Serum-free culture conditions for the generation of dendritic cells from cord blood CD34+ hematopoietic progenitors: phenotypic and functional analysis

Increasing pre-clinical and clinical data suggest the efficiency of dendritic cells (DCs) in cancer immunotherapy. Relapse after cord blood hematopoietic progenitors (CBHP) transplantation is an unresolved problem. DCs obtained from CBHP could be an interesting tool for relapse treatments, but the low number of CBHP hinder their use for DC generation.

Umbilical cord blood (UCB) has been shown to be an important alternative source of hematopoietic progenitors for un-related transplants in the treatment of hematopoietic malignancies. Using this transplantation modality, a lower incidence of graft-versus-host disease (GVHD) has been described, but possibly this is accompanied by a lesser graft-versus-leukemia effect. Current results have shown that there are still significant percentages of relapses and post-transplant immunodeficiency-related deaths. Dendritic cells (DCs) are potent hematopoietic progenitor-derived antigen presenting cells, which could be used in specific immunotherapy by activating T-lymphocytes. However, the low number of HP in a unit of UCB hampers the possibility of producing DCs from the same transplant donor UCB unit.

This study shows that a limited number of UCB hematopoietic progenitors can generate sufficient quantities of active mature DCs under serum-free conditions for use in immunotherapeutic treatment. We optimized a combination of cytokines, containing: SCF (50 ng/mL), Flt3-L (100 ng/mL), GM-CSF (50 ng/mL), TGF-b1 (0.5 ng/mL), and compared this combination with a control containing 10% FBS. After 14 days of culture in the serum-free medium, 10^5 CD34+ cells produced 1x10^7 ± 3.7x10^6 CD1a+ cells, which represented 43±16% of the total cells in culture vs 1.29±1x10^7 (39±5.5%) in 10% FCS containing medium (Figure 1). The CD1a+ cells generated in serum-free medium presented the following phenotype: CD14- (46.5%), CD80 (94%), CD86 (88%), CD40 (99%), HLA-DR (99%), HLA-ABC (100%) and CD83 (62%). Functionality was tested by endocytic activity (approximately 27% of CD1a cells) and alternatively TGF-b1 (0.5 ng/mL) and 10% fetal calf serum (positive control) (C).

TGF-β, one of the components of the cytokine combination is a recognized inhibitor of multiple immunologic functions (i.e., T-cell activities and promotes a shift towards a Th2 phenotype) and, therefore, DCs obtained in presence of TGF-β, prior to T-cell activation, require the change to a TGF-β-free medium.

These cytokine combinations have also been tested for the generation of DCs from adult sources of hematopoietic progenitors. Functional characteris-

Figure 1. Percentage and number of CD1a+ cells obtained at the indicated times of culture with the following cytokine combination (B): recombinant human SCF (50 ng/mL), Flt3-L (100 ng/mL), GM-CSF (50 ng/mL), TNF-α (3 ng/mL) and alternatively TGF-b1 (0.5 ng/mL) (T) or 10% fetal calf serum (positive control) (C).

Key words

Cord blood, CD34+ cells, dendritic cells, cytokines.

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References

9. DNA was isolated from peripheral whole blood using standard protocols. The HFE genotyping was performed by PCR-RFLP using the procedure described by Bacon et al.1 The melting temperature assay is based on the ability to distinguish between PCR amplification products by their melting temperatures. They used a single-tube fluorescent PCR assay using a specific primer set, with a high annealing temperature, and the SYBR green I fluorescent dye, able to detect double stranded DNA products. The temperature at which double-strand templates dissociate depends on the product length, GC content and sequence structure. The