Investigating PBMC Plasticity in Circulation: An analysis of Phenotypic Differentiation in Response to Sepsis Patient Serum

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Introduction

- Sepsis is a leading cause of morbidity and mortality worldwide, characterised by a dysregulated host immune response to infection that results in organ failure and is a frequent cause of critical care admission.
- Following the initial hyperinflammatory phase, a state of immunosuppression often develops, predisposing patients to secondary infection and poor outcomes.
- Expansion of myeloid-derived suppressor cells (MDSCs), a type of immunosuppressive cell comprising granulocytic (G-MDSC) and monocytic (M-MDSC) subsets, is a hallmark of this immunosuppressive phase.
- The origin of MDSCs remains uncertain, arising either from immature myeloid cells released during emergency myelopoiesis or from reprogramming of mature myeloid cells within the sepsis milieu.
- AIM: To determine whether PBMCs from healthy donors can adopt an immunosuppressive MDSC-like phenotype when exposed to sepsis serum.

Methods

1. Study design & Participants

- Sample sources: Peripheral blood collected from seven healthy volunteers and critically ill sepsis patients under existing ethical approval
- Blood fractionated into Polymorphonuclear cells (PMNs) and Peripheral Blood Mononuclear Cells (PBMCs) by Density Gradient Separation using Percoll.
- PBMCs incubated for six days in several selected conditions including sepsis patient serum stored from the INNATE study.

2. Phenotypic Analysis

- Following 6 days of incubation cells were detached from the wells
- A small sample was taken for microscopy and to confirm cell count
- Remaining cells then proceeded to flow cytometry using a large panel including HLA-DR, CD15, CD16, CD66b and CD11b.
- Comparisons between healthy volunteer baselines and patient derived populations were made.

3. Light Microscopy

- Light microscopy was primarily used to ensure accurate cell counts and adequate numbers during phenotypic testing.
- Stained and stored slides from throughout the experiment were used to provide morphological clues of cell modulation in later stages of the experiment.

Results & Discussion

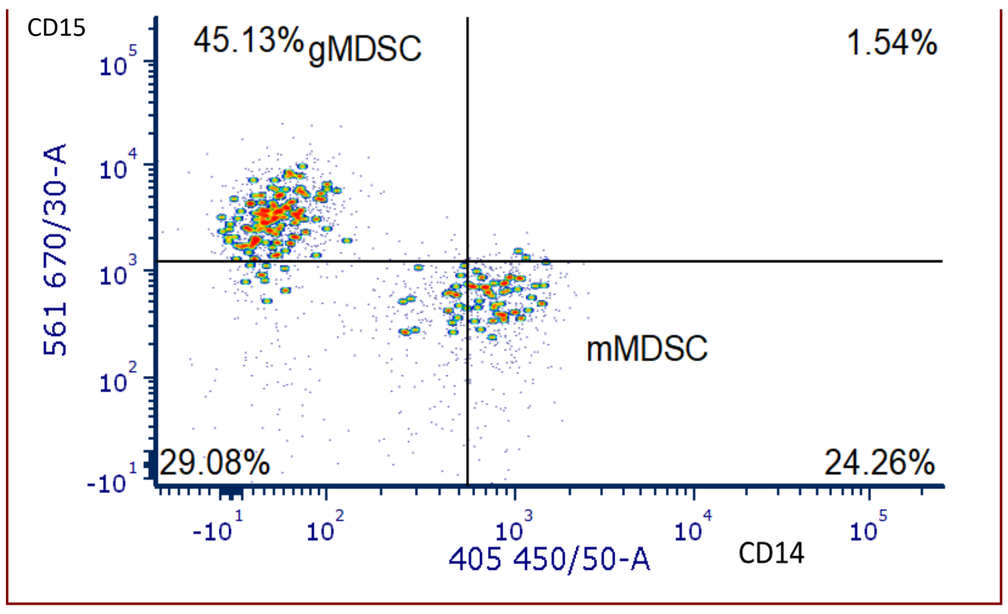

Figure 1. Sepsis Patient Sample

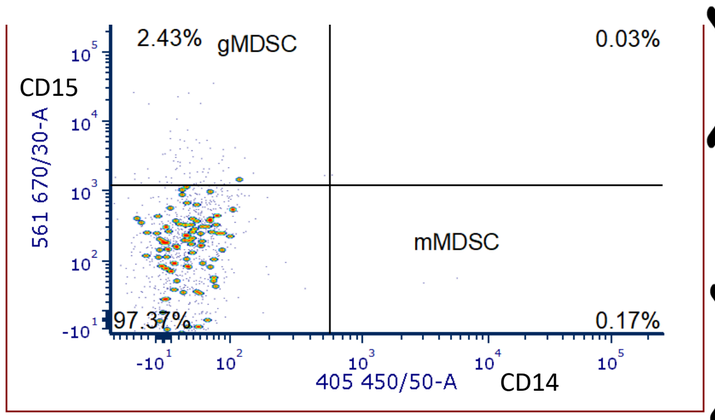

Figure 2. Healthy Volunteer A – Baseline sample

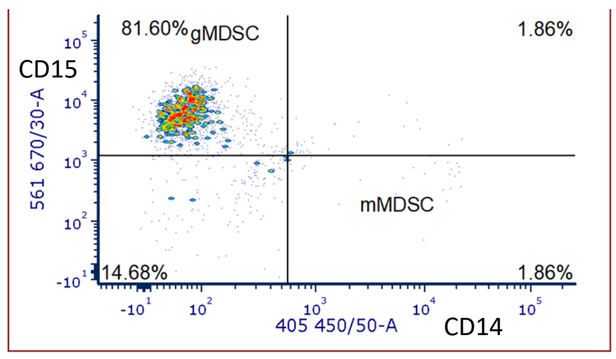

Figure 3. Healthy Volunteer A – Day 6 Sepsis

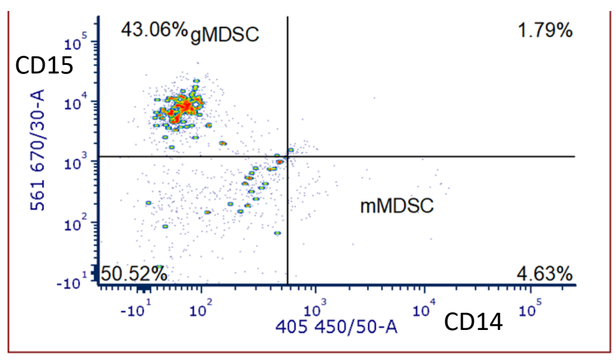

Figure 4. Healthy Volunteer A – Day 6 Critically ill (Non-sepsis)

Results:
Initial optimisation focused on incubation timing and mixing protocols, which improved assay reproducibility and reduced variability across donors.
Clotting issues were encountered during some incubations highlighting the challenges of working with sepsis patient serum. Sepsis patient serum can be hypercoagulable, with increased clotting factors (1) and the levels of these can vary individual to individual.
Future experiments could benefit from functional fibrinogen assays and calcium/thrombin challenge testing to ensure complete clot formation prior to commencing incubation. Further, consideration should be given to anticoagulation, and it may be beneficial to complete a further re-optimisation cycle trialling different anticoagulants at sample collection (citrate vs heparin tubes) and trialling a small concentration of heparin being added to the media to further prevent clot formation.

Figure 1 shows a sample taken from a patient critically ill with sepsis showing expansion of myeloid-derived suppressor cells (MDSCs) compared with healthy controls. Two main subsets are identified:

- Granulocytic MDSCs (G-MDSCs): $CD11b^{+}CD33^{+}HLA\text{-}DR^{low/-}CD15^{+}CD14^{-}$
- Monocytic MDSCs (M-MDSCs): $CD11b^{+}CD33^{+}HLA\text{-}DR^{low/-}CD14^{+}CD15^{-}$

Both populations are increased in sepsis, indicating enhanced myeloid activation and immunosuppressive potential.
Figure 2 shows the baseline of healthy volunteer A prior to a moderate shift towards a suppressive phenotype when incubated in critically ill (non-sepsis) serum (Fig. 3) and a significant shift to a gMDSC phenotype when incubated in sepsis serum (Fig. 4).

Limitations:

The small sample size limits the generalisability of these findings.

Discussion

These preliminary findings provide early evidence that mature PBMCs may undergo limited phenotypic changes when exposed to a sepsis-like environment.

These early observations align with existing literature describing PBMC plasticity in oncology.

Conclusion

Peripheral blood mononuclear cells (PBMCs) show early signs of phenotypic plasticity in response to sepsis patient serum. These preliminary observations highlight the feasibility of using in vitro models to study PBMC adaptation and provide a foundation for future studies to investigate the mechanisms and functional consequences of these changes in sepsis.

References

1. Tsantes AG, Parastatidou S, Tsantes EA, Bonova E, Tsante KA, Mantzios PG et al. Sepsis-Induced Coagulopathy: An Update on Pathophysiology, Biomarkers, and Current Guidelines. Life [Internet]. 2023 Jan 28; 13(2). Available from: https://doi.org/10.3390/life13020350