Biology of Blood and Marrow Transplantation 14:693-701 (2008)

1083-8791/08/1406-0001$32.00/0
doi:10.1016/j.bbmt.2008.04.001

Recipient-Derived Cells after Cord Blood Transplantation: Dynamics Elucidated by Multicolor FACS, Reflecting Graft Failure and Relapse

Nobukazu Watanabe,[1]* *Satoshi Takahashi*,[2]* *Masayuki Ishige*,[1] *Yumiko Ishii*,[1] *Jun Ooi*,[2] *Akira Tomonari*,[2] *Nobuhiro Tsukada*,[2] *Takaaki Konuma*,[2] *Seiko Kato*,[2] *Aki Sato*,[2] *Arinobu Tojo*,[2] *Hiromitsu Nakauchi*[3]

[1] FACS Core Laboratory; [2] Research Hospital; and [3] Center for Stem Cell and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Correspondence and reprint requests: Nobukazu Watanabe, MD, PhD, FACS Core Laboratory, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan (e-mail: nwatanab@ims.u-tokyo.ac.jp).

*The first two authors contributed equally to this work.

Received February 20, 2008; accepted April 7, 2008

ABSTRACT

Although umbilical cord blood has been increasingly used as an alternative donor source to treat hematologic malignancies, cord blood transplantation (CBT) is frequently complicated by graft failure and relapse of primary diseases. Because persistence or increase of recipient-derived hematopoietic or malignant cells has pathogenic import under these conditions, analysis of recipient-derived cells should be useful to understand the pathogenesis of graft failure and relapse of primary disease. Because most CBT involves human leukocyte antigen (HLA)-mismatched transplantation, we developed a 9-color fluorescence activated cell sorter (FACS)-based method of mixed chimerism (MC) analysis using anti-HLA antibodies to detect mismatched antigens (HLA-Flow method). Among $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, monocytes, and granulocytes, donor- and recipient-derived cells alike could be individually analyzed simultaneously in a rapid, quantitative and highly sensitive manner, making the HLA-Flow method very valuable in monitoring the engraftment process. In addition, this method was also useful in monitoring recipient-derived cells with leukemia-specific phenotypes, both as minimal residual disease (MRD) and as early harbingers of relapse. Leukemia relapse can be definitively diagnosed by cytogenetic or PCR studies using recipient-derived cells sorted for leukemia markers. Multicolor HLA-fFlow analysis and cell sorting in early diagnosis of graft failure and relapse was confirmed as valuable in 14 patients who had received HLA-mismatched CBT.

KEY WORDS

Chimerism • HLA • Flow cytometry • Cord blood transportation

INTRODUCTION

Analysis of donor-recipient mixed chimerism (MC) after allogeneic stem cell transplantation has become routine in confirming the engraftment of donor-derived cells. Polymerase chain reaction-based short tandem repeat analysis (STR-PCR) [1] and X/Y chromosome analysis using fluorescence in situ hybridization (X/Y-FISH) [2] after gender-mismatched transplantation are the methods most commonly used for mixed chimerism (MC) analysis. Persistence of recipient-derived cells early after transplantation or increases in their numbers are thought to be risk factors for relapse of leukemia [3-5]. In fact, if immunosuppression is discontinued or donor lymphocyte infusions (DLI) are given after early identification of increased numbers of recipient-derived cells, the outcome of SCT is significantly improved [6,7]. Because lineage-specific analysis of MC is thought to be important in understanding the pathogenesis of graft failure and subsequent relapse of leukemia, several research groups evaluated MC of individual leukocyte subpopulations using magnetic cell separation or fluorescence-activated cell sorting (FACS) techniques followed by PCR-based analyses [8,9]. PCR- or

FISH-based methods, however, are complicated, insensitive, and time consuming, especially in the case of lineage-specific MC analysis.

During the last decade, the technologies supporting multicolor FACS analysis have been dramatically developed [10,11]. This methodology is very useful to investigate the pathogenic conditions of human diseases by the simultaneous analysis of many phenotypes and functions of cells [12,13]. If donor- and recipient-derived cells have specific surface markers that are able to be stained by fluorescence-conjugated antibodies, respectively, chimerism analysis may be possibly done by flow cytometry in a rapid, quantitative, and highly sensitive manner.

The number of cord blood transplantations (CBTs) has increased recently. Most CBTs are carried out with human leukocyte antigen (HLA)-mismatched donor-recipient combinations, especially in adult patients [14-16]. If donor- and recipient-specific anti-HLA antibodies, respectively, can stain donor- and recipient-specific HLAs, lineage-specific MC theoretically can be analyzed by multicolor flow cytometry in the setting of HLA-mismatched transplantation [10,11].

Serologic analysis of HLA was first established by Paul I. Terasaki as a lymphocyte toxicity test using antiserum from a multiparous woman that contained anti-HLA polyclonal antibodies [17]. These polyclonal antibodies cannot be used for flow cytometric analysis because of their low affinity and complicated crossreactivity for HLAs [18]. Because the availability of these anti-HLA polyclonal antibodies is limited and it is difficult to maintain their quality, Terasaki et al. established hybridomas to produce anti-HLA monoclonal antibodies (mAbs) that can be used for serologic analysis of HLA. These anti-HLA antibodies are also useful for flow cytometric analysis [19,20].

To overcome the problems of current PCR- or FISH-based methods for MC analysis, we have developed a flow cytometry-based method, using fluorescence-conjugated anti-HLA mAbs, of MC analysis after HLA-mismatched transplantation. Here we show that this HLA-Flow method is very useful for analysis of lineage-specific MC after HLA-mismatched CBT.

RESULTS

Analysis of Recipient-Derived Cells Using the HLA-Flow Method to Monitor Engraftment Early after CBT

We analyzed lineage-specific MC in 9 patients early after CBT (Table 1; unique patient numbers [UPNs] 1 through 9). Figure 1A shows typical results of peripheral blood MC analysis at week 2 after CBT for mononuclear cells (PBMCs) and leukocyte subpopulations (UPN 1). Lineage-specific MC could be separately analyzed for $CD4^+$ T cells, $CD8^+$ T cells, NK cells, monocytes, and granulocytes. The highest frequency of recipient-derived cells existed in the $CD4^+$ T cell subset (4.62% of total $CD4^+$ T cells). In 7 patients, recipient-derived cells were consistently observed in the $CD3^+$ T cell subset before week 3 after CBT (UPNs 1, 3-5, and 7-9). These patients showed complete chimerism at week 4 after CBT (Figure 1B) and all achieved donor-type engraftment successfully (data not shown). Another patient (UPN 2) maintained

Table 1. *Patient Characteristics and Outcomes after Cord Blood Transplantation*

			Donor HLA		Recipient HLA		
UPN	Disease	Age	A	B	A	B	Outcome
1	AML (M2)	50	2, 24	52, 62	2, 11	52, 67	Relapse (day 281), 2nd CBT (day 497)
2	MDS (RAEB)	41	2, 24	13, 61	2, 26	13, 61	CR (day 434)
3	MDS (Overt AML)	44	11, 26	61, 61	24, 26	7, 61	CR (day 592)
4	AML (M2)	41	2, 31	52, 61	2, 31	61, 62	Relapse (day 203), 2nd CBT (day 314)
5	AML (M0)	33	11, 24	7, 54	2, 24	7, 39	CR (day 137)
6	AML (M3)	50	2, 24	71, 61	1, 2	38, 61	Death (day 21, HHV6B encephalitis)
7	AML (M2)	52	2, 24	35, 67	11, 24	56, 67	CR (day 112)
8	AML (M2)	55	2, 2	51, 52	24, 24	51, 52	CR (day 105)
9	AML (M4Eo)	49	11, 11	62, 71	24, 24	62, 72	CR (day 71)
10	AML (M2)	49	2, 24	46, 52	2, 26	46, 52	CR (day 1,568)
11	MDS (RAEB-T)	35	24, 33	44, 52	26, 33	61, 44	CR (day 613)
12	AML (M4)	43	2, 24	46, 62	24, 31	46, 61	CR (day 938)
13	AML (M4)	45	2, 24	61, 62	2, 24	7, 61	CR (day 844)
14	MDS (Overt AML)	42	11, 33	55, 44	24, 33	55, 44	CR (day 1,569)

Conditioning regimen consists of TBI (12 Gy) + Ara-C + CY in all patients except 2 (TBI + Ara-C + Flu in UPNs 8 and 13). GVHD prophylaxis consists of CsA + MTX in all patients except 1 (CsA + MMF in UPN 8). Subtype of HLA-A and B specifically recognized by anti-HLA antibodies are underlined. Both engraftment and minimal residual disease were monitored in UPN 1-4. Only engraftment was monitored in UPN 5-9. Only minimal residual disease was monitored in UPN 10-14. Outcomes were confirmed on August 10, 2007.

UPN indicates unique patient number; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess of blasts; RAEB-T, RAEB in transplantation; TBI, total body irradiation; Ara-C, cytosine arabinoside; G, granulocyte colony-stimulation factor; CY, cyclophosphamide; Flu, fludarabin; CsA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil.