Biol Blood Marrow Transplant 23 (2017) 103–112

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Angiogenic Factors Correlate with T Cell Immune Reconstitution and Clinical Outcomes after Double-Unit Umbilical Cord Blood Transplantation in Adults

Ioannis Politikos [1,†], Haesook T. Kim [2], Theodoros Karantanos [1], Julia Brown [1], Sean McDonough [3], Lequn Li [1,‡], Corey Cutler [3], Joseph H. Antin [3], Karen K. Ballen [4], Jerome Ritz [3], Vassiliki A. Boussiotis [1,*]

[1] *Hematology-Oncology and Cancer Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts*
[2] *Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, Massachusetts*
[3] *Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, Massachusetts*
[4] *Bone Marrow Transplant Unit, Massachusetts General Hospital, Boston, Massachusetts*

Article history:
Received 13 April 2016
Accepted 15 October 2016

Key Words:
Umbilical cord blood transplantation
Immune reconstitution
Angiogenic factors
ANG-1
ANG-2
VEGF
TM

A B S T R A C T

Umbilical cord blood (UCB) is a valuable graft source for allogeneic hematopoietic stem cell transplantation (HSCT) in patients who lack adult donors. UCB transplantation (UCBT) in adults results in delayed immune reconstitution, leading to high infection-related morbidity and mortality. Angiogenic factors and markers of endothelial dysfunction have biologic and prognostic significance in conventional HSCT, but their role in UCBT has not been investigated. Furthermore, the interplay between angiogenesis and immune reconstitution has not been studied. Here we examined whether angiogenic cytokines, angiopoietin-1 (ANG-1) and vascular endothelial growth factor (VEGF), or markers of endothelial injury, thrombomodulin (TM) and angiopoietin-2 (ANG-2), associate with thymic regeneration as determined by T cell receptor excision circle (TREC) values and recovery of T cell subsets, as well as clinical outcomes in adult recipients of UCBT. We found that plasma levels of ANG-1 significantly correlated with the reconstitution of naive $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$ T cell subsets, whereas plasma levels of VEGF displayed a positive correlation with $CD4^+CD45RO^+$ T cells and regulatory T cells and a weak correlation with TRECs. Assessment of TM and ANG-2 revealed a strong inverse correlation of both factors with naive T cells and TRECs. The angiogenic capacity of each patient's plasma, as determined by an in vitro angiogenesis assay, positively correlated with VEGF levels and with reconstitution of $CD4^+$ T cell subsets. Higher VEGF levels were associated with worse progression-free survival and higher risk of relapse, whereas higher levels of TM were associated with chronic graft-versus-host disease and nonrelapse mortality. Thus, angiogenic factors may serve as valuable markers associated with T cell reconstitution and clinical outcomes after UCBT.

INTRODUCTION

The use of umbilical cord blood (UCB) as a graft source has expanded the application of allogeneic hematopoietic stem cell transplantation (HSCT) in patients who lack a suitable HLA-matched adult donor. The use of 2 UCB units has circumvented the stem cell dose barrier and has shortened the time to engraftment in adult recipients of UCB transplantation (UCBT) [1,2]. However, despite the improved myeloid engraftment, lymphoid reconstitution after UCBT remains delayed, even with the use of 2 UCB grafts [3,4].

Neovascularization is important in the hematopoietic and immunologic reconstitution process after HSCT [5].

Financial disclosure: See Acknowledgments on page 110.

* Correspondence and reprint requests: Vassiliki A. Boussiotis, MD, PhD, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Dana 513, Boston, MA 02215.
E-mail address: vboussio@bidmc.harvard.edu (V.A. Boussiotis).
† Current address: Memorial Sloan Kettering Cancer Center, Adult Bone Marrow Transplant Service, Department of Medicine, 1275 York Avenue, Box 298, New York, NY 10065.
‡ Current address: Division of Thoracic Surgery at Tongji Hospital, Tongji Medical School, Huazhong University of Science and Technology, Wuhan, China.

http://dx.doi.org/10.1016/j.bbmt.2016.10.013
1083-8791/

Vascular endothelial growth factor (VEGF) has a regulatory role in reconstitution of hematopoiesis after bone marrow injury or transplantation, likely because hematopoietic and endothelial cells share a common progenitor and express membrane receptors (Flt-1, Flk-1, Tie 2) for VEGF and other angiogenic cytokines. Specifically, mice deficient in VEGFR2 display profound defects in both vasculogenesis and hematopoiesis, whereas inhibition of VEGFR1 signaling prevents HSC differentiation and hematopoietic recovery after bone marrow suppression [6-8]. Consistent with the mandatory role of VEGF-mediated angiogenesis in hematopoiesis, VEGF blockade at the time of transplantation in lethally irradiated mice results in failure of hematopoietic reconstitution and early death [9]. Elevated levels of VEGF and/or its receptors have been reported in patients with hematologic malignancies, where they play a role in an autocrine/paracrine fashion [10-12], and have been shown to be of prognostic significance [13-16]. Levels of VEGF before allogeneic HSCT are associated with increased risk of relapse [17], but reports regarding its role on clinical outcomes such as graft-versus-host disease (GVHD), nonrelapse mortality (NRM), or survival are conflicting [17-20].

In addition to VEGF and its receptors, the angiopoietin/Tie-2 system plays a critical role in the regulation of vasculogenesis and hematopoiesis. Angiopoietin-1 (ANG-1) is a cognate ligand that serves as an agonist by inducing phosphorylation of the Tie-2 receptor and mediates vasculoprotective properties by promoting endothelial cell survival and migration [21]. In contrast, angiopoietin-2 (ANG-2) is a natural Tie-2 antagonist that renders blood vessels unstable [22]. The ANG-1–Tie-2 axis has been reported to play an important role in the repopulating activity of HSCs and recovery of hematopoiesis after myelosuppression [23,24]. Elevated levels of ANG-2 are associated with worse outcomes after HSCT [25,26]. However, the significance of ANG-1 and ANG-2 in the setting of UCBT is not known.

Thrombomodulin (TM) is a transmembrane glycoprotein expressed on the surface of all vascular endothelial cells where it promotes thrombin-mediated activation of protein C [27-29]. A soluble form of TM is also present in the plasma, produced either by secretion or by enzymatic cleavage of tissue TM after endothelial cell injury [30]. TM levels have been used as a marker of endothelial damage after HSCT [31,32], but the relationship between TM and clinical outcomes after UCBT is not well characterized.

A significant component of immune reconstitution after UCBT involves thymic-dependent generation of T cells [33]. HSCT compromises thymopoiesis by injury of the thymic microenvironment, particularly thymic epithelial cells. Human UCB is enriched in endothelial precursors that can sustain thymopoiesis in immunodeficient mice transplanted with human thymic grafts, where they engraft and promote neovascularization and wound healing [34]. Notably, the angiopoietins ANG-1 and ANG-2 have been implicated in the proliferation of endothelial cells from UCB $CD34^+$ progenitors [35]. Furthermore, in murine studies, VEGF has been shown to play a key role in thymic reconstitution after experimental transplantation [36,37]. Therefore, we hypothesized that in addition to their role in hematopoietic recovery and clinical outcomes, angiogenic factors might also be involved in the thymic recovery and T cell reconstitution after HSCT.

In the present study we investigated the association of plasma angiogenic factors with thymic and T cell reconstitution and with clinical outcomes in a cohort of adult patients undergoing double-unit UCBT (dUCBT). We determined that plasma levels of the 2 proangiogenic factors, VEGF and ANG-1, positively correlate with T cell subsets and T cell receptor excision circles (TRECs). In contrast, patients with high TM and ANG-2, markers of endothelial injury, have inferior thymic reconstitution at 1 year after dUCBT. Further, high VEGF levels are associated with worse progression-free survival (PFS) and increased incidence of relapse, whereas TM levels are associated with chronic GVHD (cGVHD), higher NRM, and worse overall survival (OS). These findings suggest that circulating angiogenic factors may be involved in post-transplant immune reconstitution and might serve as prognostic markers for clinical outcomes after UCBT.

METHODS

Patients and UCB Units

Patients were enrolled in a phase II study of dUCBT at the Dana Farber/Harvard Cancer Center. Eligibility criteria, detailed design, and clinical outcomes of the trial were previously reported [38,39]. In brief, patients were eligible for enrollment if they were adults, had a hematologic malignancy, and lacked a suitable related (6/6 or 5/6 HLA-A, -B, and -DRB1 matched) or unrelated (10/10 HLA-A, -B, -C, -DRB1, and -DQ matched) donor. UCB units were obtained from national and international cord blood banks. Each individual UCB unit was required to have a minimum of 1.5×10^7 total nucleated cells/kg before cryopreservation, and the 2 UCB units selected for each subject were required to provide a minimum of combined pre-cryopreservation cell dose of 3.7×10^7 total nucleated cells/kg. UCB units were required to be a 4/6 match or better at the allele level for HLA-A, -B, and -DRB1 with each other and with the recipient. The UCB units were hierarchically selected on the basis of higher cell dose, greater HLA match, and younger age of the unit.

Treatment Protocol

On a research study, all subjects were conditioned with fludarabine (30 mg/m^2/day) for 6 consecutive days (days −8 through −3; total dose 180 mg/m^2), melphalan (100 mg/m^2) on day −2, and rabbit antithymocyte globulin (Thymoglobulin, Sangstat, Fremont, CA, 1.5 mg/kg/day) on days −7, −5, −3, and −1. Two UCB units were infused sequentially between 1 and 6 hours apart on day 0. After transplantation, patients received transfusion support, and filgastrim (5 μg/kg/day) was administered from day +5 until an absolute neutrophil count was higher than 2.0×10^9 cells/L for 2 consecutive days. GVHD prophylaxis began on day −3 with tacrolimus (.05 mg/kg for a target serum level of 5 to 10 ng/mL) and an oral loading dose of sirolimus (12 mg). Sirolimus was subsequently dosed orally once a day for a goal serum trough level of 3 to 12 ng/mL. Both GVHD prophylaxis agents were tapered from day 100 through day 180 for patients with no evidence of GVHD.

The research protocol was approved by the Institutional Review Board of the Dana Farber/Harvard Cancer Center. Written informed consent was obtained from all patients before enrollment to the study. The trial was prospectively registered at http://www.clinicaltrials.gov (NCT00133367).

Immunophenotyping

Patient blood samples were collected before administration of conditioning chemotherapy and at 4 weeks, 8 weeks, 100 days, 6 months, and 1 year after transplantation. Peripheral blood mononuclear cells were isolated using Ficoll-Paque Plus (GE Healthcare, Chicago, IL) and stained with fluorescence-conjugated monoclonal antibodies for lineage-specific marker analysis, using a BD FACSCanto flow cytometer (BD Biosciences, San Jose, CA).

TREC Analysis

TREC analysis was performed according to a previously described protocol [40]. DNA was isolated from peripheral blood mononuclear cells with a QIAmp DNA Mini Kit (Qiagen, Germantown, MD). Quantification of signal-joint TREC DNA was performed by quantitative-competitive PCR, using a Rotor-Gene 6000 thermal cycler (Corbett Life Science). The standard curve was prepared with 10-fold dilutions of a plasmid containing the signal-joint TREC sequence (kindly provided by Daniel Douek, National Institute of Allergy and Infectious Diseases).

Cytokine Measurements

Patient plasma was collected after centrifugation of citrated blood samples collected before transplantation and at 4 weeks, 8 weeks, 100 days, 6 months, and 1 year after transplantation. For each time point the cytokines VEGF, ANG-1, ANG-2, and TM were measured with commercial Colorimetric Sandwich ELISA kits (R&D Systems), according to the manufacturer's instructions.