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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.

Sir,

Umbilical cord blood (UCB) has been shown to be an important alternative source of hematopoietic progenitors for unrelated transplants in the treatment of hematopoietic malignancies.[1] 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.[2-4] Dendritic cells (DCs) are potent hematopoietic progenitor-derived antigen presenting cells, which could be used in specific immunotherapy by activating T-lymphocytes.[5] 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), TNF-α (3 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 $1x10^7 \pm 3.7x10^6$ $CD1a^+$ 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 label for FITC-dextran at day 10) and allogeneic T-cell stimulation at day 14 [3 fold higher (75000 cpm vs 25,000 cpm) than the CB $CD34^-$ fraction as a control].

TGF-β, one of the components of the cytokine combination is a recognized inhibitor of multiple immunologic functions (i.e., T-cell activities[6] and promotes a shift towards a Th2 phenotype[7]) 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 characteristics of these DCs are similar to those obtained from UCB hematopoetic progenitors (data not shown), however, as described for other myeloid lineages, the expansion potential of adult hematopoetic progenitors is lower.

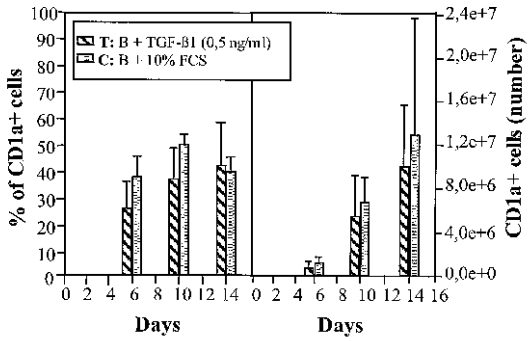

Figure 1. Percentage and number of CD1a+ cells obtained at the indicated times of culture with the following cytokine combination (B): recombinant human SCF (50 ng/mL), Flt-3L (100 ng/mL), GM-CSF (50 ng/mL), TNF-a (3 ng/mL) and alternatively TGF-b1 (0.5 ng/mL) (T) or 10% fetal calf serum (positive control)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

The results obtained with this optimized cytokine combination are comparable with those achieved with 10% FCS instead of TGF-β.[1] A schedule of DC for patient vaccination has not yet been established, but ≈$5 x10^5$ to 10^7 DCs/ infusion has been used several times in clinical trials, and clinical responses have been reported.[8-10] Our results show that expansion of a small fraction of UCB hematopoietic progenitors can produce sufficient quantities of DCs for immunological treatments.

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Key words

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

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Single tube melting temperature assay for rapid and sensitive detection of the most frequent hemocromatosis mutations, C282Y and H63D

We report the development of a single-tube assay for rapid genotyping of the two mutations C282Y and H63D of the HFE gene, commonly detected by PCR-RFLP and responsible for 80% of cases of hereditary hemochromatosis. The method, named T melting curve analysis, discriminates between amplification products by their melting temperatures.

Sir,

The eight hemochromatosis (HH) patients in this study included: two homozygotes for C282Y, two homozygotes for H63D, two compound heterozygotes and two heterozygotes for C282Y. Four healthy individuals, two heterozygotes for H63D and two without HFE mutations, were also studied.

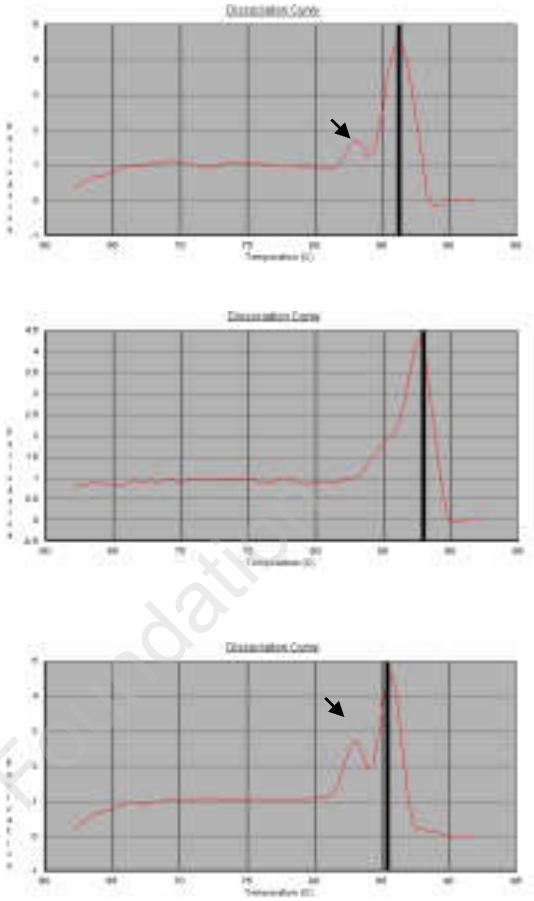

Figure 1. Melting profile of the wild type (A), mutated (B) and heterozygous (C) genotypes at position 63 of HFE gene. On the X axis there are the temperatures at which fluorescent data was collected (see materials and methods), the Y-axis shows the first derivative of the above data. The vertical bold bar highlights the melting point at which the amplicons reassociate. PCR has been optimized in order to better discriminate the different genotypes. A good Tm profile is represented in panel B because of low presence of primer dimers, still present in panels A and C, as the lower peak which is indicated by arrows. Nevertheless, even the heterozygous (C) and the wild type (A) are easily distinguishable from the primer dimer product.

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 temperature (Tm).[2] The single tube fluorescent PCR assay uses 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