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HAPLOIDENTICAL STEM CELL TRANSPLANTATION

Donor lymphocytes expressing the herpes simplex virus thymidine kinase suicide gene: detailed immunological function following add-back after haplo-identical transplantation

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Abstract

Background aims. Haplo-identical hematopoietic stem cell transplantation (HSCT) with add-back of donor lymphocytes expressing the herpes simplex virus thymidine kinase suicide gene (TK cells) is one of the most widely applied promising new gene therapy approaches. However, the immunological status of added-back TK cells after HSCT has yet to be well characterized. *Methods.* We investigated TK cells through the use of flow cytometry, T-cell receptor (TCR) Vβ repertoire spectratyping and linear amplification-mediated polymerase chain reaction followed by insertion site analysis in a patient enrolled in our clinical trial. *Results.* A comparison of onset with remission of acute graft-versus-host disease confirmed that TK cells were predominantly eliminated and that proliferative $CD8^+$ non-TK cells were also depleted in response to ganciclovir administration. The TCR Vβ-chain repertoire of both TK cells and non–TK cells markedly changed after administration of ganciclovir, and, whereas the TCR repertoire of non–TK cells returned to a normal spectratype long after transplantation, that of TK cells remained skewed. With the long-term prophylactic administration of acyclovir, TK cells oligoclonally expanded and the frequency of spliced variants of TK cells increased. Known cancer-associated genes were not evident near the oligoclonally expanded herpes simplex virus (HSV)-TK insertion sites. *Conclusions.* We demonstrate obvious differences in immunological status between TK cells and non-TK cells. In addition, we speculate that long-term prophylactic administration of acyclovir increases the risk of oligoclonal expansion of spliced forms of TK cells.

Key Words: *haplo-identical hematopoietic stem cell transplantation, herpes simplex virus thymidine kinase suicide gene, T-cell function, T-cell repertoire, insertional oncogenesis*

Introduction

Haplo-identical $CD34^+$-purified hematopoietic stem cell transplantation (HSCT) with add-back of donor lymphocytes expressing the herpes simplex virus thymidine kinase suicide gene (TK cells) is thought to be a promising strategy for patients with poor-risk leukemia, if no human leukocyte antigen (HLA)-matched donor is available [1,2]. Recently, the

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preliminary results of an ongoing randomized Phase III trial (TK008, NCT00914628) in adult patients with high-risk leukemia suggested this approach to be safe and to confer a potential survival benefit [3]. Even in the case of graft-versus-host disease (GVHD), TK cells can be promptly eliminated by administration of ganciclovir, which prevents this potentially lethal post-transplantation complication [4–7]. However, the immunological status of added-back TK cells and donor-derived untransduced lymphocytes (non–TK cells) after transplantation has not yet been studied in detail.

We conducted a clinical trial with the aim of undergoing haplo-identical $CD34^+$-purified HSCT with add-back of TK cells. We separately analyzed TK cells labeled with a truncated form of the low-affinity nerve growth factor receptor (△LNGFR) in a patient who was successfully treated with post-transplantation infusions of TK cells. The patient had grade II acute GVHD, which resolved rapidly with the administration of ganciclovir, and was followed up for more than 3 years without the recurrence of GVHD. Characteristically, the patient received long-term oral acyclovir as prophylaxis against varicella zoster virus (VZV) reactivation. Our analysis focused on two issues: first, comparison of the immunological status of $LNGFR^+$ cells with that of $LNGFR^-$ cells at the onset of acute GVHD, at its remission and after restoration of the donor-derived immune system; and second, risk evaluation of spliced variants of TK cells leading to ganciclovir/acyclovir resistance, and assessment of clonal expansion of TK cells causing insertional oncogenesis.

Case report

A 46-year-old woman was diagnosed as having Philadelphia chromosome–positive acute lymphoblastic leukemia in January 2009. During consolidation therapy after imatinib-combined induction chemotherapy, the frequency of the minor *BCR-ABL* fusion gene increased gradually. Therefore, imatinib was replaced with dasatinib and she was referred to the National Cancer Center Hospital, when her disease status was confirmed as hematological complete remission with positive minor *BCR-ABL* fusion gene by quantitative reverse transcription–polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) analysis.

Because the patient had no HLA-matched sibling or alternative donors in the Japan Marrow Donor Program, she was registered for this clinical trial after providing informed consent. The conditioning regimen consisted of total body irradiation (8 Gy in four fractions), fludarabine (40 mg/m^2/d for 5 days), melphalan (70 mg/m^2/d for 2 days) and anti-thymocyte globulin (2.5 mg/m^2/d for 5 days). The donor's peripheral blood hematopoietic stem cells were mobilized with granulocyte colony-stimulating factor (G-CSF) and collected. $CD34^+$ cells were selected by use of the CliniMacs one-step procedure [1,8,9]. The numbers of infused $CD34^+$ and $CD3^+$ cells were, respectively, 5.4×10^6 and 1.7×10^4 cells per kilogram of recipient body weight. No GVHD prophylaxis was administered. Neutrophil engraftment was documented on day 15. Cytomegalovirus anti-genemia was detected with a maximum of 39 positive cells per 50,000 white blood cells by C7-HRP on day 47, and anti-viral treatment with foscarnet was administered for a total of 13 weeks (from day 13 to day 112 after transplantation).

The first add-back of 1×10^6 gene-modified cells/kg, with 95.0% viability and 90.5% purity of △LNGFR expression, was administered to the patient on day 21 after transplantation. Because neither GVHD nor immune reconstitution (defined as $CD3^+$ counts >100 cells/μL for two consecutive observations) was present, the second and third infusions of 1×10^7 gene modified cells/kg, with viabilities of 98.3% and 97.6%, were administered on day 55 and day 89 after transplantation, respectively. Defined immune reconstitution was achieved on day 112. Simultaneously, a rapidly spreading rash developed over 50% of the body surface area. Pathological examination of skin biopsy specimens on day 113 showed observations compatible with acute GVHD involving the skin (Supplemental Figure 1). In addition, 1.2×10^4 copies/10^5 cells of the herpes simplex virus thymidine kinase (HSV-TK) DNA were detected in the same skin biopsy sample by means of quantitative RT-PCR.

Grade II acute GVHD (skin, stage 3) was diagnosed and complete resolution was achieved with ganciclovir (10 mg/kg) from day 113 until day 123 and topical steroid therapy. Immediately after the start of ganciclovir, the patient's lymphocyte counts decreased (Figure 1). Skin re-biopsy on day 125 revealed normal pathological findings with no lymphocyte infiltration (Supplemental Figure 1) and the copy number of HSV-TK DNA decreased to 8.5×10^2 copies/10^5 cells. Thereafter, the patient had no recurrence of either acute GVHD or chronic GVHD. The results of RT-PCR for the *BCR-ABL* fusion gene remained negative, and complete donor chimerism has been reconfirmed by variable tandem repeat analyses. The patient received oral acyclovir (200 mg/d) as prophylaxis against VZV reactivation from day 126 until day 595. On day 645, a vesicular eruption on her forehead was diagnosed as herpes zoster and was successfully treated with intravenous administration of acyclovir. There have been no other infectious events to date. After the completion of ganciclovir administration on day 125, the patient's peripheral $LNGFR^+$ lymphocyte counts again increased and remained elevated for more