HLA-B, HLA-C Genotypes and Red Blood Cell Content in Umbilical Cord Blood are Related to CD34⁺ Cell Viability after Thawing

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INTRODUCTION

Umbilical cord blood (UCB) is an important source of hematopoietic stem and progenitor cells (HSPC) for bone marrow reconstitution in several hematological diseases.[1] Clinical advantages such as immediate availability, higher tolerance to human leukocyte antigen (HLA) mismatching between patient and donor, lower severity of graft versus host disease (GVHD) and lesser risk of infectious disease transmission compared to other sources have been reported.[2,3] The selection of a suitable UCB unit for a patient includes two main characteristics which have proven good correlation with the transplant success: HLA matching and total nucleated cell (TNC) dose.[2] A low cell dose will cause engraftment failure and therefore, increase the mortality risk.[4] UCB banks also report additional information such as HSPC (CD34⁺ cell) and colony-forming unit (CFU) assays, but the importance of those variables on selecting an appropriate donor is not completely clear.[4] The total CD34⁺ cell content has been proposed as a selection criterion, particularly when TNC dose does not reach the optimal, since it exhibits an impact on neutrophil and platelet engraftment, mainly in adult patients with single or double unit transplant.[4–6] Therefore, it has been stated that CD34⁺ cell viability and clonogenic capacity after thawing is a very important variable when selecting a unit for transplantation and may be a good predictor of engraftment.[7] The aim of this study was to determine if any intrinsic or processing variable was associated with the post-thawing CD34⁺ cell viability and recovery when performing confirmatory testing using a CBU attached-segment.

METHODS

UCB units of our inventory were collected in public hospitals in Bogotá, Colombia by trained nurses. All mothers signed an informed consent for clinical and research purposes. UCB units were transported to the laboratory and white blood cell (WBC) content was measured by an automated hematology analyzer to determine if the units were suitable for cryopreservation (TNCs > $10x10^8$). Volume reduction was performed in a closed system with previous HES addition. After processing, quality control tests were performed using flow cytometry (CD34⁺ and CD45⁺ cell count and viability) and clonogenic assays (CFU). UCB units were cryopreserved by DMSO/Dextran 40 addition in a closed system and subsequently transferred to a controlled-rate freezer. All units were stored in vapor phase of liquid nitrogen. UCB units included in the transplant inventory were typed in high resolution in an accredited laboratory.

For confirmatory testing, the distal attached segments of 72 UCB units were removed with permanent temperature control. Thawing was performed using a HES-based media and a 30-minutes stabilization incubation as previously standardized. Flow cytometry and clonogenic assays were performed. Fifty-three UCB units were HLA typed. Multivariate analyses by a linear model were performed in R. Two-groups comparison were performed with a Mann-Whitney test and the three groups comparison was analyzed with a Kruskal-Wallis test in Graph Pad Prism.

RESULTADOS

Post-reduction variables were selected to determine their effect on CD34⁺ cell recovery after thawing by a linear model in a multivariate analysis (Table 1a). However, the model did not explain CD34⁺ cell viability with those variables. To find a suitable linear model, HLA data were included along with red blood cells (RBC), and turned out statistically significant only with HLA-B and HLA-C loci (Table 2b).

a.

CD34⁺ Cell recovery		
Variable	Estimate	p
PR CD34⁺	-1,2E-06	0,202
PR CD45⁺	1,6E-09	0,8532
PR RBC x 10^9/µl	-4,24	0,104
Vol. Red. Eff.	***-0,04***	***0,029***
PR Neut %	-0,03	0,392
R^2 = 0,12	p = 0,019	

b.

HLA Gene	# Genotypes	R^2	p
HLA-A	34	0.16	0.29
HLA-B	***47***	***0.89***	***0.004***
HLA-C	***36***	***0.59***	***0.007***
HLA-DRB1	43	0.12	0.42
HLA-DQB1	26	0.24	0.10

Table 2. Multivariate analysis of **a)** CD34⁺ cell recovery in a linear model including post-reduction variables and **b)** CD34⁺ cell viability explained in a linear model including HLA genotypes in each locus and post-reduction RBC content.

There were HLA-B (Figure 1a) and HLA-C (Figure 1b) genotypes statistically associated with a higher or lower CD34⁺ cell viability. The remaining alleles are included in the model but not shown in the figure since they were not statistically significant independently. The model with HLA-C included also neutrophil percentage after volume reduction.

a.

HLA-B		
Variable	Estimate	p
RBC 10^9/µl	-8,58	0,010
B*07:02g/B*35:43g	69,58	0,000
B*07:02g/B*48:01g	-45,9	0,002
B*07:02g/B*51:01g	31,19	0,011
B*08:01g/B*40:02g	-21,99	0,023
B*14:02g/B*15:01g	30,78	0,012
B*18:03g/B*40:02g	21,22	0,047
B*35:03g/B*35:43g	27,13	0,018
B*35:20g/B*39:10g	24,86	0,026
B*40:02g/B*45:01g	33,83	0,003
B*40:02g/B*48:01g	-33,33	0,007
B*40:02g/B*51:01g	-48,07	0,001
B*40:02g/B*51:10g	-20,27	0,026
B*08:01g/B*35:43g	16,58	0,056
R^2 = 0,89	p = 0,0042	

b.

HLA-C		
Variable	Estimate	p
RBC 10^9/µl	-7,8	0,003
PR Neut. %	-0,98	0,012
C*01:02g/C*01:02g	129,4	0,000
C*02:02g/C*04:01g	-28,86	0,019
C*03:04g/C*07:01g	-46,05	0,003
C*03:04g/C*12:03g	-37,63	0,015
C*03:04g/C*15:02g	-26,49	0,018
C*03:05/C*08:01g	-51,16	0,002
C*03:05/C*16:01g	-67,1	0,000
C*04:01g/C*06:02g	-35,54	0,023
C*07:02g/C*08:01g	-38,4	0,002
R^2 = 0,71	p = 0,0014	

Table 3. Associated genotypes with a high or low CD34+ cell viability after thawing in HLA-B (a) and HLA-C (b).

Particular alleles related to acquired aplastic anemia such as HLA-B*40:02 also exhibited a lower CD34⁺ cell viability (Figure 1).

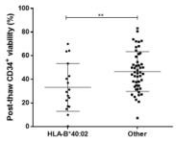

Figure 1. Differences in post-thawing CD34⁺ cell viability according to presence or absence of HLA-B*40:02

DISCUSSION AND CONCLUSIONS

It has been demonstrated that DMSO does not have high toxic effects on CD34⁺ cells if osmotic changes are controlled and kept inside "safe" limits.[1,8] Therefore, we believe that viability loss of CD34⁺ cell may be due to the cellular and processing factors found in our multivariate analysis. It has been reported that RBC reduce cell viability of bone marrow mononuclear cells when co-cultured in a dose-dependent manner, due to a soluble factor released by red cells.[9] We also believe it could be due to hemoglobin release after UCB units thawing.[10] Dead granulocytes may also impact cell viability due to their higher membrane susceptibility[1,11] and therefore release of oxygen reactive species (ROS) which may also be catalyzed by free hemoglobin. HLA may also be involved due to lymphocyte direct attack on certain allele-bearing progenitor cells, such as in aplastic anemia model, leading to sensitivity in certain UCB units. This fact could also imply an important consideration when using matching UCB units in patients with HLA-related diseases, since the pathology itself may be causing progenitor cell death and continue attacking progenitors from the graft.

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