Macrophage polarisation analysis using morphology

Noud Holshuijsen, MSc[1,2]; Anthal Smits, PhD[2].; Inge Thijssen-van Loosdregt, PhD.[1]

[1] CytoSMART Technologies B.V., Eindhoven, The Netherlands; [2] Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands

Introduction

Macrophages are key players in the body's immune system, they are involved in e.g. regulation of homeostasis, inflammation control and anti-tumour immunity.[1] Macrophages consist of different subtypes, which can be split into the classically activated or pro-inflammatory (M1) and pro-regenerative (M2) macrophages. In vitro, macrophages (M0) can be polarized using cytokines; IFN-γ activates M1 macrophages, whereas IL-4 activates M2 macrophages.[2,3]

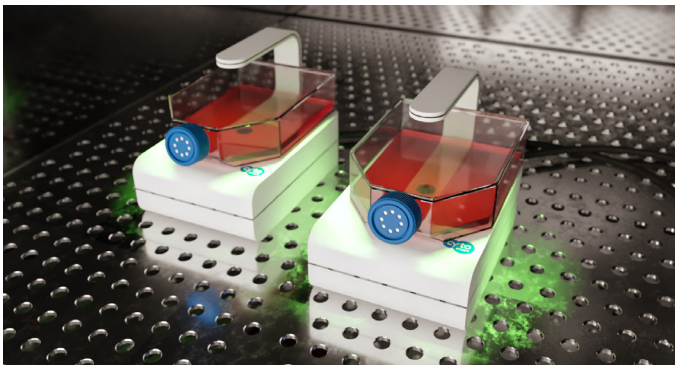
Cell morphology changes with polarization, which means that macrophage characterization could be performed using morphology analysis. M2 macrophages have shown to exhibit an elongated cell shape compared to M1 macrophages, therefore it is expected that morphological analysis can be used to identify polarization of macrophages and analyse the polarization transition in culture.[4]

This study aims to analyse macrophage polarisation using live-cell imaging and morphological analysis. The polarization is tracked using time-lapse imaging by the CytoSMART Lux2 Duo Kit and verified using qPCR to quantify M1 and M2 marker expression.
As M1 macrophages appear round and flat, therefore, they are expected to show a low aspect ratio.[5] The marker expression results are expected to show an up-regulation of TNF, MCP-1 and CCR7 and down-regulation of CD206, CD163 and IL-10.
We expect M2 polarisation to result in elongation and therefore a higher aspect ratio. Marker expression is expected to show down-regulation of TNF, MCP-1 and CCR7 and up-regulation of CD206, CD163 and IL-10.[2]

Material and methods

PBMCs (Pheripheral Blood Mononuclear Cells) were seeded in differentiation medium (Gibco RPMI 1640 with L-Glutamine (1x), 1% penicilin/streptomicin (Pen/Strep), 20% FBS and 20ng/ml M-CSF) and incubated to facilitate monocyte attachment and differentiation to macrophages. The differentiation culture was maintained for 7 days, the medium was changed every 3 days. Thereafter, the cultures were polarized using IFN-γ (20 ug/ml) for M1 and IL-4 (20 ug/ml) for M2 in growth medium (Gibco RPMI 1640 with L-Glutamine (1x), 1% Pen/Strep and 5% FBS). Cells were placed on the CytoSMART Lux2 Duo Kit (37°C and 5% CO_2) and imaged at an interval of 30 minutes for 20 hours.

Samples for RNA isolation and consequent gene expression analysis were taken after differentiation (M0) and after polarization for both M1 and M2. These were analysed for marker gene expression (qPCR) of TNF, MCP1, and CCR-7 for M1 polarization and CD206, CD163 and IL-10 for M2 polarization.



Cell contours were identified in the images obtained with the CytoSMART Lux2 Duo Kit. The aspect ratio of each cell was calculated by fitting an ellipse over the contour and dividing the major diameter by the minor diameter.

Results

The marker expression of the differentiated macrophages was normalised to the marker expression in M0 macrophages (Fig. 1). After M1 polarisation, samples showed an increase in TNF expression and very little decrease in MCP-1 compared to M0, consistent with M1 polarisation. TNF and MCP-1 decreased during M2 polarization, which is consistent with M2 polarisation. CCR-7 showed very small differences in both cases.

Macrophage polarisation analysis using morphology

In the case of the M2 markers, CD206 and CD163 showed a decrease in expression as a result of M1 polarization. After M2 polarisation, CD206 expression increased, while CD163 decreased. However, CD163 expression after M2 polarisation was still higher compared to expression after M1 polarisation. IL-10 expression was not taken into account as the differences were too small.

As shown in figure 2, the average M1 marker expression was higher after M1 polarisation than after M2 polarisation. Whereas M2 marker expression was higher after M2 polarisation than after M1 polarisation.

The morphological analysis (Fig. 3 and 4) showed a 0.59% increase in aspect ratio after M1 polarisation (not significant). M2 polarisation, however, caused a significant increase in the aspect ratio of 11.2% (P=0.0003 Mann-Whitney).

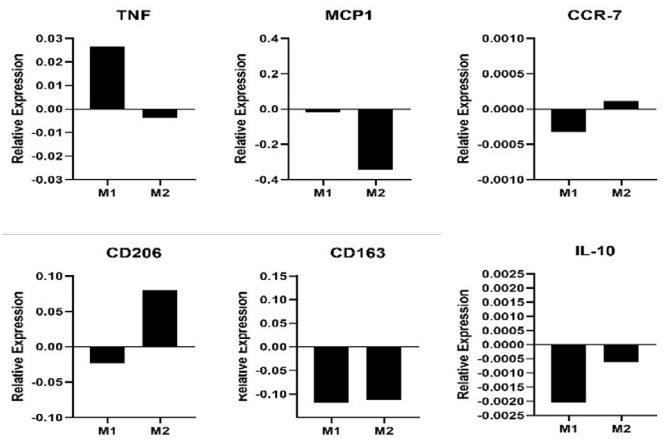


Figure 1. Normalised relative expression of M1 (top row) and M2 (bottom row) polarization associated genes. Samples were normalised by subtracting the relative expression of M0 samples.

Discussion

M1 polarisation

M1 marker genes showed a higher average expression after M1 polarisation compared to M2 polarisation, as expected.[2] Although MCP-1 did not show upregulation compared to the expression in M0 samples, expression was higher after M1 polarisation compared to M2 polarisation.

M2 marker genes showed a lower expression after M1 polarisation compared to M2 polarisation, as expected.[2] CD163 expression showed little difference between M1 and M2, which was not consistent with expectations. IL-10 showed little difference overall.

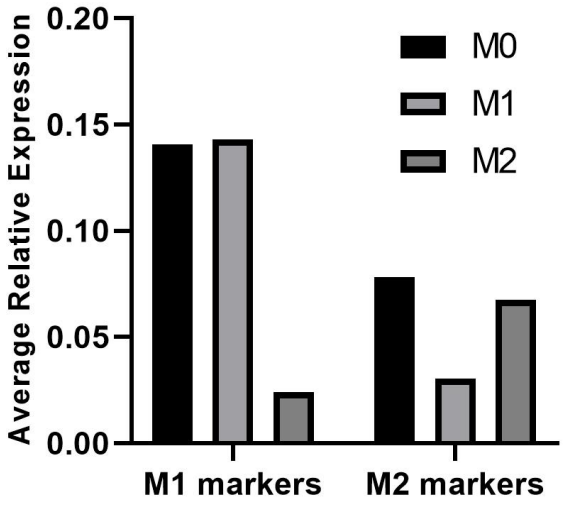


Figure 2. Average expression of marker gene expression per macrophage type.

M2 polarisation

M1 markers showed a low average expression after M2 polarisation, compared to after M1 polarisation. TNF and MCP-1 both showed downregulation as a result of M2 polarisation, as expected. Whereas, the difference in CCR-7 expression was very low.

M2 markers showed a higher average expression after M2 polarisation compared to after M1 polarisation. And although, IL-10 and CD163 showed downregulation after M2 polarisation, CD206 was upregulated, as expected.[2]

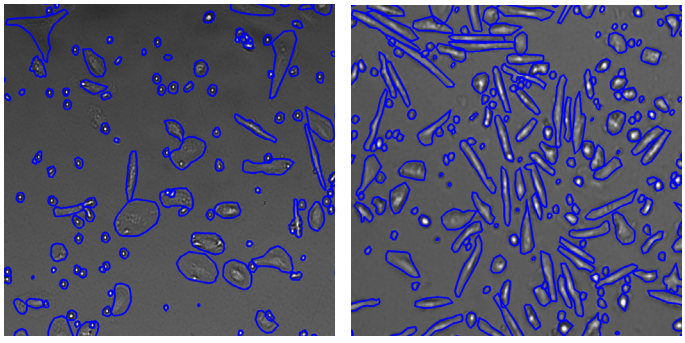


Figure 3. Representative images of M1 (left) and M2 (right) polarized cells obtained with the CytoSMART Lux2 Duo Kit. Overlay of the cell contours in blue.

Marker gene expression showed some unexpected results, which are most likely due to the polarisation protocol. This experiment used a simple polarisation culture, whilst other studies have also used a large range of different polarisation protocols, which results in differences in marker gene expression.[6]

Macrophage polarisation analysis using morphology

Elongation

M1 polarisation did not show a significant difference in mean aspect ratio, whereas M2 polarisation did, indicating elongation of macrophages due to M2 polarisation, as expected.[4]

The aspect ratio data also showed a high variation within each population. Individual cell polarisation analysis is therefore not recommended using the aspect ratio only. However, the data does indicate a significant difference between the two different polarisation cultures as a whole.

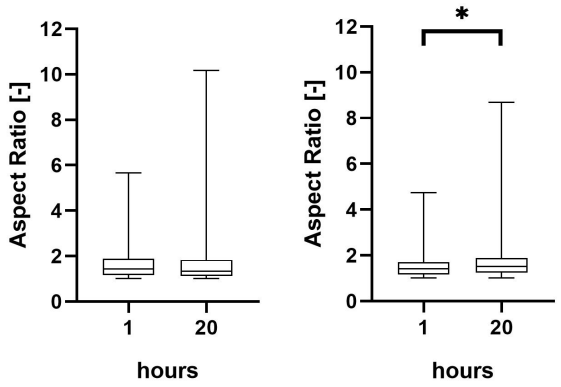


Figure 4. Aspect ratio boxplot of M1 and M2 polarisation. *: significantly higher aspect ratio after polarization (p=0.0003).

Conclusion

The data showed cell elongation after M2 polarisation, consistent with what was expected. The CytoSMART Lux2 Duo Kit enabled the use of live-cell imaging and analysis of macrophage morphology. This provides a tool to analyse macrophage polarisation and can therefore be used for continuous monitoring of macrophage polarisation without interfering the culture.

References

[1] Yu T, Gan S, Zhu Q, et al. Modulation of M2 macrophage polarization by the crosstalk between Stat6 and Trim24. Nat Commun. 2019;10(4353). doi:10.1038/s41467-019-12384-2

[2] Ahmed I, Nahed I. M1 and M2 Macrophages Polarization via mTORC1 Influences Innate Immunity and Outcome of Ehrlichia Infection. J Cell Immunol. 2020;2(3). doi:10.33696/immunology.2.029

[3] Wissing TB, Bonito V, Haaften EE van, et al. Macrophage-driven biomaterial degradation depends on scaffold microarchitecture. New Front. 2019;7(April). doi:10.3389/fbioe.2019.00087

[4] McWhorter FY, Wang T, Nguyen P, Chung T, Liu WF. Modulation of macrophage phenotype by cell shape. Proc Natl Acad Sci U S A. 2013;110(43):17253-17258. doi:10.1073/pnas.1308887110

[5] Vereyken EJ, Heijnen PD, Baron W, de Vries EH, Dijkstra CD, Teunissen CE. Classically and alternatively activated bone marrow derived macrophages differ in cytoskeletal functions and migration towards specific CNS cell types. J Neuroinflammation. 2011;8(1):58. doi:10.1186/1742-2094-8-58

[6] Gordon S, Martinez FO. Alternative Activation of Macrophages: Mechanism and Functions. Immunity. 2010;32(5):593-604. doi:10.1016/j.immuni.2010.05.007

Contact information
General: info@cytosmart.com
Author: inge.thijssen@cytosmart.com