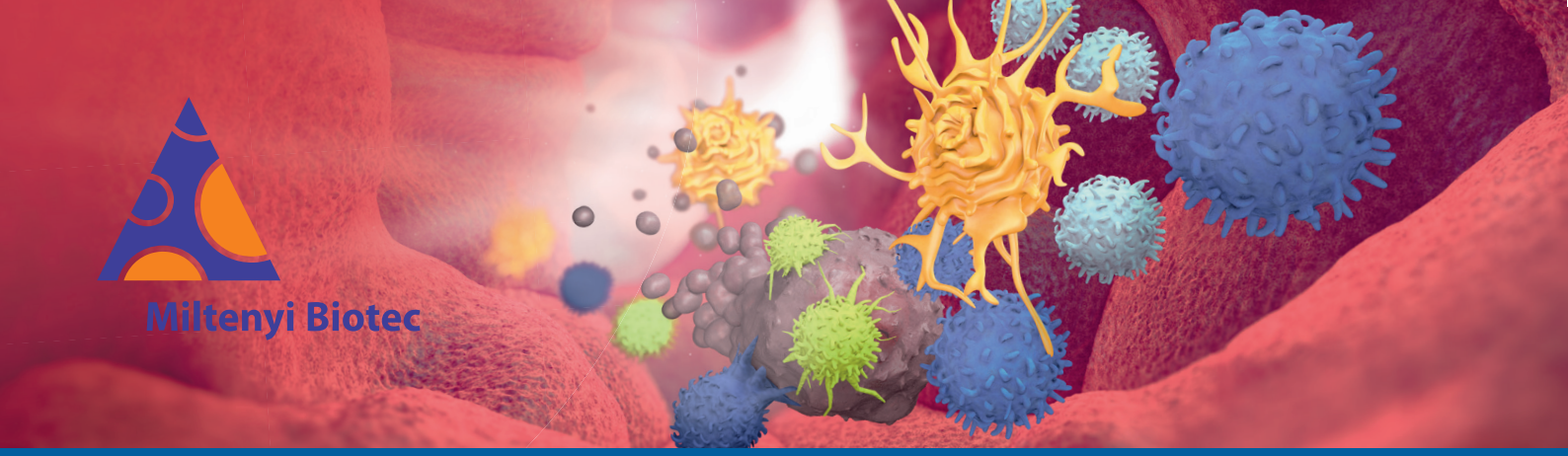


Miltenyi Biotec

MACS® Technology facilitates mixed lymphocyte reaction

Background

The mixed lymphocyte reaction (MLR) has become a valuable tool in basic and translational immuno-oncology reasearch for e.g. assessing the effect of therapeutic agents on the antigen presenting cell (APC)-mediated T cell activation or for the development of monocyte-derived dendritic cell (Mo-DC) vaccines. Several independent processing steps including density gradient centrifugation, cell separation, cell cultivation, and cell analysis require especially in translational settings fast and reliable protocols as well as highest quality and sensitivity. Here we show that cells isolated and cultured either manually, fully automated on the autoMACS® Pro Separator, or in a closed, clinical-grade system operated by an automated cell processing instrument, the CliniMACS Prodigy®, meet these requirements and can be used for demanding downstream applications like the MLR assay. This report shows the activation of $CD4^+$ T cell proliferation by cocultured Mo-DCs as well as the phenotypical and functional characterization of differentiated and matured Mo-DCs using MACS® Technology.

Materials and methods

$CD14^+$ monocytes and naive $CD4^+$ T cells were isolated from human peripheral blood mononuclear cells (PBMCs) using $CD14^+$ MicroBeads, human, and Naive $CD4^+$ T Cell Isolation Kit II, human, respectively. Monocytes were differentiated into immature Mo-DCs (imMo-DCs) during a 6-day culture in the presence of GM-CSF and IL-4. Maturation of imMo-DCs into mature Mo-DCs (mMo-DCs) was performed over the course of 24 hours in the presence of IL-1β, IL-6, TNF-α, and PGE_2. Allogenic cocultures of monocytes or differentiated Mo-DCs with freshly isolated naive $CD4^+$ T cells were performed for 7 days.

Results

In vitro generated Mo-DCs assumed the typical DC phenotype (fig. 1). During differentiation, monocytes down-regulated the expression of CD14. In contrast, mMo-DCs expressed various DC markers that are involved in the formation of immunological synapses between DCs and naive T cells, including CD80, CD86, and MHC II (HLA-DR). Mature Mo-DCs also expressed DC activation markers CD83, CD40, and CCR7.

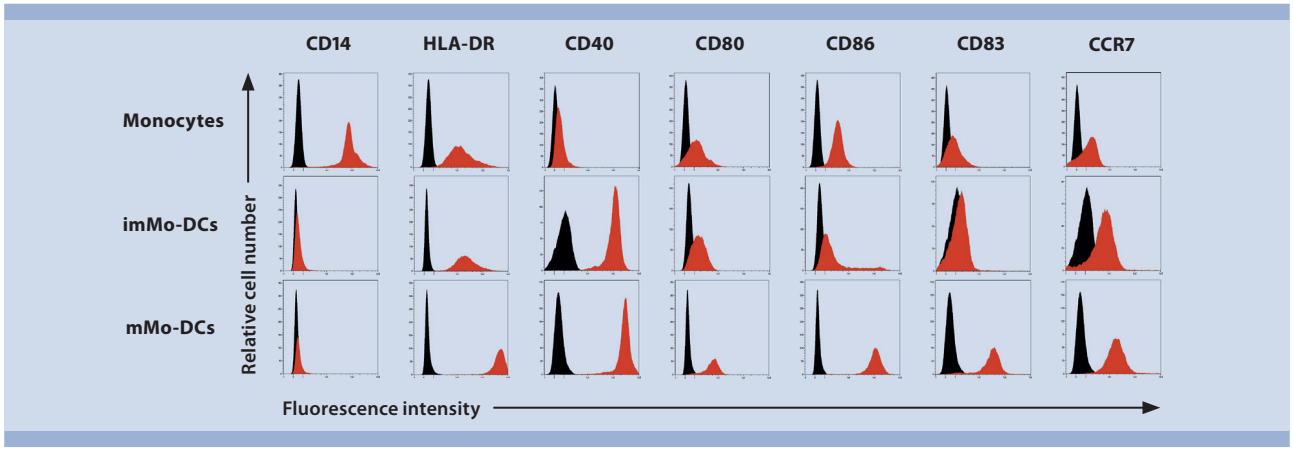


Figure 1: Immunophenotyping of monocytes, imMo-DCs, and mMo-DCs. Cells were labeled with MACS® antibodies specific for the respective markers and analyzed with the MACSQuant® Analyzer. Black: isotype controls, red: marker-specific antibodies.

Allogeneic naive CD45RA$^+$CD45RO$^-$ T cells, labeled with cell-tracking dye, were cocultured with monocytes, imMo-DCs, or mMo-DCs (fig.2 A, B). After 7 days, cell numbers and the CellTrace™ Violet staining intensity of the T cells were analyzed (fig. 2C). In contrast to monocytes, both, imMo-DCs and mMo-DCs induced T cell proliferation as indicated by the increase in cell count and by the reduction of CellTrace™ Violet staining. T cell proliferation was highest when mMo-DCs were used as antigen-presenting cells (fig. 2D).

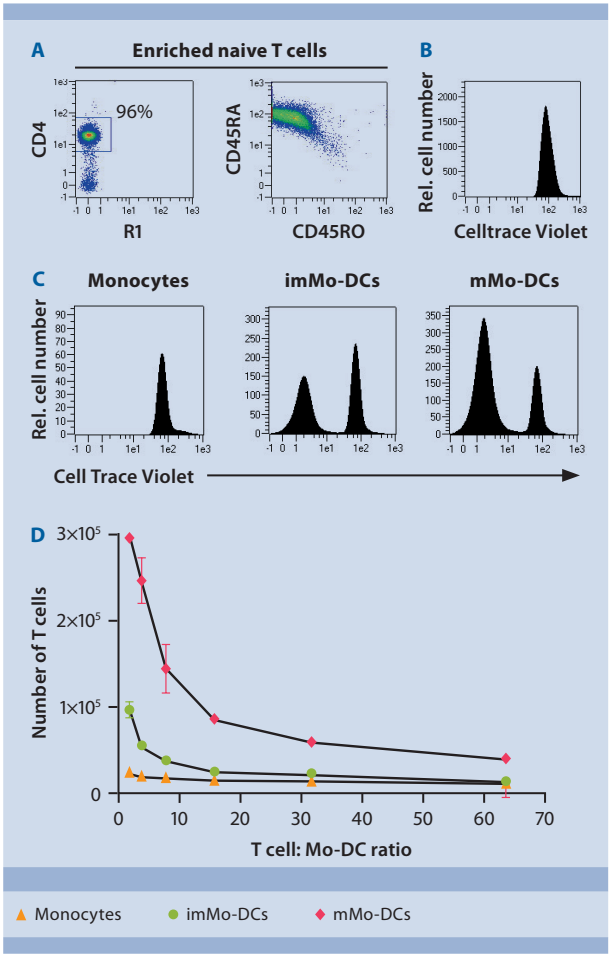


Figure 2: T cell priming capacity of monocytes and Mo-DCs in a MLR. (A) Flow cytometry analysis of enriched naive T cells. (B) CellTrace Violet™ staining of T cells. (C) CellTrace Violet™ staining of T cells cocultured with monocytes, imMo-DCs, or mMo-DCs. (D) T cell proliferation in indicated cocultures and cell ratios.

Conclusion

CD4$^+$ T cells and CD14$^+$ monocytes isolated with MACS® Isolation Kits and MicroBeads on e.g. the autoMACS® Pro Separator and cultured with MACS Cytokines can be successfully used for MLR assays.

For more data, download our poster

Monocyte-derived dendritic cells (Mo-DCs) generated with CliniMACS Prodigy® are functional and fulfill requirements for cancer vaccines at **www.miltenyibiotec.com/Mo-DCs**

Product	Order no.
Cell isolation	
autoMACS® Pro Separator	130-092-545
Naive CD4$^+$ T Cell Isolation Kit II, human	130-094-131
CD14 MicroBeads, human	130-050-201
MACS Cytokines for Mo-DC differentiation	
Human GM-CSF, premium grade (50 µg)	130-093-865
Human IL-4, premium grade (25 µg)	130-093-921
MACS Cytokines for Mo-DC maturation	
Human IL-1β, premium grade (25 µg)	130-093-563
Human IL-6, premium grade (25 µg)	130-093-931
Human TNF-α, premium grade (50 µg)	130-094-023
Human PGE_2	not available
Flow cytometry	
MACSQuant® Analyzer 10	130-096-343
MACS Antibodies	Various order nos. please refer to **www.miltenyibiotec.com/antibodies**

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