Biol Blood Marrow Transplant 20 (2014) 1501–1507

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Impaired Interferon-Alpha Production by Plasmacytoid Dendritic Cells after Cord Blood Transplantation in Children: Implication for Post-transplantation Toll-Like Receptor Ligand–Based Immunotherapy

Emily Charrier [1,2], Paulo Cordeiro [1], Rose-Marie Brito [1], Michaël Harnois [1,3], Samira Mezziani [1], Sabine Herblot [1], Françoise Le Deist [1,3,4], Michel Duval [1,2,3,4,*]

[1] *Groupe de Recherche en Transplantation et Immunologie du Sang de Cordon (GRETISC), Centre de Cancérologie Charles Bruneau, Centre de Recherche du CHU Sainte-Justine, Montréal, Québec, Canada*
[2] *Département de Sciences Biomédicales, Université de Montréal, Québec, Canada*
[3] *Département de Microbiologie et d'Immunologie, Université de Montréal, Québec, Canada*
[4] *Département de Pédiatrie, Université de Montréal, Québec, Canada*

Article history:
Received 13 February 2014
Accepted 5 June 2014

Key Words:
Cord blood transplantation
Plasmacytoid dendritic cells
Interferon-alpha
Toll-like receptor (TLR)-9 agonist

A B S T R A C T

Plasmacytoid dendritic cells (pDCs) initiate both innate and adaptive immune responses, making them attractive targets for post-transplantation immunotherapy, particularly after cord blood transplantation (CBT). Toll-like receptor (TLR) agonists are currently studied for pDC stimulation in various clinical settings. Their efficacy depends on pDC number and functionality, which are unknown after CBT. We performed a longitudinal study of pDC reconstitution in children who underwent bone marrow transplantation (BMT) and single-unit CBT. Both CBT and unrelated BMT patients received antithymocyte globulin as part of their graft-versus-host disease prophylaxis regimen. pDC blood counts were higher in CBT patients than in healthy volunteers from 2 to 9 months after transplantation, whereas they remained lower in BMT patients. We showed that cord blood progenitors gave rise in vitro to a 500-fold increase in functional pDCs over bone marrow counterparts. Upon stimulation with a TLR agonist, pDCs from both CBT and BMT recipients upregulated T cell costimulatory molecules, whereas interferon-alpha (IFN-α) production was impaired for 9 months after CBT. TLR agonist treatment is thus not expected to induce IFN-α production by pDCs after CBT, limiting its immunotherapeutic potential. Fortunately, in vitro production of large amounts of functional pDCs from cord blood progenitors paves the way for the post-transplantation adoptive transfer of pDCs.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the unique hope for cure for patients with chemotherapy-refractory leukemia through the combined antileukemic effects of the conditioning regimen and the donor-derived immune response [1]. This immune graft-versus-leukemia (GVL) effect is altogether due to T cells and natural killer (NK) cells [2–5]. Many patients still relapse after transplantation and the prognosis for high-risk patients remains dismal [6]. Several approaches are, thus, currently under investigation to enhance this GVL effect. Among them, the activation of immune cells involved in the early steps of the immune cascade, such as plasmacytoid dendritic cells (pDCs), is particularly appealing, as these cells are expected to activate both innate and adaptive immunity. Indeed, upon activation of their toll-like receptors (TLR), pDCs first secrete high amounts of interferon-alpha (IFN-α) and other cytokines, leading to NK cell activation, and then they upregulate T cell costimulatory molecules, allowing them to act as antigen-presenting cells [7–11].

Synthetic DNA oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs) have been shown to induce strong immune stimulation and increase antitumor responses both in animal models and in humans [12–14]. CpG ODNs mimic viral or bacterial DNA and bind TLR-9 in the endosomal compartment of pDCs [12,14]. The safety and activity of TLR-9 agonists are being explored in a wide range

Financial disclosure: See Acknowledgments on page 1506.
* Correspondence and reprint requests: Michel Duval, Centre de Cancérologie Charles Bruneau, CHU Sainte-Justine, 3175 Côte Sainte-Catherine, Room A-12-52, Montréal, Québec H3T 1C5, Canada.
E-mail address: michel.duval@umontreal.ca (M. Duval).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.06.007

of tumor types as part of a variety of therapeutic strategies aimed at harnessing the immune response to fight cancer [15]. In particular, TLR-9 agonists could be used after HSCT to increase the GVL effect [16]. To be effective, TLR-9 agonists need the presence of functional pDCs to initiate immune responses. It has been shown that pDC blood counts remain below normal levels for 1 year after bone marrow (BM) transplantation (BMT) and even lower in patients experiencing acute graft-versus-host disease (GVHD) [17,18]. However, the functional properties of post-HSCT pDCs have not been explored and pDC count recovery has never been studied after cord blood (CB) transplantation (CBT).

Indeed, CB is increasingly being chosen as a graft source because of its immediate availability [19] and greater tolerance to HLA disparity, as well as its lower incidence of GVHD combined with a preserved GVL effect [20]. Enhancing the GVL effect after CBT poses specific challenges, as donor lymphocytes are not available for adoptive immunotherapy. Therefore, targeting cells involved early in the immune cascade may be particularly well suited for post-CBT immunotherapy.

To assess post-HSCT pDC reconstitution, we undertook the first longitudinal study of the recovery of pDC counts and function in CBT and BMT patients. We enrolled 53 patients to explore the recovery of pDC count and function after BMT and CBT. The 2 main functional hallmarks of pDCs after TLR-9 stimulation were assessed: the production of IFN-α, and the upregulation of T cell costimulatory molecules CD40, CD54, CD80, and CD86.

METHODS

Patients

Written informed consent was obtained in accordance with the Declaration of Helsinki after CHU Sainte Justine's institutional review board approval. Blood samples were collected at 1, 2, 3, 6, 9, and 12 months after transplantation. Fifty-three patients (19 BMT and 34 CBT) were enrolled between 2005 and 2010 (Table 1). Patients with donor chimerism < 90% at 1 month after transplantation were excluded from the study (2 BMT and 6 CBT patients). pDC blood counts were, thus, analyzed for 17 BMT and 28 CBT patients. In case of leukemia relapse, data were censored at the time of relapse. Grafts were not T cell depleted. All patients received myeloablative reparative regimens, except for 3 patients in CBT group and 3 patients in BMT group; 10 patients who underwent CBT and 3 who underwent BMT received total body irradiation. GVHD prophylaxis for related BMT patients included a short course of methotrexate and cyclosporine for 6 months after transplantation. Unrelated BMT patients received a short course of methotrexate, cyclosporine until 10 months after transplantation, and rabbit antithymocyte globulin (ATG, Genzyme, Cambridge MA), 2 mg/kg, on days −2, −1, +1, +2. All CBT patients were unrelated. They received ATG, methylprednisolone followed by an oral

Table 1
Patient and Transplantation Characteristics

Characteristic	Bone Marrow Transplantation	Cord Blood Transplantation	*P* Value*
No. of patients	17	28	
Age at transplantation, median (range), yr	11.6 (6-17.4)	9.9 (.5-19.9)	.06
Sex, n (%)			.22
Female	9 (53)	9 (32)	
Male	8 (47)	19 (68)	
Diseases, n (%)			.16
Malignant	10 (59)	23 (82)	
ALL	3	12	
AML	5	4	
CML	1	1	
MDS/JMML	1	5	
Neuroblastoma	0	1	
Nonmalignant	7 (41)	5 (18)	
Donors, n (%)			<.0001
Related	12 (71)	0 (0)	
Unrelated	5 (29)	28 (100)	
Number of HLA mismatches, n (%)			<.0001
0	16 (94)	3 (11)	
1	1 (6)	16 (57)	
2	0 (0)	9 (32)	
Total nucleated cells infused, median (range), · 10^8/kg	4.1 (2.5-5.3)	.5 (.3-2.5)	<.0001
$CD34^+$ cells infused, median (range), · 10^6/kg	5.0 (.1-12.5)	.2 (.1-1.8)	<.0001
Length of neutropenia, median (range), d	22 (10-25)	22 (8-34)	.42
Length of thrombopenia, median (range), d	24 (0-82)	47 (0-80)	.0006
Acute GVHD (stages II-IV), n (%)	0 (0)	3 (11)	.28
Chronic GVHD, n (%)	0 (0)	1 (4)	1.00
Relapse, n (%)	4 (24)	9 (32)	.74
Time from transplantation (range), d	(210-668)	(68-1310)	
Patients with EBV viremia, n (%)	11 (65)	19 (68)	1.00
Patients with CMV viremia, n (%)	0 (0)	6 (21)	.07
Deaths, n (%)	1 (6)	7 (25)	.13
From relapse	1	6	
Other causes	0	1	

ALL indicates acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myeloblastic leukemia; MDS, myelodysplastic syndrome; JMML, juvenile myelomonocytic leukemia; EBV, Epstein-Barr virus; CMV, cytomegalovirus.

* *P* value: Fisher test for categoric variables and Mann-Whitney for continuous variables.