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 grown 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 monocyte 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 ± 1.8 × 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 ± 26.2 × 10^5 (10-fold), 143.8 ± 78.9 × 10^5 (36 fold), 197.5 ± 145.5 × 10^5 (49-fold), 241.5 ± 167.4 × 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 allogeneic 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 that 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.
because of its immune responsiveness. Yet, the reason(s) remain(s) unclear. We have previously suggested that a promising strategy for the understanding of melanoma immune responsiveness could consist of the study of tumor/host interactions ex vivo through genetic profiling of serial fine needle aspirate biopsies that allow direct correlation between experimental results and clinical outcome. By prospectively studying the transcriptional profile of melanoma metastases during immunotherapy we observed that immune responsiveness is pre-determined by an immune reactive micro-environment. Interestingly, the addition of systemic interleukin-2 therapy to active specific immunization seems to increase the frequency of immune rejections of cancer. Functional profiling of the effect of interleukin-2 in tumors suggested that this cytokine induces or enhances the effector function of immunization-induced T cells by causing an acute inflammatory process at the tumor site that can in turn recruit and activate T cells. Thus, we hypothesize that effective immune responses occur when a pro-inflammatory inflammatory threshold is reached at tumor site capable of maintaining active immunization induced-T cells. To search for the reason for the erratic behavior of metastatic melanoma, we analyzed 62 melanoma metastases to identify functional signatures possibly responsible for immune responsiveness. Melanoma metastases were biopsied with a 23 gauge needle and anti-sense RNA was amplified to produce single stranded cDNA for hybridization to custom-made cDNA arrays. Genes specific for the tumor microenvironment were sorted (Wilcoxon test p-value < 0.001). Eisen's hierarchical clustering was applied to the resulting gene pool and two subsets of melanomas were identified. A smaller cluster including 15 samples (24%) was characterized by significantly higher expression of the inflammatory cytokines GRO-α, MIP-1α and β, MPBF, IL-1β and IL-8, RANTES, Lymphotactin and Lymphotocxin. This signature strongly correlated with up-regulation of IFN-responsive elements. The same cluster displayed a higher expression of MMP-9, 11 and 15 (cytokine-dependent metalloproteinases), genes encoding growth and angiogenic factors and cell cycle regulatory sequences. These findings suggested that some melanoma metastases display a very heterogeneous immune environment that could variably modulate T cell function at the receiving end of the immune response against cancer and could co-operate with the pro-inflammatory effects of the systemic administration of interleukin-2. Although these results are needed to be confirmed in larger patient populations this report suggests that strategies are presently available for the efficient screening of biological principles and related biomarkers using high-throughput technology. References: 1. Wang F, Marincola FM. A natural history of melanoma: serial gene expression analysis. Immunol Today 2000; 21:619-23. 2. Wang F, Miller LD, Ohnmacht GA, Mocellin S, Petersen D, Zhao Y, et al. Prospective molecular profiling of subcutaneous melanoma metastases suggests classifiers of immune responsiveness. Cancer Res 2002;62: 3581-6. 3. Panelli MC, Wang E, Phan G, Puhlman M, Miller L, Ohnmacht GA, et al. Genetic profiling of peripheral mononuclear cells and melanoma metastases in response to systemic interleukin-2 administration. Genome Biol 2002;3: RESEARCH0035. 4. Marincola FM, Wang F, Herlyn M, Seliger B, Ferrone S. Tumors as elusive targets of T cell-directed immunotherapy. Trends Immunol 2003;24:344-41. 5. Wang E, Miller L, Ohnmacht GA, Liu E, Marincola FM. High fidelity mRNA amplification for gene profiling using cDNA microarrays. Nature Biotech 2000;17:457-9.

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Cell Transplantation to Improve Heart Function: Cell or Matrix

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Current attempts to regenerate the damaged myocardium after myocardial infarction have primarily focused on therapies directed at increasing regional perfusion and reducing cell loss. Accumulating evidence suggests that implantation of healthy muscle cells into the damaged myocardium replaces the fibrotic tissue. In addition to muscle cells, stem cells in circulation, from bone marrow or in the myocardium, have recently been documented to have great potential to differentiate into myogenic cells. These neo-myogenic cells in the myocardial scar tissue prevented ventricular dilation and delayed cardiac dysfunction. Early clinical trials show encouraging data for cellular cardiomyoplasty.

Although the beneficial effects of cell therapy for myocardial regeneration after an infarction have lead to phase I clinical trials, the mechanism of the novel therapy is often questioned. Replacing the scar tissue with muscle cells and stimulating neo-vein formation in the implanted area have been proposed. However, a number of studies recently demonstrated that the survival rate of implanted cells was too low and that number of implanted cells decreased with time after transplantation. The number of surviving cells may not be enough to form adequate new muscle tissue to repair the damaged myocardium.

We recently found that extracellular matrix in the myocardium plays an important role in maintaining the ventricular chamber size, and disruption of the matrix network may contribute to the apoptosis of cardiomyocytes leading to dilated cardiomyopathy. We implanted smooth muscle cells into the heart with dilated cardiomyopathy prior to ventricular dilatation.