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Deciphering *in vitro* polarization of THP-1 monocytes upon cytokine treatment

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Introduction

In Chronic Lymphocytic Leukemia (CLL), the accumulation of malignant B-cells depends largely on their support by the microenvironment. *In vitro* modeling of this support can be mimicked by growth of CLL cells together with tumor associated macrophages (TAMs), which can have an immune suppressive phenotype, referred to as M2, helping the tumor cells to evade immune surveillance, or they can have a pro-inflammatory phenotype (M1), which augments the clearance of tumor cells by the immune system.

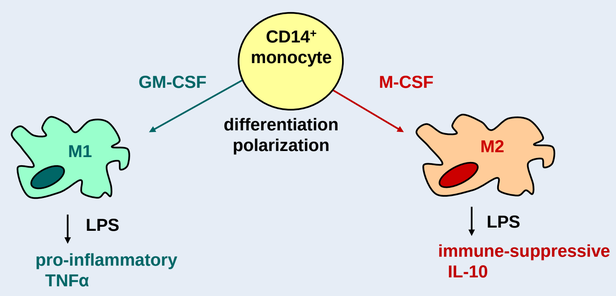

Figure 1: Peripheral blood-derived monocytes can be differentiated and polarized to M1 and M2 phenotypes by M-CSF and GM-CSF. Macrophage activation can be achieved by lipopolysaccharide (LPS) treatment.

Materials and Methods

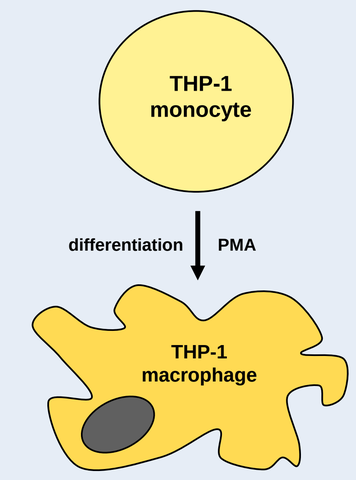

Figure 2: Differentiation of THP-1 monocytes, which grow in suspension; cells can be stimulated by Phorbol-12-myristat-13-acetat (PMA) to become adherent macrophages.

THP-1 cells, which are a cell line derived from a primary tumor of an acute myeloid leukemia (AML) patient, offer a suitable tool for *in vitro* assessment of TAMs effect's on CLL cells.

After successful differentiation of THP-1 monocytes into macrophages, supernatants were taken for cytokine assessment by means of enzyme-linked immunosorbent assay (ELISA) and cells were stained for flow-cytometric analysis (FACS).

The THP-1 cells were incubated in medium (RPMI + 10% FCS + 1% Pen/Strep) at 37°C with 5% CO_2.

For the experiment one million cells/well were plated out in 5 ml medium on a six well plate. The concentration of the cytokines amounts to 10 ng/ml.

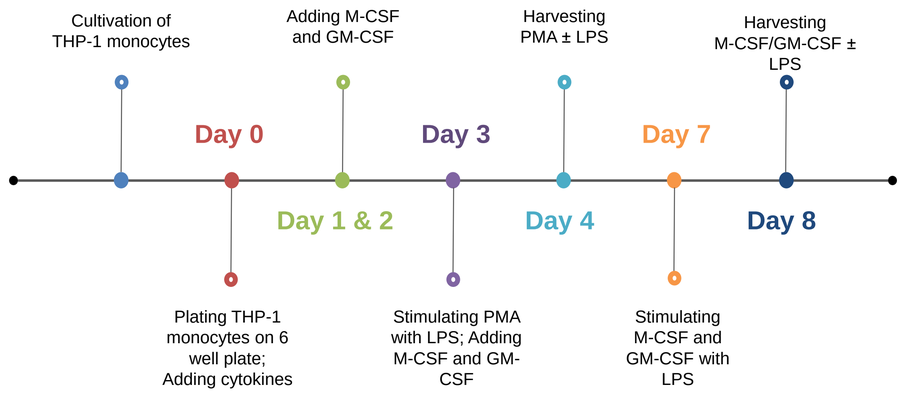

Figure 3: Workflow timeline.

Results

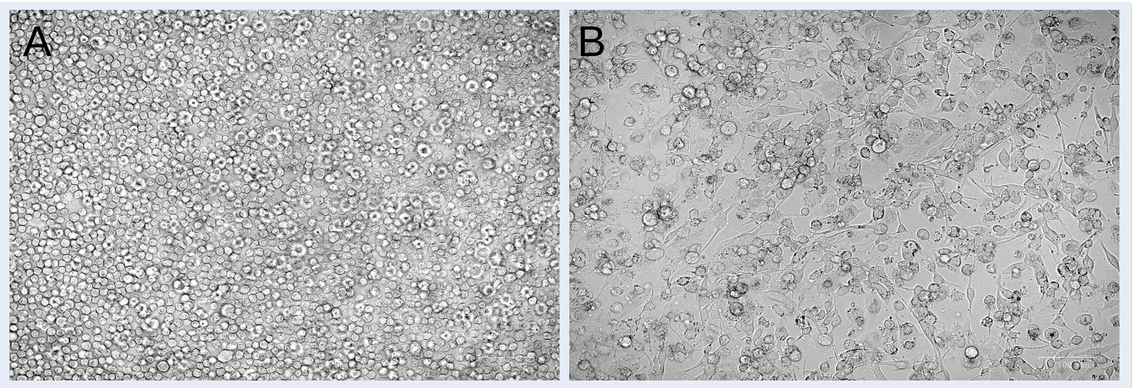

Figure 4: Monocytic THP-1 cells grow in suspension and are single round cells before PMA treatment (**A**). After four days of PMA treatment cells adhere to the surface and form elongations and can be described as 'needle-shaped' (**B**).

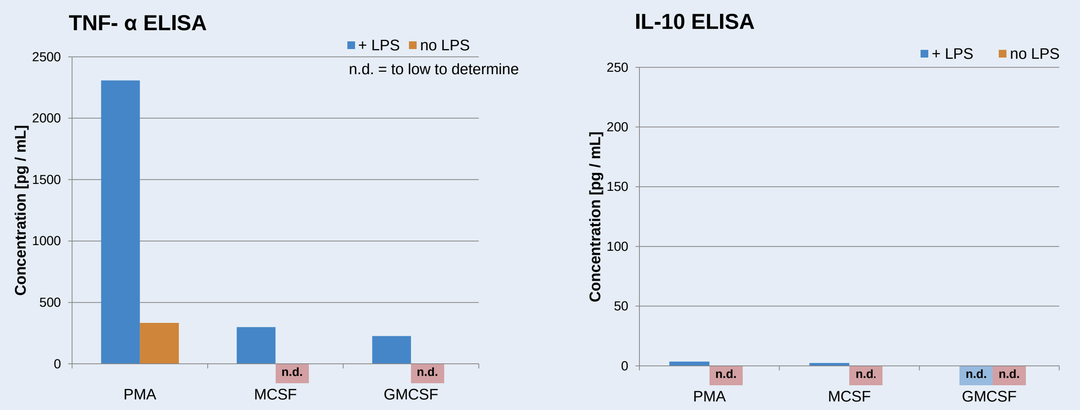

Figure 5: Assessment of two typical cytokines secreted by the M1 (TNF-α) and M2 (IL-10) phenotype of macrophages. PMA differentiation yields higher levels of TNF-α compared to M-CSF and GM-CSF stimulation. IL-10 could only be detected in very low amounts.

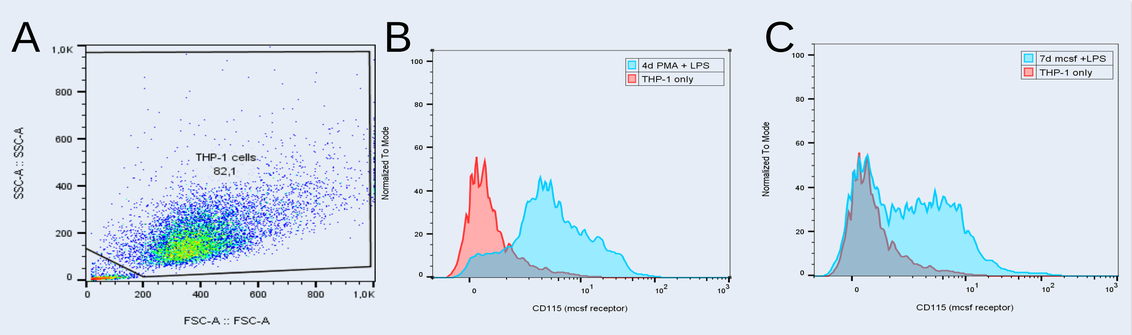

Figure 6: Flow cytometry of THP-1 cells differentiated with PMA for four days (**B**) or seven days (**C**) show high levels of CD115, which is the receptor for M-CSF. (**A**) shows the gating strategy of the THP-1 cell analysis using the forward scatter to represent the size of the cells and the sideward-scatter to represent the granularity.

Conclusion

- THP-1 cells have M1 phenotype when differentiated with PMA
- M-CSF receptor is present on THP-1 cells
  - Further research is needed to show whether this is time dependent
  - The GM-CSF receptor needs to be analyzed
- Phenotypically no THP-1 macrophages
  - When differentiated with M-CSF and GM-CSF

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