250 U/L (normal range: 106 to 211 IU/L) and serum total bilirubin (T-Bil) level was 4.6 mg/dL (normal range: 0.3 to 1.0 mg/dL), of which the indirect bilirubin (I-BiL)

value was 3.6 mg/dL (normal range: 0.3 to 0.7 mg/dL). Other findings included increased reticulocytes at 86%, and a decreased haptoglobin level of <10 mg/dL (normal range: 36 to 195 mg/dL). Tests for a series of autoantibodies related to collagen diseases were negative. Infections with cytomegalovirus, Epstein-Barr virus, hepatitis A/B/C virus, HIV, and parvovirus, as well as *Mycoplasma pneumoniae* were excluded by means of serological investigations. Direct and indirect Coombs tests were positive, the cold hemagglutination test was negative, and serum monoclonal immunoglobulin was absent. The direct antiglobulin test (DAT) was positive for C3d and immunoglobulin G (IgG). Bone marrow showed marked erythroid hyperplasia and megakaryocytic hyperplasia without lymphoma cell infiltration. A cytogenetic study of bone marrow was normal, showing 46, XY. The immunophenotyping of bone marrow nuclear cells showed a reversed CD4:CD8 ratio (CD4 5.2%; CD8 11.6%) (Figure 2b). Neither the relapse of lymphoma nor splenomegaly was evident in systemic PET/CT scans. He had never received a RBC, PC, or FFP transfusion. His blood type was B and his Rh blood group was confirmed to be CcDEe. In the presence of polyethylene glycol (PEG) enhancement, anti-D antibodies were 516 and 256-fold higher in plasma and antibody-dissociated solution from erythrocytes, respectively, suggesting a large amount of anti-D in his plasma after a reaction to RBCs. Additional screening tests detected no other autoantibodies in the plasma. RhD antigen typing using the Advanced Partial RhD Typing kit indicated that the patient did not have a partial D or weak D phenotype. The reactivities of plasma against RhD-negative panel RBCs were all negative, suggesting the absence of an antibody against Rh-D-mimicking antigens, such as the Landsteiner-Wiener (LW) antibody. A diagnosis of AIHA due to anti-D antibody was made. He was administered prednisolone at 1 mg/kg. However, since anemia worsened, steroid pulse therapy was initiated at 1 g/day of methylprednisolone for three days and two units of packed blood cells lacking D-antigen were transfused. However, anemia continued to worsen, and he died 2 days later.
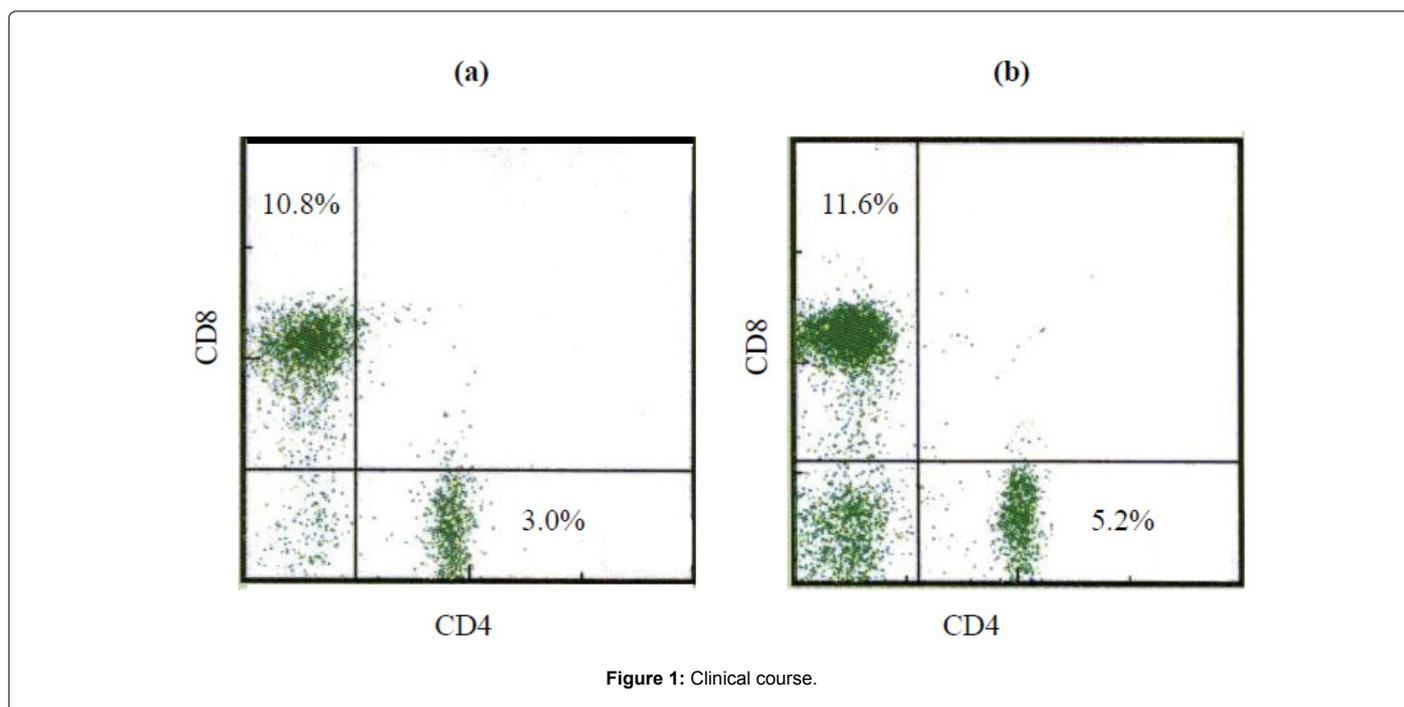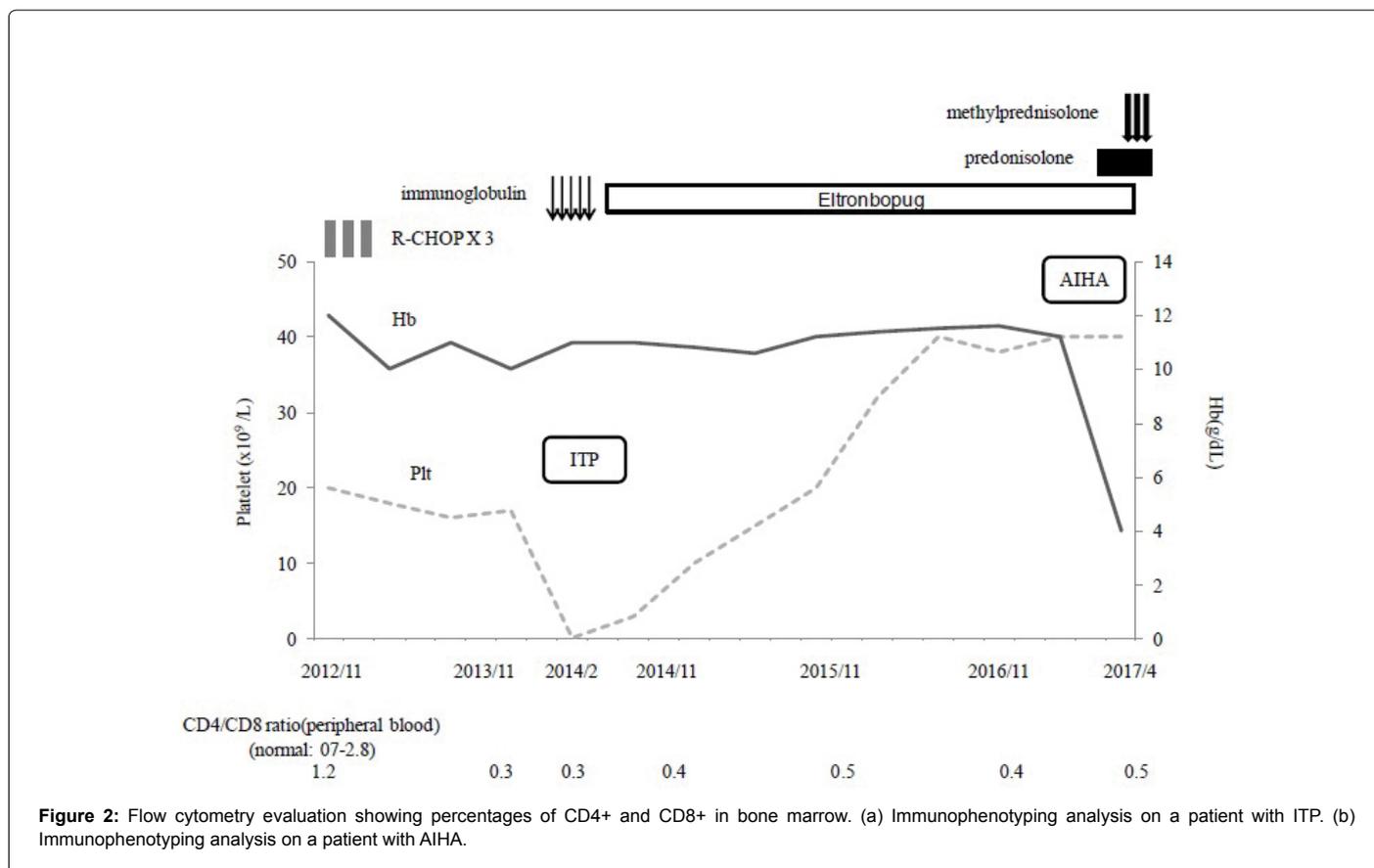

Figure 1: Clinical course.

Figure 2: Flow cytometry evaluation showing percentages of CD4+ and CD8+ in bone marrow. (a) Immunophenotyping analysis on a patient with ITP. (b) Immunophenotyping analysis on a patient with AIHA.

Discussion

Various autoimmune disorders, including AIHA, ITP, autoimmune neutropenia, and pure red cell aplasia, have been described in patients with NHL (1-4). Evans syndrome is a rare immunological abnormality that is characterized by a combination of AIHA and ITP without an underlying etiology. This immunological abnormality also occurs in patients with lymphoproliferative disorders, collagen diseases, and autologous or allogeneic stem cell transplantation (1). The incidence of AIHA associated with NHL is reported to be between 0.23% and 6.2% [13-15]. The underlying mechanisms may vary between entities and patients. The current hypothesis for chronic lymphocytic lymphoma is that polyclonal antibodies cause AIHA (16). The pathogenesis of AIHA or all autoimmune phenomena that complicate the course of NHL remain a matter of considerable controversy. Autoimmune phenomena in NHL may occur before or upon a diagnosis and during or after treatments without the recurrence of lymphomas [4]. In some cases, anti-red cell or platelet antibodies are produced by lymphoma cells because the production of an antibody by lymphoma cells has been demonstrated or AIHA or ITP resolved after surgical removal of the lymphoma [5]. However, since our patient maintained the complete remission of lymphoma at the occurrence of AIHA, the relationship between NHL and AIHA was not considered to be a factor. The relationship between autoimmune diseases and Hodgkin disease may be attributed to immune dysfunctions, particularly those involving T cells [8]. Imbalances in helper/suppressor T-cell functions lead to the over-reactivity of B cells and, hence, autoimmune phenomena [8-10]. Immune-mediated cytopenia has also been described in patients after allogeneic and autologous bone marrow transplantation [9]. Although the mechanisms responsible for this relationship remain unclear, several theories have been proposed. Chemoradiotherapy and bone marrow reinfusion may lead to a dysfunction in the immune system, and this may be related to impaired suppressor T-cell function observed in the post-transplant period [10]. Suppressor T cells are important regulators of immune responses to self antigens and defective suppressor T-cell function may allow the emergence of autoreactive lymphocytes with consequent autoantibody production. Immune dysregulation may also be caused by damage to thymic function as a result of irradiation and chemotherapy [16,17]. It has been shown in experimental models that various organ-specific autoimmune diseases may develop by depleting T-cell subsets including CD4 and CD8 cells [18]. Impaired B-cell regulation as a result of quantitative or qualitative T-cell abnormalities may also lead to autoantibody formation. Klumpp et al. [19] proposed that the random selective deletion of certain T suppressor clones may cause the appearance of autoantibodies. Winiarski et al. [8] reported four cases of Evans syndrome among 28 children undergoing bone marrow transplantation. These patients received antithymocyte globulin or OKT3 as part of their conditioning regimens, in addition to chemotherapy and/or radiation. The high incidence of Evans syndrome in their study may be related to vigorous T-cell suppression, leading to B-cell dysregulation and autoimmune diseases. It is interesting to note that our case, with anti-red cell or platelet antibodies, showed evidence of a T-cell imbalance, such as a reversed CD4:CD8 ratio in bone marrow and peripheral blood. Michinton et al. [20,21] suggested that viral infections that occur during the post-transplant period may play a role by damaging or combining with self antigens and forming "altered self" antigens. In this case, infection may be one of the triggers for immune dysregulation.

Imbalances in helper and suppressor T-cell populations have been reported after R-CHOP chemotherapy [11,12]. The effects of rituximab alone on the restoration of immunity after treatments remain unknown. Among the cytotoxic agents of R-CHOP, cyclophosphamide and doxorubicin have well-documented detrimental effects on immunoglobulins and lymphocytes. However, the effects of rituximab in R-CHOP therapy on immune function have not yet been elucidated. Ito et al. [11] reported the recovery of serum IgG and CD4+ counts more than 2 years after R-CHOP therapy in patients with B-cell lymphoma. Our patient, who had anti-red cell or platelet antibodies, showed evidence of a T-cell imbalance more than 5 years after R-CHOP chemotherapy. Although weaker immunity in older patients is a plausible explanation, further investigations are needed in order to reach a definitive.

In this case, severe hemolytic anemia was caused by acquired autoanti-D antibody. D antigen is the most immunogenic antigen in the setting of the development of alloantibodies following exposure; however, it is not commonly associated with autoantibody development. Antibodies against antigens in the Rh system, such as anti-e, anti-E, and anti-c, are more commonly implicated in warm AIHA [22-24]. The presence of only anti-D antibodies is rare in AIHA, and autoanti-D antibodies have been detected in only a few cases, such as in the setting of myelodysplasia, Burkitt's lymphoma, and a paraneoplastic syndrome associated with breast cancer [25-28]. However, the underlying mechanisms remain unclear.

Conclusion

In conclusion, we encountered a case of ITP and AIHA more than two years after R-CHOP therapy without the recurrence of lymphoma. Our patient showed reversed imbalances in helper/suppressor T-cell populations, and an immune system imbalance that resulted from chemotherapy may have contributed to immunological abnormalities. Although the relationship between imbalances in helper/ suppressor T cell populations and the development of auto-antibodies production after chemotherapies remains unclear, immunosuppressive effect of the rituximab-containing chemotherapy may be contributing factor. To ascertain the phenomenon, the long-term monitoring of T-cell populations after rituximab-containing chemotherapies is important and attention to infections is needed. Further studies will be necessary in order to confirm immune system imbalances after chemotherapy or transplantation especially in older patients.

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