

## ***Ex vivo* expansion of human hematopoietic stem cells**

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Human cord blood (CB) has gained attention as one of the sources for hematopoietic stem cell transplantation (HSCT). Successful expansion of hematopoietic stem cells (HSC) would overcome the limitations of cord blood transplantation to adopt for adult patients. We have previously reported a novel culture system in which the murine stromal cell line HESS-5 dramatically supports the rapid expansion of CB CD34<sup>+</sup> cells in synergy with thrombopoietin, Flk-2 ligand, and stem cell factor. Within 5 days of serum-free culture in this system, a 20- to 30-fold increase in CD34<sup>+</sup> cells was obtained; colony-forming units in culture (CFU-C) and mixed colonies (CFU-GEMM) were amplified by 10- to 30-fold and 10- to 20-fold, respectively. To further assess the ability of long-term repopulation and multi-lineage differentiation of those expanded cells, we performed SCID-repopulating cells (SRC) assay using CD34<sup>+</sup> cells cultured in this system. SRC and their multilineage differentiation were detected in NOD/SCID mice 7 weeks after injection of these cultured cells. Within 5 days of culture, 10 fold amplification of the SRC was obtained.

We then evaluated functional capacity of B cells developed from *ex vivo* expanded HSC by investigating the antigen-specific antibody production in NOD/SCID mice. CB CD34<sup>+</sup> cells were cultured for 5 days and transplanted into irradiated NOD/SCID mice. These mice, reconstituted with human hematopoietic cells, were challenged with T-cell-independent (TI)

or -dependent (TD) antigens after CD19<sup>+</sup> cells appeared at 6 weeks. Three months later, anti-DNP specific antibody was detected in both mice immunized with DNP-Ficoll(TI) and those immunized with DNP-KLH or DNP-OVA(TD). The anti-DNP antibody was mainly IgM but a small amount of IgG was also detected. In the spleen, the majority of CD19<sup>+</sup> cells expressed mature B cell markers such as CD40, IgM, IgD, and cytoplasmic C $\mu$ ,  $\kappa$  and  $\lambda$ . These results indicate that human B cells develop from CD34<sup>+</sup> cells in NOD/SCID mice to produce antigen specific antibody with *in vivo* primary stimulation. This system provides a powerful and versatile tool for studying the entire process of human B-lymphocyte development and producing specific human monoclonal antibodies.

T cells developed from *ex vivo* expanded HSC were evaluated by transplantation into newly developed NOD/SCID, IL-2R $\gamma$  null (NOD/SCID/ $\gamma$ c<sup>null</sup>) mice. When these mice were transplanted with human cord blood CD34<sup>+</sup> cells, the mice reproductively developed human T cells in their thymus and migrate into peripheral lymphoid organs. Furthermore, these T cells bear polyclonal  $\alpha\beta$  TCR, and respond not only to mitogenic stimuli, such as PHA and IL-2, but to allogenic human cells. These results indicate that functional human T lymphocytes can be reconstituted from CD34<sup>+</sup> cells in mice.

These results indicated that *ex vivo* expanded CB CD34<sup>+</sup> cells maintained the capacity to differentiate into functional hemato-lymphoid cells.