Biology of Human Umbilical Cord Blood-Derived Hematopoietic Stem/Progenitor Cells

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ABSTRACT

Reported in 1989, studies by Broxmeyer, Gluckman, and colleagues demonstrated that umbilical cord blood (UCB) is a rich source of hematopoietic stem/progenitor cells (HSPC) and that UCB could be used in clinical settings for hematopoietic cell transplantation. Since then, a great interest has been generated on the biological characterization of these cells. Over the last nine years, several groups have focused on the study of UCB HSPC, addressing different aspects, such as the frequency of these cells in UCB, the identification of different HSPC subsets based on their immunophenotype, their ability to respond to hematopoietic cytokines, the factors that control their proliferation and expansion potentials, and their capacity to reconstitute hematopoiesis in animal models. Most of these studies have shown that significant functional differences exist between HSPC from UCB and adult bone marrow (i.e., the former possess higher proliferation and expansion potential than the latter). It is also noteworthy that genetic manipulation of UCB HSPC has been achieved by several groups and that genetically modified UCB cells have already been used in the clinic. In spite of the significant advances in the characterization of these cells, we are still in the process of trying to fully understand their biology, both at the cellular and the molecular levels. In the present article, we describe and discuss what is currently known about the biology of UCB HSPC.

INTRODUCTION

The presence of relatively mature hematopoietic progenitor cells (HPC) in human umbilical cord blood (UCB) was demonstrated by Knudtzon in 1974 [1]. About ten years later, Ogawa and colleagues documented the presence of primitive HPC in UCB [2, 3]. However, it was not until 1989 that experimental and clinical studies were published indicating that human UCB could be used in clinical settings. Broxmeyer et al. showed experimental evidence that UCB is a rich source of hematopoietic stem/progenitor cells (HSPC) [4]. That same year, Gluckman et al. reported on the first hematopoietic cell transplant in which UCB was used instead of bone marrow (BM) as the source of hematopoietic cells. They were able to reconstitute the hematopoietic system of a child with Fanconi anemia by means of UCB from an HLA-identical sibling [5].

Since then, there has been an expanding interest in the use of UCB as an alternate source of HSPC for transplantation [6]. To date, more than 500 of these transplants have been performed worldwide, for patients—mostly children—with different hematological and genetic diseases, including lymphoid and myeloid leukemia, Fanconi anemia, aplastic anemia, Hunter syndrome, Wiskott-Aldrich syndrome, β-thalassemia, and neuroblastoma. Furthermore, an international UCB transplant registry has been planned and UCB banking is already in progress [7, 8].

These findings prompted several research groups to investigate the in vitro and in vivo characterization of UCB cells, with the ultimate goal of both optimizing and expanding their clinical use. During the last eight years, a vast number of studies have been published addressing the purification, biologic characterization, ex vivo expansion...
and genetic manipulation of UCB HSPC. Several questions regarding the biology of these cells have been answered, but even more new questions have been raised, so that the study of UCB HSPC has become a topic of great interest in hematology due to its relevance both at the basic and clinical levels. The main goal of the present article is to review the literature published on the biology of human UCB HSPC.

IN VITRO CHARACTERIZATION OF UCB HSPC

Frequency of HSPC in UCB

Several groups have reported the colony-forming cell (CFC) content in human UCB. Such studies indicate that in 1 ml UCB there are about 8,000 erythroid progenitors (BFU-E), between 13,000 and 24,000 myeloid progenitors (colony-forming units-granulocyte/macrophage [CFU-GM]), and between 1,000 and 10,000 multipotent progenitors (CFU-granulocyte/erythroid/macrophage/megakaryocyte [CFU-GEMM]) [9, 10]. Although in general, the CFC content observed by these groups was similar, some variations have been reported, particularly in terms of CFU-GM and CFU-GEMM. These differences seem to result from differences in culture conditions. In this regard, Broxmeyer et al. have demonstrated that CFC detection depends on the cytokine combination used in culture. In fact, these authors have shown that the addition of stem cell factor (SCF) to semisolid cultures significantly increases detection of myeloid and multipotent progenitor cells [11].

Studies comparing the total content of HPC in UCB and BM have shown that the frequency of these cells is very similar in both sources. However, important differences in the frequency of particular HPC subsets have been observed: A) several groups have found higher levels of BFU-E in UCB than in BM [12-14]; B) the total numbers of myeloid progenitors per 10^5 mononuclear cells (MNC) are similar both in UCB and BM; however, there seems to be a higher proportion of immature, bipotent granulomonocytic progenitors in UCB [14], and C) immature megakaryocytic progenitors (CFU-megakaryocyte [CFU-MK])—those containing more than 200 cells/colony—are also more abundant in UCB [12]. Finally, the number of CFU-GEMM/10^5 MNC has been shown to be significantly higher (up to fourfold) in UCB than in BM [14]. Thus, taken together, the above reports indicate that UCB possess a higher proportion of immature CFC (i.e., CFU-GEMM, BFU-E, CFU-GM and CFU-MK > 200 cells) than adult BM. Table 1 summarizes some of the results described above.

The frequency of more primitive progenitors than those mentioned above has also been determined in UCB. McNiece et al. have described a type of primitive HPC capable of giving rise to colonies containing large numbers of hematopoietic cells. Such cells are known as high proliferative potential colony-forming cells (HPP-CFC) [15]. Lu et al. have shown that these progenitors are present in about eightfold higher frequency in UCB than in adult BM [16]. Sutherland et al. [17] and Verfaillie et al. [18] have characterized a cell type unable to form colonies in semisolid cultures but capable of giving rise to CFC after several weeks in Dexter-type long-term cultures. These cells, referred to as long-term culture-initiating cells (LTC-IC), are one of the best approximations to stem cells in humans. Studies by Pettengell and colleagues have shown that the frequency of LTC-IC in UCB is very similar to that in adult BM [19]. They found that LTC-IC giving rise to CFC after five weeks of culture are present in a proportion of 1/13,000 MNC, whereas LTC-IC giving rise to CFC after eight weeks of culture are present at a concentration of 1/34,000 MNC. Thus, these results suggest that the incidence of putative stem cells in UCB is at least comparable with that of BM [19].

Immunophenotype of UCB HSPC

The CD34 antigen, an integral membrane glycoprotein of 90-120 kD, is a defining hallmark of HSPC [20-23]. It has been suggested that this molecule functions as a regulator of hematopoietic cell adhesion to stromal cells of the hematopoietic microenvironment [24]. The frequency of CD34+ cells in adult BM has been estimated to be 1%-3% of all nucleated cells [20-23]. The CD34+ cell content in UCB has also been shown to be around 1% of nucleated cells [25-28]. Interestingly, the frequency of CD34+ cells in UCB decreases with gestational age; that is to say, at 17 weeks of gestation, the number of CD34+ cells is approximately eightfold lower in UCB than in adult BM [21]. These cells are referred to as long-term culture-initiating cells (LTC-IC), are one of the best approximations to stem cells in humans. Studies by Pettengell and colleagues have shown that the frequency of LTC-IC in UCB is very similar to that in adult BM [19]. They found that LTC-IC giving rise to CFC after five weeks of culture are present in a proportion of 1/13,000 MNC, whereas LTC-IC giving rise to CFC after eight weeks of culture are present at a concentration of 1/34,000 MNC. Thus, these results suggest that the incidence of putative stem cells in UCB is at least comparable with that of BM [19].

Table 1. HPC content in UCB and BM

<table>
<thead>
<tr>
<th>HPC/10^6 nucleated cells</th>
<th>Myeloid</th>
<th>Erythroid</th>
<th>Multipotent</th>
</tr>
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<tbody>
<tr>
<td>UCB</td>
<td>132 ± 69</td>
<td>153 ± 78</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>BM</td>
<td>160 ± 58</td>
<td>155 ± 67</td>
<td>5 ± 3</td>
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<table>
<thead>
<tr>
<th>Relative proportion of myeloid progenitors (%)</th>
<th>CFU-G</th>
<th>CFU-M</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCB</td>
<td>33 ± 9</td>
<td>40 ± 12</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>BM</td>
<td>62 ± 8</td>
<td>32 ± 11</td>
<td>9 ± 6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative proportion of erythroid progenitors (%)</th>
<th>CFU-E</th>
<th>BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCB</td>
<td>7 ± 8</td>
<td>94 ± 11</td>
</tr>
<tr>
<td>BM</td>
<td>35 ± 15</td>
<td>62 ± 16</td>
</tr>
</tbody>
</table>

Results represent mean ± SD of the absolute numbers or the relative proportion of myeloid, erythroid and multipotent progenitor cells present in UCB or BM (data from [14]).

Table 1 summarizes some of the results described above.
weeks' gestation, CD34+ cells comprise 11% of all small MNC, whereas by week 38 they comprise only 1% [29].

As in adult BM, CD34+ cells present in UCB constitute a very heterogeneous cell population. The vast majority of CD34+ cells express both HLA-DR and CD38 antigens [10, 30-32]. It has been previously demonstrated that the most primitive HSPC lack the expression of HLA-DR and CD38 [17, 18, 33, 34]. It is noteworthy that the frequencies of CD34+HLA-DR- and CD34+CD38- cells in UCB are higher than in adult BM (e.g., CD34+CD38- cells in UCB account for 4% of the CD34+ fraction, compared with only 1% in BM) [32]. This supports the notion that UCB possess a higher proportion of immature HPC than adult BM. Interestingly, Traycoff and colleagues have recently reported that, in contrast to BM LTC-IC, LTC-IC from UCB express both CD34 and HLA-DR [10], indicating that the phenotype of functionally similar cells may vary. The biological significance of the difference in phenotype between UCB and BM LTC-IC remains to be determined.

Mayani and Lansdorp have reported that UCB CD34+ cells can be separated based on the expression of both CD45RA and CD71, and this results in the identification of three major cell subpopulations enriched for multipotential, myeloid, and erythroid progenitor cells, respectively [35, 36]. UCB CD34+ cells expressing low/undetectable levels of both CD45RA and CD71 are enriched for CFU-GEMM, which correspond to up to 47% of the CFC in this cell subpopulation. CD34+CD45RA-CD71low cells are enriched for granulomonocytic progenitors, which correspond to 90% of the CFC. Finally, in the CD34+CD45RA-CD71low cell subpopulation, more than 70% of the CFC corresponded to erythroid progenitors (BFU-E and CFU-erythroid (CFU-E)).

Studies by Baum et al. [37] have demonstrated that CD34+ hematopoietic cells capable of reconstituting human hematopoiesis in severe combined immunodeficient (SCID) mice express the Thy-1 antigen (CD90). A significant proportion of UCB CD34+ cells express Thy-1 [38, 39]; interestingly, all of the LTC-IC present in UCB express both CD34 and Thy-1. Among UCB CD34+CD45RA+CD71low cells, 20%-30% also express Thy-1 [40]. Such a CD34+CD45RA+CD71lowThy-1+ cell subpopulation (Fig. 1) is enriched for HPP-CFC. The function of Thy-1 on HSPC is currently unknown; however, it has been suggested that Thy-1 is involved in hematopoietic cell development, possibly by mediating a negative signal that results in inhibition of cell proliferation [40].

The c-kit proto-oncogene encodes a transmembrane receptor (CD117) with tyrosine kinase activity, which is expressed on HPC [41-43]. The ligand for c-kit, steel factor (SCF), mast cell growth factor, or M-CSF plays a relevant role in HPC viability and proliferation [42-44]. Sixty percent of UCB CD34+ cells coexpress c-kit [45]. Such a CD34+c-kit+ population possesses a higher cloning efficiency in semisolid cultures than CD34+ cells lacking the expression of c-kit; moreover, all of the UCB CD34+Thy-1+ cells also express c-kit, although at low levels [38].

The vast majority of UCB CD34+ cells (90%) coexpress FLT3 (CD135), the receptor for the early-acting cytokine FLT3-ligand (FL), whereas only a small proportion (5%) express the receptor for M-CSF (CD115) [39]. Eighty percent of UCB CD34+ cells have the ability to take up rhodamine-123 (Rh123) [48], a supravital fluorochrome dye that binds to mitochondria of living cells [49]. UCB CD34+Thy-1+c-kitlow cells are also Rhlow [38] and it seems that all of the LTC-IC are present within this cell subpopulation [48]. Thus, taking all of the above data together, it can be concluded that the most primitive HSPC are present in UCB at a frequency of approximately 1/30,000 nucleated cells and that they are small MNC with the following immunophenotype: CD34+CD38-CD45RA+CD71+Thy-1+c-kitlow Rhlow.

Expression of lymphoid- and myeloid-associated antigens on UCB CD34+ cells has also been documented. Saeland et al. found that, in contrast to adult BM in which 25% of the CD34+ cells express CD10 and 18% express CD19, CD10+CD19+ cells are rare within the CD34+ cell population from UCB [50]. CD13 seems to be expressed on the majority of UCB CD34+ cells, and CD33 is also widely expressed on these cells, although it is absent on the most primitive CD34+ cells. The same authors have observed that several cell adhesion molecules are present on UCB CD34+ cells, similar to those observed in BM CD34+ cells. Virtually all CD34+ cells express CD11a and CD18 integrin chains, indicating the presence of leukocyte-function-associated antigen (LFA-1) heterodimers. All CD34+ cells express the LFA-1 ligand, ICAM-1. Similarly, LFA-3 (CD58) is strongly expressed on UCB CD34+ cells. Integrins of the β1 family, important in cell-extracellular matrix adhesion, are also present on CD34+ cells, particularly the very late activation antigen (VLA)-4 and 3.
VLA-5 integrins. Finally, CD44 and LAM-1, adhesion receptors involved in the homing of hematopoietic cells, have been found to strongly expressed on UCB CD34+ cells [50]. Table 2 summarizes some of the data described above.

**Lineage Commitment of UCB Multipotent Progenitors**

One of the major problems in stem cell biology has been the definition of the mechanisms by which a multipotent cell selects a particular differentiation pathway [51]; this, indeed, is a central question in hematopoiesis [52-54]. Several studies, the majority using BM-derived hematopoietic cells, have been carried out to address the actual role of hematopoietic cytokines in this process. Although several conclusions can be drawn, the results remain controversial.

**Metcalf** has presented evidence indicating that certain cytokines can act directly on multipotent or bipotent progenitor cells by inducing their differentiation into a particular lineage [55, 56]. On the other hand, results by **Ogawa** and colleagues suggest that stem cells commit to a particular lineage independently of the action of cytokines, which act subsequently to promote the survival and proliferation of the committed progenitors [57-59]. Similar conclusions were obtained by the same group in experiments using primitive progenitor cells from UCB [60]. Working on this same issue, **Mayani et al.** [61], cultured single UCB CD34+ CSF4RA+Co CD71b cells, enriched for multipotent HPC, in liquid cultures supplemented with different cytokine combinations and assessed the action of such cytokines in HPC commitment. Their results support the notion that hematopoietic cytokines play a permissive, rather than a deterministic, role in hematopoietic cell commitment.

**Proliferation and Expansion of UCB HSPC**

The proliferation potential of HSPC (defined as the capacity to divide and generate new daughter cells) as well as their expansion potential (defined as the capacity to produce more progenitor cells) appear to be biologic features that depend upon intrinsic factors. These are related to whether the cell is already committed or not to a particular lineage of differentiation and, if so, the specific hematopoietic lineage to which it belongs and its stage of maturation. However, the ability of a cell to exhibit such potentials depends on extrinsic factors that include the different cell types and cytokines that form part of the microenvironment in which the cell develops [62]. In vitro proliferation and expansion of HSPC also depend on variables such as type of culture medium, medium change schedule, temperature, presence or absence of serum, number of cells plated per culture, etc. [63].

Several groups have addressed the in vitro expansion and proliferation of UCB progenitors using either total CD34+ cells or CD34+ cell subsets. In general, it is clear that primitive subpopulations of CD34+ cells (e.g., CD34+ Rhlow

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% positive cells</th>
<th>Antigen</th>
<th>% positive cells</th>
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<tbody>
<tr>
<td>CD13</td>
<td>70 ± 13</td>
<td>CD54</td>
<td>&gt; 98</td>
</tr>
<tr>
<td>CD18</td>
<td>94 ± 5</td>
<td>CD58</td>
<td>&gt; 98</td>
</tr>
<tr>
<td>CD19</td>
<td>4 ± 2</td>
<td>CD71</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>CD33</td>
<td>78 ± 12</td>
<td>CD90</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>CD38</td>
<td>97 ± 2</td>
<td>CD115</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>CD44</td>
<td>&gt; 98</td>
<td>CD117</td>
<td>61 ± 17</td>
</tr>
<tr>
<td>CD45RA</td>
<td>55 ± 11</td>
<td>CD135</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>CD51</td>
<td>4 ± 2</td>
<td>HLA-DR</td>
<td>89 ± 8</td>
</tr>
</tbody>
</table>

Results represent mean ± SD of the relative proportion of cells (contained within the CD34+ cell population present in UCB) expressing the indicated antigen. CD34+ cells, in turn, correspond to 0.8%-1% of the total number of MNC in UCB (data based on [10, 28-36, 38-40, 47, 50]).
GM progenitors were identified based on their expansion and proliferation potentials. Interestingly, the progenitor cell type with the highest expansion/proliferation potential was a subtype of GM progenitor capable of giving rise to up to $46 \times 10^6$ total cells and up to 91,000 CD34+ cells [65]. Such a progenitor cell may correspond to the HPP-CFC described by McNiece et al. [15].

The ability of HPC to express an intrinsic expansion and proliferation potential in vitro will depend on the cytokines present in culture (Fig. 2). In this regard, several cytokines (including SCF, interleukin 1 (IL-1), IL-3, IL-6, GM-CSF, G-CSF, M-CSF, erythropoietin (Epo), thrombopoietin (Tpo), and FL) have been assessed, either alone or in combination, in cultures of UCB-derived CD34+ cells [10, 40, 48, 66-77]. In terms of HPC expansion, the best results have been obtained when cytokines are used in combinations that include early-acting factors, such as SCF, FL, and Tpo. Indeed, the greatest expansion of UCB-derived CD34+ cells reported to date (146,000-fold expansion in CD34+ cell numbers and $2 \times 10^6$-fold expansion in CFC numbers) has been achieved by using both FL and Tpo [77]. Addition of late-acting factors, such as Epo, usually contribute to the production of large numbers of mature cells, however, they do not seem to have an effect on HPC expansion [35, 36]. Table 3 summarizes some of the studies reported to date on the in vitro expansion of UCB-derived CD34+ cells.

In contrast to the cytokines mentioned above, hematopoietic inhibitors, such as transforming growth factor-β, tumor necrosis factor-α, and macrophage inflammatory protein-1α have been shown to significantly reduce both expansion and proliferation of different CD34+ cell subpopulations from UCB [78]. Indeed, some investigators have used antitransforming growth factor-β monoclonal antibody, together with stimulatory cytokines, to achieve a significant expansion of primitive progenitor cells [32].

![Figure 2. In vitro expansion of CD34+ cells depends on both intrinsic (biologic properties of the cell subpopulation) and extrinsic (cytokines and culture conditions) factors. Serum- and stroma-free liquid cultures were initiated with 10,000 UCB-derived CD34+ CD45RA+ CD71+ cells (A), CD34+ CD45RA+ CD71− cells (B), and CD34+ CD45RA− CD71+ cells (C). Cultures were supplemented with SCF + IL-6 + IL-3 + Epo (hatched bars) or SCF + IL-6 + PIXY (a GM-CSF-IL-3 fusion protein) + M-CSF + G-CSF (filled bars). Complete culture medium change was performed at weekly intervals. After 20 days of culture, CD34+ cell number was evaluated in all three subpopulations. Note that (1) the CD34+ CD45RA+ CD71+ cell subpopulation (containing the most primitive progenitors) showed the highest expansion potential of all three subsets, and (2) regardless of the cell subpopulation cultured, the cytokine combination that included SCF + IL-6 + PIXY + M-CSF and G-CSF was always more efficient in promoting expansion of CD34+ cells (data based on [36]).](image-url)

<table>
<thead>
<tr>
<th>Input cells</th>
<th>Cytokines</th>
<th>Days</th>
<th>CFC Expansion</th>
<th>CD34+ Expansion</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+</td>
<td>SF, IL-3</td>
<td>30</td>
<td>5,000</td>
<td>nr</td>
<td>[68]</td>
</tr>
<tr>
<td>CD34+</td>
<td>SF, IL-1, IL-3</td>
<td>42</td>
<td>160</td>
<td>nr</td>
<td>[73]</td>
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<tr>
<td>CD34+</td>
<td>SF, IL-1, IL-3, IL-6</td>
<td>28</td>
<td>10</td>
<td>nr</td>
<td>[71]</td>
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<tr>
<td>CD34+</td>
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<td>21</td>
<td>2,364</td>
<td>nr</td>
<td>[75]</td>
</tr>
<tr>
<td>CD34+</td>
<td>FL, TPO</td>
<td>175</td>
<td>$2 \times 10^4$</td>
<td>146,000</td>
<td>[77]</td>
</tr>
<tr>
<td>CD34+4HCα</td>
<td>SF, IL-3</td>
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<td>92</td>
<td>nr</td>
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<tr>
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<td>10</td>
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<tr>
<td>CD34+Rhβ</td>
<td>SF, E</td>
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<td>2.5</td>
<td>nr</td>
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<td>94</td>
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<td>[48]</td>
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<tr>
<td>CD34+45RA+71Thy-1α</td>
<td>SF, IL-6, PX, G, M, E</td>
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<td>241</td>
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<td>CD34+45RA+71Thy-1α</td>
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<td>40</td>
<td>4,719</td>
<td>32,000</td>
<td>[40]</td>
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</table>

Results represent mean fold expansion in CFC and CD34+ cell numbers in liquid cultures of CD34+ cells supplemented with the cytokine combinations shown and for the culture period (days) indicated. nr = not reported; E = Epo; G = G-CSF; M = M-CSF; PX = PIXY-321.
Expansion and proliferation of UCB HPC has also been assessed in the presence of plasma both from UCB and adult peripheral blood (PB). Interestingly, it was found that UCB plasma induced significantly higher expansion/proliferation of CD34+ cells than adult PB plasma, suggesting that the former contains a factor(s), absent in PB plasma, important for UCB HPC growth [72]. It has also been reported that expansion and proliferation of UCB HPC is influenced by the cell density in culture. Xiao et al. compared the expansion and proliferation capacity of CD34+++ cells when plated at 1 or 5,000 cells per well, in the presence of recombinant cytokines. They found that proliferation was greater in cultures of single cells, whereas expansion was favored in cultures of 5,000 cells [73]. They suggested that a feeder effect occurred when CD34+++ cells were plated at higher cell densities and that either cell-cell contact was necessary, probably due to stimulation from membrane-bound cytokines, or that cytokines in addition to those supplied were required for optimal progenitor cell expansion.

Production of Lymphoid and Endothelial Cells from UCB CD34+ Cells

Most of the studies described to date regarding the in vitro development of UCB CD34+ cells have focused on the generation of erythroid, myeloid, and megakaryocytic cells. However, it has been shown that under particular culture conditions, involving the use of certain murine stromal cell lines, UCB CD34+ cells can also generate B lymphoid cells. Rawlings and colleagues [79] seeded UCB CD34+ cells onto the murine stromal cell line S17 and followed their development for more than 12 weeks. They observed a transient growth of myeloid cells followed by the development of early B cell progenitors. Cultures containing more than 90% early B cells (CD10+CD19+CD20-CD22-CD23-CD38+CD45+ cells) were maintained for more than 12 weeks without addition of growth factors. Interestingly, these cells were unresponsive to the addition of the pre-B cell cytokines IL-7 and SCF, suggesting that growth support was provided by a cross-reactive factor produced by the S17 cells. In this culture system, more than 10^8 B lymphoid cells could be generated from a typical UCB sample [79].

DiGiusto et al. also studied the production of CD10+CD19+ B cells in cultures of UCB CD34+ Lin- cells seeded onto the Sys-1 murine stromal cell line [80]. However, in contrast to the study by Rawlings et al., IL-6 and leukemia inhibitory factor were added into these cultures. From limiting-dilution analysis, the authors estimated that approximately 1% of UCB CD34+ Lin- cells are capable of producing both myeloid and B cells [80]. Coulombel’s group has recently shown that, when cocultured individually during six weeks with the MS-5 murine stromal cell line in the absence of exogenous cytokines, 7% of CD34+ CD38-CD19- cells generated both B lymphoid (CD19+) and myeloid (CD11b+) cells [81]. These results conclusively demonstrate the presence of bipotent, lymphomyeloid cells within this cell subpopulation.

It is noteworthy, however, that in none of the studies mentioned above fully mature B cells were generated (i.e., all of the B cells produced lacked surface expression of IgM), thus suggesting that the murine stromal cell lines used in these studies may not supply the critical regulatory molecules for the downstream transition between early B and cIgM+ cells.

More recently, production of endothelial cells from UCB CD34+ cells has also been documented. Hows and colleagues observed that when cultured in the presence of recombinant IL-2 and conditioned medium from the 5637 human bladder carcinoma cell line, UCB CD34+ cells give rise to an adherent cell layer consisting of endothelial cells expressing von Willebrand factor, CD31, CD54, and CD62. These results indicate that endothelial cell progenitors are derived from the UCB CD34+ cell fraction [82].

Functional Differences between UCB and BM HSPC

From studies described in previous sections, it is evident that quantitative differences exist between HPC from UCB and BM (i.e., UCB possesses higher frequencies of primitive HPC than adult BM). However, important functional differences between UCB and BM HPC have also been described.

Several investigators have demonstrated that UCB-derived HSPC possess higher expansion and proliferation potentials than their BM counterparts [12, 82-88]. Such differences have been observed both in cytokine-supplemented liquid cultures of total CD34+ cells and CD34+ cell subsets (Fig. 3) [83-89], as well as in Dexter-type long-term marrow cultures [12]. By using semisolid cultures, a higher replating potential of UCB-derived CFU-GEMM, than of

Figure 3. CD34+ cells from UCB possess a higher expansion capacity in vitro than BM-derived CD34+ cells. Serum- and stroma-free liquid cultures were initiated with 10,000 CD34+ CD45RA+ CD71+ cells from adult BM (hatched bars) and UCB (filled bars). Cultures were supplemented with SCF + IL-6 + IL-3 + Epo (data based on [83]).
CFU-GEMM from BM has been documented [46, 90]. Furthermore, differences in the responses of UCB and adult BM progenitors to inhibitory cytokines have also been reported, indicating that UCB HPC are less sensitive to the effects of certain hematopoietic inhibitors, such as tumor necrosis factor-α and interferon-α [91].

Currently, it is not clear what the actual mechanisms are that account for the higher proliferation/expansion potential of UCB HPC over their BM counterparts. However, several studies have been published that give some insights into the possible origin of the functional differences between both types of progenitors.

Traycoff and colleagues reported that 97% of the UCB CD34+ HLA-DR+ cells is in G0/G1 phases of the cell cycle, at the moment of initiating the culture. However, after 36 h of incubation in the presence of SCF, only 55% of the cells remained in G0/G1. Interestingly, even in the absence of exogenous cytokines, there was a significant decrease in the proportion of dormant cells, from 97% to 84%, after 36 h [87]. The authors also found that, at the onset of culture, 88% of CD34+ HLA-DR+ cells from BM were in G0/G1. After 36 h of culture with SCF, the proportion of dormant cells decreased to only 72%, and in the absence of exogenous cytokines the proportion of resting cells remained around 90% [87]. Thus, it seems that the ability to exit from G0/G1 more rapidly could be one of the mechanisms that give UCB HPC a higher proliferation/expansion potential, as compared to BM HPC. It is noteworthy that in the study by Traycoff et al., UCB plasma also promoted UCB cells to exit from dormancy. This observation may be explained by the high levels of cytokines, such as IL-6, G-CSF, and GM-CSF, present in UCB plasma [92, 93]. Furthermore, other—possibly novel—cytokines present in UCB plasma may also be part of such a stimulatory activity [72, 90].

Schibler et al. have found that 6% of UCB CD34+ cells are capable of forming colonies in semisolid cultures in the absence of exogenous recombinant growth factors. In contrast, BM CD34+ cells fail to produce any colonies under such conditions [94]. Spontaneous growth of UCB megakaryocytic colonies in the absence of added cytokines has also been documented by Deutsch et al. [95]. In the former study, Schibler and colleagues individually analyzed the growth-factor-independent colonies and found transcripts for both GM-CSF and IL-3, suggesting that UCB HPC are capable of producing some hematopoietic cytokines [94]. Cytokine production by UCB CD34+ cells has also been documented by Watari et al., who demonstrated both at the mRNA and protein levels the production of IL-1β by CD34+ CD45RA0 CD71lo cells from UCB [96, 97]. Moreover, IL-1β converting enzyme, necessary for the production of IL-1β, was also detected at the mRNA level [96]. These studies indicate that the autocrine production of hematopoietic cytokines may be another mechanism that gives UCB HPC their elevated proliferation/expansion potential. Such an autocrine mechanism could also explain the spontaneous exit of UCB CD34+ HLA-DR+ cells from G0/G1 phases of the cell cycle [87].

Recent studies suggest that the functional differences observed between UCB and BM HPC could also be related to differences in telomere length. Telomeres, consisting of both DNA and proteins, are the physical ends of eukaryotic chromosomes [98]. Several investigators have shown that the length of telomeric repeats (TTAGGG)n in human cells decreases with in vivo and in vitro cell divisions. Indeed, somatic human cells lose 50-100 basepairs of telomeric DNA in each cell division [99, 100]. Furthermore, studies with cultured fibroblasts indicate that there is a direct correlation between telomere length and the replicative capacity of the cells [101]. Vaziri et al. purified CD34+CD38- cells from UCB and adult BM and measured their telomere length before and after 25 days in liquid culture with a selected cocktail of cytokines. They observed that, prior to the cultures, the mean length of telomere restriction fragments (TRF) of UCB cells was about 12 kb, which was significantly longer than in BM cells (8 kb) [102]. After the culture period, and as a result of cell proliferation, the mean length of the TRF decreased 1.5 kb, both in UCB and BM cultures. It is noteworthy that in this study, purified CD34+CD38- cells from fetal liver were also analyzed, and their mean TRF was even longer than in UCB cells. Thus, this study supports proliferation-dependent loss of telomeric DNA in cells of hematopoietic origin. Furthermore, the direct correlation between telomere length and developmental stage of human HPC suggests that changes in proliferation/expansion potential could be related to changes in telomere length [102-104].

**In Vivo Characterization of UCB HSPC**

Mice with SCID (lacking T- and B-cell function) and those in which the SCID mutation has been backcrossed onto the nonobese diabetic background (lacking T- and B-cell function, showing low natural killer cell activity and with defective macrophage function) have served as excellent models for in vivo studies on the biology of human HSPC both from BM and UCB [105-109].

Vormoor et al. have reported stable human long-term hematopoiesis in SCID mice transplanted with total or MNC from human UCB. Both myeloid and lymphoid cells of human origin were observed in the animal’s tissues and human CFC, including multipotent, myeloid, and erythroid progenitors, were also detected by in vitro cultures [107]. These results indicate the engraftment of an early hematopoietic progenitor/stem cell. Interestingly, the authors observed no difference in the levels of engraftment in UCB-transplanted mice treated...
with or without human cytokines (SCF and PIHY321 [a GM-CSF-IL-3 fusion protein]). This was in contrast to studies with adult BM cells, in which there is a clear improvement in the level of engraftment in mice treated with human cytokines [110]. This observation indicates that human UCB cells may be able to provide their own cytokines in a paracrine (or autocrine) fashion, thus rendering themselves independent from the exogenous supply of human cytokines. These results demonstrate that functional differences between UCB and BM HPC are also observed in vivo.

The engraftment cells characterized in these studies have been defined as SCID-repopulating cells (SRC) [111]. By using limiting dilution analysis, Wang et al. have estimated the frequency of SRC in human UCB, adult BM and mobilized peripheral blood (mPB). They observed that the frequency of SRC in UCB is threefold higher than in BM and sixfold higher than in mPB (1 SRC per 9.3 x 10^6 cells in UCB as compared to 1 SRC per 3.0 x 10^6 cells in BM and 1 SRC per 6.0 x 10^6 cells in mPB) [111]. These quantitative differences support the notion from in vitro studies that the proportion of primitive HSPC in UCB is higher than in adult BM and mPB.

More recently, Hogan and colleagues have transplanted nonobese diabetic SCID mice with purified CD34+ cells from human UCB, observing engraftment and long-term reconstitution. Once again, treatment with human cytokines had no effect on the level of engraftment [112]. Fraser et al. [113], transplanted SCID-human mice with CD34+ Thy-1+ Lin- cells derived from human UCB and observed reconstitution of human hematopoiesis. Thus, these studies demonstrate that the human UCB cell capable of reconstituting long-term hematopoiesis into SCID mice is comprised within the CD34+ Thy-1+ Lin- cell subpopulation. It is noteworthy that, in both studies, the authors observed repopulation of secondary recipients by BM cells from primary recipients, demonstrating maintenance of an extensive proliferation capacity of the input human progenitor cell population [112, 113].

An alternate in vivo model for studies of human HSPC has been developed by Zanjani and colleagues, who described the engraftment and long-term expression of human HSPC in sheep following transplantation in utero [114]. In this animal model, the immunologically naive environment of the early gestational-age fetus and the availability of sites for homing/engraftment of donor HSPC permit the engraftment of human HSPC without the need for cytoablative procedures [115].

Unfractionated and CD34+ cells from UCB have been transplanted into fetal lambs (54- to 65-day gestation). In both cases, engraftment was achieved, and detection of human hematopoietic cells was observed for over one year post transplant. However, most of the animals that received unfractionated UCB cells developed graft-versus-host-disease, which seemed to be associated with donor mature T cells. In contrast, long-term engraftment and reconstitution without graft-versus-host-disease was achieved in animals that received purified CD34+ cells from UCB [116]. These studies demonstrate that purified HSPC from human UCB are capable of developing in the fetal sheep marrow microenvironment. Further studies will be necessary to characterize in more detail the cells responsible for the hematopoietic engraftment and reconstitution in this large animal model.

**GENETIC MANIPULATION OF UCB CELLS**

Genetic manipulation of human cells (including HSPC) has been of great biomedical interest because of its potential relevance in the treatment of specific disorders [117]. Several investigators have reported on the successful introduction of particular genes into primitive hematopoietic cells from BM [118–120], and similar approaches are being used with UCB cells.

Moritz et al. compared the efficiency of gene transfer into both UCB and BM MNC. Two genes were analyzed, TK-neo (which confers resistance to the drug G418, a neomycin analog) and adenosine deaminase (ADA), involved in the development of SCID. Both genes were successfully transferred into primitive HPC, including CFU-MIX/ BFU-E, CFU-GM and LTC-IC [121]. Interestingly, these authors observed that the proportion of CFU-MIX/BFU-E, CFU-GM and LTC-IC resistant to G418 was significantly higher in UCB cultures (53%, 36%, and 25%, respectively) than in similar cells from BM (24%, 13%, and 10%, respectively). These results indicate that UCB HPC are better targets for gene transfer than HPC from BM, and suggest that UCB cells may have distinct advantages in some protocols of human gene therapy.

Lu et al. evaluated the in vitro transduction of the TK-neo gene directly into single CD34+++ UCB cells that were cultured with Epo, SCF, IL-3, and GM-CSF, and in the absence of accessory or stromal cells. They observed that the TK-neo gene was transduced at very high efficiency into primitive CFC, including CFU-GEMM and HPP-CFC, and that the proviral integration was also detected in cells from replated colonies derived from primary CFU-GEMM and HPP-CFC [122]. In a related study, Zhou et al. used adeno-associated virus 2 (a viral vector that, in contrast to the most commonly used retroviral vectors, can efficiently integrate into slow or noncycling cells) to transduce the TK-neo gene into CD34+++ UCB cells. As in the previous study, they observed that a significant proportion of CFU-GM, BFU-E, and CFU-GEMM (containing the appropriate gene) were resistant to G418 [123]. This observation suggests that, in the clinical setting, adeno-associated virus may prove to be useful for the transduction of primitive, noncycling HPC, without the need for cytokine stimulation,
which could potentially lead to differentiation of these cells before transplantation.

Genetically manipulated CD34+ UCB cells have been recently used in the treatment of patients with SCID [124]. Three women who were known to be heterozygous for ADA deficiency were identified to be carrying ADA-deficient fetuses. After term delivery of each neonate, UCB was collected, and CD34+ cells were isolated by standard methods. The CD34+ cells were transduced with the retroviral vector LASN, which carried the TK-neo gene and a normal human ADA cDNA. On the fourth day after birth, the transduced CD34+ cells were reintroduced to their respective donors by i.v. infusion. The efficiency of retroviral-mediated transduction of clonogenic myeloid progenitors contained within the UCB cells before transplantation was assessed by colony assays. From the three patients, 21.5%, 12.5%, and 19.4% of the myeloid progenitor cells were resistant to G418. After 18 months post transplant, all three patients showed the presence of BM granulocytes and MNC containing the LASN vector at approximately 1/10,000 cells. A similar frequency was observed in leukocytes present in the PB. Myeloid progenitors were cultured to assess the expression of the retroviral vector, and it was found that 4% to 6% of myeloid CFC were G418-resistant. According to this report, there have been no adverse effects from the administration of gene-modified UCB cells after 18 months post transplant. The continued administration of ADA enzyme replacement therapy has allowed the patients to develop normal immune function and to remain free of infections [124]. Obviously, in this study, SCID patients were not cured by the introduction of genetically manipulated UCB HSPC; however, these results are very encouraging, since they demonstrate the feasibility of using genetically modified UCB cells in clinical settings.

CONCLUDING REMARKS

Since the reports by Broxmeyer Gluckman in 1989 indicate that UCB constitutes an excellent source of HSPC for transplantation, a great deal of interest has been generated in the in vitro and in vivo characterization of such cells.

To date, it has been demonstrated that UCB contains a higher proportion of primitive hematopoietic cells, including multipotent CFC as well as in vivo (SCID) repopulating cells, than adult BM. Furthermore, in vitro and in vivo studies have shown that UCB-derived HSPC possess higher proliferation and expansion potentials than their adult BM counterparts. Several research groups have focused on trying to explain, at the molecular and cellular levels, the functional differences observed between UCB and BM cells. The actual reasons are still not completely understood; however, it has been shown that UCB HSPC A) exit G0/G1 phases of the cell cycle, in response to cytokines, more rapidly than BM progenitors; B) are able to produce some of the cytokines they need (possible autocrine loop), and C) possess longer telomeres than BM cells. From a practical point of view, it has been documented that UCB cells are more adequate for genetic manipulation than BM progenitors, thus, making them a very attractive target for gene therapy.

Several questions regarding the biology of human UCB-derived hematopoietic stem/progenitor cells remain to be answered. In view of the biological properties of such cells both in vitro and in vivo, careful delineation of the differences between UCB and adult HSPC remain of great interest. Moreover, due to the tremendous potential of UCB HSPC in the clinic [125], such studies should be encouraged.

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