Development of *in vitro* assays using macrophage-like cell lines for the screening of novel anti-cancer therapeutic antibodies

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Poster 28

Introduction

- Tumour associated macrophages (TAMs) are the most abundant immune cell within many tumours types with a high infiltration often correlating to a poor prognosis[1].
- TAMs express an immunosuppressive and anti-inflammatory profile, similar to that of 'M2' polarised macrophages, which contributes to their prominent role in tumourigenesis and progression.
- Consequently, TAMs are under the spotlight as a novel target for new cancer therapeutics.
- THP-1 and U937 cells were used to develop models of macrophage function to assist with the screening of in-house therapeutic antibodies able to modulate macrophage activity in cancer.

Differentiation and polarisation of 'macrophage-like' cells

THP-1 and U937 monocytic cells were cultured in medium supplemented with PMA for a period of 48 h and 7 days, respectively, to differentiate them into 'macrophage-like' cells. Following this they were polarized using specific cytokine cocktails into 'M1' and 'M2' like cells.

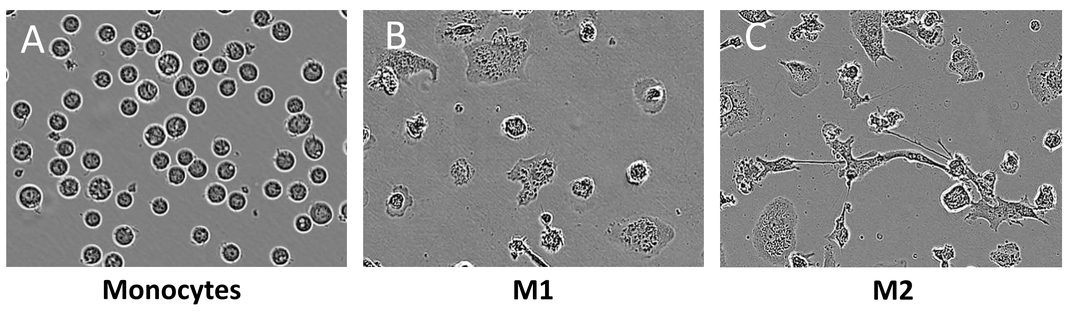

Figure 1. Morphology of THP-1 cells throughout differentiation **A)** THP-1 cultured in RPMI + 10% FBS only (monocytes), **B)** THP-1 cultured in RPMI + 10% FBS, supplemented with IFN-γ adopt a 'fried egg' morphology (M1) whilst **C)** THP-1 cultured in RPMI + 10% FBS supplemented with IL-13 and IL-4 were characterised by an elongated body and numerous processes (M2).

THP-1 and U937 derived 'macrophage-like' cells express some key macrophage markers

The phenotypes of the M1 and M2-like cells generated were evaluated for expression of characteristic macrophage markers via flow cytometric analysis. Surface markers such as HLA-DR and CD86 were used to distinguish 'M1' polarised macrophages whilst CD206 was used for 'M2' polarised macrophages.

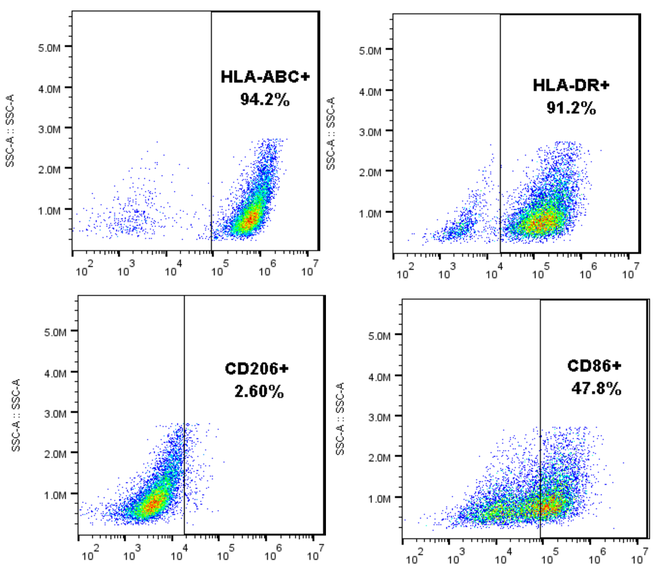

Figure 2. Representative flow cytometry analysis of THP-1 differentiated with PMA and polarised with IFN-γ (M1)

Table 1. Phenotypes from flow cytometry analysis of THP-1 and U937 differentiated with PMA and polarised with IFN-γ or IL-4 and IL-13 (n=3)

Cell type and condition	HLA-ABC	HLA-DR	CD86	CD206
THP-1 + IFN-γ (M1)	+	+	+/-	-
THP-1 + IL-4 and IL-13 (M2)	+	-	+/-	-
U937 + IFN-γ (M1)	+	-	+	-
U937 + IL-4 and IL-13 (M2)	+	-	+	-

Although the polarisation didn't result in the expected expression profiles seen for M1 and M2 primary macrophages, both cell lines did express some key macrophage surface markers such as HLA-ABC and CD86.

References

1. Gentles et al. (2015), *Nat. Medicine* (21), 938–945.
2. Leidi et al. (2009), J Immunol. ;182(7):4415-22.

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THP-1 and U937 derived cells show expected 'M1' and 'M2'-like cytokine and chemokine profiles

Cytokine and chemokine production by THP-1, U937 and their derived macrophages were analysed using MSD and ELISA.

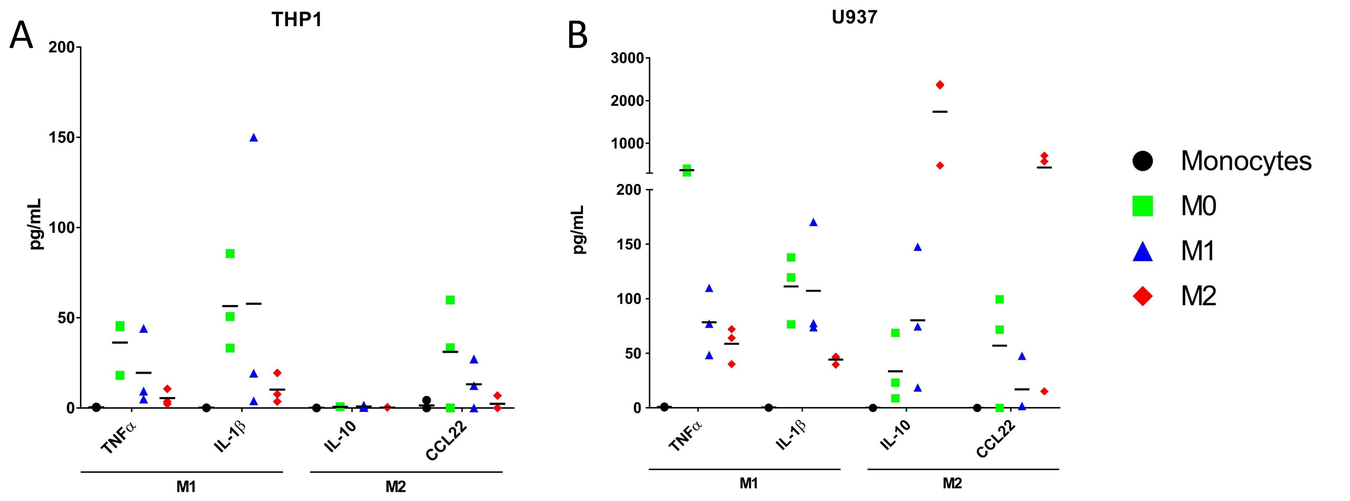

Figure 3. Example cytokine and chemokine production by THP-1 **(A)**, U937 **(B)** and their derived macrophages as quantified by ELISA and MSD. (n=3 individual repeats).

Cytokine and chemokine production was expected to vary between cell subtype with M1 macrophages secreting more pro-inflammatory cytokines (e.g. TNF-α and IL-1β) whist M2 macrophages secrete more anti-inflammatory cytokines (e.g. IL-10 and CCL22).
Data generated showed the cytokine profiles of polarised THP-1 and U937 were in line with the expected cytokine and chemokine profiles.

THP-1 and U937 cells are able to phagocytose pHrhodo® Red *E.coli* bioparticles

Phagocytosis is a key macrophage function. Therefore, a method to evaluate phagocytosis was developed, which quantified the ability of polarised U937 and THP-1 cells to phagocytose pHrhodo® Red *E.coli* using the IncuCyte® live imaging platform. When the bioparticles are engulfed by the cells, the lower pH in the phagosome causes them to emit a red fluorescence.

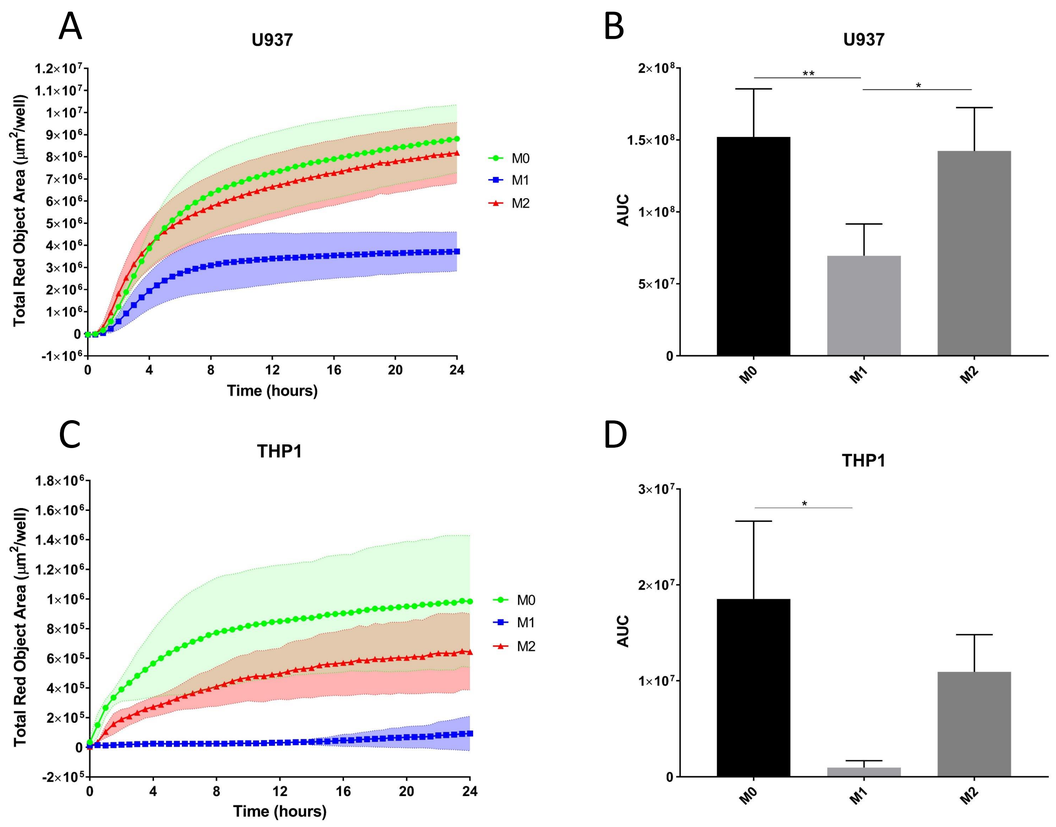

Figure 5. Quantification of the phagocytic activity of THP-1 and U937 cells. **A&C)** Total red object area per well was calculated using the IncuCyte software. Results were normalised by subtracting red object area calculated in control wells containing only the bioparticles. An increase in red correlates with an increase in phagocytosis. **B&D)** Area under the curve (AUC) values of curves in A&D were calculated in GraphPad. Statistical analysis using a one way-ANOVA followed by a post hoc Tukey test showed there to be a significant difference between phagocytosis in M0 macrophages (PMA only treated) compared to M1 macrophages for both cell lines and between M2 and M1 in U937 (* =p<0.05, **=p<0.01). Data are the average ± SD of four different experiments (n=4).

M0 and M2-polarised cells in both cell lines were shown to have higher phagocytic potential, as reported in the literature[2]. It is thought that M2-polarised macrophages are more phagocytic than M1-polarised macrophages due to their role in tissue repair and resolution after infection.

Conclusions and next steps

- We have established culture conditions to differentiate and polarise THP-1 and U937 monocytes into macrophage-like cells.
- We have determined that THP-1 and U937 cells are relevant models that present some key aspects found in primary human macrophages such as certain cytokines and surface markers.
- We have developed a method with the potential for high throughput functional screening of novel therapies designed to modulate macrophage function.
- Furthermore, this work will enable a better understanding of macrophage biology.