Journal of Clinical Virology 56 (2013) 331–335

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

HHV-6 cell receptor CD46 expression on various cell subsets of six blood and graft sources: A prospective series

Patrice Chevallier[a,*], Nelly Robillard[b], Marina Illiaquer[c,d], Julie Esbelin[e], Mohamad Mohty[a], Celine Bodin-Bressollette[c,d], Thierry Guillaume[a], Veronique Stocco[f], Fabienne Auffray[f], Sophie Derenne[f], Lucie Planche[g], Marie-Christine Bene[b], Berthe-Marie Imbert-Marcille[c,d]

[a] Service d'Hématologie Clinique, CHU Hôtel Dieu, Université de Nantes, Centre d'Investigation Clinique en Cancerologie (CI2C) and INSERM U892, Nantes, France
[b] Service d'Hematologie/Biologie, CHU de Nantes, France
[c] Service de Virologie, CHU de Nantes, Nantes, France
[d] UPRES-EA4271, PRES L'UNAM, Nantes University, France
[e] Service de gynecologie/obstetrique, CHU de Nantes, Nantes, France
[f] Unité d'ingénierie cellulaire, EFS Pays de la Loire, Nantes, France
[g] Cellule de Promotion à la Recherche Clinique, CHU de Nantes, Nantes, France

ARTICLE INFO

Article history:
Received 6 August 2012
Received in revised form 23 October 2012
Accepted 6 December 2012

Keywords:
HHV-6
CD46
Cord blood
Allo-SCT
Leukapheresis
GCSF

ABSTRACT

Background: Cord Blood (CB) are increasingly used as an alternative stem cells source in adults for allogeneic Stem Cell Transplantation (allo-SCT). The risk of *human herpesvirus* (HHV-6) reactivation is significantly higher after CB transplant vs unrelated peripheral blood stem cells (PBSC) allo-SCT. Higher HHV-6 cell receptor CD46 expression on progenitor cells in CB may explain this difference.
Objectives: To prospectively compare the HHV-6 cell receptor CD46 expression on various cell subsets of three freshly harvested blood sources on one hand and of three graft sources on the other hand.
Study design: 52 samples were used for the purpose of this study. They were issued from peripheral blood (PB, $n = 10$), G-CSF mobilised PB (GCSF-PB, $n = 10$), cord blood (CB, $n = 10$), unmanipulated bone marrow (uBM, $n = 5$), leukapheresis product (LP, $n = 10$) and thawed CB graft ($n = 7$). CD46 expression was assessed by FACS analysis on total lymphocytes, monocytes, NK cells, T and B cells subsets, plasmacytoid (pDCs) dendritic cells and stem cells.
Results: As all cell subsets were found CD46 positive, CD46 mean fluorescence intensity (MFI) was then considered for comparison between the three blood sources and the three graft sources. The most impressive result observed was that HHV-6 cell receptor CD46 expression was significantly reduced in almost all cell components of thawed CB graft compared to other graft sources.
Conclusions: This original study shows strong differences in term of quantitative CD46 expression between several blood and grafts samples. Our results suggest that other factors than the qualitative CD46 expression play a role in the higher HHV-6 reactivation observed after CB transplant in adults.

1. Background

Cord Blood (CB) is increasingly used as an alternative stem cells source in adults for allogeneic stem cell transplantation (allo-SCT).[1] Collection of CB is very easy and there is no risk for the donor. CB has the advantage of immediate availability and of an acceptable partial HLA mismatches due to the immaturity of newborn cells and of a lower risk of transmissible diseases for the recipient.[2] Feasability of CB transplant, especially in adult acute myeloid leukaemia (AML) patients, has been already demonstrated.[3] Nevertheless, the proportion of infections-related deaths within 100 days after allo-SCT is significantly higher when using CB compared to other sources.[4,5] We have also shown recently that the risk of *human herpesvirus 6* (HHV-6) reactivation was significantly higher after CB transplant vs unrelated peripheral blood stem cells (PBSC) allograft while the incidences of *cytomegalovirus* (CMV) or *Epstein–Barr virus* (EBV) reactivations were similar between both groups.[6] According to the International Committee of Taxonomy of viruses, HHV-6 is described under the order of the herpesvirales and as belonging to the family of herpesviridae, the subfamily

* Corresponding author at: Service d'Hématologie Clinique, Centre Hospitalier Universitaire de Nantes, Place Alexis Ricordeau, 44093 Nantes cedex 01, France. Tel.: +33 240083271; fax: +33 240083250.
E-mail address: patrice.chevallier@chu-nantes.fr (P. Chevallier).

1386-6532/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2012.12.005

Table 1
Characteristics of the donors according to the blood or graft sources.

Donors	Median age (range)	Sex: male	Type of donor: related
PB [$n = 10$]	50 years (29–67)	6 (60%)	7 (70%)
GCSF-PB [$n = 10$]	54 years (33–67)	7 (70%)	8 (80%)
CB [$n = 10$]	0	5 (50%)	NA
uBM [$n = 5$]	40 years (20–50)	4 (80%)	4 (80%)
LP [$n = 10$]	54 years (33–67)	6 (60%)	6 (60%)
Thawed CB graft [$n = 7$]	0	3 (43%)	NA

Abbreviations: PB: peripheral blood; GCSF-PB: granulocyte colony stimulating factor-stimulated PB; CB: cord blood; uBM: unmanipulated bone marrow graft; LP: leukapheresis product; NA: not applicable.

of betaherpesvirinae (such as CMV and HHV-7) and the genus of roseolovirus (such as HHV-7). Finally, HHV-6 must be currently distinguished as two distinct species: HHV-6A and HHV-6B. HHV-6 is a double-stranded DNA beta-herpesvirus which is the causative agent of exanthema subitum (roseola) in children and reactivates in immunocompromised hosts such as transplanted or HIV-seropositive patients.[7] HHV-6B seems to be the sole species detected in stem cell transplanted patients.[6,8] CD46, a ubiquitous type-1 glycoprotein belonging to the family of regulators of complement activation, has been identified as one of the HHV-6 cell receptor for both species.[9] However, one HHV-6B strain (HST) has been reported to be unable to induce syncytia formation mediated by CD46, suggesting the existence of an alternative receptor for at least some HHV-6B strains.[10] This could explain the differences observed in term of cell tropism between the two species. If HHV-6A and HHV-6B can be detected in CD4+ T cells, which are the primary target cells for both species, or in monocytes/macrophages, dendritic cells or progenitors cells, HHV-6B does not replicate in CD8+ T cells, NK cells or gamma/delta T cells.[11]

One explanation for higher HHV6 reactivation after CB transplant is that the expression of the HHV6 cell receptor CD46 has been found significantly higher on hematopoietic progenitor cells in CB compared to PBSC grafts.[12] This hypothesis is further strengthened by the fact that CD46 is also a receptor for adenovirus, which reactivation is also more frequent after CB allograft.[13]

2. Objectives

In order to confirm higher CD46 expression in CB cells and to better understand the mechanism of HHV-6 reactivation after CB transplant, we have conducted a prospective study with the aim to compare the CD46 expression within various cell subsets on six different blood and graft sources.

3. Study design

3.1. Collection of blood and graft samples

Freshly harvested peripheral blood (PB, $n = 10$), PB from donors mobilised with G-CSF before (indicated hereinafter as "GCSF-PB" samples, $n = 10$) and after leukapheresis (indicated hereinafter as leukapheresis product or "LP" samples, $n = 10$) and unmanipulated bone marrow (uBM, $n = 5$) samples were collected from 25 healthy donors after they gave informed consent between April and October 2011. Donors (male $n = 14$; female $n = 11$) were healthy siblings ($n = 17$) or unrelated donors ($n = 8$) with a median age of 41 years (range: 20–67). PBSC donors received 10 μg/kg of G-CSF (Neupogen, Amgen, France) subcutaneously during four days and leukapheresis was started on day 5 after G-CSF administration. Donor venous access was obtained through bilateral cubital veno puncture and PBSCs harvested using a continuous flow blood cell separator (Cobe Spectra, Cobe BCT, Lakewood, CO, USA). The uBM grafts were collected from the posterior iliac crest under general anaesthesia. Collection of CB samples ($n = 10$) was performed at time of delivery in 10 pregnant women followed at the obstetric department of the CHU de Nantes after informed consent. Frozen CB grafts ($n = 7$) from patients who died before their programmed allograft were a gift from the EFS of Nantes. Blood sources (PB, GCSF-PB and CB) were collected (1–7 ml) and analysed within 24 h of harvest. Graft sources (uBM, LP, thawed CB (indicated hereinafter by "tCB graft")) were collected from the graft packet (around 1 ml) and analysed immediately. Overall, 52 samples (blood $n = 30$; graft $n = 22$) were analysed for the purpose of this study. Characteristics of donors according to each sample source are given in Table 1. The protocol was approved by the institutional review board of the CHU de Nantes.

3.2. Immunofluorescence staining and flow cytometry analyses

The different cellular subsets were assessed by four/five or six-colour immunofluorescence analyses using a FACS CANTO II (BD Biosciences, San Jose, CA, USA). Briefly, 10–50 μl samples from PB, GCSF-PB, CB, uBM, LP and tCB graft were incubated for 10 min at room temperature in the dark with the FITC-, PE-,APC-, PerCP-, PECy7 and the V500- conjugated monoclonal antibodies (MoAbs) according to the seven combinations shown in Table 2. MoAbs were all purchased from Beckton Dickinson (BD Biosciences) except IgG1-FITC, CD10-PE, CD19-APC and CD27-PECy7 which were obtained from Beckman-Coulter (Villepinte, France) and CD303-APC (BDCA-2) which was obtained from Myltenyi Biotec Inc (Auburn, CA, USA).

Then, erythrocytes were lysed (FACS lysing solution, BD) and samples were washed once with phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) (Sigma, St-Louis, MO, USA). Finally cells were fixed in PBS/0.5% paraformaldehyde (Sigma) and analysed directly on the FACS CANTO II. Forty thousand lymphocytes were acquired and all events stored using BD FACSDiva V6.1.3 (BD Biosciences) which is the operating software on the FACS CANTO II. Data were analysed using this same analysis software.

3.3. Definition of cell populations

Total lymphocytes, monocytes, NK cells, T and B cells subsets, plasmacytoid (pDCs) dendritic cells and stem cells were the cell components considered for the purpose of this study. Phenotypic definitions of cell subsets are given in Table 3.[14–19] A example of FACS analysis is given in Supplemental File 1.

3.4. CD46 expression

CD46 expression and mean fluorescence intensity (MFI) were assessed on all considered population subsets (see definition of cell populations above). As lin- and CD46 MoAbs were both FITC-conjugated, CD46 expression and MFI on pDCs was assessed looking at only a DR(+)/BDCA-2(+)/CD123(+) cell population. This was possible as we have found that the percentage of lin-/DR(+)/BDCA2(+)/CD123(+) cells (Table 2) was similar to the