Hematopoietic stem cell expansion: when less is better than more

The major disadvantage of using umbilical cord blood (CB) as an alternative source of clinically transplantable hematopoietic stem cells (HSC) is the relatively low number of cells present in a single unit of cord blood. In fact, the success of a cord blood transplant is related with the number of cells infused: the higher the number the lower the risk of transplantation failure.[1] This has resulted in CB being used for HSC transplantation mainly in patients with low body weight. However, compared to bone marrow or to mobilized peripheral blood, CB remains a much better souce of HSC for transplantation because of the easy collection and ready availability of the units, the less stringent requirement for HLA matching between donor and recipient, and the low severity of GVHD.[1,2] The major disadvantages of CB are, as already mentioned, the smaller number of transplantable cells and the delayed engraftment which result in an increased peri-transplant morbidity and mortality.[3] It is therefore not surprising that scientists in the last years have been directing major efforts toward the *ex vivo* expansion of CB derived-HSC. Greater availability of HSC would make extensive use of CB in HSC transplantation possible.

It is well known that the major problem in expanding HSC is to maintain the *stem cell nature* of HSC while amplifying their number. However, besides the expansion of true stem cells, an expansion of committed hematopoietic progenitor cells (colony-forming units, CFU) would also be desirable because increased availability of these cells could help in overcome the delayed engraftment observed after CB transplantation. In other words, an ideal expansion protocol should be able to increase both the number of HSC, without inducing their differentiation, and the number of CFU. In addition to these points, we must also keep in mind that obtaining *true* HSC expansion may not be enough to CB for transplantation in adults. In fact, the effects of the expansion procedures on the telomere length of expanded HSC and more in general on accelerated HSC aging, due to the high number of cell divisions, have not been yet completely clarified.[4-7] Indeed, it is still matter of debate whether telomere shortening of stem cells, if any, can compromise their long-term repopulating ability.[8,9]

Although some studies have been published on HSC expansion *in vitro*, most of them have used their expanded HSC to transplant NOD/SCID mice, as a test to prove that expansion of HSC had been obtained without loss of these cells' long-term engraftment and repopulating capacity,[10-12] whereas there have not been any large scale-clinical studies in humans using *ex vivo* expanded HSC for transplantation. The results of transplantation of *ex vivo* expanded cells in the animal model have been controversial because, although in some cases encouraging outcomes were obtained,[13,14] in other cases expanded cells failed to produce long-term engraftment.[15]

In recent years, despite the limitation imposed by the low number of HSC, the use of non-expanded CB for HSC transplantation in adult patients with hematologic malignancies who lack an HLA-matched bone marrow donor[16-18] has increased. This has been made possible by the selection units of CB very rich in $CD34^+$ cells from CB banks. In this particular setting, CB has proven to be an alternative source to bone marrow, giving similar outcomes to those of bone marrow but, as expected, causing a lower incidence of acute GVHD and slower neutrophil recovery.

In this last regard, the paper published by Levac *et al.*[19] in this issue of the journal (see page 166) could have some intriguing implications. Levac and colleagues show that the cocktail of stem cell factor (SCF), Flt3-ligand, and thrombopoietin (TPO) does not expand HSC (assayed as SRC) to a greater extent than SCF + Flt3-ligand alone. Surprisingly, the addition of TPO to SCF and Flt3-ligand greatly expands committed hematopoietic progenitor cells (assayed as CFU) to an extent similar to that induced by interleukin-3, granulocyte colony-stimulating factor and interleukin-6 (IL-3+G-CSF+IL-6), when the latter are added to SCF and Flt3-ligand. This observation, if confirmed, could pave the way for designing clinically relevant protocols aimed at improving the efficiency of CB as a source of transplantable HSC, at least in the clinical setting such as in pediatric patients and in adults lacking HLA-matched bone marrow donors. In fact, the expansion of committed progenitor induced by such a combination could greatly help in overcoming the delayed engraftment which characterizes CB transplantation. At the same time, the lack of any effect on the number of HSC of the SCF/Flt3-ligand/TPO cocktail could be useful because it would spare the cells from the potentially harmful consequences related to the expansion procedure, such as telomere shortening, without affecting their long-term repopulating capacity. This potential application is also supported by the recent observation that CB-derived HSC can provide better reconstitution of the hematopoietic reservoir than can their bone marrow counterpart in humans.[20] It was found that at 1 year after transplant patients who had received a CB graft had a higher number of LTC-IC in their bone marrow than those who were transplanted with bone marrow-derived HSC, despite the fact the formers had had a delayed neutrophil and platelet recovery. Two other minor but not negligible favorable aspects of an expansion protocol based on the results of Levac *et al.* are that it would be very simple and cost-saving, because based on the use of only three cytokines.

The results reported by Levac *et al.* do, of course, need to be confirmed before one could really envisage a practical clinical application. In fact, some of the data reported, although encouraging and interesting, should be made stronger by further confirmatory experiments. For instance, they assess the number of HSC by using the SCID repopulating assay; however, they do not perform secondary or tertiary transplants in order to prove the conserved long-term repopulating activity of the $CD34^+CD38^-$ cells exposed to their cytokine cocktail. Also, the absence of effects of TPO on more immature progenitor cells associated with its capacity of replacing the activity of the IL-3/G-CSF/IL-6 combination on committed progenitor cells is in disagreement with previous reports showing that TPO exerts its effects on HSC rather than on CFU.[21-23] However, in regard to this latter consideration, it should be underlined that differences in the experimental protocols dealing with HSC expansion, even when based on the same cytokines, could account for discrepancies in the results obtained from different groups. Starting cell population, cytokine concentration, time of exposure of cells to cytokines, and different ways of assessing cell phenotype and/or function should be taken into account when comparing results from different groups. Nevertheless, despite these necessary caveats, the paper by Levac *et al.* presents an alternative

way of thinking of CB-derived HSC (non-) expansion which could, if confirmed, be quickly translated into practical and useful clinical applications.

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Thrombosis and cancer: clinical relevance of a dangerous liaison

Venous thromboembolism (deep vein thrombosis and pulmonary embolism) and cancer are linked by a *two-way clinical association*. Indeed, an idiopathic or unprovoked episode of venous thromboembolism (VTE) may be the first clinical manifestation of an occult cancer while patients with clinically overt cancer are prone to have a thromboembolic complication.[1] The pathophysiology of thrombosis in cancer patients is rather complex. Cancer cells can activate the clotting system directly, by generating thrombin, or indirectly, by stimulating endothelial cells and circulating mononuclear cells to synthesize and express a number of procoagulant factors, including the tissue factor. An activation of blood coagulation has been consistently demonstrated in patients with cancer, but the correlation between these laboratory findings and clinical outcomes has been only partially elucidated.

Idiopathic VTE and occult cancer

Patients presenting with an idiopathic or unprovoked VTE have an approximately 10% probability of developing a cancer in the two years after the index event.[2] Because of the concerns about this adverse outcome, extensive screening for underlying cancer has been advocated in patients presenting with idiopathic VTE. In a recently published randomized trial the clinical benefit of extensive screening for occult cancer was compared with the management of no screening in patients with idiopathic VTE. The sensitivity of the extensive screening was found to be approximately 90%.[3] During the 2-year follow-up period, a newly diagnosed cancer was found in 10 of the 102 patients randomized to no-screening and 1 of the 99 patients randomized to extensive screening. After a follow-up of 2 years from the index event, no significant difference in cancer-related mortality was observed between the two groups (2.0% in the screened group and 3.9% in the not-screened group). In a second recently published study, Monreal *et al.*[4] prospectively evaluated the clinical benefit of a limited screening for occult cancer (abdominal ultrasound and laboratory markers of malignancy) in patients with acute VTE. This limited diagnostic work-up showed a sensitivity of approximately 50%, leading the authors to conclude for the opportuneness of a more extensive diagnostic screening. Based on the currently available data, there is not sufficient evidence to recommend routinely either exten-