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Individual and combined effects of mesenchymal stromal cells and recombinant stimulatory cytokines on the *in vitro* growth of primitive hematopoietic cells from human umbilical cord blood

Patricia Flores-Guzmán[1], Eugenia Flores-Figueroa[2], Juan J. Montesinos[3], Guadalupe Martínez-Jaramillo[1], Veronica Fernández-Sánchez[1], Ignacio Valencia-Plata[1], Guadalupe Alarcón-Santos[4] and Hector Mayani[1]

[1]Hematopoietic Stem Cells Laboratory, [2]Hematopoietic Microenvironment Laboratory and [3]Mesenchymal Stem Cells Laboratory, Oncology Research Unit, Oncology Hospital, National Medical Center, IMSS, Mexico City, Mexico, and [4]Troncoso General Hospital, IMSS, Mexico City, Mexico

Background aims

We have previously characterized the in vitro *growth of two cord blood-derived hematopoietic cell populations in liquid cultures supplemented with recombinant cytokines. In the present study, we assessed the effects of bone marrow-derived mesenchymal stromal cells (MSC) on the growth of such cells.*

Methods

$CD34^+$ $CD38^+$ Lin^- and $CD34^+$ $CD38^-$ Lin^- cells were obtained by negative selection, and cultured in the presence of marrow-derived MSC and/or early- and late-acting cytokines. Hematopoietic cell growth was assessed throughout a 30-day culture period.

Results

In the presence of MSC alone, both populations showed significant proliferation. Direct contact between MSC and $CD34^+$ cells was fundamental for optimal growth, especially for $CD34^+$ $CD38^-$ Lin^- cells. In the presence of early-acting cytokines alone, cell growth was significantly higher than in cultures established with MSC but no cytokines. In cultures containing both MSC and early-acting cytokines, a further stimulation was observed only for $CD34^+$ $CD38^-$ Lin^- cells. The cytokine cocktail containing both early- and late-acting cytokines was significantly more potent at inducing hematopoietic cell growth than the early-acting cytokine cocktail. When cultures were supplemented with early- and late-acting cytokines, MSC had no further effect on the growth of hematopoietic cells.

Conclusions

MSC seem to play a key role, particularly on more primitive ($CD34^+$ $CD38^-$ Lin^-) cells, only in the absence of cytokines or the presence of early-acting cytokines. When both early- and late-acting cytokines are present in culture, MSC seem to be unnecessary for optimal development of CFC and $CD34^+$ cells.

Keywords

cell expansion, cell proliferation, cytokines, hematopoietic progenitors, in vitro, *mesenchymal stromal cells.*

Introduction

Ex vivo expansion and manipulation of primitive hematopoietic cells, from either adult or neonatal sources, has become a major goal in the experimental hematology field, because of its potential relevance in the development of therapeutic strategies aimed at treating a diverse group of hematologic disorders, such as leukemia, myelodysplasia and aplastic anemia [1]. To date, several *in vitro* systems have been established to assess the biologic characteristics of different hematopoietic cell populations, and to develop appropriate

Correspondence to: **Hector Mayani,** PhD, National Medical Center, IMSS, Tallo 2, D-102, San Pablo Tepetlapa, Coyoacan, Mexico City DF 04620, Mexico. E-mail: hmayaniv@prodigy.net.mx

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conditions for the clinically oriented culture of such cells [2,3]. In this regard, stroma-free liquid cultures supplemented with combinations of early- and late-acting stimulatory cytokines have proven to be particularly important, as they allow and promote the expansion of progenitor cells [4–6]. Interestingly, this procedure has already been used in clinical settings with promising results; indeed, different groups have demonstrated that *in vitro*-expanded hematopoietic cells can be safely introduced into patients for the treatment of hematologic and non-hematologic disorders [7–10].

In keeping with the fact that the *in vivo* development of hematopoietic stem and progenitor cells (HSC and HPC, respectively) takes place in close association with stromal cells [11–14], *in vitro* culture systems have been established in which stromal cells are used as feeder cells, to allow the expansion of primitive hematopoietic cells. For this purpose, different types of stromal cells have been assessed, including primary whole bone marrow (BM) stroma, endothelial cells, stroma cell lines and mesenchymal stromal cells (MSC), the latter from different tissues [15–21]. These studies have demonstrated that stromal cells are capable of promoting the *ex vivo* expansion of stem and progenitor cells, a process that may involve both cell-to-cell-contact and cytokine secretion. These observations, in turn, suggest that stromal cells may be included as part of experimental protocols aimed at the expansion of HSC and HPC for clinical purposes. To date, however, many questions still remain regarding the role that stromal cells may play in the *in vitro* development of primitive hematopoietic cells, and the mechanisms involved in the interaction between HSC/HPC and stromal cells.

We have previously reported on the characterization of two different cell populations, obtained by negative selection from human umbilical cord blood (UCB), that are enriched for $CD34^+$ $CD38^+$ Lin^- and $CD34^+$ $CD38^-$ Lin^- cells, respectively [22]. Both cell populations possess significant proliferation and expansion potentials *in vitro*, and show the ability to generate mature cells of different lineages [23,24]. It is noteworthy that the particular behavior of such cells in culture, that is to say their ability to proliferate, expand and differentiate, is highly dependent on the presence of specific combinations of recombinant stimulatory cytokines.

In trying to understand in greater detail the *in vitro* biology of primitive hematopoietic cells (particularly their interaction with their microenvironment), and to assess the potential role of stromal cells within protocols aimed at the *ex vivo* expansion of HSC/HPC, in the present study we characterized the effects of human marrow-derived MSC on the proliferation, expansion and differentiation capacities of the two UCB cell populations previously described by us. As soluble cytokines are key elements of the hematopoietic microenvironment and play fundamental roles in the regulation of stem cell biology, in the present study we assessed the effects of human MSC both in the presence and absence of recombinant stimulatory cytokines.

Methods

Cell collection and processing

UCB cells, collected according to institutional guidelines, were obtained from normal full-term deliveries at the Troncoso Hospital (IMSS, Mexico City, Mexico) as described in detail previously [22–24].

Primitive cell enrichment

Cells expressing the CD34 antigen (Ag) ($CD34^+$ cells) were enriched from Mononuclear Cells (MNC) by negative selection [25] using the StemSep™ system according to the manufacturer's instructions (manufacturer's Technologies Inc., Vancouver, Canada) and as described in detail previously [22–24].

Two different cell populations were obtained by this procedure. The first one was obtained by using an antibody (Ab) cocktail that included monoclonal antibodies (MAb) to the following cell-surface Ag: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b and glycophorin A. Thus this cell population, referred to as population I, was enriched for $CD34^+$ lineage-negative (Lin^-) cells, which corresponded to $59 \pm 10\%$ (mean ± SD from 11 samples, as determined by immunocytochemistry) of the total cells included in this fraction. The second population was obtained by using Ab to the following cell-surface Ag: CD2, CD3, CD14, CD16, CD19, CD24, CD36, CD38, CD45RA, CD56, CD66b and glycophorin A. Thus this population was also enriched for $CD34^+$ Lin^- cells. However, the presence of Ab against CD36, CD38 and CD45RA resulted in the recovery of a cell population (referred to as population II) with a more primitive immunophenotype: $CD34^+$ $CD36^-$ $CD38^-$ $CD45RA^-$ Lin^- cells, which corresponded to $76 \pm 4\%$ (mean ± SD from 11 samples, as determined by immunocytochemistry) of the cells included in this fraction. It is noteworthy that 100% of the $CD34^+$ cells in population II corresponded to $CD38^-$ Lin^- cells.