

**GENERATION OF LONG LIVED DENDRITIC CELLS FOR ALLERGENICITY STUDIES AND MUCOSAL TISSUE ENGINEERING.** Ayehunie, S., Lamore, S., Lappen, R., Bellevance, K., Cannon, C., Klausner, M., and Sheasgreen, J. MatTek Corporation, 200 Homer Avenue, Ashland, MA 01721.

**ABSTRACT**

Dendritic cells (DC) residing in the skin, mucosa, and lymphoid tissues play a key role in sensitization and viral infection. However, difficulty in harvesting, short survival time in culture, rapid phenotypic changes, and variability in cytokine release patterns by cultured DC have limited the use of human dendritic cells (DC) for fundamental cell based studies in allergenicity, immunotherapy, and microbial infections. Here we report an improved method of generating DC (an average of  $205 \pm 86.02$  fold increase;  $n = 15$ ) from CD34+ progenitor cells derived from umbilical cord blood (UCB). The generated DC express CD1a, HLA-DR, CD80, CD86, CD40, CD54, CD11c, and CD206 (mannose receptor). Unlike monocyte derived DC, the cells express Birbeck granules and have certain degree of expansion capacity. The lifespan of these cells has been increased to 41 days with detectable Birbeck granules and very little phenotypic changes and express detectable Birbeck granules. Upon stimulation with lipopolysaccharide (LPS) and phorbol-12-myristate-13-acetate (PMA), the DC show a reproducible ( $n = 14$ ), high level protein release for IL-6, IL-12, MIP-1 $\alpha$ , MIP-3 $\alpha$ , and RANTES. Functionally, these DC can: 1) initiate allogeneic T cell proliferation, 2) induce primary and secondary T-cell responses to a strong allergen (FITC) but not for an irritant (SDS). In addition, these DC can be incorporated into in vitro reconstructed three-dimensional, ectocervical-vaginal tissue model. The DC containing vaginal tissue model is infectable with HIV-1 subtypes B and C. In summary, the generated DC alone or the DC containing tissue models will likely be useful in a variety of studies related to: 1) allergenicity, 2) microbial infection and transmission, 3) neutralizing antibodies and anti-microbial agents, 4) antigen presentation, and 5) immuno-therapy, amongst others.

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