

Plasmacytoid Dendritic Cells



Features

- **Normal human, immature dendritic cells**
- **FACS sorted (CD123+ / CD11c-)**
- **Key markers present: CD1a^{low}, CD80^{low}, CD83^{low}, CD86^{low}, CD40^{high}, and HLA-DR^{high}**
- **Non-adherent suspension cells**
- **Excellent for studies related to: Viral infection, Innate and adaptive immunity, allergenicity**

Dendritic cells (DC) play a key role in the immunological reactions throughout the body. Dendritic cells (DC) and their immature counterparts, Langerhans cells (LC), are highly specialized antigen-presenting cells (APC) located in the skin, mucosa, and lymphoid tissues. DC and LC play a key role in the induction phase of contact allergenicity, and it is likely that these cells can be used to develop in vitro assays for contact sensitization and other immunological reactions of the body. At least two distinct subsets of DC have been identified based on phenotypic and functional differentiation. These are 1) plasmacytoid DC (pDC) expressing high level of interleukin-3 (IL-3) receptor (CD123) and lack CD11c antigen expression and 2) myeloid DC (mDC) which expresses myeloid markers such as CD11c antigen but not the IL-3 receptor. A novel method has been developed at MatTek Corporation for generating plasmacytoid dendritic cells. The method also increases the survival time of pDC in culture. In summary, MatTek's new pDC culture methodology enables us to produce pDC for basic and experimental research.

CHARACTERIZATION of cells:

Dendritic cells generated from umbilical cord blood were characterized for:

1. FACS sorting of DC (Figure 1A)
2. Morphology - using light microscopy (Figure 1B).
3. Phenotype - using FACS analysis of surface marker expression (Table 1).
4. Functional analysis following stimulation with exogenous factors (Table 2 and Figure 3).

1. Fractionation of DC-100 cells into pDC and mDC

Figure 1A: FACS sorting of DC/LC into pDC (CD123+CD11c-) and mDC (CD123-CD11c+).

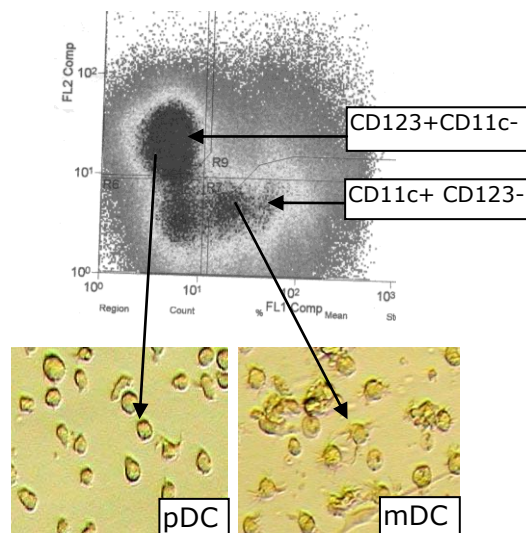


Figure 1B: Morphology of two subsets of DC following a five day culture period. pDC are less dendritic than mDC as observed using light microscopy (40X objective).

2. Phenotypic characterization of pDC

Table 1: Phenotypic analysis of pDC using FACS. Cells were cultured in pDC-MM prior to FACS analysis.

Antibody	% Expression
No Antibody	1
CD1a	12
HLA-DR	79
CD40	48
CD80	11
CD83	6
CD86	22

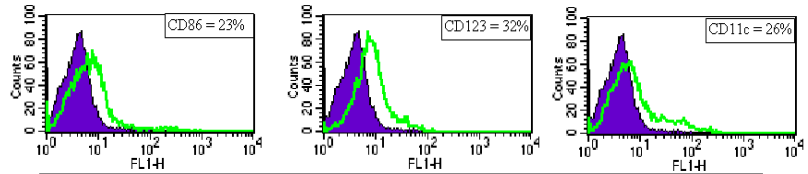


Figure 2: Surface marker expression by pDC. pDC were isolated by FACS sorting as CD123+CD11c-. Isolated pDC were cultured in DCP-MM medium (MatTek Corporation) for 3 days and analyzed for CD86, CD123, and CD11c

3. FUNCTIONAL ANALYSIS: RESPONSE TO IMMUNO-STIMULANTS

To examine the functionality of generated pDC, cells were stimulated with lipopolysaccharide (LPS) and Tumor necrosis factor-alpha (TNF- α) as follows.

Stimuli: LPS (5 μ g/ml) and TNF- α (30 ng/ml)	
Incubation time	18 hr
Plate	96-well plates
Cell number used	100,000
Medium	300 μ l

Table 2: Surface marker expression by LPS+ TNF- α pulsed pDC		
Surface Markers	Expression (%)	
	PBS	LPS + TNF- α
Control	0.9	1.3
HLA-DR	76.7	93.3
CD80	23.7	59.9
CD83	12.5	43.4
CD86	24.3	50.7

After 18 hr of exposure, cells were harvested, RNA was isolated, and cytokine gene expression levels were monitored using RT-PCR (Figure x). RT-PCR shows that expression levels of IL-12p40, IL-6, and IL-1beta were increased following exposure to LPS and TNF- α .

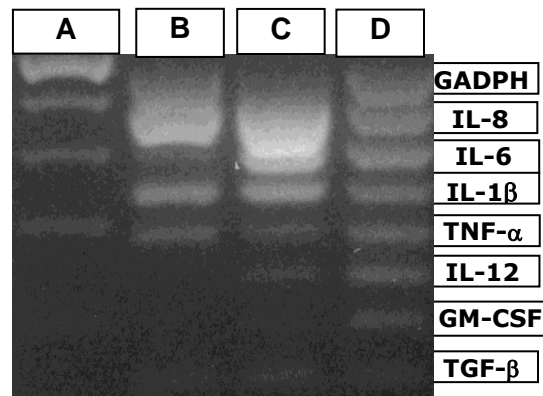


Figure 3: Gene expression by pDC using multiplex primers. The different lanes represent: A) DNA marker, B) unstimulated pDC, C) LPS and TNF- α stimulated pDC, and D) positive control for PCR reaction. To activate pDC LPS (5 μ g/ml) and TNF- α (30 ng/ml) were added to culture medium and cells were incubated at 37°C for overnight.