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CASE REPORT

Persistent hypogammaglobulinemia due to immunoglobulin class switch impairment by peri-transplant rituximab therapy

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Abstract

Post-transplant lymphoproliferative disorder (PTLD) is one of the most serious complications of allogeneic hematopoietic stem cell transplantation (HSCT). Rituximab is effective for PTLD; however, rituximab can produce adverse effects, including hypogammaglobulinemia. Here, we present the case of an 18-year-old female with refractory cytopenia of childhood who developed persistent selective hypogammaglobulinemia with low immunoglobulin G (IgG) 2 and IgG4 levels and monoclonal protein after rituximab therapy against probable PTLD. Despite B-cell recovery, the serum IgG levels gradually declined, reaching < 300 mg/dL at 33 months after rituximab treatment. In addition, class-switched memory ($CD27^+IgD^-$) B cells were limited in phenotypic analysis. These findings suggest that peri-HSCT rituximab may contribute to an abnormal B-cell repertoire induced by impaired immunoglobulin class switch.

Keywords Rituximab · Post-transplant lymphoproliferative disorder · Allogeneic hematopoietic stem cell transplantation · Hypogammaglobulinemia · Monoclonal protein

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for hematological malignancies and nonmalignant disorders. However, it is associated with various complications, including infection due to insufficient immune reconstitution under immunosuppressive therapy or graft-versus-host disease (GVHD). Defects in humoral and cellular immunity may persist for months (even years) after allo-HSCT [1]. In a retrospective study of 278 adult patients undergoing allo-HSCT, the cumulative incidence of hypogammaglobulinemia at 1 year was 24.1%. In stable courses of allo-HSCT without GVHD, serum immunoglobulin G (IgG) levels reach their lowest levels at 6 months and recover after around 1 year [2].

Risk factors for long-term hypogammaglobulinemia after allo-HSCT have been studied. Previous retrospective studies identified acute GVHD, unrelated donor, and hypogammaglobulinemia before allo-HSCT as risk factors for long-term hypogammaglobulinemia [2–5]. On the other hand, long-lasting hypogammaglobulinemia was reported in patients treated with rituximab for post-transplant lymphoproliferative disorder (PTLD) and autoimmune hemolytic Hodgkin after allo-HSCT [6–9]. PTLD is one of the most serious complications of allo-HSCT and has a high mortality rate [10]. Fujimoto et al. reported that the probability of PTLD at 2 years was 0.79% in a large retrospective analysis using the Japanese national transplant registry [11]. Although EBV DNA monitoring and preemptive rituximab therapy improve the outcome of patients with PTLD, it is important to consider the side effects of rituximab, such as long-lasting hypogammaglobulinemia due to delayed humoral immune reconstitution [12].

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Here, we describe a patient with persistent hypogammaglobulinemia accompanied by monoclonal protein after rituximab therapy against probable PTLD. Serum IgG levels in our patient were maintained at > 400 mg/dL early after rituximab therapy, but declined to their lowest levels more than 2 years after treatment. The patient still requires immunoglobulin supplementation 3 years after allo-HSCT.

Case report

An 18-year-old female without remarkable medical history was referred to our hospital due to headache and exertional dyspnea. Laboratory blood analysis revealed severe pancytopenia (white blood cells, 0.24×10^9/L; hemoglobin, 7.1 g/dL; and platelets, 46×10^9/L). Blood chemistry analysis showed no particularly abnormal findings The IgA levels (196.8 mg/dL) were normal, while the levels of IgG (715.9 mg/dL) and IgM (37.5 mg/dL) were slightly below the normal range. Monoclonal protein was not detected by serum protein electrophoresis and immunofixation electrophoresis. Bone marrow aspiration revealed hypocellular marrow with mild megaloblastoid changes in erythroid precursors, and megakaryocytes showed multiple widely separated nuclei. The bone marrow cell karyotype was normal. The patient was diagnosed with refractory cytopenia of childhood. She was treated with cyclosporine A (CsA); however, no improvement in blood cell count was observed, even after 2 months of treatment.

Figure 1 shows the clinical course, B-cell recovery, and immunoglobulin levels. HSCT from a fully HLA-matched unrelated donor was performed after a conditioning regimen with fludarabine (25 mg/m^2, 5 days), melphalan (70 mg/m^2, 2 days), anti-thymocyte globulin (2.5 mg/kg, 2 days), and total body irradiation (2 Gy). The clinical course was uneventful without serious complications, including acute GVHD or infection, and the patient was discharged on day 55. However, she was readmitted due to high-grade fever and pharyngalgia on day 76. A computed tomography scan revealed tonsillar hypertrophy

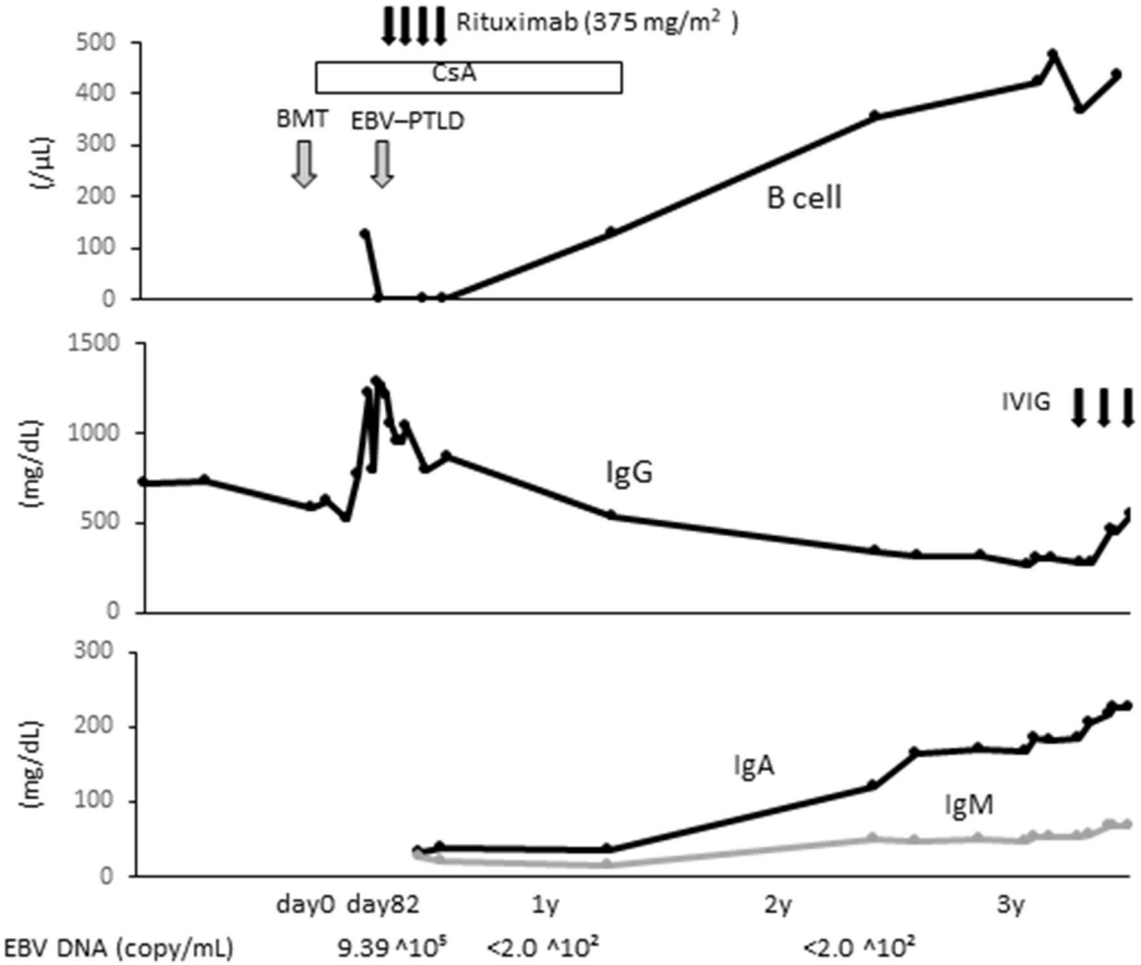

Fig. 1 Clinical course including B-cell recovery and levels of IgG, IgA, and IgM. Levels of EBV DNA load are indicated below the *x*-axis

and splenomegaly, and the peripheral blood Epstein–Barr virus (EBV) DNA levels increased to 9.36×10^5 copies/mL. Because a pre-transplant serological analysis revealed she was seropositive for antibodies to EBV (the serostatus of donor was not available), we diagnosed probable EBV–PTLD and administered four weekly doses of rituximab 375 mg/m^2 from day 83. The patient's symptoms improved immediately, and the EBV DNA levels declined to undetectable levels at day 117. Because there was no evidence of chronic GVHD, immunesuppression was gradually tapered and finally discontinued on day 439.

B-cell aplasia lasted for 6 months and B-cell recovery was confirmed at 2 years after rituximab administration (18% of total lymphocytes, 358/μL). Following B-cell recovery, the serum IgM and IgA levels increased to within the normal range. In contrast, the serum IgG levels declined gradually, and finally reached < 300 mg/dL at 33 months after rituximab treatment. A blood cell count showed no abnormal findings. Chimerism analysis showed that both CD3$^+$ T cells and CD19$^+$ B cells were completely donor type (Fig. 2a). Immunoglobulin subtype analysis revealed low IgG2 (55.5 mg/dL) and IgG4 (< 2.0 mg/dL) levels. Serum protein immunofixation electrophoresis suggested the

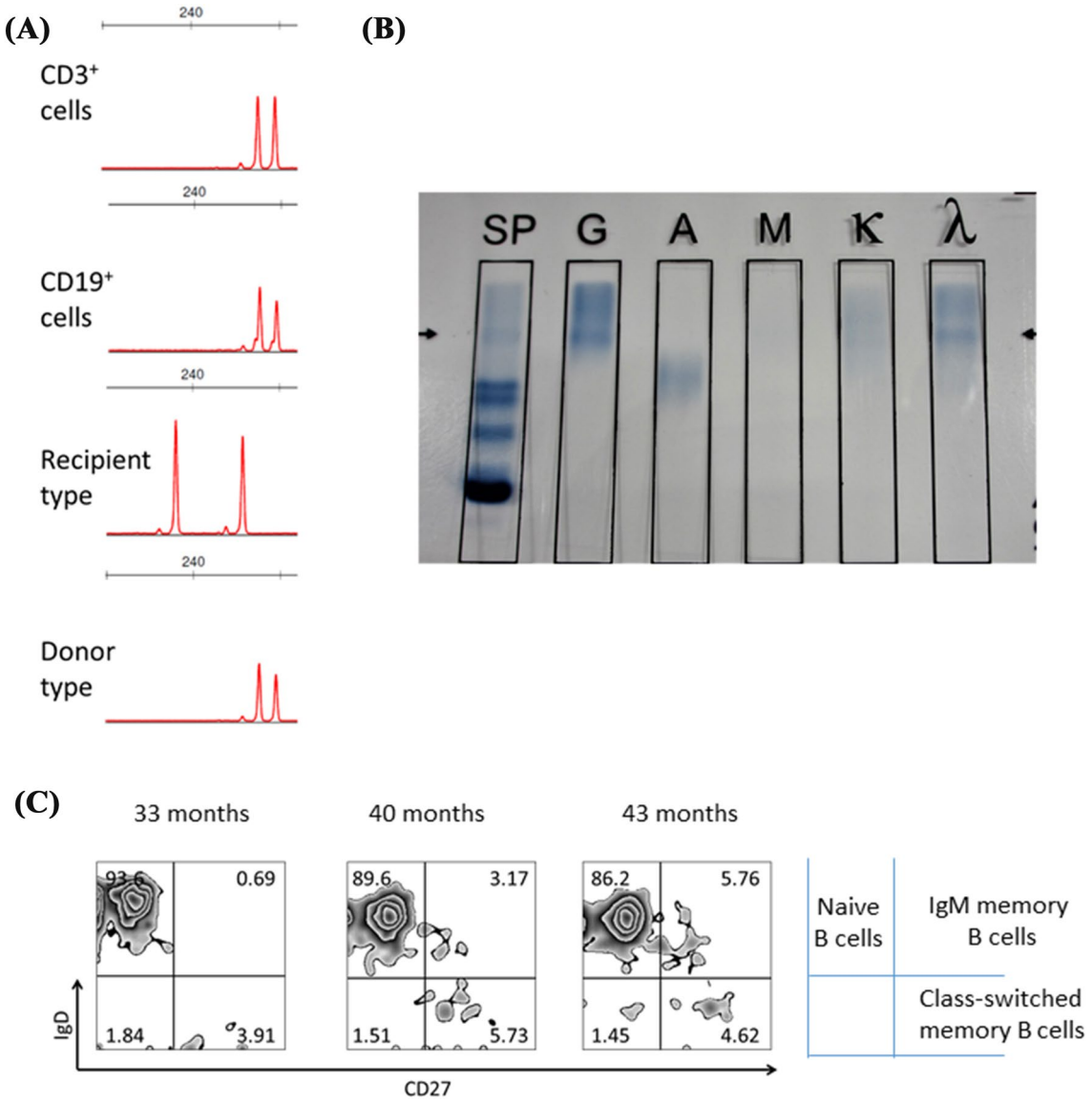

Fig. 2 Characteristics of B cells and immunoglobulins. **a** Chimerism analysis using short tandem repeat for CD3$^+$ and CD19$^+$ cells at 34 months after transplant. **b** Serum protein immunofixation analysis at 28 months after transplant. Arrows indicate monoclonal band suggesting IgG lambda. **c** Phenotypic analysis using IgD and CD27 at 33, 40, and 43 months. CD27$^-$IgD$^+$, CD27$^+$IgD$^+$, and CD27$^+$IgD$^-$ cells indicate naive, IgM memory, and class-switched memory B cells, respectively

presence of IgG lambda-type monoclonal protein (Fig. 2b). Monoclonal protein was detected at 14 months after transplant and continued to be detected even at 42 months after transplant as the latest follow-up. She has been in complete remission and never experienced severe infection so far. The EBV serostatus has been positive. Due to the persistent severe hypogammaglobulinemia (IgG < 400 mg/dL), she still requires immunoglobulin supplementation. Phenotype analysis of T cells showed that they were almost identical to those of healthy individuals (data not shown). However, analysis of the B cells showed that the $CD27^{+}IgD^{-}$ phenotype (class-switched memory B cells) was limited, even in the sample taken at 3 years after rituximab therapy (Fig. 2c). These results suggest that, in this patient, hypogammaglobulinemia was induced by a B-cell class switch abnormality.

Discussion

Long-lasting hypogammaglobulinemia after rituximab treatment against PTLD following HSCT has previously been reported in two case reports and one case series [6, 7, 9]. In the present case, our patient showed persistent progression to lowering of IgG2 and IgG4 levels, accompanied by monoclonal protein after rituximab therapy against PTLD. To the best of our knowledge, there has been no previous report of hypogammaglobulinemia with monoclonal protein induced by rituximab against PTLD.

Rituximab is a humanized anti-CD20 monoclonal antibody used in the treatment of B-cell non-Hodgkin's lymphoma (NHL) and PTLD. While B-cell deficiency may persist for up to 6 months after rituximab treatment, severe hypogammaglobulinemia is not usually observed in a non-HSCT setting [13]. However, rituximab maintenance for B-cell NHL after autologous HSCT is reported to prolong severe hypogammaglobulinemia [14–17]. It is speculated that peri-transplant rituximab and additional cellular immunosuppressive background associated with HSCT affect the B-cell differentiation into plasma cells [15].

Agematsu et al. reported that the percentage of B-cell subsets was: $19.4 \pm 3.2\%$ for $CD27^{+}IgD^{-}$ class-switched memory B cells and $12.3 \pm 2.0\%$ for $CD27^{+}IgD^{+}$ IgM memory B cells [18]. In other report, $CD27^{+}IgD^{-}$ class-switched memory B cells comprised 6.5% of total B cells in HSCT recipients who neither received rituximab and experienced chronic GVHD with median post-transplant follow-up of 48 months [19]. In our patient, the number of $CD27^{+}IgD^{-}$ class-switched memory B cells was lower than that in healthy population and long-term survivors after allo-HSCT even 3 years after rituximab treatment, suggesting peri-HSCT rituximab induces a delayed recovery of memory B cells. Because class-switched memory B-cell deficiency is reported to correlate with serum IgG level in allo-HSCT recipients [20], we believe it was one of the possible causes for persistent hypogammaglobulinemia.

In the present case, abnormal immune reconstitution was observed. First, the IgA and IgM levels decreased immediately after rituximab and recovered following B-cell recovery. Although the patient remained in remission and experienced no other transplant-related complications, the IgG levels continued to decrease, and no improvement was observed for more than 3 years after the last rituximab administration. In a previous study of 17 patients who received rituximab within 6 months of allo-HSCT, the median IgG levels recovered at 24 months after HSCT [21]. Thus, the period of hypogammaglobulinemia, especially low IgG level, observed in our patient was long, compared with that of previous reports. Second, selective IgG2 and IgG4 deficiencies with IgG lambda-type monoclonal protein were observed in our patient. Nishio et al. reported that patients with B-cell NHL treated with rituximab after autologous HSCT showed defective expression of one or more IgG isotypes and delayed recovery of $CD27^{+}IgD^{-}$ class-switched memory B cells among patients with hypogammaglobulinemia [14]. Yamazaki et al. reported that history of rituximab administration in the peri-HSCT period was a significant risk factor for decreased IgG2 levels and IgG2/IgG ratio after allo-HSCT [22]. The both above-mentioned reported may suggest peri-HSCT rituximab can also impair immunoglobulin class switch and induce abnormal B-cell repertoire. The limited number of $CD27^{+}IgD^{-}$ class-switched memory B cells and unbalanced recovery of gammaglobulin with monoclonal protein observed in our patient may reflect impaired immunoglobulin class switch, resulting in abnormal B-cell repertoire.

In conclusion, the findings from the current case suggest that peri-HSCT rituximab may induce persistent hypogammaglobulinemia, as well as abnormal B-cell repertoire. Regardless of whether relapse or transplant-related complications including GVHD are observed, long-term monitoring of immunoglobulin including subclass analysis and the proportion of lymphocyte subsets should be performed in allo-HSCT recipients treated with rituximab during the peri-HSCT period. Hypogammaglobulinemia observed in our patient may be a novel pattern of rituximab-induced abnormal B-cell reconstitution, and further accumulation of cases is required.

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Compliance with ethical standards

Conflicts of interest The authors report no potential competing conflicts of interest.

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