



Excerpt from

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Cell separation: human

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Monocytes

Monocytes and *ex vivo*-generated dendritic cells

Monocytes, together with dendritic cells (DCs) and B cells, belong to the classical antigen-presenting cells. Antigen-uptake takes place via phagocytosis or receptor-mediated endocytosis. Therefore, they express specific pattern-recognition receptors, e.g. toll-like receptors, which recognize highly conserved structures such as that of bacterial lipopolysaccharides (LPS) or viral dsRNA. After stimulation by antigen-uptake, monocytes produce a variety of inflammatory cytokines, like TNF- α , IL-1, and IL-12, which simulate cells of the innate and adaptive immunity. Monocytes differentiate to macrophages when they leave the blood and enter the tissue. This differentiation can also be performed *ex vivo* by culturing purified monocytes in medium supplemented with M-CSF. However, most often monocytes are isolated for *ex vivo* generation of cells with myeloid dendritic cell-like phenotype, so called monocyte-derived dendritic cells (Mo-DCs).

Ex vivo generation of monocyte-derived dendritic cells

Large numbers of Mo-DCs can be generated *ex vivo* from purified CD14⁺ monocytes. Highly pure monocytes are easily isolated from bone marrow, cord blood, peripheral blood, or peripheral blood mononuclear cells (PBMCs) by using **CD14 MicroBeads** or the **Monocyte Isolation Kit II**, respectively. Excellent purity and recovery of selected monocytes provide a pure and consistent cell source. Contamination with unwanted cells (e.g. platelets) that may interfere with a controlled differentiation process is markedly reduced. The combined action of, for example, GM-CSF and IL-4 can direct monocytes to differentiate into a homogenous population of immature Mo-DCs. To produce stable mature Mo-DCs, the

immature Mo-DCs can be cultured, for example, with LPS or TNF- α alone or with a cocktail of inflammatory stimuli: IL-6, IL-1 β , TNF- α , and PGE₂.

Ex vivo generation of hematopoietic progenitor cell-derived dendritic cells (HPC-DCs)

Cells with a DC-like phenotype can also be generated *ex vivo* from CD133⁺ or CD34⁺ hematopoietic progenitor cells. Highly pure hematopoietic progenitor cells are easily isolated from bone marrow, cord blood, peripheral blood, or PBMCs by using the **CD34 MicroBead Kit** or the **CD133 MicroBead Kit**. If purified hematopoietic progenitor cells are induced to differentiate into HPC-DCs, e.g. by the combined action of GM-CSF and TNF- α , only a subset of the final cell culture shows the DC phenotype. These HPC-DCs can further be enriched to highest purity by a final separation using **CD1a MicroBeads**.



Figure 1: CD14⁺ monocytes isolated by using CD14 MicroBeads, May-Grünwald/Giemsa stained.

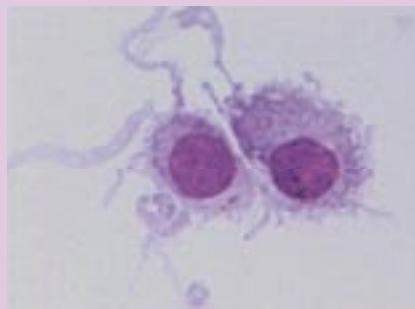


Figure 2: Immature Mo-DCs after culture of monocytes in IL-4 and GM-CSF, May-Grünwald/Giemsa stained. (Courtesy of Pickl, Vienna, Austria.)

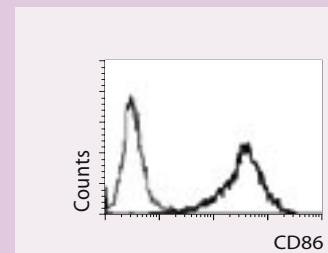
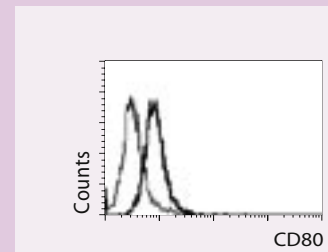
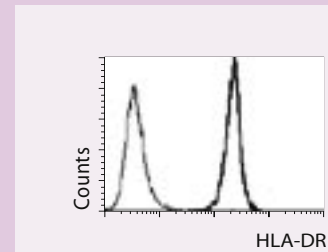
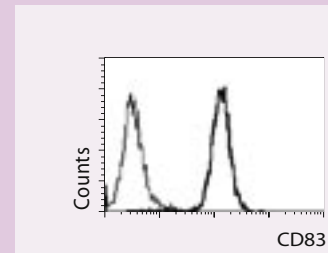
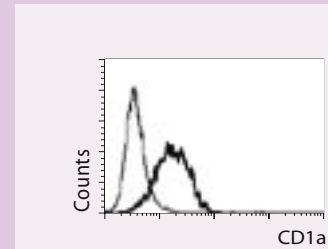


Figure 3: Monocytes were isolated from PBMCs using CD14 MicroBeads and subsequently cultured for seven days with IL-4 and GM-CSF to generate immature Mo-DCs and were, finally, cultured for additional three days with TNF- α for maturation. Mature Mo-DCs were stained for expression of CD1a, co-stimulatory molecules (CD80, CD86), CD83, and HLA-DR.