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We make the difference in efficacy and safety

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In vitro Pharmacology: Human Differentiated Leukocyte Assays

Leukocyte Differentiation

Leukocytes are white blood cells that patrol the body and possess a potent arsenal of bactericidal agents and chemical messengers that regulate inflammation, immune responses, blood vessel formation and wound healing. Once exposed to a specific antigen or nonspecific mitogens, leukocytes respond with changes in morphology and behaviour, macromolecular synthesis and production of cytokines followed by proliferation and differentiation of the progeny into various effector and memory cells like macrophages, dendritic cells, Th1 and Th2 cells etc.

In addition to standard assays on primary leukocytes Fidelta developed assays with differentiated leukocyte subpopulations as an important follow up within anti-inflammatory assays cascade. The portfolio of *in vitro* assays with differentiated leukocytes covers various types of immune responses with multiple readouts and endpoints. Most of the assays are highly flexible in terms of set up and are adaptable. Applying experience and expertise, we offer customized assay development as need.

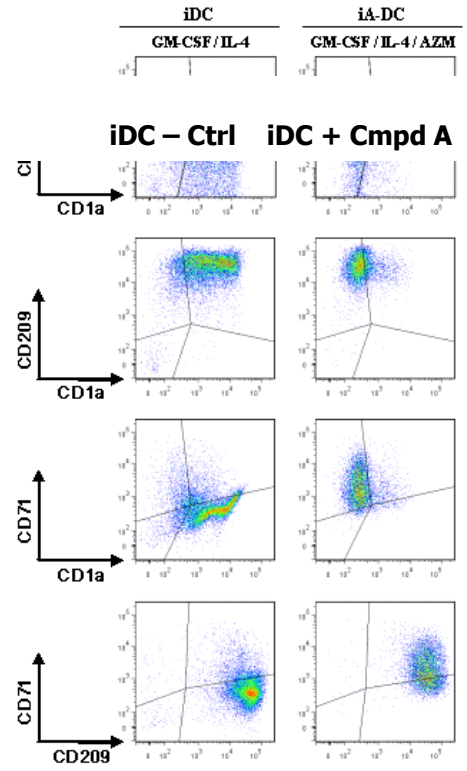
Differentiated cells

- Monocyte-derived macrophages
- Monocyte-derived dendritic cells
- Th1/Th2 Differentiation

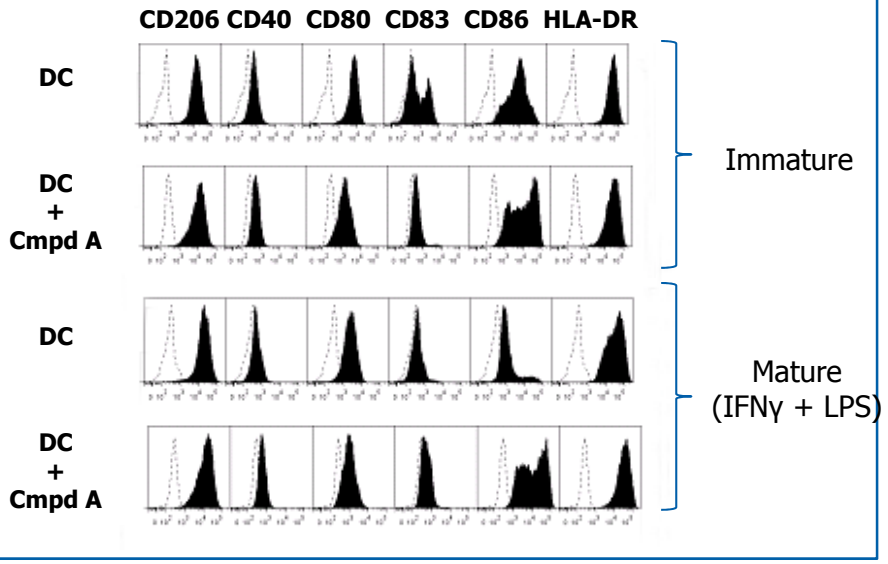
Available read-outs

- ^{3}H-thymidine incorporation
- Chemiluminiscence
- Flow cytometry
- RT-qPCR
- Multiplex cytokine/chemokine assays

Monocyte-Derived Dendritic Cells



Differentiation of monocytes toward classical dendritic cells (CD1a^{+} CD14^{-} CD71^{-} CD209^{high}) was redirected toward non-classical tolerogenic-like immunophenotype (CD1a^{-} CD14^{-} CD71^{+} CD209^{high}) characterized by high expression of co-stimulatory receptors CD86 and HLA-DR upon maturation with pro-inflammatory stimuli.
Stupin Polancec et al. 2012



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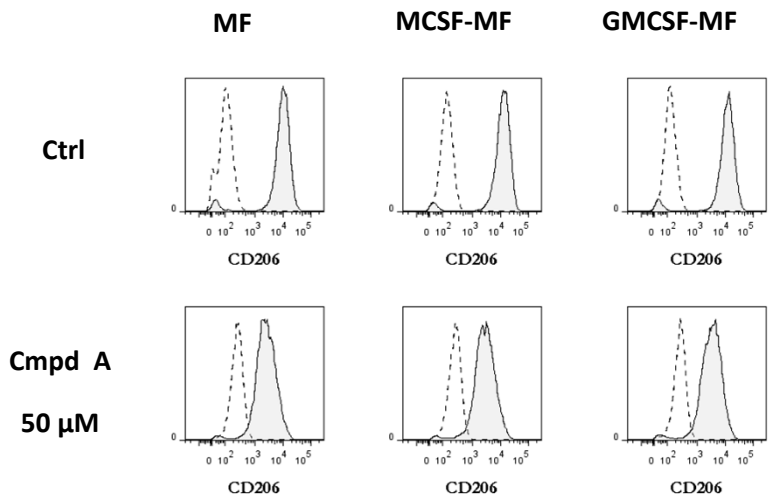
Monocyte-Derived Macrophages

➢ Long-Term Differentiation

Influence of the presence of Cmpd A during the differentiation process was examined on three types of monocyte- derived macrophages:

- MF:
  spontaneous differentiation of plated monocytes toward macrophages
- MSCF-MF:
  M-CSF-induced differentiation of monocytes toward macrophages
- GMSCF-MF:
  GM-CSF-induced differentiation of monocytes toward macrophages



Polychromatic flow cytometry was used for the measurement of the surface expression of various lineages-related receptors:
CD1a, CD14, CD16, CD71, CD163, CD206, CD209.
Presented is example for CD206 analysis.

Monocyte-Derived Macrophages

➢ Short-Term Differentiation

Influence of the presence of Cmpd A on human monocytes, classically activated *in vitro*. Human blood monocytes were primed with IFN-γ and activated with LPS.
Quantitative real-time PCR was used for measurement of the mRNA expression of various surface markers and cytokines.

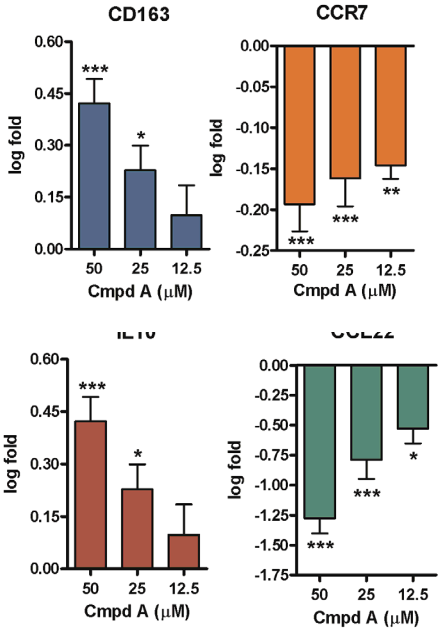
- M1 macrophage markers:

  CCR7, CXCL 11, IL-12p70, CCL2, TNF-α, IL-6.
- M2 macrophage markers:

  IL-10, CCL18, CCL22.
- Pan macrophage marker:

  CD163

Vrancic et al. 2012



References

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