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Plasmacytoid dendritic cells (pDC) and myeloid DC (mDC) are the two major human dendritic cell populations. Each subset comprises only a small fraction of peripheral blood mononuclear cells (PBMC) and current protocols for their isolation are time consuming, involve multiple steps and often require special equipment. We have developed a rapid and efficient method for the isolation of pDCs from normal blood that yields excellent cell purities and recoveries. Briefly, PBMCs are isolated by Ficoll-Paque PLUS density gradient sedimentation and pDCs are isolated using immuno-magnetic, column-free, negative selection (EasySep®). Our EasySep® technology involves specifically labeling unwanted cells with a cocktail of bi-specific tetrameric antibody complexes and dextran-coated magnetic particles. Using a hand-held magnet, unwanted cells can then be easily removed from the unlabeled pDCs. Flow cytometric assessment of EasySep® isolated pDCs (Lin$^-$, HLA-DR$^+$, BDCA-4$^+$) demonstrate purities of 93.8% ± 3.8% with cell recoveries of 65.6% ± 16.2% (n=9). Using our RoboSep® cell separator, the entire separation procedure can be fully automated with equivalent cell purities and recoveries.

A simple one-step method for isolating highly purified plasmacytoid dendritic cells from human peripheral blood

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Summary

We report here the successful development of rapid and simple method for the enrichment by negative selection of human plasmacytoid dendritic cells (pDC). Starting with peripheral blood mononuclear cells (PBMC), pDC purities of 93.8% ± 3.8% and cell recoveries of 65.6% ± 16.2% (n=9) were obtained. Functional assessment of EasySep® and RoboSep® isolated pDC demonstrated robust IFNα responses following treatment with TLR7 agonists and live viruses.

Methods

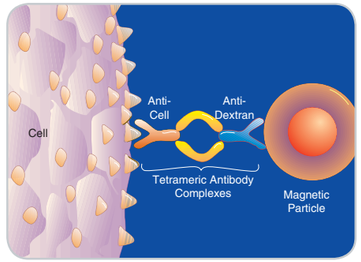

Figure 1: EasySep® labeling of human PBMCs
Unwanted cells are specifically labeled with dextran-coated magnetic particles using bi-specific tetrameric antibody complexes (TAC). These TAC recognize both dextran and the cell surface antigen expressed on the unwanted cells. The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep® procedure.

Figure 2: EasySep® procedure for column-free enrichment of plasmacytoid dendritic cells

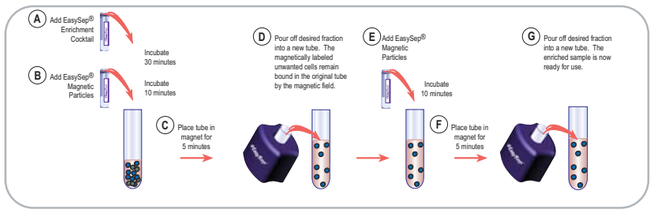

Figure 2: The EasySep® human pDC enrichment kit is designed to isolate untouched pDCs from fresh or previously frozen PBMC samples by depletion of all other non-pDCs (STEMCELL Technologies, Inc. Cat #19062). A) PBMCs are incubated for 30 minutes with the EasySep® pDC enrichment cocktail; B) followed by a 10 minute incubation with EasySep® magnetic particles. C) The tube containing the labeled PBMCs is then placed in an EasySep® magnet for 5 minutes. D) EasySep® enriched pDCs are poured off into a new tube while the magnetically labeled non-pDCs cells are held in the original tube by the EasySep® magnet. E-G) An additional round of magnetic cell separation enables the isolation of highly purified pDCs that are ready to use in functional assays. The entire procedure requires only 60 minutes of incubation time and can be fully automated with the RoboSep® cell separator.

Results

Table 1: Purity and recovery of Lin$^-$HLA-DR$^+$CD304$^+$ pDCs enriched by negative selection from PBMC using EasySep® or RoboSep®

	n	Purity	Recovery
EasySep®	9	93.8 ± 3.8	65.6 ± 16.2
RoboSep®	5	92.8 ± 2.6	76.8 ± 20.6

Values expressed as means ± SD
Purity and recovery determined by flow cytometry. All samples gated on viable (PI negative) cells.

Figure 3: Typical EasySep® and RoboSep® enrichment profiles

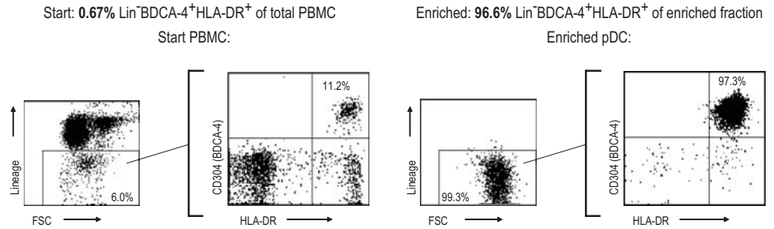

Figure 3: Starting with 0.2 - 0.9% pDC in PBMC, the pDC content of the enriched fraction typically ranges from 87 - 97% purity based on the pDC phenotype of lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD304 (BDCA-4) positive.

Figure 4: Virtually all EasySep® enriched pDCs express IFNα following *in vitro* TLR7 stimulation.

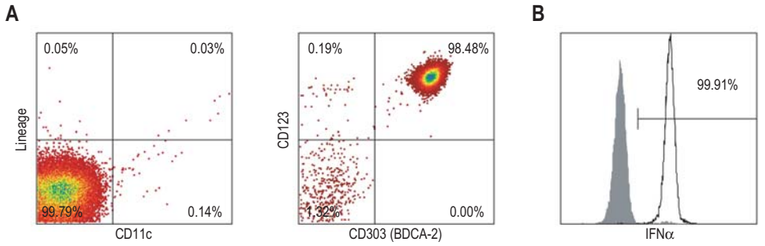

Figure 4: Untouched human pDCs express IFNα following *in vitro* activation. A) EasySep® enriched human pDCs were analyzed for the expression of lineage markers, CD11c, CD123 and CD303 (BDCA-2). B) EasySep® enriched pDCs were cultured for 20 hours in serum-free media supplemented with 5µg/mL Imiquimod-R837, a small synthetic TLR7 ligand. Monensin was added during the final 2 hours of culture followed by flow cytometric assessment of intracellular IFNα. Open and shaded histograms represent staining with anti-human IFNα (clone: MMHA-11) or an isotype control, respectively.

Figure 5: RoboSep isolated pDCs secrete IFNα following stimulation with live viruses.

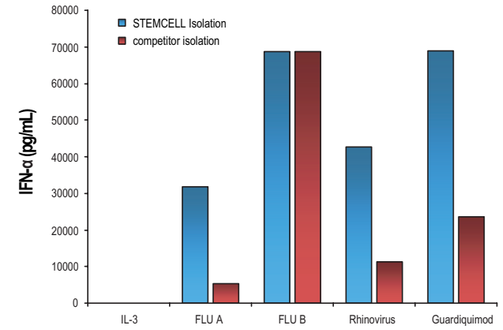

Figure 5: Compared to competitor isolated pDCs, RoboSep® isolated pDCs secrete increased levels of IFNα following treatment with type A and B Influenza, Rhinoviruses or a TLR7 agonist, Gardiquimod™. Data kindly provided by Dr. Michelle Gill from The Department of Pediatrics, University of Texas Southwestern Medical Center.

Conclusions

- Untouched human pDCs can be rapidly isolated from PBMC samples using column-free negative selection.
- Excellent purity and recovery can be achieved with the EasySep® and RoboSep® platforms.
- Virtually all EasySep® enriched pDCs express IFNα following *in vitro* TLR7 stimulation with Imiquimod-R837 in serum free media.
- RoboSep® isolated pDCs secrete increased levels of IFNα following *in vitro* treatment with live viruses or a TLR7 agonist compared to competitor isolated pDCs.

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