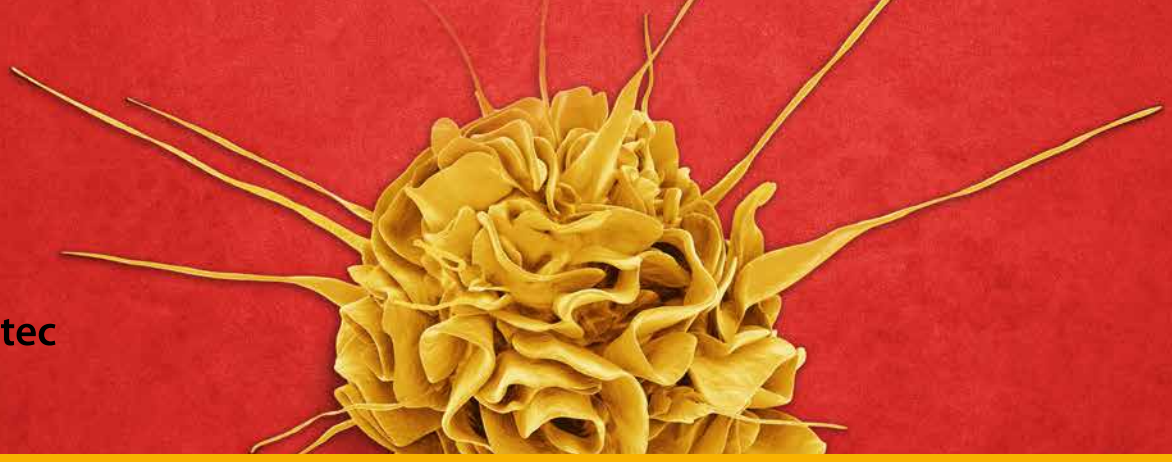

Miltenyi Biotec

From monocyte isolation to DC characterization

Generation of Mo-DCs

Introduction

As professional antigen-presenting cells, dendritic cells (DCs) are a cornerstone of the adaptive immune system. Various clinical trials have shown the great immunotherapeutic potential of DC-based vaccines in the treatment of tumors.[1]

Immature DCs residing in peripheral tissues, including blood, have the capacity to take up tumor-associated antigens, for example. Following maturation, DCs up-regulate their antigen processing machinery, express a variety of cell surface markers that are involved in the formation of immunological synapse, and migrate from the periphery to draining lymph nodes where they present the antigen to T cells[2]. Both antigen presentation and triggering of CD28 on T cells by the DC costimulatory markers, CD80 and CD86[3], are required to induce an antigen-specific response. Upon stimulation T cells up-regulate the expression of CD40 ligand (CD40L) and proliferate to counteract the disease that is associated with the particular pathogenic antigen. Binding of CD40L on activated T helper (TH) cells to CD40 on mature DCs provides a feedback signal that leads to the secretion of IL-12 by DCs[4], which in turn drives T cell polarization towards the TH1 lineage and the antitumor response mediated by cytotoxic T cells.

Large numbers of DCs for basic research and immunotherapies can be generated *in vitro* from monocytes[5,6]. Differentiation/maturation protocols have been described by various groups[7–10]. To obtain functional monocyte-derived DCs (Mo-DCs) that possess the distinct phenotype and function of natural DCs, it is crucial to follow reliable procedures using high-quality reagents. This application note describes all the steps for the generation of Mo-DCs and their phenotypic and functional characterization.

Material and methods

Isolation of monocytes

$CD14^+$ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors by MACS® Technology using CD14 MicroBeads (Miltenyi Biotec). Purity and recovery were determined by labeling cells with CD14-FITC antibodies and subsequent flow cytometry analysis on a MACSQuant® Analyzer 10 (Miltenyi Biotec). Cell viability was determined according to propidium iodide (PI) staining.

Differentiation and maturation of monocyte-derived dendritic cells (Mo-DCs)

The generation of Mo-DCs can be achieved by using various protocol formats, manually and automatically, at various scales. Here we describe the procedure for 6-well plates as an example. On day 0, isolated monocytes (3×10^6 cells) are cultured in 3 mL of complete medium (RPMI 1640, 2 mM L-glutamine, 1% autologous plasma), supplemented[7,8] with 250 IU/mL IL-4 and 800 IU/mL GM-CSF, and incubated at 37 °C and 5% CO_2. On day 2, a volume of 1.5 mL is removed from the culture and centrifuged. Cells are resuspended in 1.5 mL of complete medium supplemented with the 2-fold concentration of IL-4 and GM-CSF and put back into the original culture. On day 6, a volume of 1.5 mL is removed from the culture and centrifuged. Cells are resuspended in 1.5 mL medium supplemented[9,10] with 2000 IU/mL IL-6, 400 IU/mL IL-1β, 2000 IU/mL TNF-α, and 2 µg/mL PGE_2, put back into the original culture, and cultured for another 24 hours. On day 7, viability, yield, and absolute cell count of mature Mo-DCs (mMo-DCs) is determined by flow cytometry via light scatter signals and PI fluorescence. For the gating strategy see figure 1.

For all experiments shown in this application note, Mo-DCs were generated using cytokines from Miltenyi Biotec.

Analysis of cell morphology

Monocytes as well as immature Mo-DCs (imMo-DCs) and mMo-DCs were placed in a 6-well plate and allowed to sediment. Images of the cells were captured using a light microscope with phase-contrast at a 400× magnification.

Immunophenotyping of monocytes and Mo-DCs by flow cytometry

To analyze cell surface expression of various maturation markers, co-stimulatory molecules, and receptors for chemokines and antigens, the monocytes, imMo-DCs, and mMo-DCs were labeled with specific monoclonal

antibodies (table 1). Non-specific antibody binding was assessed using appropriate isotype controls. The mean fluorescence intensity (MFI) of the respective markers was determined by flow cytometry on a MACSQuant Analyzer 10 using the MACSQuantify™ Software (Miltenyi Biotec). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. All antibodies and Propidium Iodide Solution were from Miltenyi Biotec.

Cell surface antigen	Clone	Fluorochrome
CD1a	HI149	PE
CD14	TÜK4	FITC
CD25	3G10	APC
CD40	HB14	APC
CD54	REA266	PE
CD80	REA661	PE
CD83	REA304	APC
CD86	FM95	PE
CD206	DCN228	APC
CD197 (CCR7)	REA108	APC
CD209 (DC-SIGN)	REA617	APC
HLA-DR	AC122	FITC
HLA-ABC	REA230	FITC

Table 1: Antibodies used for monocyte and Mo-DC immunophenotyping. All antibodies were from Miltenyi Biotec.

Antigen uptake capacity

To assess the pinocytosis capacity, 1×10^5 monocytes, imMo-DCs, or mMo-DCs were incubated with FITC-labeled dextran (1 mg/mL) in complete medium (RPMI, 2 mM L-glutamine, 1% autologous plasma) for 5, 10, 20, 30, and 60 minutes at 37 °C. To check for non-specific binding of FITC-dextran to the cell surface, a control sample was kept on ice for 60 min. After 60 min, all samples were washed twice (centrifugation at 300×g, 5 min, 4 °C) with ice-cold PBS supplemented with 1% fetal calf serum (FCS) and finally suspended in PBS supplemented with 0.5% BSA. Samples were kept on ice until flow cytometry analysis. Uptake of the FITC-dextran was determined by measuring the mean fluorescence intensity (MFI) of FITC by flow cytometry. Dead cells were excluded from the analysis by PI fluorescence. Specific uptake of FITC-dextran was calculated by subtracting the MFI of the control sample that was incubated on ice from the MFI of the samples incubated at 37 °C.

Migratory capacity of mMo-DCs

CCR7-dependent migration of mMo-DCs towards CCL19 was tested in 24-well Transwell® Plates (Corning; pore size 5 µm). The mMo-DCs were resuspended in RPMI 1640 with 10% FCS at a density of 5×10^5 cells/mL, and 200 µL were placed in the upper compartment of a Transwell Plate. The lower compartment was filled with 600 µL of complete medium supplemented with Human CCL19 (MIP-3β) (Miltenyi Biotec) at different concentrations. After 3 h the cells contained in the lower compartment were harvested and counted on a MACSQuant® Analyzer 10.

Isolation of human naive CD4⁺ T cells for mixed lymphocyte reaction (MLR)

Naive allogeneic CD4⁺ T cells were isolated from PBMCs using the Naive CD4⁺ T Cell Isolation Kit II, human (Miltenyi Biotec) according to the instructions provided in the data sheet. Purity of the isolated cells was determined by labeling with i) CD4-PE or ii) CD45RO-APC and CD45RA-PE antibodies (all from Miltenyi Biotec) and subsequent analysis by flow cytometry using the MACSQuant Analyzer 10.

Assessment of the T cell priming capacity of Mo-DCs in MLR

The capacity of Mo-DCs to induce proliferation of naive T cells was measured in MLR. To this end, 5×10^6 purified naive CD4⁺ T cells were suspended in 400 µL PBS, and labeled with 100 µL of a 10 µM CellTrace™ Violet solution (Life Technologies®) for 5 min at RT. Subsequently, the cells were washed once with 1 mL FCS and three times with 2 mL MLR medium (RPMI 1640, 2 mM L-glutamine, non-essential amino acids, 0.1 mM sodium pyruvate, 5% human AB serum). Finally, the CD4⁺ T cells were suspended in MLR medium at a density of 5×10^5 cells/mL.
Monocytes, imMo-DCs, and mMo-DCs were suspended in MLR medium at a density of 1×10^6 cells/mL and serially diluted according to table 2. The different cell dilutions (100 µL per well) were placed in a 96-well plate. Subsequently, the labeled T cells (100 µL per well) were added and cultured for 7 days at 37 °C, 5% CO_2.
Proliferation of CD4⁺ T cells was determined by measuring the fluorescence of CellTrace Violet by flow cytometry. Cell debris and dead cells were excluded from the analysis by scatter signals and PI fluorescence.

Mo-DC/monocyte density (cells/mL)	Number of Mo-DCs/ monocytes per well	Ratio of Mo-DCs/ monocytes to T cells
2.5×10^5	25,000	1:2
1.25×10^5	12,500	1:4
6.25×10^4	6,250	1:8
3.13×10^4	3,125	1:16
1.56×10^4	1,562	1:32
7.81×10^3	781	1:64

Table 2: Serial dilution of Mo-DCs/monocytes for MLR assay. The number of naive T cells was constant at 50,000.

Quantitation of IL-12 and IL-10 secreted by mMo-DCs after stimulation with CD40L

The mMo-DCs were suspended in medium (RPMI, 2 mM L-glutamine, 1% autologous plasma) at a density of 1×10^6 cells/mL, and 5×10^5 cells were used for the assay performed in 96-well plates.
To measure the secretion of IL-12p70 and IL-10, mMo-DCs were stimulated with soluble Human CD40-Ligand (16 µg/mL; Miltenyi Biotec) or in a co-culture with J558L cells expressing CD40L (7×10^4, 14×10^4, or 28×10^4 cells/well) for comparison. Mo-DCs were stimulated for 24 h at 37 °C, 5% CO_2. Subsequently, the cell supernatants were collected and centrifuged (300×g; 10 min) to remove any cells. The concentrations of IL-12p70 and IL-10 were determined by

specific ELISAs (eBioscience). The CD40L-expressing J558L cell line was kindly provided by Professor Marina Cella (Washington University, St. Louis, MO, USA)

Results

Isolation of monocytes to high purities

CD14⁺ monocytes were isolated from PBMCs by MACS Technology to a purity of 96.8±3.8%. The proportion of CD14⁺ cells that were recovered from the PBMC fraction amounted to 81.3±8.8%%, and the viability was 97.5±1.4% (fig. 1).

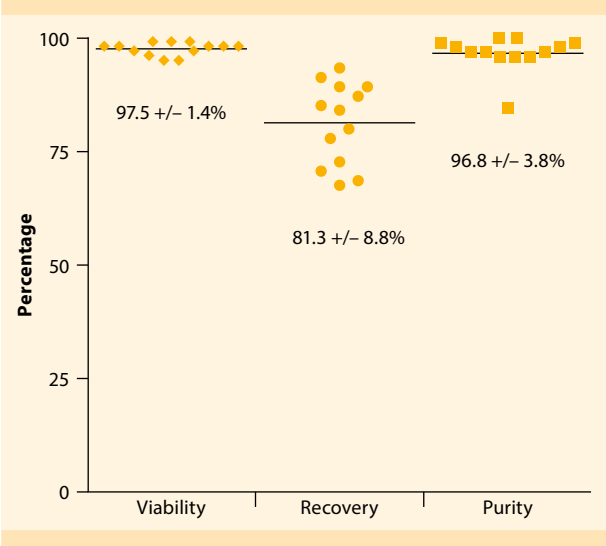

Figure 1: Isolation of CD14⁺ monocytes. Purity, recovery (i.e., percentage of CD14⁺ cells in the purified fraction in relation to the original fraction), and viability of enriched CD14⁺ cells are shown. Cells were labeled with a CD14-FITC antibody and analyzed by flow cytometry.

Differentiation of monocytes into mature Mo-DCs

Purified monocytes were used to generate Mo-DCs according to the protocol for 6-well plates. On day 7, cells were analyzed by flow cytometry using the gating strategy depicted in figure 2. The viability of mMo-DCs, evaluated by excluding PI⁺ dead cells, amounted to 95.4±2.4%. The recovery of mMo-DCs was 23%, calculated based on the number of originally seeded monocytes (fig. 2).

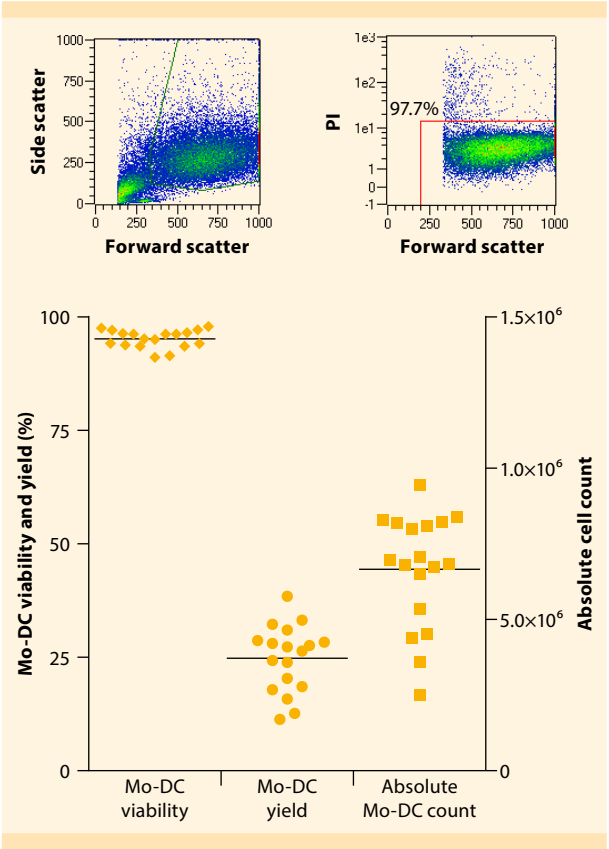

Figure 2: Generation of Mo-DCs. Viability, yield (i.e., mMo-DC count in relation to initial monocyte count), and total count of mMo-DCs generated by using the protocol for 6-well plates (n= 18). Data were obtained by flow cytometry on the MACSQuant Analyzer 10 following the gating strategy shown: First cells were gated according to their scatter properties. Subsequently, dead cells were excluded from the analysis by PI fluorescence.

Mo-DCs assume the characteristic DC morphology during differentiation

Dendritic cells have a distinct morphology characterized by many cellular processes. To evaluate whether monocytes assume this morphology during differentiation and maturation to Mo-DCs, we analyzed the cells on days 0, 6, and 7 of cell culture, both by microscopy and flow cytometry. On day 0, the cells had a spherical shape, which is normal for monocytes. After 6 days the cells showed cytoplasmic protrusions, which were even more pronounced after maturation on day 7 (fig. 3A). Moreover, size and granularity of the cells increased during differentiation and maturation, which is reflected in increased forward and side scatter signals in the flow

cytometry analysis (fig. 3B).

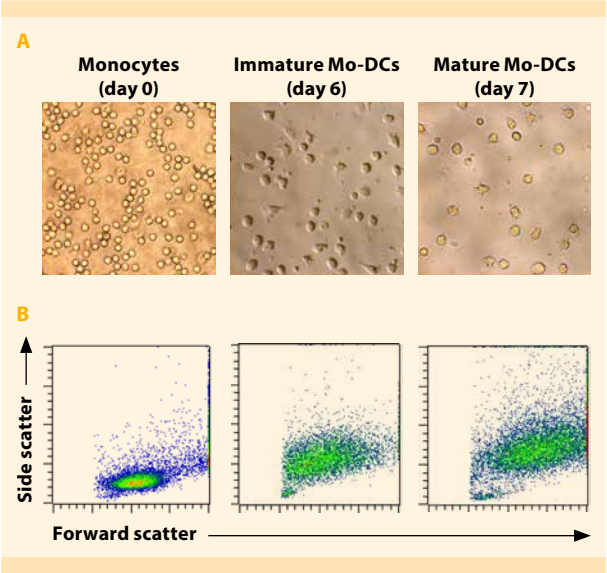

Figure 3: Morphology of monocytes, imMo-DCs, and mMo-DCs. Cells were analyzed on days 0, 6, and 7 of differentiation/maturation. (A) Images were captured on a light microscope using phase contrast at a 400× magnification. (B) Assessment of relative cell size and granularity by flow cytometry using forward and side scatter channels, respectively. 10,000 events are shown for each dot plot.

Mature Mo-DCs express characteristic DC surface markers

Flow cytometry analysis of cell surface markers showed that *in vitro* generated Mo-DCs assumed the typical DC phenotype (fig. 4). During differentiation monocytes down-regulated the expression of CD14. In addition, mMo-DCs expressed various DC markers that are involved in the formation of immunological synapse between DC and naive T cells, including the costimulatory proteins CD80 and CD86, the cell adhesion molecule CD54, and antigen-presenting molecules MHC I (HLA-ABC) and MHC II (HLA-DR). Mature Mo-DCs also expressed the DC activation markers, CD83, CD25, and CD40, which were up-regulated accordingly. CCR7, which is required for migration of DCs to draining lymph nodes, was also up-regulated. The highest expression levels of the antigen uptake receptors, CD209 and CD206, were detected on imMo-DCs (fig. 5), corresponding to their antigen uptake function.

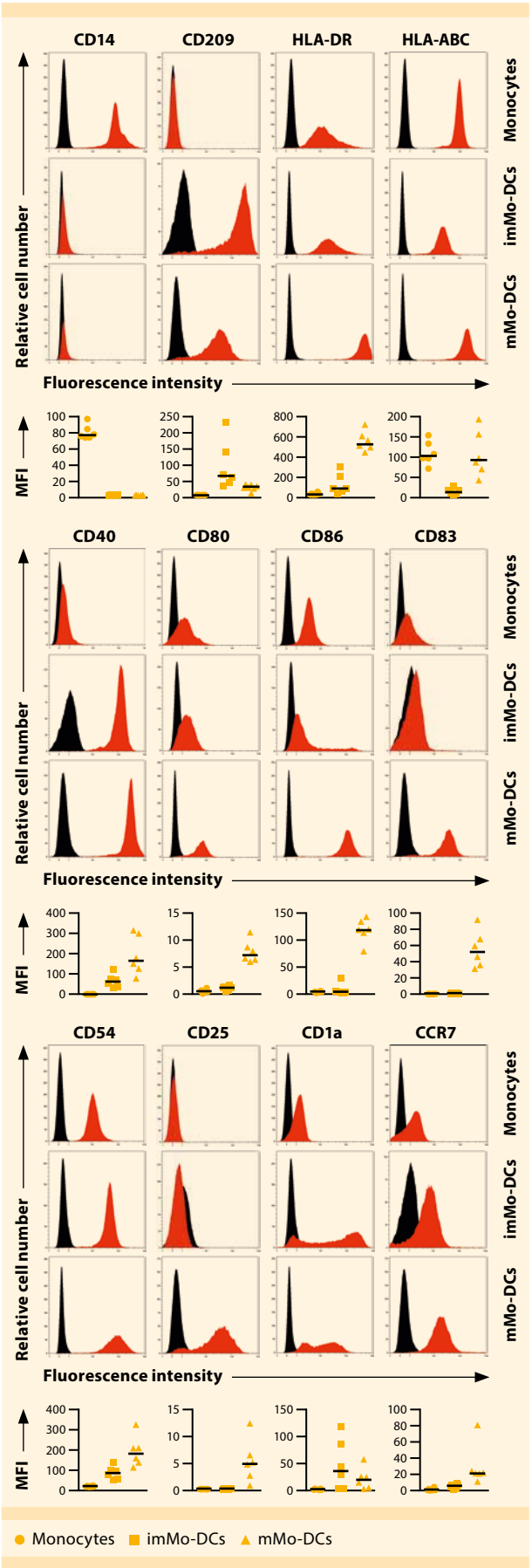

Figure 4: Immunophenotyping of monocytes, imMo-DCs, and mMo-DCs. Cells were labeled on days 0, 6, and 7 with antibodies specific for the respective markers and analyzed by flow cytometry. The histograms show the results from one representative experiment. Marker-specific and isotype control labeling are shown in red and black, respectively. The plots below the histograms depict the MFI for each marker on monocytes, imMO-DCs, and mMo-DCs in six independent experiments.

Immature Mo-DCs have the highest antigen uptake capacity

Antigen uptake by antigen-presenting cells can occur specifically via receptor-mediated endocytosis or non-specifically via pinocytosis or phagocytosis. To assess the pinocytosis capacity of *in vitro* generated Mo-DCs in comparison to monocytes, cells were cultured at 37 °C for up to 1 h in the presence of FITC-dextran. Non-specific binding of FITC-dextran to the cell surface was determined by incubating samples on ice throughout the procedure. The MFI of FITC was analyzed by flow cytometry after various time points (fig. 5A). Subtraction of MFI_{Ice} (samples on ice) from MFI_{37} (samples at 37 °C) allowed for assessment of the relative antigen uptake capacities (fig. 5B). Immature Mo-DCs had the highest antigen uptake capacity, reflected in a continuous increase in MFI over the entire time course. Mature Mo-DCs from day 7 showed decreased antigen uptake activity compared to imMo-DCs throughout the time course. The antigen uptake was further decreased when Mo-DCs were matured for additional 2 days (day 9). Monocytes showed a negligible antigen uptake capacity compared to Mo-DCs.

Additionally, flow cytometry analysis showed that imMo-DCs exhibited the highest expression of CD206 and CD209 (fig. 5C). These results correlate with the finding that imMo-DCs lose their antigen uptake function upon maturation.

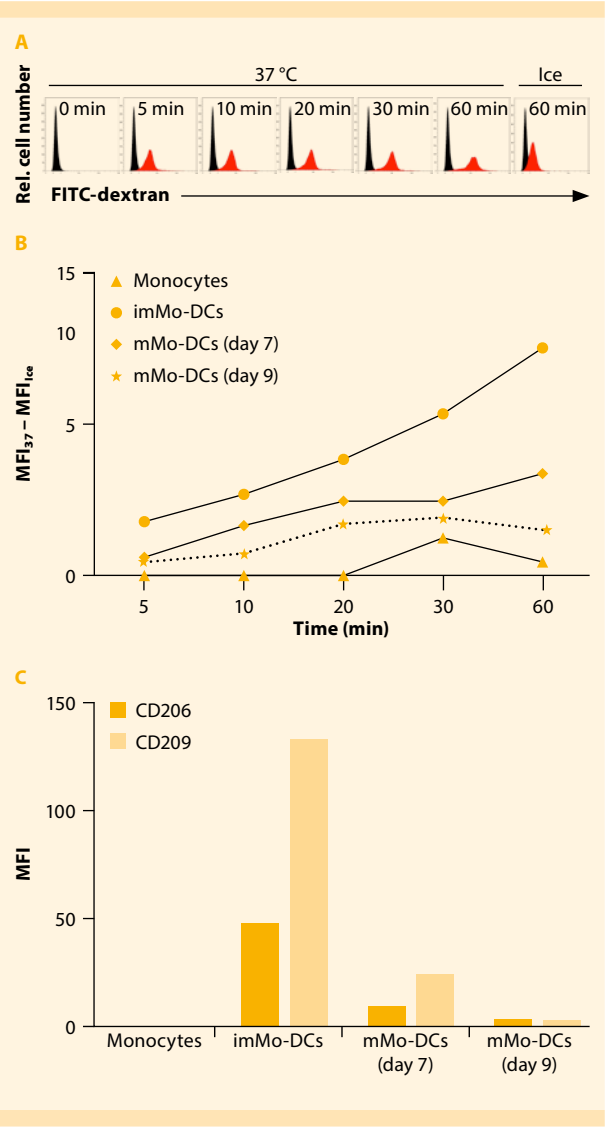

Figure 5: Antigen uptake capacity of monocytes and Mo-DCs. (A) Flow cytometry results from a representative experiment with imMo-DCs cultured at 37 °C in the presence (red) or absence (black) of FITC-dextran for the indicated amounts of time. Non-specific binding was determined by incubating cells on ice. (B) Relative FITC-dextran uptake was calculated by subtracting the MFI of cells incubated for 60 minutes on ice (MFI_{Ice}) from the MFI of cells incubated at 37 °C (MFI_{37}). Results are shown for monocytes, imMo-DCs, and mMo-DCs (days 7 and 9). (C) Monocytes and Mo-DCs were labeled with CD206-APC or CD209-APC antibodies and analyzed by flow cytometry.

Mature Mo-DCs migrate towards CCL19

Upon activation of DCs in the periphery, the cells migrate to the draining lymph nodes where they encounter naive T cells. Migration depends on the expression of CCR7 on the DC surface[11]. The CCR7 ligand CCL19 acts as a chemoattractant for DCs and is expressed in the lymph node areas characterized by high T cell densities. Figure 4 indicates that CCR7 expression was up-regulated during Mo-DC maturation. To measure the migration capacity of *in vitro* generated mMo-DCs, we used Transwell Plates. Figure 6 shows that mMo-DCs migrated towards a CCL19 stimulus in a dose-dependent manner.

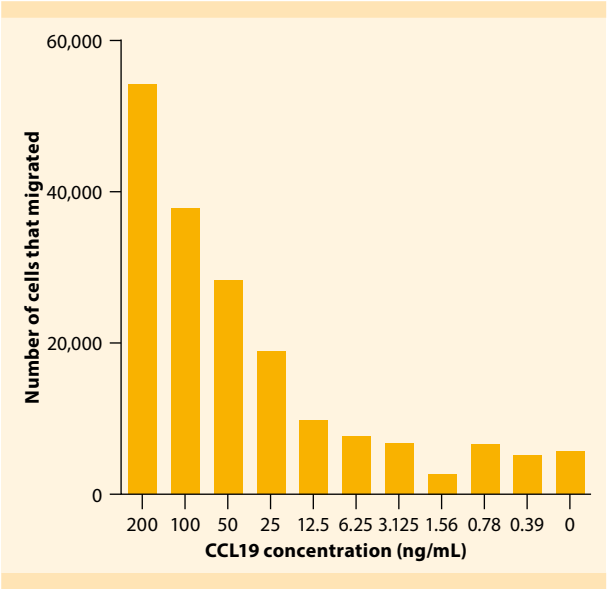

Figure 6: Migration of mMo-DCs towards a CCL19 stimulus. The mMo-DCs (1×10^5 cells) were placed in the upper compartment of a 24-well Transwell Plate. Media containing different concentrations of CCL19 were placed in the lower compartment. After 3 h the total number of cells that migrated to the lower compartment was determined by flow cytometry.

Mature Mo-DCs have the capacity to prime naive T cells

After migration to the lymph nodes, DCs can induce the proliferation of naive T cells. We tested the capacity of monocytes and Mo-DCs to induce T cell proliferation in an MLR. To this end, we isolated allogeneic naive $CD45RA^+CD45RO^-$ T cells to high purities by MACS® Technology (fig. 7A) and labeled their plasma membrane with CellTrace Violet (fig. 7B). After coculturing the labeled T cells with monocytes or Mo-DCs for 7 d, we determined the cell numbers of T cells and analyzed the CellTrace Violet staining by flow cytometry. As with each division of the T cells the dye gets more diluted in the plasma membrane, an increase in unlabeled cells indicates a high T cell proliferation rate. In contrast to monocytes both imMo-DCs and mMo-DCs increased the number of CellTrace Violet–negative T cells in the culture (fig. 7C). This correlated with higher T cell numbers, as determined by cell counting. T cell proliferation was highest when mMo-DCs were used as antigen-presenting cells (fig. 7D), which is in line with the aforementioned result (fig. 4A) showing that receptors involved in T cell priming are up-regulated on Mo-DCs upon maturation.

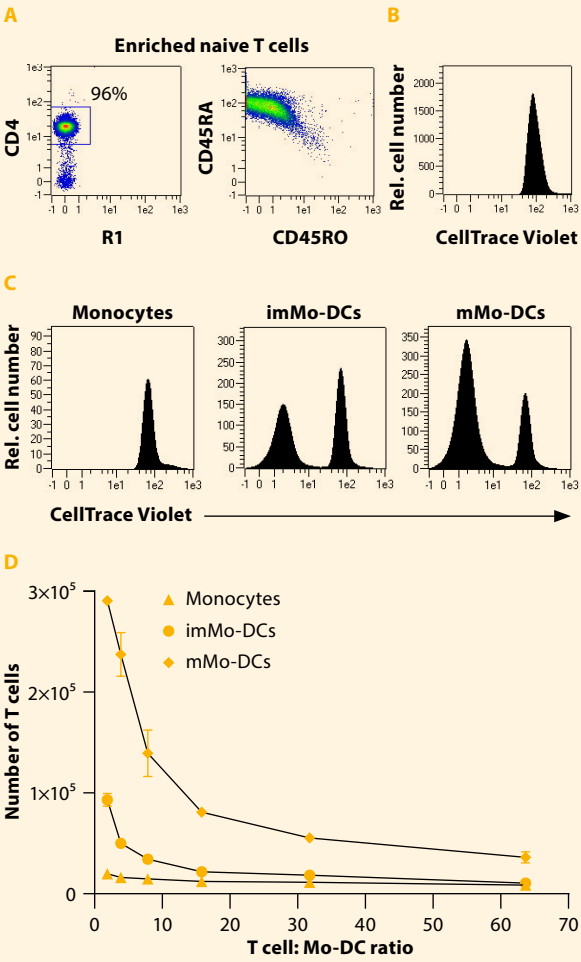

Figure 7: Induction of T cell proliferation by Mo-DCs. (A) Naive $CD4^+$ T Cells were isolated from PBMCs by MACS Technology. Isolated cells were stained with CD4-PE or CD45RA-PE/CD45RO-APC antibodies and analyzed by flow cytometry using the MACSQuant Analyzer 10. (B) Isolated naive T cells were labeled with CellTrace Violet and analyzed by flow cytometry. (C) CellTrace Violet–labeled naive T cells were cocultured with monocytes, imMo-DCs, and mMo-DCs at a ratio of 16:1 for 7 d and analyzed by flow cytometry. (D) Naive T cells were cocultured with imMo-DCs and mMo-DCs at various ratios. After 7 d the numbers of T cells were determined by flow cytometry based on scatter signals. Dead cells were excluded from the analysis by PI fluorescence. One of three representative experiments is shown.

Mature Mo-DCs secrete IL-12 upon stimulation with soluble CD40L

In general, DCs have the capacity to secrete both pro-inflammatory and immunoregulatory cytokines, depending on the stimulus received. T cell–mediated CD40L stimulation of Mo-DCs induces the production of T_H1-polarizing IL-12, but also the secretion of the immunosuppressive IL-10[12-14]. However, for studies towards the development of cancer therapies it is desirable to generate Mo-DC populations secreting high amounts of IL-12 and low amounts of IL-10 as these cells generate a more effective antitumor response via induction of T_H1 cells.

To determine the capacity of *in vitro* generated Mo-DCs to secrete IL-12 and IL-10, we stimulated mMo-DCs under various conditions for one day: i) incubation with soluble Human CD40-Ligand (Miltenyi Biotec), which forms multimers *in vitro*, and ii) coculture with a J558L cell line expressing CD40L. Stimulation with soluble CD40L led to production of high levels of IL-12 and low levels of IL-10. Co-culture with CD40L-expressing J588L cells resulted in an overall increase in secretion of both IL-12 and IL-10 (fig. 8A). However, the ratio of IL12/IL-10 was higher after stimulation with soluble CD40L than after coculture with the cell line (fig. 8B). Thus, recombinant human CD40L multimers represent an attractive alternative to CD40L-transfected cell lines, allowing for CD40 stimulation of Mo-DCs under defined conditions.

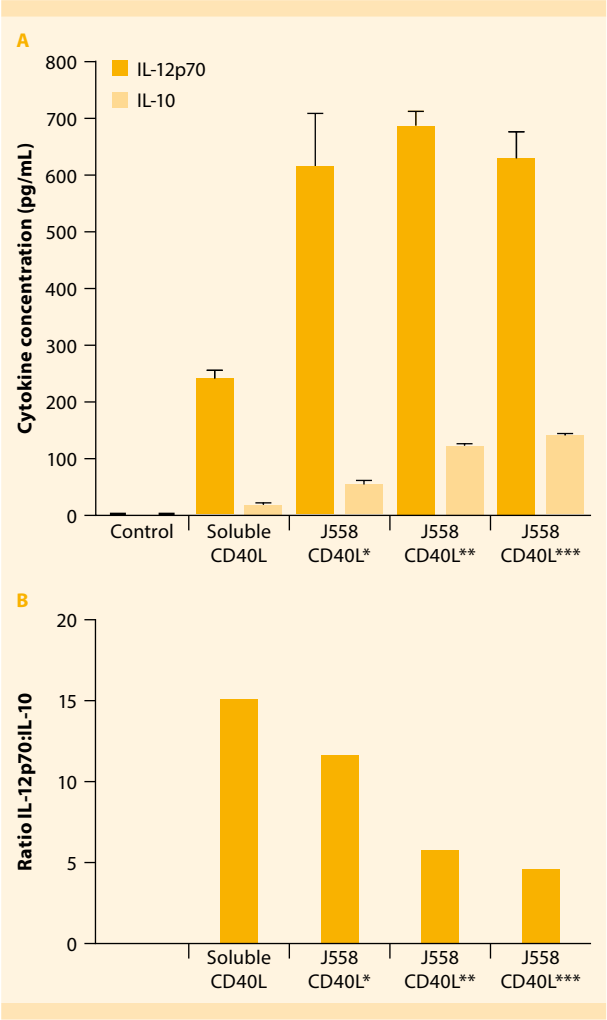

Figure 8: IL-12 and IL-10 secretion by mMo-DCs upon stimulation with CD40L. (A) The mMo-DCs were cultured for 24 h in the absence or presence of soluble CD40L (16 µg/mL) or a J558 cell line expressing CD40L (*7×10^4 cells/well, **14×10^4 cells/well, ***28×10^4 cells/well). IL-12p70 and IL-10 concentrations in the culture supernatant were determined by ELISA. (B) The ratios of IL-12p70 vs. IL-10 were calculated based on the results shown in (A).

Conclusion

- This applications note describes procedures that cover a complete workflow for the generation and phenotypic and functional analysis of mMo-DCs.
- CD14 MicroBeads enable the isolation of viable CD14$^+$ monocytes to high purities, with high yields. The isolated monocytes can be easily differentiated into Mo-DCs.
- A straightforward and reliable differentiation/maturation protocol based on cytokines from Miltenyi Biotec enables the effective generation of mMo-DCs.
- *In vitro* generated imMo-DCs and mMo-DCs possess the characteristics of DCs in terms of i) morphology, ii) surface marker expression, iii) antigen uptake capacity, iv) migration towards a CCL19 stimulus, v) induction of T cell proliferation, and vi) capacity to secrete IL-12 in response to CD40L stimulation.
- Comprehensive phenotypic analysis is accomplished using flow cytometry tools from Miltenyi Biotec, including the powerful MACSQuant Analyzer 10 and a wide range of MACS Antibodies.
- Recombinant human CCL19 from Miltenyi Biotec is a potent chemoattractant for CCR7-expressing Mo-DCs.
- CD40L from Miltenyi Biotec effectively stimulates Mo-DCs to secrete IL-12. The resulting ratio of IL-12:IL-10 is at least as high as with a CD40L-expressing cell line.

References

1. Palucka, K. and Banchereau, J. (2012) Cancer immunotherapy via dendritic cells. Nat. Rev. Cancer 12: 265–277.
2. Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. Nature 392: 245–252.
3. Acuto, O. and Michel, F. (2003) CD28-mediated co-stimulation: a quantitative support for TCR signalling. Nat. Rev. Immunol. 3: 939–951.
4. van Kooten, C. and Banchereau, J. (2000) CD40-CD40 ligand. J. Leukoc. Biol. 67: 2–17.
5. Figdor, C.G. *et al.* (2004) Dendritic cell immunotherapy: mapping the way. Nat. Med. 10: 475–480.
6. Hubo, M. *et al.* (2013) Costimulatory molecules on immunogenic versus tolerogenic human dendritic cells. Front. Immunol. 4: 51–63.
7. Romani, N. *et al.* (1994) Proliferating dendritic cell progenitors in human blood. J. Exp. Med. 180: 83–93.
8. Caux, C. *et al.* (1994) Activation of human dendritic cells through CD40 cross-linking. J. Exp. Med. 180: 1263–1272.
9. Jonuleit, H. *et al.* (1997) Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur. J. Immunol. 27: 3135–3142.
10. Feuerstein, B. *et al.* (2000) A method for the production of cryopreserved aliquots of antigen-preloaded, mature dendritic cells ready for clinical use. J. Immunol. Methods. 245: 15–29.
11. Martín-Fontecha, A. *et al.* (2003) Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J. Exp. Med. 198: 615–621.
12. Moser, M. and Murphy, K.M. (2000). Dendritic cell regulation of TH1-TH2 development. Nat. Immunol. 1: 199–205.
13. Trinchieri, G. (1994) Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. Blood. 84: 4008–4027.
14. Iwasaki, A. and Kelsall, B.L. (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. J. Exp. Med. 190: 229–239.

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