EX-VIVO EXPANSION AND DIFFERENTIATION OF HUMAN HEMATOPOIETIC STEM CELLS SUPPORTED BY HUMAN STROMAL-BASED CULTURE SYSTEMS

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ABSTRACT

The role of a human stromal-based (hu-ST) serum-free culture system on the *ex-vivo* expansion/maintenance of hematopoietic stem/progenitor cells from adult bone marrow (BM) and umbilical cord blood (CB) was investigated. Significant expansion of BM and CB cells occurred in the hu-ST system. In stroma-free cultures, BM cells showed a significant lower expansion, whereas CB cells simply could not be expanded. A simple kinetic modeling study using two parameters (expansion rate, μ, and death rate, k_k), allowed to conclude that total cell expansion is similar for hematopoietic cells from both BM and CB, whereas cell death seemed to be related with the presence/absence of hu-ST *ex-vivo*. A predictive model was also developed accounting for hematopoietic cell expansion, differentiation, and death, which estimated adequately total cell numbers and relative amounts of the different phenotypes (*e.g.* $CD34^+$, $CD34^+CD38^-$), allowing the prediction of expansion/differentiation pathways and identification of key steps in the hematopoiesis scheme.

Keywords: hematopoietic stem cells, expansion, stroma, kinetic, modeling

INTRODUCTION

The *ex-vivo* expansion of human hematopoietic cells for clinical applications has been the focus of considerable research in the last decades, with potential applications in a variety of clinical settings such as bone marrow (BM) transplantation, immunotherapy, gene therapy and production of mature blood cells[1-4]. Hematopoietic stem cells (HSC) have the dual potential for self-renewal and differentiation into progenitors of all the mature blood lineages. HSC biology research has been directed towards the identification of cell populations that have stem cell characteristics and the study of mechanisms that regulate stem cell self-renewal and differentiation[5]. Because stem cells are rare, one of the major focuses in experimental hematology is the *in-vitro* manipulation of HSC with the ultimate goal of expanding long-term transplantable HSC, since the difficulties associated with inadequate numbers of HSC collected for transplantation from autologous or allogeneic sources such as BM or umbilical cord blood (CB) would be largely eliminated by the *ex-vivo* expansion of those cells[6,7]. Although substantial advances have been made in identifying cytokine combinations that are able to maintain or expand to some extent HSC[3,8-13] or important stromal derived factors[14,15], an ideal system that allows extensive expansion of these cells while maintaining their engraftment capability remains to be defined[16,17]. Regardless of the source, the HSC content of the initial hematopoietic product can be enriched by selecting for cells expressing the surface antigen CD34, which is found on many of the primitive HSC[18].

Hematopoiesis depends upon a complex interaction of growth and regulatory factors within the BM microenvironment, where specific cellular interactions between primitive hematopoietic cells and mesenchymal stromal tissue of non-hematopoietic origin take place[9,10,19]. The evaluation of the expansion kinetics in hematopoietic cell cultures is complicated by the cell distribution over the various stages of differentiation ranging from stem, to progenitors, precursors, and mature cells, potentially comprising cells from both myeloid and lymphoid lineages. Therefore, an observed response in the culture system is an integral response from several cell populations[20]. Kinetic modeling, as it has been applied to many other biochemical systems, can provide a significant insight into the "limiting steps" involved in the hematopoiesis scheme and on how the various factors (cytokines, stroma, feeding rates...) influence the outcome of the expansion process[4]. In this context, several kinetic models have been established predicting specific cell subsets or distributed populations by incorporating data such as rates of proliferation, death, differentiation, cytokine concentration, glucose uptake and lactic acid production rates, dissolved oxygen, and pH[4,21-23]. The information retrieved by those models is useful not only for the *in vitro* expansion itself, but also for the design and operation of stem cell bioreactors[20]. In the present studies, we evaluated the ability of a human stromal-based (hu-ST) serum-free culture system to support the *ex-vivo* expansion/maintenance of human BM and CB HSC and the expansion results obtained were modeled based on kinetic models: (i) considering hematopoietic cells as a global; or (ii)

considering the hematopoietic phenotypic distribution within cell culture. Both models were found to describe adequately the expansion results.

Material and Methods

Human donor cell preparation

Heparinized human bone marrow was obtained from healthy donors after informed consent. Umbilical cord blood samples were obtained from the Pediatric Stem Cell Transplant Program, at Duke University Medical Center, Durham, NC and from the Serviço de Ginecologia e Obstetrícia, Hospital D. Estefânia, Lisboa, Portugal. After obtaining maternal donor consent, fresh blood was collected from the umbilical cord vein using the method previously described[24]. Low density bone marrow mononuclear cells (BM MNC) or cord blood mononuclear cells (CB MNC) were separated by a Ficoll density gradient (1.077g/ml) (Sigma, St. Louis, MO, USA) and washed twice in Iscove's Modified Dulbecco's Medium (IMDM), (Gibco Laboratories, Grand Island, NY, USA). BM MNC and CB MNC from each donor were enriched for $CD34^+$ cells using magnetic cell sorting (Miltenyi Biotec, Inc., Auburn, CA, USA).

Human bone marrow stromal cell cultures

Isolated BM MNC obtained from healthy donors were magnetically based on Stro-1 expression and cultured in gelatin coated T-25 flasks with Mesenchymal Stem Cell Growth Medium (MSCGM) (Poietics™, Cambrex Bioscience, Baltimore, MD, USA). Stromal layers (hu-ST) were obtained after culture for 10 days and then γ-irradiated (14 Gy) with a ^{137}Cs source. The irradiated stromal layers were maintained at 37° C under 5% CO2 humidified air and then used within 1-5 days.

Ex-vivo expansion of $CD34^+$ enriched cells

BM and CB $CD34^+$ enriched cells (1 to 3×10^6 for BM and 0.75 to 1.5×10^6 for CB) were cultured in T-25 flasks (5 ml) for several days in QBSF-60 serum free medium (Quality Biological, Inc., Gaithersburg, MD, USA) in the presence or absence of hu-ST with the following cytokines: SCF (100 ng/ml), bFGF (5 ng/ml), LIF (10 U/ml) and Flt-3 (100 ng/ml) (Peprotech, Rocky Hill, NJ, USA)[3]. Cultures were half-fed every 2-4 days with half of the cultures being harvested and used for analysis, and the same volume being replaced with fresh medium.

Proliferative and phenotypic analysis

The *ex-vivo* expansion of the $CD34^+$ enriched populations was determined at each time point by counting the content of hematopoietic cells in each culture flask using Trypan Blue Stain 0.4% solution (GibcoBRL, Grand Island, NY, USA) and analyzed for stem cell and lineage content by flow cytometry (FACScan equipment, Becton Dickinson, San Jose, CA, USA) using monoclonal antibodies against CD3, CD7, CD14, CD15, CD19, CD33, CD34 and CD38 (Becton Dickinson Immunocytometry Systems, San Jose CA, USA) as described[3].

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Comparisons between experimental results were determined by two-sided non-paired Student's *t*-test analysis. A p value < 0.05 was considered statistically significant.

Kinetic modeling

The total hematopoietic cell (HC) expansion of BM and CB cells was characterized by means of a Monod-type kinetic model involving only two parameters: the specific cell expansion rate, μ, and the death constant, k_k. The parameters obtained were used to evaluate the influence of the presence/absence of a human feeder layer on the overall HC expansion as recently published[25]. In addition, a predictive kinetic model was developed for BM cells, based on the hematopoietic hierarchy which comprises stem, progenitors and fully mature cells[4]. This model computes the concentration of each type of cells (*e.g.* Y) as a function of time, including: a self-expansion term (k_e^Y); a term for cell death (k_k^Y); and a term accounting for the differentiation, which includes a generation term involving production of Y cells from its precursor and a consumption term involving the differentiation of cells *Y*, themselves, into *Z* (the next cells along the differentiation line), with a rate constant k_d^Z as previously described[4].

Results

Evaluation of ex-vivo expansion of CD34⁺ enriched cells in serum-free medium with selected cytokines, in the presence and absence of human stroma

The ex-vivo expansion of human CD34⁺ enriched cells derived from either BM or CB in the presence of stroma was evaluated in comparison to stroma-free BM and CB cultures as recently published[3]. Figures 1A and 1B show the fold increase in total number of cells with the time in culture obtained when BM and CB CD34⁺ enriched cells were cultured either with or without stroma.

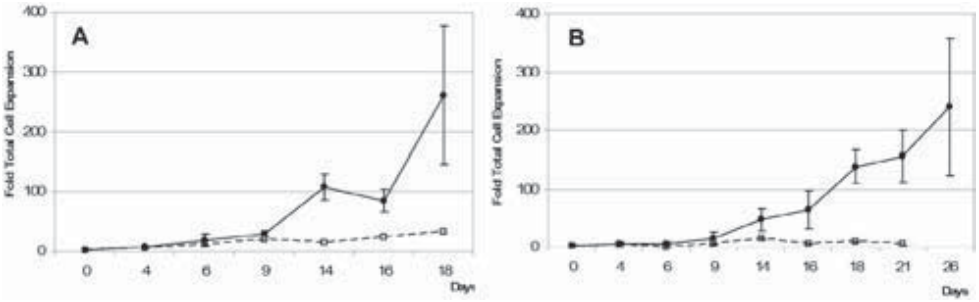

Fig. 1 – Total cell expansion of CD34⁺ enriched cells co-cultured in the presence/absence of human stromal layers. Cell numbers for hu-st system (black line, ●) and in the absence of stromal layer (dashed line, □) are expressed as mean fold expansion ± SEM. (**A**) BM cells (1-2.5×10⁶) were cultured for 2-3 weeks in the presence (n=4), or absence (n=2) of stroma (**B**) CB cells (0.1-1.8×10⁶) were cultured for 3-4 weeks in the presence (n=4) or absence (n=3), of stroma.

In BM cultures, the total expansion as assessed by cell number in both stromal and stroma-free system was minimal and similar until day 9 of the culture. However, after this time point, and as can be seen starting at day 14 of culture, $CD34^+$ enriched cells cultured in the presence of stroma began to proliferate more effectively when compared with stroma-free cultures (Figure 1A). As it was expected, in the absence of stromal layers, CB cultures were unable to expand (Figure 1B), since the combination of cytokines selected was anticipated to exert their effect through stromal or accessory cells. In the presence of stromal layers, CB $CD34^+$ enriched cells were able to proliferate to the same extent as their BM $CD34^+$ counterpart grown in the same conditions, although at a later time point[3].

Effect of culture conditions on differentiation potential of expanded cells.

The results of the differentiative potential of the BM and CB cell populations expanded in the presence of stroma are presented in Figure 2[3].

The differentiative potential of both BM and CB cells co-cultured with huST was primarily shifted towards the myeloid lineage, with the presence of CD14, CD15 and CD33 positive cells. BM $CD34^+$ enriched cells cultured in the absence of stroma also differentiated towards a myeloid phenotype and no $CD3^+$ or $CD19^+$ cell populations were detected upon day 6-8 in both BM and CB cultures[3]. Of importance was a significant increase in the percentage of $CD7^+$ cells with time in culture of both BM and CB cells in the cultures grown on stromal layers, showing that in our culture system we were also able to expand cells with an early lymphocytic phenotype.

Two-parameter kinetic modeling of total hematopoietic cell expansion

A simple kinetic modeling study using two parameters only (the specific cell expansion rate, μ, and specific death rate, k_k) was applied to total cell expansion results of both BM (in the presence and absence of stroma) and CB cells (in the presence of stroma only)[25].

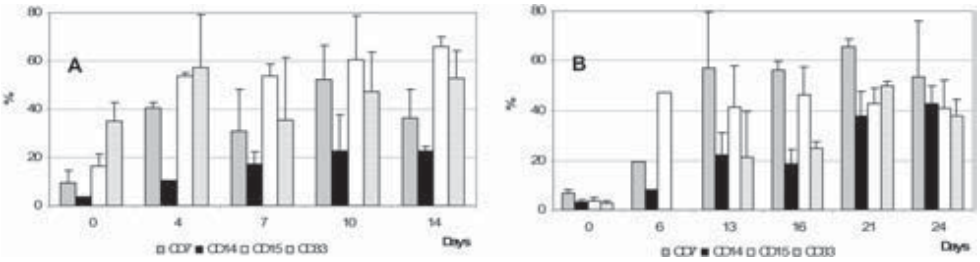

Fig. 2 – Differentiative potential of BM and CB expanded cells expanded in the presence of human stroma. BM (**A**) and CB (**B**) $CD34^+$ enriched cells were cultured for 2-4 weeks. Non adherent cells were harvested periodically and analyzed by flow cytometry. Each bar represents the mean expression±SEM for each phenotype (n=4 for both BM and CB experiments).

Table I shows BM and CB expansion parameters estimated when culturing different initial cell numbers in the presence and absence of human stromal layers.

Since CB cells could not be expanded under stroma-free conditions, only the expansion parameters for CB CD34$^+$ enriched cells expanded over human feeder cells were estimated. Figure 3 illustrates the model fitting to the experimental values for representative experiments of BM (A) and CB (B) CD34$^+$ enriched cells in the presence of hu-ST.

It is possible to conclude that there is a good agreement between experimental values and theoretical values estimated by the kinetic model for both BM (Figure 3A) and CB cells (Figure 3B)[25]. The estimated μ and k_k parameters are represented in Figure 4.

Interestingly, within the range of starting cell concentration the specific expan-

Table I – CB and BM expansion parameters.

	Starting population			hu-ST	Expansion parameters	
	#	X_0 (cells)	% CD34$^+$CD38$^-$		k_e (day^{-1})	k_k (day^{-1})
CB	1	1.2×10^6	n.d.	+	0.21 ± 0.13	0.013 ± 0.003
CB	2	9.0×10^4	n.d.	+	0.24 ± 0.04	0.035 ± 0.011
CB	3	7.5×10^5	n.d.	+	0.19 ± 0.05	0.031 ± 0.006
CB	4	1.27×10^6	n.d.	+	0.15 ± 0.03	0.011 ± 0.003
CB	5	8.5×10^5	0.23	+	0.14 ± 0.05	0.004 ± 0.001
BM	1	3.25×10^6	23	-	0.36 ± 0.03	0.057 ± 0.005
BM	1	3.25×10^6	23	+	0.30 ± 0.16	0.020 ± 0.003
BM	2	1.76×10^6	8.2	-	0..24 ± 0.03	0.057 ± 0.006
BM	2	1.76×10^6	8.2	+	0.21 ± 0.02	0.024 ± 0.008
BM	3	6.2×10^6	7.4	+	0.13 ± 0.02	0.009 ± 0.003
BM	4	2.5×10^6	1.7	+	0.14 ± 0.02	0.005 ± 0.001
BM	5	9.3×10^5	0	+	0.17 ± 0.03	0.008 ± 0.001
BM	6	4.3×10^6	0.65	+	0.11 ± 0.03	0.014 ± 0.003
BM	7	1.7×10^6	0.63	+	0.30 ± 0.06	0.015 ± 0.002

Expansion parameters were estimated when culturing different initial cell numbers in the presence (+)/absence(-) of human stromal layers (n.d., not determined). The estimated parameters are given together with their 95% confidence interval.

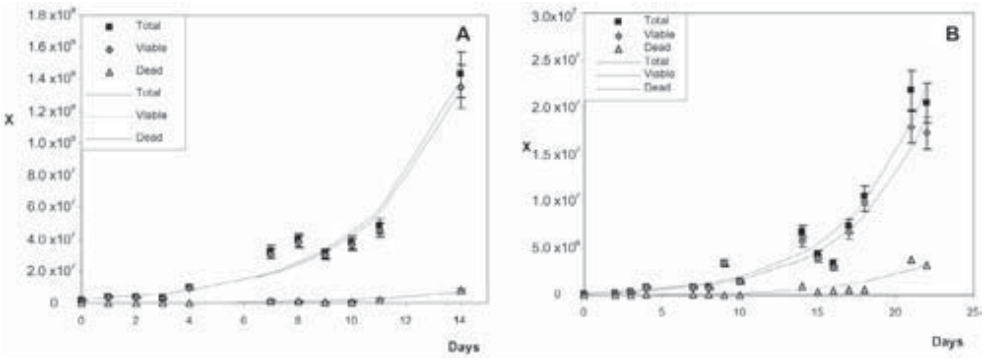

Fig. 3 – Example of the two-parameter model fitting (lines) to the experimental time course values of total (■), viable (◆) and dead (▲) cell numbers (X) for the BM#7 experiment (A) and CB#2 experiment (B) expanded over stromal layers.

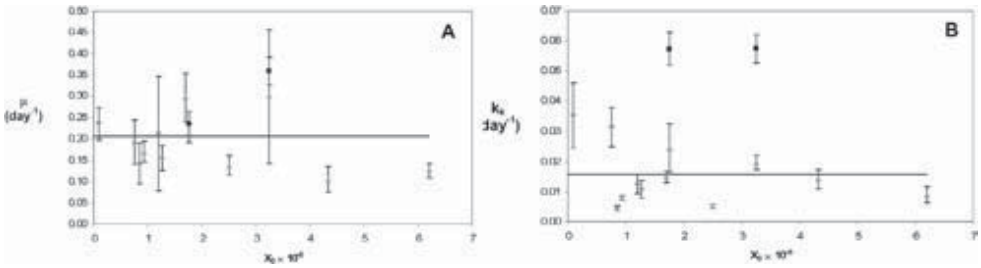

Fig. 4 – Total cell expansion and death rates as a function of the initial cell number (X_0) for BM and CB cultures. (◆) CB with stromal layer, (■) BM with no stromal layer and (▲) BM with stromal layer. Horizontal line corresponds to the average of all values. Vertical bars correspond to estimated errors in the parameters[25].

sion rates of BM CD34+ enriched cells (both in presence and absence of a stromal layer) are similar to the CB CD34+ enriched cells, and an average *ke* value of 0.21 day^{-1} can be estimated for both CB and BM CD34+ enriched cells (Figure 4A). On the other hand, the values of k_k for BM experiments in the presence of human stroma fall within the same range as those for CB, and an average of 0.016 day^{-1} was estimated (Figure 4B). In addition, k_e values for BM experiments in the absence of stroma seem to be slightly higher than the corresponding experiments using a stromal layer, but still fall within the same range (Figure 4A). However, cell death rates seem to be significantly higher (0.057 day^{-1}) in the absence of a stromal layer, which explains why total expansion is lower when no stromal layer is present[3,25].

Predictive modeling of the expansion of hematopoietic stem/progenitor cells

In addition, a predictive model was developed accounting for hematopoietic cell expansion, differentiation, and death[4]. This type of kinetic modeling can provide a significant insight into the "limiting steps" involved in the hematopoietic hierarchy and into the influence of conditions affecting the expansion process. Total expansion results for a representative BM experiment are depicted in Figure 5, showing a more efficient expansion in the presence of a stromal layer (Figure 5A), compared to stroma-free conditions.

Data displayed in Figure 5 show a good agreement between experimental and theoretical values predicted by this

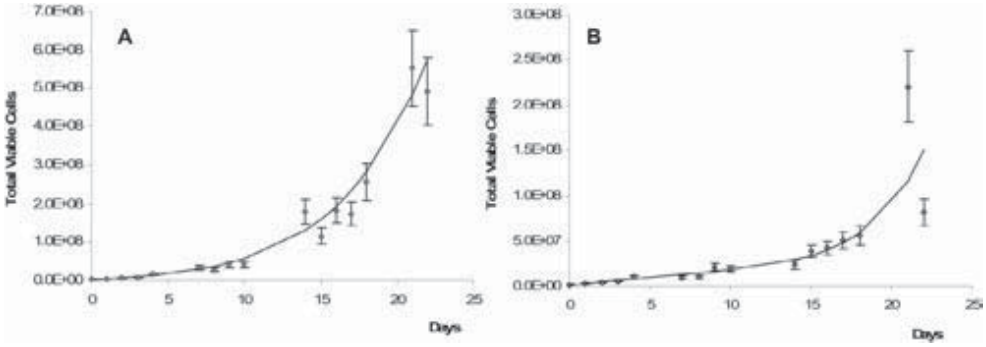

Fig. 5 – Example of the predictive model fitting (lines) to the experimental time course values of total viable cells. BM total cell numbers in the presence (**A**) and absence (**B**) of hu-ST are represented.

model for BM CD34$^+$ enriched cells in both stroma- and stroma-free conditions. As previously shown, the differentiative potential of BM CD34$^+$ cells co-cultured with and without human stroma was primarily shifted towards the myeloid lineage. From this kinetic model, the relative amounts of the different phenotypic cells (*e.g.* CD34$^+$, CD34$^+$CD38$^-$, CD33$^+$) can also be estimated, with the aim of predicting the hematopoietic cell pathways for expansion/differentiation and identifying the key steps in the production of specific types of cells. Typical fittings of this model to experimental data are depicted in Figures 6 for more primitive cells, CD34$^+$ (A) and CD34$^+$CD38$^-$(B) and for more mature cells, CD33$^+$ (Figure 6C). The fitting of the model to the CD15$^+$ cells was similar to the CD33$^+$ [4].

Discussion

Most potential clinical applications of hematopoietic/progenitor stem cells (HSC) involve their *ex-vivo* expansion and/or controlled differentiation, to obtain a therapeutically significant amount of cells. There is a need for predictive kinetic models to optimize scale-up strategies for the *ex-vivo* maintenance, expansion and differentiation of stem cells for cell therapy.

Presented results demonstrate that HSC from both BM and CB, can be efficiently expanded in the human-stromal based serum-free culture system previously established, preserving both myeloid and early lymphocytic potential[3]. In addition, it was found that, contrarily to BM cultures, CB expansion results are strictly dependent on the presence of an hu-ST system, in the present culture conditions[3]. Two kinetic modeling approaches were considered to evaluate hematopoietic cell expansion. First, a simple kinetic modeling study using two parameters only (the specific cell expansion rate, μ, and specific death rate, k_d) allowed to characterize BM and CB cell expansion in terms of total cells. For both CB and BM cells cultured with hu-ST, μvalues were similar (around 0.21 day^{-1}), despite the wide range of inoculum. In addition, it was found that the expansion rates of the BM cells are not affected by the presence or absence of a feeder layer, whereas the specific death rates (k_d) for BM cells were 2 to 3 times higher in stroma-free conditions, compared to the

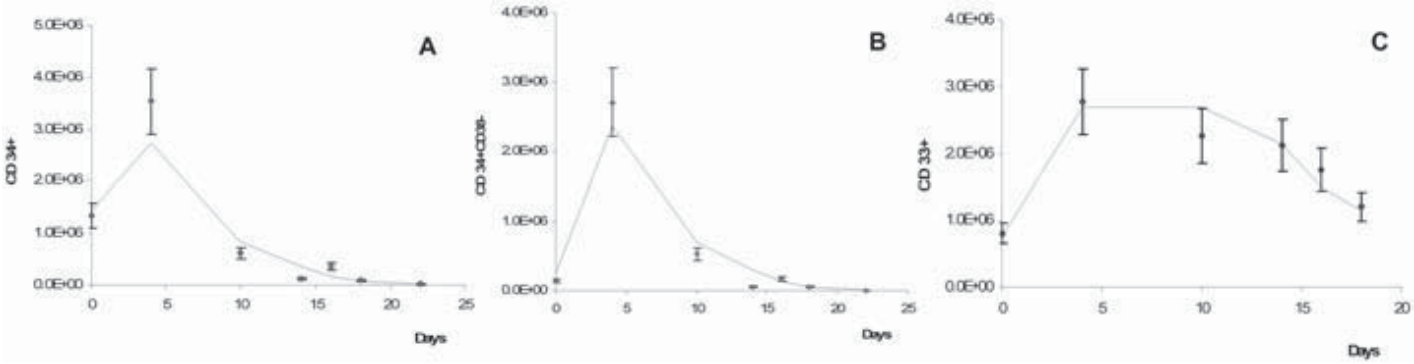

Fig. 6 – Example of the predictive model fitting (lines) to the experimental time course values of specific cell phenotypes. BM CD34+ cells (**A**), CD34+CD38- cells (**B**), both in the presence of hu-ST, and BM CD33$^+$ cells in the absence of a feeder layer (**C**).

corresponding experiments using hu--ST. This simple study provides a global measure of the ex-vivo culture system performance in terms of total cell expansion and quantitatively address a dependency of hematopoietic cell death on the environment in which cells are cultured (hu-ST presence/absence); however, this study is limited by the fact the cells expand but also differentiate and, thus, the expansion rate is likely to change as the cells evolve. Therefore, a predictive model was developed accounting for hematopoietic cell expansion, differentiation, and death. The model described adequately the total cell number and estimated the relative amounts of the different phenotypic cells (*e.g.* $CD34^+$, $CD34^+CD38^-$, $CD33^+$). This predictive model also quantitatively demonstrated the important role of hu-ST for the efficient *ex-vivo* expansion/maintenance of BM HSC by: enhancing the expansion of the majority of the more mature cells; reducing the death rate constant for the more primitive cell of the hematopoietic hierarchy[4]; and reducing the differentiation for the more mature cells. This kind of modeling approach potentially allows the prediction of the hematopoietic cell expansion/differentiation pathways and identification of the key and limiting steps in the production of specific types of cells in highly controlled bioreactor systems.

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References

1. Douay, L. Experimental culture conditions are critical for ex vivo expansion of hematopoietic cells. J Hematother Stem Cell Res 2001; 10 341-346.
2. Heath, C.A. Cells for tissue engineering. Trends Biotechnol 2000; 18 17-19.
3. Da Silva, C.L., Gonçalves, R., Crapnell, K.B., Cabral, J.M., Zanjni, E.D., and Almeida-Porada, G. A human stromal-based serum-free culture system supports the ex vivo expansion/maintenance of bone marrow and cord blood hematopoietic stem/progenitor cells. Exp Hematol 2005, 33 828-835.
4. Da Silva, C.L., Gonçalves, R., Lemos, F., Lemos, M.A., Zanjani, E.D., Almeida-Porada, G., and Cabral, J.M. Modelling of ex vivo expansion/maintenance of hematopoietic stem cells. Bioprocess Biosyst Eng 2003; 25 365-369.
5. Huang, S., Law, P., Francis, K., Palsson, B.O., and Ho, A.D. Symmetry of initial cell divisions among primitive hematopoietic progenitors is independent of ontogenic age and regulatory molecules. Blood 1999; 94 2595-2604.
6. Almeida-Porada, G., Brown, R.L., Mackintosh, F.R., and Zanjani, E.D. Evaluation of serum-free culture conditions able to support the ex vivo expansion and engraftment of human hematopoietic stem cells in the hu-

man-to-sheep xenograft model. J Hematother Stem Cell Res 2000; 9 683-693.

7. Traycoff, C.M., Abbound, M.R., Laver, J., Brandt, J.E., Hoffman, R., Law, P., Ishizawa, L., and Srour, E.F. Evaluation of the in vitro behavior of phenotypically defined populations of umbilical cord blood hematopoietic progenitor cells. Exp Hematol 1994; 22 215-222.
8. Lazzari, L., Lucchi, S., Rebulla, P., Porretti, L., Puglisi, G., Lecchi, L., and Sirchia, G. Long-term expansion and maintenance of cord blood haematopoietic stem cells using thrombopoietin, Flt3-ligand, interleukin (IL)-6 and IL-11 in a serum-free and stroma-free culture system. Br J Haematol 2001; 112 397-404.
9. Kashiwakura, I., and Takahashi, T.A. Basic fibroblast growth factor-stimulated ex vivo expansion of haematopoietic progenitor cells from human placental and umbilical cord blood. Br J Haematol 2003; 122 479-488.
10. Shih, C.C., Hu, M.C., Hu, J., Weng, Y., Yazaki, P.J., Medeiros, J., and Forman, S.J. A secreted and LIF-mediated stromal cell-derived activity that promotes ex vivo expansion of human hematopoietic stem cells. Blood 2000; *95* 1957-1966.
11. Gammaitoni, L., Bruno, S., Sanavio, F., Gunetti, M., Kollet, O., Cavalloni, G., Falda, M., Fagioli, F., Lapidot, T., Aglietta, M., and Piacibello, W. Ex vivo expansion of human adult stem cells capable of primary and secondary hemopoietic reconstitution. Exp Hematol 2003; 31 261-270.
12. Podesta, M., Piaggio, G., Pitto, A., Zocchi, E., Soracco, M., Frassoni, F., Luchetti, S., Painelli, E., and Bacigalupo, A. Modified in vitro conditions for cord blood-derived long-term culture-initiating cells. Exp Hematol 2001; 29 309-314.
13. Brandt, J.E., Bartholomew, A.M., Fortman, J.D., Nelson, M.C., Bruno, E., Chen, L.M., Turian, J.V., Davis, T.A., Chute, J.P., and Hoffman, R. Ex vivo expansion of autologous bone marrow CD34(+) cells with porcine microvascular endothelial cells results in a graft capable of rescuing lethally irradiated baboons. Blood 1999; 94 106-113.
14. Broxmeyer, H.E., Kohli, L., Kim, C.H., Lee, Y., Mantel, C., Cooper, S., Hangoc, G., Shaheen, M., Li, X., and Clapp, D.W. Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells. J Leukoc Biol 2003; 73 630-638.
15. Liu, L.Q., Sposato, M., Liu, H.Y., Vaudrain, T., Shi, M.J., Rider, K., Stevens, Z., Visser, J., Deng, H.K., and Kraus, M. Functional cloning of IGFBP-3 from human microvascular endothelial cells reveals its novel role in promoting proliferation of primitive CD34+CD38-hematopoietic cells in vitro. Oncol Res 2003; 13 359-371.
16. Lewis, I.D., Almeida-Porada, G., Du, J., Lemischka, I.R., Moore, K.A., Zanjani, E.D., and Verfaillie, C.M. Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system. Blood 2001; 97 3441-3449.
17. Mcniece, I.K., Almeida-Porada, G., Shpall, E.J., and Zanjani, E. Ex vivo expanded cord blood cells provide rapid engraftment in fetal sheep but lack long-term engrafting potential. Exp Hematol 2002; 30 612-616.
18. Koller, M.R., Palsson, M.A., Manchel, I., and Palsson, B.O. Long-term culture-initiating cell expansion is dependent on frequent medium exchange combined with stromal and other accessory cell effects. Blood 1995; 86 1784-1793.
19. Kahn, J., Byk, T., Jansson-Sjostrand, L., Petit, I., Shivtiel, S., Nagler, A., Hardan, I., Deutsch, V., Gazit, Z., Gazit, D.,

KARLSSON, S., AND LAPIDOT, T. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. Blood 2004; 103 2942-2949.

20. CABRITA, G.J., FERREIRA, B.S., DA SILVA, C.L., GONÇALVES, R., ALMEIDA-PORADA, G., AND CABRAL, J.M. Hematopoietic stem cells: from the bone to the bioreactor. Trends Biotechnol 2003; 21 233-240.
21. COLLINS, P.C., NIELSEN, L.K.,WONG, C-K, PAPOUTSAKIS, E.T., AND MILLER, W.M. Real-time method for determining the colony-forming cell content of human hematopoietic cell cultures. Biotechnol Bioeng 1997; 55 693-700.
22. HEVEHAN, D.L., WENNING, L.A., MILLER, W.M., AND PAPOUTSAKIS, E.T. Dynamic model of ex vivo granulocytic kinetics to examine the effects of oxygen tension, pH, and interleukin-3. Exp Hematol 2000; 28 1016-1028.
23. YANG, H., PAPOUTSAKIS, E.T., AND MILLER, W.M. Model-based estimation of myeloid hematopoietic progenitor cells in ex vivo cultures for cell and gene therapies. Biotechnol Bioeng 2001; 72 144-155.
24. CHRYSLER, G., MCKENNA, D.H., SHIERMAN, T., KADIDLO, D., ASKARI, S., MILLER, J., CLAY, M., and MCCULLOUGH, J. Umbilical cord blood banking. In Cord Blood - Biology, Immunology, Banking and Clinical Transplantation, Hal E. Broxmeyer (ed). AABB Press. 2004. pp 219-258.
25. GONÇALVES, R., DA SILVA, C.L., FERREIRA, B.S.., LEMOS, M.A.N.D.A., LEMOS, F., ALMEIDA PORADA, G., and CABRAL, J.M.S. Kinetic analysis of the ex-vivo expansion of human hematopoietic stem/progenitor cells. Biotechnol Lett 2006; *accepted for publication*.