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Abstract

Expansion and Differentiation of Dendritic Cells in Clinical Scale from Human Cord CD34+ Progenitor Cells

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Dendritic cells are the most potent antigen presenting cells and appear to be the only cell type capable of limiting a primary T-cell dependent immune response. Recently, progress in the understanding of DCs biology has been relatively fast and systems using CD34+ stem cells stimulated with GM-CSF and tumor necrosis factor-α (TNF-α) have been described. These systems have been further modified by others to increase the diversity and the yield. Indeed, several studies have shown distinct clinical responses after vaccination with tumor antigen-loaded, autologous DC. Despite this progress, the total number of DC available for immunotherapy remains limited. *In vitro* human DC can be generated from human CD34+ bone marrow and peripheral blood progenitor cells after culture with different cytokine combinations or from peripheral blood CD14+ monocytes when grow in the presence of GM-CSF and IL-4. Here, we have explored another source of DC precursors, human CD34+ cord blood cells, which in contrast to monocytic precursors, expand when cultured in the presence of GM-CSF and TNF-α.

The CD34+ cells were purified using MACS and expanded in culture with cytokine mixtures (SCF, Flt-3 TPO, IL-3, and IL-6). The CD34+ cells ($4.0 \pm 1.8 \times 10^5$) isolated from cord blood cultured for 1, 2, 3, and 4 weeks resulted in a mean increase of total cell number of $41.5 \pm 26.2 \times 10^5$ (10-fold), $143.8 \pm 78.9 \times 10^5$ (36 fold), $197.5 \pm 145.5 \times 10^5$ (49-fold), $241.5 \pm 167.4 \times 10^5$ (60-fold), respectively. The precursor cells progressively lose most of the CD34 expression in culture and are over 95% positive for CD38 and low expression for CD3/CD19 indicating that all precursors are from myeloid origin. The percentage of CD14 positive precursors was significantly increased according to the expansion duration. The CD1a expression of expanded DC precursors was all negative (0.15-0.57%).

The CD1a expression, which were in immature DCs, was high (28-78%), and CD40, CD80, CD11c and HLA-DR was positive after expanded precursor DCs were cultured for 1 weeks using GM-CSF and IL-4. The immature DC derived from all precursor culture conditions were negative for CD83. TNF-α activated DCs derived from the four precursor culture condition according to the day of culture were used as stimulator cells in allogenenic MLR. When the total DC population was used, the expanded DCs for 2 weeks induced a slightly but reproducibly stronger MLR than those for 4 weeks. In this study, we show the sequential culture method after expansion is particularly appropriate for immunotherapeutical approaches, because relatively large numbers of DC can from cord blood be generated to overcome the limitation of cell count, which are needed for repetitive vaccination.

Key Words: CD34+ cell, cord blood, dendritic cell, *ex vivo* expansion

Immune Monitoring of Cancer Vaccines

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The recent progress in tumor immunology exemplifies the successful application of modern biotechnology for the understanding of the complex natural or therapy-induced phenomenon of immune-mediated rejection of cancer. Tumor antigens recognized by T cells were identified and successfully utilized in active immunization trials for the induction of tumor-antigen specific T cells. This achievement has left, however, the clinicians and researchers perplexed by the paradoxical observation of the immunization-induced T cells can recognize tumor cells in standard assays but most often cannot induce tumor regression. In this presentation, we will argue that successful immunization is one of several steps required for tumor clearance but more work needs to be done to understand how T cells can localize and be effective at the receiving end within a tumor microenvironment in most cases not conducive to the execution of their effector function. In fact, metastatic melanoma stands out among human cancers