Long-term Sustained Mixed Chimerism after Allogeneic Stem Cell Transplantation in a Patient with Severe Aplastic Anemia

Akiko M. Saito, Shigeru Chiba, Seishi Ogawa, Yoshinobu Kanda, Hisamaru Hirai and Mineo Kurokawa

Abstract

Mixed chimerism in a post-transplant patient with severe aplastic anemia (SAA) is generally considered to be a status preceding donor-cell rejection and bone marrow failure. Here, we report on a rare, prolonged mixed chimerism in a patient with SAA who showed a full recovery in hematological and immunological status after transplantation. The analysis in this patient showed about 20% and 80% recipient-type cells of total blood cells and T cells, respectively, at two years post-transplantation, and 14% and 25% of total blood cells and T cells, respectively, at four years post-transplantation. This report describes the most comprehensive case study known to date.

Key words: long-term sustained mixed chimerism, severe aplastic anemia, allogeneic bone marrow transplantation, young patient, immunological tolerance

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Introduction

Allogeneic bone marrow transplantation (BMT) remains an important treatment for young patients with severe aplastic anemia who have an HLA-compatible sibling donor. Most centers now report long-term survival rates of 60% to 100% (1, 2), leaving the persistent high frequency of graft rejection as a serious complication. Emergence of recipient cells has been thought to be a sign preceding graft rejection, but there have been suggestions that stable mixed chimerism could be established.

Case Report

A 17-year-old man complained of easy fatigability, palpitation and shortness of breath, and was found to have pancytopenia (white blood cell count 2.6×10⁹/L, hemoglobin 6.6 g/dL and platelet count 20×10⁹/L) in January 1999. The bone marrow was extremely hypocellular, being comprised mainly of lymphocytes. Karyotyping of 20 cells that were in metaphase exclusively showed 46, XY. There was no history of anomaly, nor was any family member diagnosed Fanconi anemia. Consequently, a diagnosis of aplastic anemia was made. Shortly thereafter, the patient developed a progression of anemia and thrombocytopenia, resulting in bilateral retinal hemorrhage; he began to receive transfusions. His 12-year-old brother demonstrated identical HLA haplotypes (HLA A11/33(19), B61(40)/44(12), Cw3 and DR9/6) and matching ABO and Rh blood typings. The patient was referred to our hospital for allogeneic hematopoietic stem cell transplantation. When transferred, his laboratory data continued to show severe pancytopenia (white blood cell count 1.4×10⁹/L, neutrophil count 0.2×10⁹/L, hemoglobin 5.9 g/dL, reticulocyte count 5.6×10⁹/L, and platelet count 8.0×10⁹/L).

Informed consent for transplantation and bone marrow harvest was obtained from the patient, and from his brother and parents.

Before transplantation, the patient had been transfused with 18 units (more than 9 times) and 105 units (more than 10 times) of red blood cells and platelet concentrate, respectively. Three months after the diagnosis, 1.3×10⁹ bone mar-
Row cells, corresponding to $2.4 \times 10^8$ kg patient weight, were obtained from the brother, and transplanted to the patient without manipulation. The conditioning regimen consisted of 50 mg/kg cyclophosphamide (CY) on days -7 through -4 (total 200 mg/kg) and 2.5 mg/kg rabbit antithymocyte globulin (thymoglobulin™) (ATG) on days -5 through -2 (total 10 mg/kg), together with prednisolone. Cyclosporine A and short-course methotrexate were given as a prophylaxis for acute graft-versus-host disease (GVHD) (3). No cytokine support was given. Regimen-related toxicities were confined to grade I stomatitis and mild abnormalities in liver enzymes. A neutrophil count of $>0.5 \times 10^9$/L was achieved on day 28 and the platelet count was $>50 \times 10^9$/L without transfusion on day 35 and the reticulocyte count was 59.3 $>20.0 \times 10^9$/L on day 24, respectively. A bone marrow biopsy on day 14 showed tri-lineage engraftment. Recovery of bone marrow function was prompt after engraftment (hematological data is summarized in Fig. 1). The patient was discharged on day 51 without any sign of acute GVHD. No chronic GVHD was observed, and cyclosporine A was discontinued 6 months after transplantation. There have been no documented infections, and a cytogenetic study, performed 11 months posttransplantation was normal. After bone marrow recovery, results of all hematological tests were within normal range for 4 years. Serum immunoglobulin levels, the absolute and relative numbers of peripheral blood lymphocytes, and the T/B ratio, have all been normal. The absolute number of CD4 T cells ranged from 0.4 to $0.8 \times 10^9$/L; the number of CD8 T cells was slightly higher. The patient resumed actively playing tennis 10 months after the transplantation and is now enjoying a full range of physical activity.

**Results of Chimerism Studies**

Bone marrow and blood chimerism was repeatedly evaluated by a quantitative method that uses fluorescence-based polymerase chain reaction amplification of short tandem repeat markers (4). The proportion of recipient-type cells in both bone marrow and peripheral blood stayed below 10% of the total cells until day 78 posttransplantation, when the proportion of recipient-type cells detected in the peripheral blood (lymphocyte count on day 78 was $1.5 \times 10^9$/L) unexpectedly rose to 18%. Thereafter, the proportion of recipient-type cells in the total peripheral blood has remained at levels between 12% and 34%. At 18 months posttransplant, the proportion of recipient-type cells was analyzed in the peripheral blood T cells as well. Surprisingly, it was as high as 67% (lymphocyte count=$2.3 \times 10^9$/L; proportion of T cell=72.4%) and increased to 77% (lymphocyte count=$1.7 \times 10^9$/L; proportion of T cell=72.2%) when monitored subsequently. The proportion remained at 25% on the latest follow-up study, performed 4 years posttransplant (lymphocyte count=$2.3 \times 10^9$/L; proportion of T cell=63.1%). However, the proportion of recipient-type cells in the bone marrow remained ≤10% until the latest bone marrow follow-up, which was performed 11 months after transplantation (Fig. 2). Although we did not evaluate chimerism of non-T cells including granulocytes posttransplantation for this patient, the proportion of recipient-type cells of total peripheral blood was consistently low compared to that of peripheral T cells, which accounted for 20-30% of total peripheral blood. The proportion of recipient-type cells of non-T cells is considered lower than that of T cells.
Discussion

Stable mixed chimerism has been commonly described in canine models for non-myeloablative transplantation (5, 6). In a vast majority of clinical settings of non-myeloablative transplantation in humans, however, mixed chimerism appears to represent a temporary status prior to full donor chimerism or rejection of donor cells (7). Mixed chimerism has also been demonstrated posttransplant in patients with aplastic anemia, but it reportedly precedes donor cell rejection and bone marrow failure (8). Prolongation or appearance of posttransplant mixed chimerism is often related to T-cell depletion, transplantation for aplastic anemia, younger recipient ages, complete matching of HLAs, and non-TBI conditioning regimens in the literature (7, 9-11). In the 1980s and early 1990s, mixed chimerism in posttransplant patients with aplastic anemia was frequently associated with donor-cell rejection (8), though some authors described stable mixed chimerism persisting for >2 years (9-11). However, limited information on individual cases is available in those reports. Stable mixed chimerism seems to represent rare events even in patients with aplastic anemia, especially since a combination of total lymph node irradiation or ATG with CY is now being used as a preparative regimen (1, 2, 12), although the true incidence of stable mixed chimerism is unclear due to the lack of detailed molecular analyses. The present patient exhibited long-term sustained mixed chimerism after transplantation, accompanied by normal hematological and immunological parameters, and otherwise good health life for four years after transplantation for severe aplastic anemia, considered to be an immune mediated disease. In this patient, recipient-derived T cells were dominant in the posttransplant period, but did not cause a cytotoxic immune reaction against the hematopoietic stem-progenitor cells of this patient. This might be a result of the change in the T cell repertoire, or because of differences in the antigens on hematopoietic stem cells between the patient and the donor.

Patients with non-myeloablative transplantation, described in several studies, showed posttransplant stable mixed chimerism persisting for over 2 years (9-11); they were aged 7, 19 and 24 years. Imamura et al also suggest that mixed chimerism in older patients, 30 years or older, is associated with rejection and relapse, while this was not true of patients less than 30 years old (13). These reports, along with the present study, may indicate that immunological tolerance can be induced more easily in humans if the recipients are younger.

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References