Generation of mesenchymal stromal cells in the presence of platelet lysate: a phenotypic and functional comparison of umbilical cord blood- and bone marrow-derived progenitors

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Online Supplementary Table S1. Kinetics of cytokine production in culture supernatants.

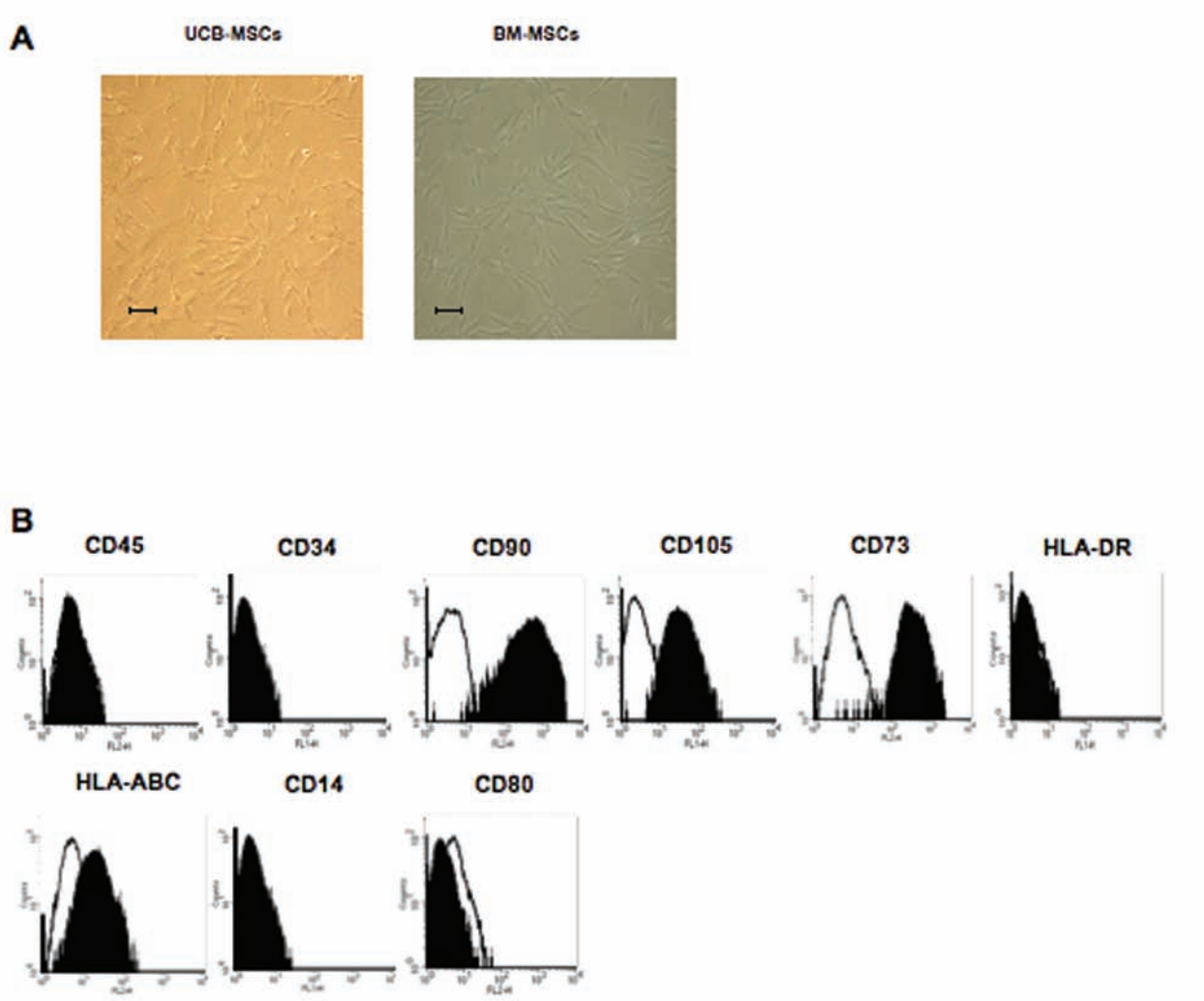
	12-h	24-h	48-h
Interferon-γ			
Control-MLC	2	6	107
MLC+UCB3-MSC	1	4	77
MLC+UCB6-MSC	4	9	71
Interleukin-10			
Control-MLC	9	11	16
MLC+UCB3-MSC	8	20	17
MLC+UCB6-MSC	10	20	20
Interleukin-6			
Control-MLC	868	1029	1000
MLC+UCB3-MSC	44,000	41,000	44,000
MLC+UCB6-MSC	48,000	45,000	46,000

Concentrations of IFN-γ, IL-10, IL-6 were quantified in MLC supernatants collected after 12, 24 and 48-hour (-h) culture in the absence (Control-MLC) or presence of UCB3-MSC (MLC+UCB3-MSC) and UCB6-MSC (MLC+UCB6-MSC). Results are reported as pg/mL. IFNγ, and IL-10 were undetectable in the supernatants of UCB-MSC simultaneously cultured in the absence of peripheral blood mononuclear cells. Both UCB3- and UCB6-MSC were able to constitutively secrete IL-6 in culture supernatants (peak of constitutive secretion at 48 h was 2,425 pg/mL and 4,569 pg/mL, respectively).

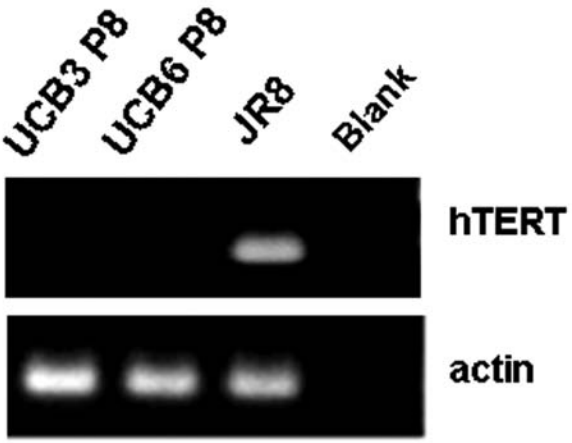
Online Supplementary Table S2. Constitutive expression of HLA-G in UCB-derived and BM-derived MSC at passage 3.

	mHLA-G		iHLA-G		sHLA-G
	%	MFI-R	%	MFI-R	U/mL
Exp 1					
UCB3-MSC	73	2.8	100	11.2	31
BM1-MSC	10	3.4	100	14.2	30
Exp 2					
UCB6-MSC	78	4.3	100	10.2	30
BM2-MSC	31	3.6	98	11.0	49

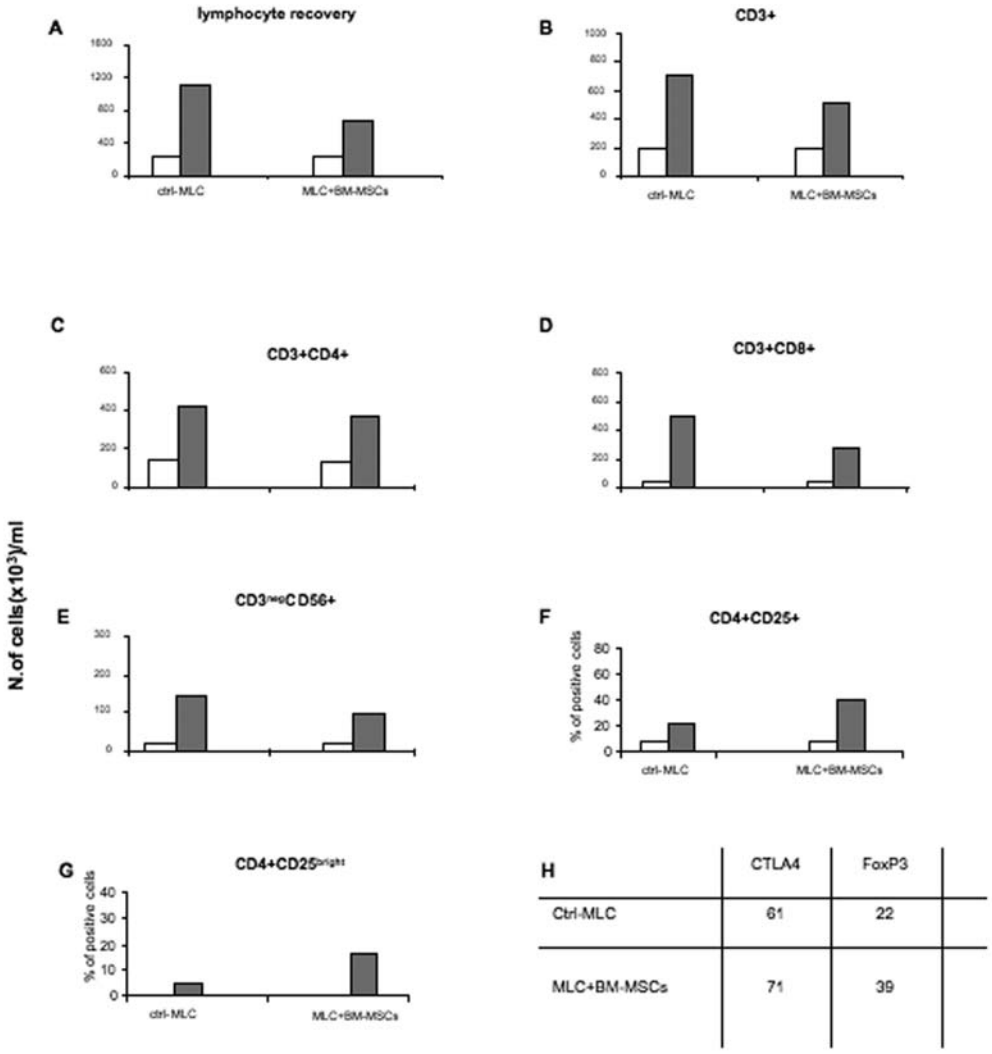
mHLA-G = membrane HLA-G; iHLA-G: intracellular HLA-G; sHLA-G: soluble HLA-G; %: percent of positive cells; MFI-R: mean fluorescence intensity ratio. Soluble HLA-G levels are expressed in U/mL. Two independent experiments are presented, in which UCB3-MSC and BM1-MSC from donor 2[27] (Exp 1) and UCB6-MSC and BM2-MSC from donor 5[27] (Exp 2) were tested.



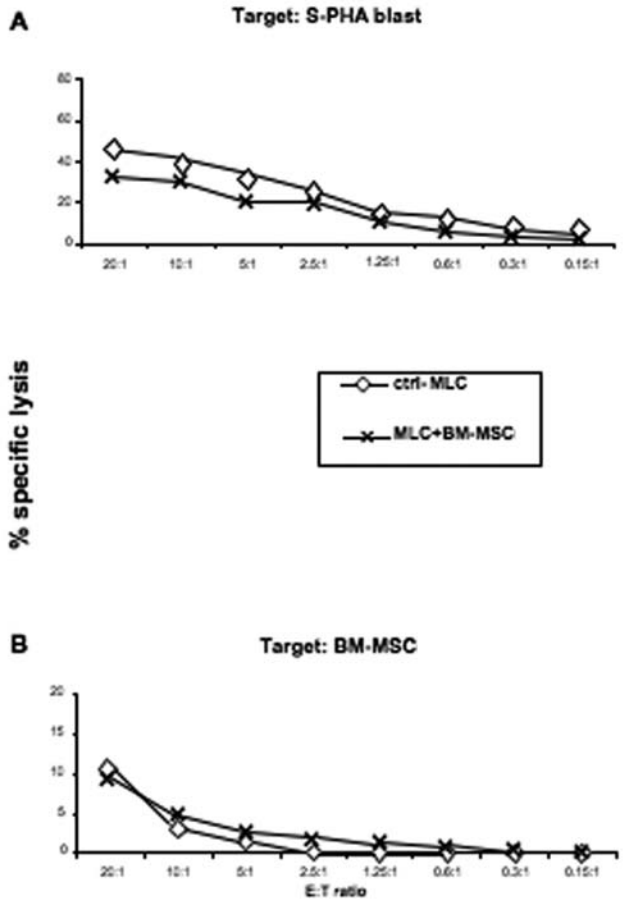
Online Supplementary Figure S1. (A) A representative photograph of MSC derived from UCB N.3 (UCB3-MSC) at passage (P) 2, expanded in the presence of PL. UCB-MSC display the typical spindle-shaped morphology, similar to that of BM-derived MSC (BM-MSC) cultured in 5% PL-supplemented medium (BM-MSC from donor 2, Bernardo *et al*.[27] Magnification x10. Scale bar indicates 50 μm. (B) Immunophenotypic characterization of UCB3-MSC at P2 by flow cytometry. UCB-MSC express CD90, CD73, CD105 and HLA-class I surface antigens, whereas they are negative for CD34, CD45, CD14, CD80 and HLA-DR.



Online Supplementary Figure S2. Expression of h-TERT mRNA, as detected by RT-PCR in UCB3- and UCB6-MSC cultures at P8. β-actin was used as the external standard. The telomerase-positive cell line JR8 was used as a positive control. The blank represents a negative control to which no RNA was added.



Online Supplementary Figure S3. Effect of BM-MSC, expanded in the presence of PL and previously reported,[27] on T and NK-lymphocyte subset expansion induced by allogeneic stimulus. Recovery of total number of lymphocytes (A), $CD3^+$ (B), $CD3^+CD4^+$ (C), $CD3^+CD8^+$ (D), $CD3^{neg}CD56^+$ NK cells (E), $CD4^+CD25^+$ (F), $CD4^+CD25^{bright}$ (G) T-lymphocyte subsets and with respect to the initial number (white columns), was assessed after 10-days primary culture (gray columns). Percentages of $CTLA4^+$ and $Foxp3^+$ cells were calculated on gated $CD4^+CD25^+$ T cells (H). MLC was performed in the absence (Ctrl-MLC) or presence of third-party BM-MSC cultured in 5% PL (MLC+BM-MSC). The MSC were added at a responder (R)-peripheral blood mononuclear cell/MSC ratio of 10:1; results are expressed as number of cells/mL of culture. The mean of two independent experiments (Exp 1, Exp 2)[27] is reported.



Online Supplementary Figure S4. Effect of third-party BM-MSC, expanded in the presence of 5% PL and previously reported,[27] on cell-mediated cytotoxic activity induced by allogeneic stimulus. ^{51}Cr-labeled target cells included phytohemagglutin-activated stimulator peripheral blood mononuclear cells (S-PHA) (A) and the same lots of BM-MSC (B) added to MLC. Effector to target (E:T) ratios ranged between 20:1 and 0.15:1. Results are expressed as percent specific lysis of target cells. The mean of two independent experiments (Exp 1, Exp 2)[27] is reported.