CD34+ Cells: A Comparison of Stem and Progenitor Cells in Cord Blood, Peripheral Blood, and the Bone Marrow

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Introduction:

Hematopoietic Stem Cells (HSCs) are defined by two characteristics: multipotency and self renewal. Multipotency refers to ability of HSCs to differentiate into any hematopoietic cell types without restriction. Self renewal is defined as the ability of an HSC to divide and produce an identical multipotent progeny cell. While the defining characteristics of stem cells are well known, there is no known cell surface protein unique to stem cells, making it difficult to sort them out of the larger, more differentiated population. Work is currently underway using a variety of cell surface antigens to better define the HSC population. For the purpose of therapeutic hematopoietic reconstitution, however, the transmembrane phosphoglycoprotein CD34 as a marker for a diverse group of non-differentiated stem and progenitor cells, many of which are from the hematopoietic compartment (Ivanovic, 2010). CD34 was first identified in 1984, and was long considered to be a marker for hematopoietic stem and progenitor cells, although subsequent characterization indicated that it was also expressed in multipotent mesenchymal stromal cells, interstitial dendritic cells, epithelial progenitors and several other cell types. Despite its expression across different cell subsets, no common function for CD34 has been found (Sidney, 2014). Its most commonly reported ligand is L-Selectin, but it has also been shown to bind the adaptor protein CrkL, which regulates adhesion. The cell type-specific functions of CD34 have been primarily investigated in hematopoietic cells, where CD34 appears to play a role in cytoadhesion, regulation of differentiation and proliferation, and trafficking of hematopoietic stem cells (HSCs) to niches within the bone marrow (Sidney, 2014).

Reconstitution of the hematopoietic compartment requires the extraction and purification of hematopoietic stem and progenitor cells. While reconstitution is theoretically possible using a single hematopoietic stem cell (HSC), there is a strong correlation between number of stem and progenitor cells infused and the time required for engraftment and reconstitution of the hematopoietic compartment. Therapeutic hematopoietic reconstitution has helped define the hierarchy within the hematopoietic lineage. Sorted CD34+ cells contain not only the quiescent HSCs that allow long term maintenance of the reconstituted hematopoietic compartment, but also the multipotent progenitor cells that have lost the ability for self renewal, as well as the oligopotent progenitor cells that are lineage restricted (Figure 1). Progenitor cells allow short term reconstitution of the immune compartment while HSCs allow its long term maintenance. The frequency of various types of stem and progenitor cells within the CD34+ subset is constant enough that CD34+ cell number can be used as an accurate predictor of time to hematopoietic engraftment (Chao, 2004). Research has shown that 5x10^6 CD34+ cells per kilogram of patient weight is the optimal number for hematopoietic reconstitution.

Because there is no phenotypic assay to directly measure the number of CD34+ multipotent stem cells in a given sample, researchers rely on functional assays to determine the number of HSCs in a sample. One such assay is the Long Term Culture Initiating Cell (LTC-IC) assay, which determines the number of primitive cells based on the ability to produce myeloid progeny over at least five weeks in culture. LTC-IC assays can be used to measure single cell proliferative potential (generative capacity) after sorting cells using FACS and plating one cell per well (Liu, 2013). Such an assay actually determines the number of intermediate progenitor cells in the sample; researchers have shown that these
intermediate progenitors play a very small role in long term reconstitution of the hematopoietic compartment. However given the consistent inverse correlation between CD34+ cell number and time to engraftment, it is likely that the ratio of intermediate progenitor cells to primitive HSCs is maintained between samples, and CD34+ cell numbers are an accurate reflection of total HSCs infused during the transplant process (Chao, 2004).

This paper will compare the phenotypes, frequency, and function of CD34+ hematopoietic stem and progenitor cells found in the cord blood, bone marrow and peripheral blood.

**Cord Blood CD34+ cells**

Umbilical cord blood has been found to be a rich source of CD34+ cells with high proliferative potential. Various published studies have found the percentage of CD34+ cells in the cord blood to be between 0.02% and 1.43% (reviewed in Hordyjewska, 2015). The CD34+ cell population in cord blood is heterogeneous. Roughly 30-50% of CD34+ cells can be classed as progenitor cells (CFU-GM, BFU-E, CFU-Mk, and CFU-mix), while only less than 0.1% of the CD34+ population can be classed as “primitive” CD34+CD38- HSCs. Half the CD34+ population exhibits neither stem nor progenitor cell functional properties (Ivanovic 2010). It has been shown that CD34+CD38- cord blood cells have a greater proliferative response to cytokines and are less dependent on stromal cells than their counterparts in the bone marrow or peripheral blood (Hao, 1995). In addition, the hematopoietic lineage hierarchy is altered in cord blood vs. adult bone marrow. In bone marrow, CD34+HLA-DR⁻/lo cells are enriched for long term culture-initiating cells and therefore more primitive than the CD34+ subpopulation that expresses HLA-DR. Conversely, in cord blood, CD34+HLA-DR+ cells are the the more primitive population, with an enriched population capable of long term culture initiation (Hao, 1995).

![Figure 1: Expression of surface markers on hematopoietic stem and progenitor cells. CLP: common lymphoid progenitor; CMP: common myeloid progenitor; MEP: myeloid-erythroid progenitor; GMP: Granulocyte-macrophage progenitor.](image)
Early work indicated that CD34+ cells represent a smaller percentage of total mononuclear cells (MNC) in the cord blood (0.36%) than in the bone marrow (1.63%) (Hao, 1995). Among the total population of CD34+ cells, the more primitive CD34+CD38- cells were enriched in the cord blood, with 4% of total CD34+ cells in the cord blood lacking CD38 and only 1% of total BM CD34+ cells lacking CD38 (Hao 1995, Hordyjewska, 2015). Cord blood CD34+ cells (both CD38+ and CD38-) have greater generative capacity than BM-derived CD34+ cells.

Cord blood continues to be an excellent source of HSCs for transplantation, especially for pediatric recipients. However, the cell number is limited and repeat collections from the same donor are impossible. Ex vivo expansion of CD34+ umbilical cord blood cells has proven difficult, since current protocols result in the expansion of progenitor cells instead of primitive stem cells.

**Bone Marrow CD34+ cells**

Bone marrow is the traditional source of CD34+ stem and progenitor cells used in stem cell therapies. The CD34+ cells that reside in the bone marrow microenvironment are associated with stromal cells, and their egress from this niche is tightly controlled by the interaction between cell surface receptors and chemokines and cytokines produced by the stromal cells. CD34+ cell surface proteins such as Kit and CXCR4 interact with stem cell factor (SCF) and stromal cell derived factor 1α (SDF-1α) respectively to keep CD34+ cells localized in the bone marrow microenvironment.

Various published studies have placed the percentage of CD34+ cells in the bone marrow at between 0.5% and 5% of total mononuclear cells (MNCs). While it is thus a rich source of CD34+ cells, the extraction of these cells requires an invasive procedure. CD34+ cells from the bone marrow show a 10x lower generative capacity than those found in the cord blood (Hao, 1995) and produce half as many total cells as cord blood CD34+ cells after 10 days in culture (Van Epps, 1994). Fewer of the primitive CD34+CD38- cells are cycling (0.4% BM vs 5.4% cord blood), resulting in a later onset of proliferation (21 days for BM vs. 7 days for cord blood) in culture (Hao, 1995). The reduced proliferative capacity is likely due to the presence of more partially differentiated progenitor cells in the BM as compared to the cord blood.

**Peripheral Blood (unmobilized Leukopak) CD34+ cells**

Peripheral blood remains the most accessible source of CD34+ HSCs, although the circulating number of steady state peripheral blood stem cells (PBSCs) is much lower than that found in the bone marrow (roughly 0.15% of cells are CD34+ in the peripheral blood vs. 2% in the BM). (Hao, 1995, Van Epps, 1994, and To, 1994). A series of papers in the 1990s examined the phenotype and proliferative capacity of PB CD34+ cells as compared to BM and cord blood-derived CD34+ cells, and found that BM and PB CD34+ cells produced similar numbers of nucleated cells after 21 days in culture, but that steady state PB CD34+ cells produced roughly 10-fold fewer granulocyte-macrophage colony forming units (CFU-GM) than CD34+ cells derived from the bone marrow (To, 1994).

Due to the low numbers of steady state circulating CD34+ cells, they remain a poor choice for therapies requiring large numbers of CD34+ cells. Fortunately protocols have been developed to increase the number of CD34+ cells in the peripheral blood. These include mobilization by G-CSF, chemotherapy, or drugs such as Plerixafor (Mozobil) that induce CD34+ trafficking into the circulatory system. The first two mobilization protocols act by increasing both the number of stem and progenitor cells in the bone marrow and the number of CD34+ cells released into the peripheral blood.

**Peripheral Blood (G-CSF-mobilized Leukopak) CD34+ cells**

At steady state, the concentration of CD34+ cells in the peripheral blood is 10-100x less than that found in the bone marrow. Thus in order to effectively collect CD34+ cells for use in transplantation, cells must be mobilized to leave the bone marrow microenvironment and move into the peripheral blood. Mobilization can be accomplished by using the cytokines G-CSF or GM-CSF, or by the administration of myeloablative chemotherapy drugs. G-CSF increases peripheral blood CD34+ cell concentration by decreasing SDF-1α gene expression and protein levels on
stromal cells while at the same time increasing the production and release of proteases that can cleave the bond between CD34+ cell surface proteins and those found on stromal cells. It has been shown that part of the mobilization is achieved by cleaving the Kit receptor (CD117) using one of several proteases released into the extracellular medium by macrophages and neutrophils after stimulation with G-CSF (Levesque, 2003). Reduced levels of Kit on CD34+ PBSCs following mobilization by G-CSF, GM-CSF, chemotherapy, or a combination of these were noted in the early 1990s (To, 1994). Researchers also found that mobilization protocols increased the percentage of PB CD34+ cells to numbers similar to those seen with BM CD34+ cells (To, 1994).

Mobilized CD34+ cells also show reduced levels activation markers, indicating that many of the CD34+ cells exiting from the BM after G-CSF stimulation are maintained in a quiescent state (To, 1994). Quiescence is a mark of HSCs, which are a slow cycling population, and the reduction in activation markers in mobilized CD34+ PB cells relative to the population of CD34+ cells in the bone marrow indicates that primitive HSCs are in particular being mobilized after stimulation. This makes mobilized PBSCs an excellent choice for hematopoietic reconstitution. Notably, G-CSF mobilized cells had the greatest percentage of CD38- cells, which correlates to an increased percentage of HSCs and multipotent progenitor cells (To, 1994). In addition, mobilized PB CD34+ cells showed a similar fold increase in cell number compared to BM-derived CD34+ cells after 10 days in culture (Van Epps, 1994).

Summary

CD34+ acts as an easily identifiable phenotypic marker for stem and progenitor cells in the hematopoietic system. Total CD34+ cell number is tightly correlated with success of hematopoietic reconstitution, and there is a strong inverse correlation between total CD34+ cell number infused and time to engraftment. Within the CD34+ cell population there is a hierarchy of cells ranging from the primitive HSCs, capable of self renewal and differentiation into any of the blood cell types, to multipotent progenitor cells (capable of differentiation into any hematopoietic cells type but lacking the ability to self renew); oligopotent progenitor cells; lineage restricted progenitor cells; and finally the mature effector cells of the hematopoietic system. CD34+ cells are commonly collected from the umbilical cord blood, the bone marrow or the peripheral blood following mobilization. CD34+ cells are found in the bone marrow and cord blood in approximately equal percentages; however CD34+ cells from the cord blood have a greater frequency of primitive HSCs (and correspondingly greater capacity to multiply and form colonies). CD34+ cells collected from the peripheral blood of donors after mobilization with G-CSF offer an easily accessible source of HSCs that can be collected via apheresis over multiple sessions. Mobilized peripheral blood HSCs are phenotypically similar to BM HSCs in terms of proliferative capacity, but they have a greater percentage of CD38- cells (and therefore more primitive HSCs), making them a good choice for subsequent transplant and reconstitution of the hematopoietic compartment.
References


