

## **Harvest, Proliferation, and Functional Testing of Human Dendritic Cells**

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### **Purpose.**

Difficulty in harvesting large numbers of cells, short survival time, and rapid phenotypic changes in culture have prevented the widespread use of human dendritic cells (HDC) for many fundamental studies applicable to the initial stages of pharmaceutical discovery and development. The ability to overcome these problems would enable a broad variety of extremely useful in vitro assays. The purpose of this study was to develop an improved means of generating HDC.

### **Methods.**

Dendritic precursor cells were harvested from umbilical cord blood samples and proliferated with a newly developed medium. Cells were characterized phenotypically by flow cytometry (FACS), ultrastructurally by transmission electron microscopy (TEM), and functionally by the mixed lymphocyte reaction and RT-PCR. In addition, the ability of HDC to induce T-cell (TC) proliferation following exposure to allergens and the infectability of HDC with pathogenic viruses was investigated.

### **Results.**

Use of the new culture medium allowed increases of 250 fold in HDC number. FACS analysis showed that the HDC expressed CD1a, HLA-DR, CD11c, CD40, CD80, CD83, and CD86 for up to 53 days in culture; Birbeck granules were also observed over this culture period by TEM. Upon stimulation, the HDC showed gene and protein responsiveness in terms of IL-12, MIP-1 $\alpha$ , MIP-3 $\alpha$ , IL-6, and TNF- $\alpha$  expression and HDC were able to stimulate allogeneic TC. Finally, HDC exposed to allergens enhanced both primary and secondary TC responses and the HDC were infectable with HIV.

### **Conclusion.**

Improved culture conditions have allowed the harvest and expansion of functional HDC. These cells will be useful in: 1) allergenicity, 2) viral infection, 3) antigen presentation, 4) immuno-therapeutic, and numerous other studies related to the development of prophylactic and therapeutic pharmaceuticals.