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Single-cell mRNA sequencing analysis of the synergistic impact of double-stranded RNA (dsRNA) and IFNα on human monocyte-derived macrophages

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INTRODUCTION

Single-cell mRNAseq (scRNAseq) is a promising technology allowing for unbiased analysis of gene expression at the cellular level. In this study, scRNAseq was used to identify cellular sub-types and differential gene expression functional units in monocyte-derived macrophages in response to double-stranded RNA (dsRNA, viral mimic, TLR3 ligand), IFNα or their combination.

METHODS

Human monocyte-derived macrophages were cultured overnight in the absence (U) or presence of dsRNA (rintatolimod/Ampligen-A), IFNα (I) or their combination (IA) (Figure 1), which we previously identified as synergistic in induction of chemokines attracting CTLs, Th1 and NK cells [1, 2]. The cells were harvested after 1 day, captured at the single-cell level and immobilized in a vertical flow array chip (VFAC), which contains 100-microchambers packed with 105 beads immobilizing 1010 oligo(dT) probes with unique cell-ID, UMI, and PCR-tags. Each single cell was captured and lysed on the small hole (3µm in diameter) above the microchamber on the VFAC and cDNA libraries were synthesized and tagged with unique cell-IDs post mRNAs hybridization. ScRNAseq using a custom 94-gene inflammation RNA-seq panel was then performed. Post-clustering (Scanpy), unsupervised cell-type identification (louvain), and downstream analyses were performed on the gene expression data to determine impact of A, I and IA treatment on the cellular composition of the induced macrophages (Figures 2-5).

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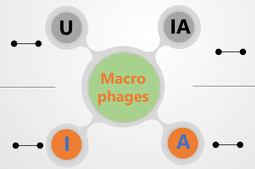
Study Design

scRNA-Seq



Untreated (U)
Cultured overnight in the absence of any treatment

IFNα (I)
Cultured overnight with interpheron alpha

Combination (IA)
Cultured overnight with combination of IFNα + Ampligen

Ampligen (A)
Cultured overnight with dsRNA (rintatolimod/Ampligen-A)

Figure 1: Study design presenting various conditions for human monocyte-derived macrophages.

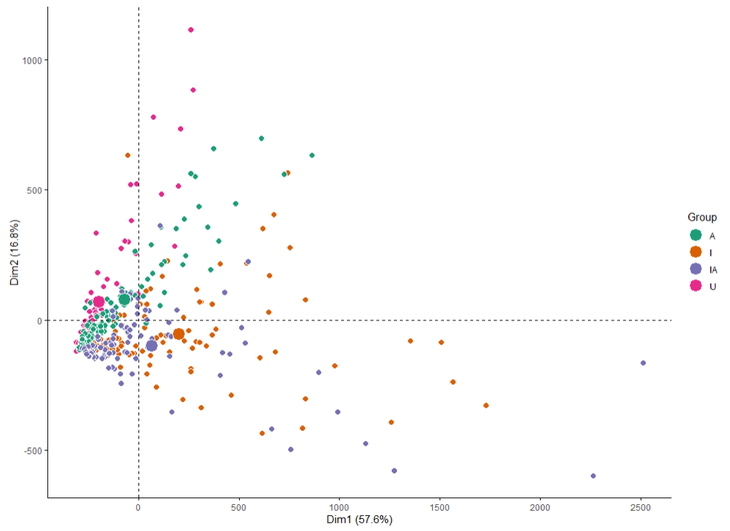


Figure 2: Principal component analysis plot showing first and second principal component of all gene features for macrophages under four conditions. "I" and "I+A" conditioned cells cluster together and unloaded "U" and Ampligen "A" loaded cells cluster together in patterns of gene expressions.

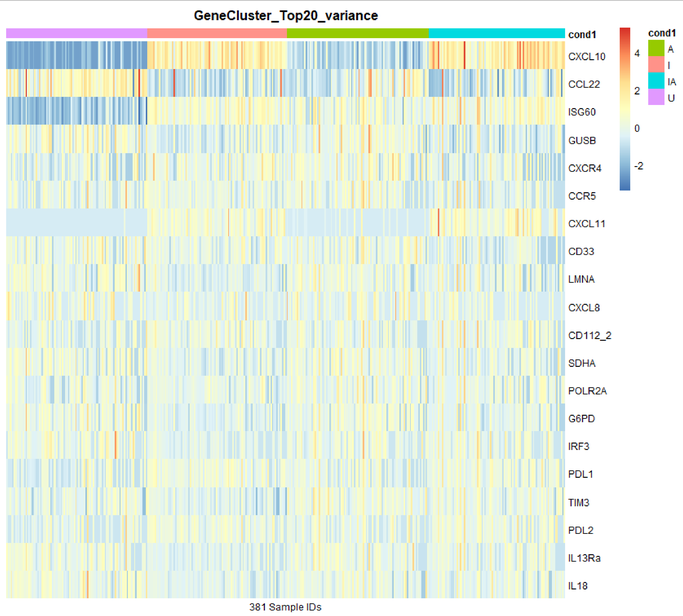


Figure 3: Heatmap of gene expression showing top20 most variable genes between four tested conditions. For instance, CXCL10 is overexpressed in "I" and "I+A" conditions compared to "A" alone or unloaded macrophages.

Figure 4: Fold change of differentially expressed inflammation genes (p<0.05) in monocyte-derived macrophages for: a) "I" versus "A" treated macrophages; b) "I" versus "U" untreated macrophages; and c) "I+A" versus "I" treated macrophages.

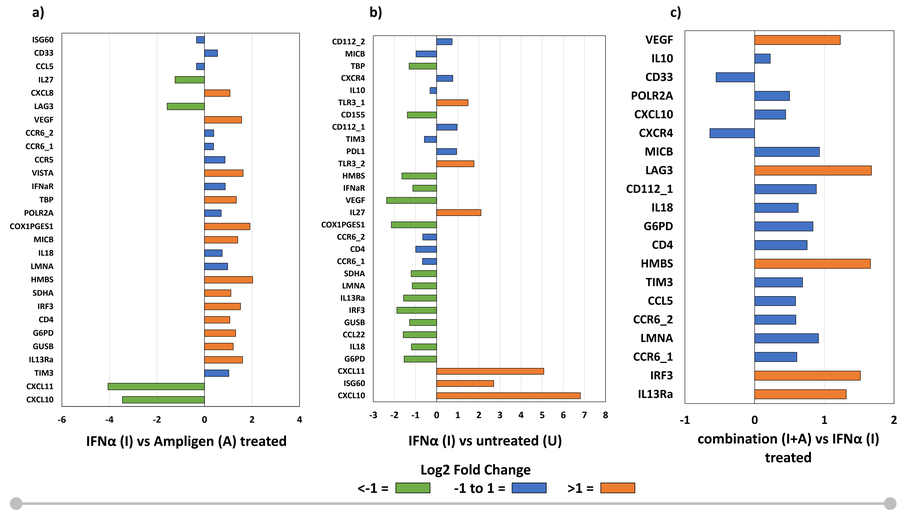


Figure 5: Fold change of differentially expressed inflammation genes (p<0.05) in monocyte-derived macrophages for: a) "A" versus "U" untreated macrophages; and b) "I+A" versus "A" treated macrophages.

CONCLUSION

scRNAseq was able to identify distinct single-cell macrophage phenotypes caused by induction of dsRNA and IFNa. Of the 5 subclusters identified, activation by IFNα alone or in combination with TLR3 identified subsets of cell populations representing activation markers of type-1 immunity and CTL/Tk1/attracting chemokines. These observations facilitate the identification of intracellular signaling pathways underlying the heterogenous response of individual myeloid cells to exogenous and endogenous activators, helping to develop improved vaccine adjuvants and cancer immunotherapies.

REFERENCES

1. Theodoraki MN, Yerneni S, Sarkar SN, Orr B, Muthuswamy R, Voyten J, Modugno F, Jiang W, Grimm M, Basse PH, Bartlett DL, Edwards RP, Kalinski P. Helicase-Driven Activation of NFκB-COX2 Pathway Mediates the Immunosuppressive Component of dsRNA-Driven Inflammation in the Human Tumor Microenvironment. Cancer Res. 2018 Aug 1;78(15):4292-4302. doi: 10.1158/0008-5472.CAN-17-3985.
2. Muthuswamy R, Berk E, Junecko BF, Zeh HJ, Zureikat AH, Normolle D, Luong TM, Reinhart TA, Bartlett DL, Kalinski P. NF-κB hyperactivation in tumor tissues allows tumor-selective reprogramming of the chemokine microenvironment to enhance the recruitment of cytolytic T effector cells. Cancer Res. 2012 Aug 1;72(15):3735-43. doi: 10.1158/0008-5472.CAN-11-4136.