Domino donor lymphocyte infusion for secondary poor graft function after HLA-mismatched allogeneic stem cell transplantation between HLA-identical sibling pairs with congenital immunodeficiency

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Abstract

Poor graft function (PGF) is a major obstacle to successful allogeneic stem cell transplantation after achieving normal bone marrow function. We report a successful treatment of domino DLI for poor graft function in younger brother with hyper IgM syndrome from HLA-identical elder brother with the same disorder with a history of HLA-mismatched unrelated bone marrow transplantation using same donor. Immunological profiling revealed that DLI-induced T-regulatory cells could correct skewed immune responses in the BM microenvironment due to secondary PGF pathogenesis. Immunological tolerized domino DLI can be a new therapeutic option for secondary PGF in HLA-identical sibling pairs with congenital immunodeficiency.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a curative option for malignant and non-malignant diseases. Poor graft function (PGF) is characterized by persistent pancytopenia with hypocellular marrow and full donor chimerism is a major obstacle to successful transplantation after achieving normal bone marrow (BM) function. PGF, including primary PGF and secondary PGF, occurs in 5-27% of all patients and is associated with considerably higher rates of infectious and hemorrhagic complications.[1] The pathogenesis of PGF has not been well documented, and it is possible that the immune system damages the hematopoietic compartment via exposure to inflammatory cytokines during graft versus host disease (GvHD) or viral infection.[2,3,4] One successful approach to treat PGF is by re-grafting CD34+-selected peripheral blood cells without preconditioning, or by donor lymphocyte infusion (DLI).[5,6] Successful serial transplantation in brothers with X-linked primary immunodeficiencies has been described previously, where the recipient of HLA-mismatched donor cells act as a functionally “HLA-matched” donor for subsequent affected siblings.[7] Here, we report a successful treatment of domino DLI for poor graft function in younger brother with hyper IgM syndrome from HLA-identical elder brother with the same disorder with a history of HLA-mismatched unrelated bone marrow transplantation using same donor. To understand the mechanism of hematological recovery, we monitored immune recovery.

BRIEF REPORT

Our patient, a 3-year-old boy, presented at age 1 year with recurrent fever and refractory otitis media. He was diagnosed with hyper-IgM syndrome (HIGM), which was confirmed by the absence of CD40L expression on activated T cells. His family history of HIGM included an HLA-identical 5-year-old brother had also undergone HLA C1 allele mismatched unrelated bone marrow transplantation for HIGM 23 months ago Our patient underwent unrelated bone marrow transplantation (BMT) with grafts from HLA C1 allele mismatched donor and ABO mismatched; the elder brother had also received grafts from the same donor. The conditioning regimen comprised fludarabine (30 mg/m^2/day for 6 days), melphalan (70 mg/kg/day for 2 days), rabbit anti-thymoglobulin (1.5 mg/kg/day for 4 days), and total body irradiation (2 Gy), followed by the

administration of 4.3×10^8/kg nucleated cells. Graft-versus-host disease (GvHD) prophylaxis consisted of short-term methotrexate (15 mg/m^2 on day 1 and 10mg/m^2 on days 3, 6, and 11 after BMT) and tacrolimus (0.02 mg/kg/day, continuous i.v. infusion). Engraftment occurred on day 19 with complete chimerism; however, he developed hypoxia on day 22. Echocardiography revealed pericardial effusion (PE) and we administered prednisolone at 1 mg/kg; but as the PE persisted, he underwent pericardiocentesis on day 34. He had no other complications such as sinusoidal obstructive syndrome (SOS), thrombotic microangiopathy (TMA), or hepatic/renal insufficiency. Steroid therapy was initiated and tapered off by 1 month. During tapering, PE did not recur but he developed grade Ⅱ acute GvHD (skin) on day 60 which was treated with a topical steroid. Cytomegalovirus (CMV) infection was monitored by CMV pp65 antigenemia and as he tested positive on day 35, he was treated with ganciclovir for 2 weeks. Concurrently, he developed a persistent fever with progressive pancytopenia. Bone marrow aspiration on day 47 showed profound hypocellularity and a marked increase in the proportion of hemophagocytic cells (12%). He was diagnosed with hemophagocytic syndrome, which was successfully managed using dexamethasone palmitate. His platelet count increased to 107×10^9/L on day 112, but decreased gradually and parallelly with progressive anemia and neutropenia from day 134 onwards. This persistent pancytopenia required weekly RBC and platelet transfusion. Further, marrow cellularity was less than 10% with full donor chimerism and there was no evidence of infections, including EB virus or HHV-6 DNA (polymerase chain reaction), or CMV pp65 antigenemia. Plasma thrombopoietin level (27.4 fmol/mL) was significantly high but anti-platelet antibody was negative. Immunosuppressant therapy and G-CSF were administered concomitantly, but failed to recover hematopoiesis. Therefore, to boost hematopoiesis, he received donor lymphocytes infusion (DLI) at 1×10^6 CD3$^+$ cells/kg without conditioning on day 198 from the elder brother, who had a history of HCT on day 700 from the same donor. Pancytopenia markedly improved after 1 month but he developed grade Ⅰ cutaneous GvHD on day 212 that was responsive to topical steroid application. Since then, he has not required transfusion during a follow-up period of 2 years (Figure 1A). In summary, CMV-related hemophagocytic syndrome occurred in our patient after engraftment, followed by grade Ⅱ acute GvHD in the skin and subsequent donor-type pancytopenia. Flow cytometric analyses of peripheral lymphocyte subsets showed that circulating B cell

numbers were higher before progression of cytopenia. (Figure 1B). Bone marrow suppression without infection was thought to result from BM dysfunction because the BM could be a target of GvHD. Incidentally, a previous report has described BM GvHD due to BM microenvironment damage with $CD4^+$ T cell involvement.[8] Additionally, delayed naïve B cell reconstitution might have resulted in expanded autoreactive B cells through high B-cell activating factor (BAFF) levels.[9] Therefore, we first analyzed the kinetics of peripheral B cell subsets and found that, after DLI, $CD19^+CD27^-IgD^+$ naïve B cells gradually increased while, parallelly, $CD19^+CD27^+IgD^-$ switched memory B cell numbers decreased. Next, as regulatory T cells (Tregs) have been known to maintain tolerance to the bone marrow niche in allogeneic lymphocyte transfer models,[10] we analyzed the kinetics of Tregs and conventional $CD4^+$T cells (Tcons) after DLI to understand if Treg skewing was involved in the pathogenesis of PGF in our patient. Tregs were defined as $CD4^+CD25^{med\text{-}hi}CD127^{lo}$ while Tcons were $CD4^+CD25^{neg\text{-}lo}CD127^{med\text{-}hi}$ [11], and we found that the frequency of Tregs gradually increased during the first month after DLI, while, inversely, the frequency of Tcons decreased. Further, the Treg:Tcon ratio increased to more than three times the level before DLI from 0.11 to 0.36 (Figure 2). Thus, Treg numbers recovered after DLI and that it was associated with hematopoietic recovery. The immunological basis of PGF was determined to be BM microenvironment disruption due to destruction of osteoblasts by allo-$CD4^+$T cells during GvHD in the BM. Previously, peripheral autoreactive B cell accumulation has been described in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) patients due to the absence of Tregs.[12] This observation supports the hypothesis that, in our patient, $CD19^+CD27^+IgD^-$ switched memory B cells, possibly including autoreactive B cells, were modified by the increase in Treg numbers after DLI. Additionally, naïve B cell recovery indicates the possibility of BM microenvironment restoration after GvHD resolution. This assumption is based on the observation that expanded Tregs correlated with greater numbers of endosteal osteoblastic cells and expanded B-cell precursors in the BM in a chronic GvHD mouse model.[13] A potential limitation in our case study is that, owing to the lack of cellular elements in the BM, we could not show impairment of the BM niche with endothelial cells, perivascular cells, and endosteal cells. However, DLI-induced Tregs might suppress the allo-$CD4^+$ T cells, causing destruction of BM niche.

As successful serial transplantation of mismatched donor between HLA-identical sibling pairs with congenital immunodeficiency by the “tolerized” graft has been reported,[7] our case suggests that the “tolerized” domino donor lymphocyte can lead to sufficient hematological recovery in patients with mild GvHD.

In conclusion, we report that domino DLI-induced Tregs can correct skewed immune responses in the BM microenvironment due to secondary PGF pathogenesis. Immunological tolerized domino DLI can be a new therapeutic option for secondary PGF in HLA-identical sibling pairs with congenital immunodeficiency.

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Conflict-of-interest

The authors declare that there is no conflict of interest.

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Figure legends

Figure 1. Recovery of hematopoiesis after DLI. (A) Clinical course and changes in white blood cells, hemoglobulin, and platelets. (B) Peripheral absolute

lymphocyte counts of $CD3^+CD4^+$ T lymphocytes, $CD3^+CD8^+$ T lymphocytes and $CD19^+$ B lymphocytes before and after DLI. *URBMT, unrelated bone marrow transplantation; G-CSF, granulocyte colony stimulating factor; HPS, hemophagocytic syndrome; DLI, donor lymphocyte infusion

Figure 2. Dot plots of $CD19^+$ B cell subsets defined by the expression CD27 and IgD (top panel), $CD3^+$ T cells defined by CD4 and CD8 (middle panel), $CD4^+$T cell defined by CD127 and CD25 (bottom panel), at days 0, 30, 50, and 75 after DLI, respectively.