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Expansion of Human Cord Blood Hematopoietic Stem Cells for Transplantation

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Abstract

A recent *Science* paper reported a purine derivative that expands human cord blood hematopoietic stem cells in culture (Boitano et al., 2010) by antagonizing the aryl hydrocarbon receptor. Major problems need to be overcome before *ex vivo* HSC expansion can be used clinically.

The use of human umbilical cord blood (CB) as a source of hematopoietic stem cells (HSC) for transplantation continues to increase, with over 3,000 CB transplants performed annually around the world (Foeken et al., 2010). Recent growth in CB transplantation has been fuelled by the availability of these cells in banks as well as strong clinical data supporting the use of HLA mismatched transplants with low risk of graft versus-host disease (GVHD) (Hwang et al., 2007). However, wide utilization of CB is limited by the relatively low number of HSCs per unit, and most CB units have insufficient stem cells for adults. Significant effort has gone into developing technologies for *ex vivo* expansion of HSCs in order to enable CB transplants for adults, who comprise the majority of patients who need such intervention.

Boitano and colleagues recently reported a purine derivative termed SR1 that significantly expands human CB HSCs in culture (Boitano et al., 2010). The authors screened a chemical library of 100,000 heterocyclic compounds for their ability to expand the numbers of CD34⁺ CB cells in culture. They showed that SR1 supported a fifty-fold increase in CD34⁺ cells and, more importantly, a 17-fold increase in cells that retain the ability to engraft immunodeficient mice. Strikingly, SR1 is an antagonist of the aryl hydrocarbon nuclear receptor protein (AhR), which normally mediates xenobiotic responses but has also been implicated in regulating hematopoietic stem/progenitor cells. Knockdown of the AhR resulted in sustained proliferation of CD34⁺ cells in culture.

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This study is significant because it demonstrates that small synthetic chemicals can be used to identify signaling pathways that govern HSC fate decisions and modulate them to allow HSC expansion for clinical use.

An important caveat to consider regarding the capacity of SR1 to expand HSCs is that this factor is not effective if added alone to the culture medium. The results presented by Boitano and colleagues were done in a serum-free medium supplemented with four cytokines: Thrombopoietin (Tpo), Stem Cell Factor (SCF), Flt3 ligand, and IL-6. Their observed maximal HSC expansion was similar to the 20-fold expansion of human CB HSCs obtained in a serum-free culture medium containing a different growth factor cocktail: SCF, Tpo, FGF-1, Angiopoietin-like 5, and Insulin-Like Growth Factor Binding Protein 2 (IGFBP2) (Zhang et al., 2008). Many other factors and cytokines may promote *ex vivo* HSC expansion, including Notch ligands, Wnts, Bone Morphogenetic Proteins, Insulin-like growth factor 2 (IGF-2) (Zhang and Lodish, 2008), pleiotrophin (Himburg et al., 2010), and prostaglandin E2 (North et al., 2007), but none of these signal activators have been successful when added alone.

Since few laboratories have attempted to systematically optimize the combination and concentrations of cytokines and other factors such as SR1, or the time of culture or the O₂ tension, it is likely that one can significantly increase the extent of HSC expansion *ex vivo*. Methods that improve HSC homing and survival after injection of HSCs could also be combined with *ex vivo* HSC expansion to further enhance the efficiency of transplantation.

In any case, in addition to optimizing HSC expansion protocols, many other hurdles remain to be overcome before these important laboratory discoveries can be converted into human therapies. One important example is that CB units are stored frozen, and thawing typically reduces the number of viable nucleated cells by half. Improvements in cryopreservation are essential.

Another important point to consider regarding the various protocols that are used to expand CB HSCs is the relative merits of pre-isolation of a subpopulation of CB cells prior to the culture period. Most of these assays begin with magnetic bead isolation of CD34+ or CD133+ cells. These populations contain most of the hematopoietic progenitors and long-term reconstituting HSCs, which are necessary for a successful clinical graft. Preisolation of CD34+ or CD133+ cells improves cell expansion *ex vivo*, possibly due to the absence of more mature populations or other non-hematopoietic cells that may inhibit HSC or progenitor proliferation. Working with purified progenitor cell populations would also facilitate eventual gene therapy approaches that require selection of HSCs that have incorporated a transgene into a desired chromosomal location.

On the other hand, preisolation of CD34+ or CD133+ cells presents several potential disadvantages, particularly given that many, if not most of the HSCs present in the original unfrozen CB unit may be lost during this fractionation process. Cell loss may in part be due to failure to capture the desired cells, since positive selection always leads to cell loss due to inefficient binding to the beads, and some HSCs may not express enough CD34 or CD133 on their surface to be selected. There may also be inadvertent cell loss due to stress and eventual death during the various manipulation steps.

In the clinical setting, the final number of HSCs and progenitors in the transplant is the essential parameter. Given the likely loss of a substantial number of HSCs prior to manipulation of the CB unit, even the current, improved, degree of HSC expansion that has been achieved may not result in an appreciative net increase in HSC number when compared to unmanipulated CB units. Thus, any isolation process that results in extensive HSC loss

should clearly be avoided in order to maximize the number of stem cells transplanted into the patient.

In addition to the risk of losing unselected cells, the use of positive selection methods to enrich for HSCs also involves tagging the cells of interest with antibodies, which could affect cell function or activation state. Furthermore, this practice also raises quality control issues, since the antibodies remain attached to the HSCs after transfer to the patient. The cost of sufficient clinical grade antibody used to select greater than 10^9 cells per CB unit is also a concern, especially since many of the red blood cells that are present will nonspecifically bind the antibody and make the process inefficient. One option to overcome these limitations is to use a negative depletion method to remove non-HSCs, such as lineage positive, differentiated, hematopoietic cells. Negative depletion methods should also minimize the loss of HSCs relative to positive selection, although the degree of enrichment for HSCs in the selected population will be lower.

Therefore, given the current clinical situation, expansion of CB cells without positive selection seems most practical and cost-effective. It remains to be seen whether SR1, in combination with other cytokines, can still expand HSCs efficiently from a coarsely purified population of HSCs, or even from unpurified total CB cells.

In addition to various combinations of soluble factors, several protocols for HSC expansion involve coculture with mesenchymal stem/stromal cell (MSC) lines (Robinson et al., 2006). This approach offers several advantages, particularly that preisolation of CD34+ or CD133+ cells does not appear to be required to yield a significant expansion of primitive cells, and that cell viability is retained during the expansion process. Despite these positive features, there is little evidence that MSC co-culture supports expansion of long-term reconstituting HSCs, although these conditions do increase the number of hematopoietic progenitors. Furthermore, challenges in production and quality control of clinical grade MSCs under Good Manufacturing Protocols (GMP) will hinder the therapeutic use of these cocultures, as will the inevitable risks to the patient following inadvertent infusions of the feeder cells, possibly including graft reactions.

Besides HSCs, successful grafts must include less primitive hematopoietic progenitors in order to generate mature myeloid and lymphoid cells during the first weeks following transplantation. Immune cells such as T cells in the graft also play a role in the engraftment process. A recent clinical study (Delaney et al., 2010) showed that Notch-mediated *ex vivo* expansion of human CD34+ CB progenitors resulted in a marked increase in the absolute number of stem/progenitor cells. When infused in myoablated patients, the time to neutrophil recovery was substantially shortened. This finding is clinically important, as delayed engraftment can result in a higher incidence of infection, which is one of the major causes of mortality after transplantation.

Finally, bioprocess and scale-up issues are relevant even if MSC co-culture is not required. Milliliter volume cell cultures are very different from multi-liter cultures that will be necessary for generating sufficient cell numbers for clinical transplants. Issues concerning absolute sterility and Good Manufacturing Practices are needed for clinical translation (Ahrlund-Richter et al., 2009), but foreign to most laboratory investigators.

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