Cytokine-induced chromatin accessibility in whole blood neutrophils links to sepsis transcriptional states

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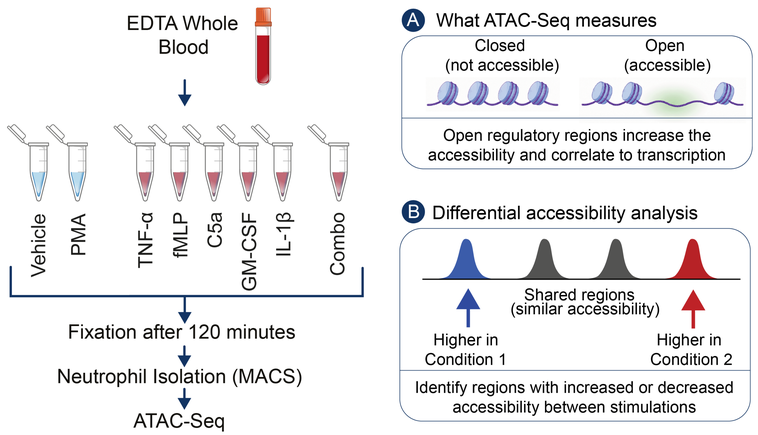
INTRODUCTION

Neutrophils form neutrophil extracellular traps (NETs) through chromatin decondensation and extracellular DNA release. While PMA is commonly used to study NET biology, it bypasses many physiological signaling pathways. In inflammatory diseases such as sepsis, neutrophils are exposed to complex combinations of cytokines, chemokines, complement fragments, and microbial products. How these physiologically relevant inflammatory signals remodel neutrophil chromatin accessibility, and whether these regulatory programs relate to clinical sepsis states, remains poorly defined.

AIM

Define how physiologically relevant inflammatory stimulation remodels neutrophil chromatin accessibility in whole blood and determine whether these accessibility programs correspond to transcriptional states associated with sepsis severity.

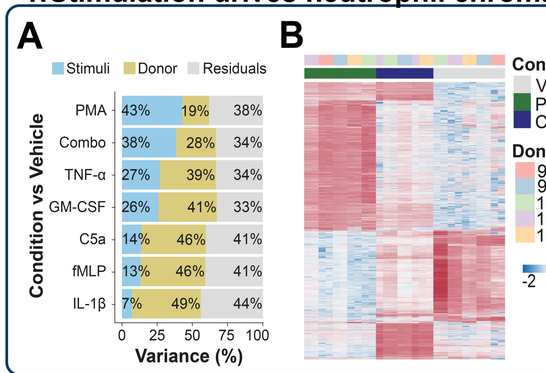
METHOD



Whole blood from healthy donors was stimulated for 120 minutes with vehicle, PMA, individual natural factors, or a combined inflammatory stimulus. Samples were fixed, neutrophils were isolated, and chromatin accessibility was profiled by ATAC-Seq. Differentially accessible regions (DARs) were identified between stimulation conditions and integrated with motif enrichment and sepsis transcriptomic analyses.

RESULTS

1. Stimulation drives neutrophil chromatin changes

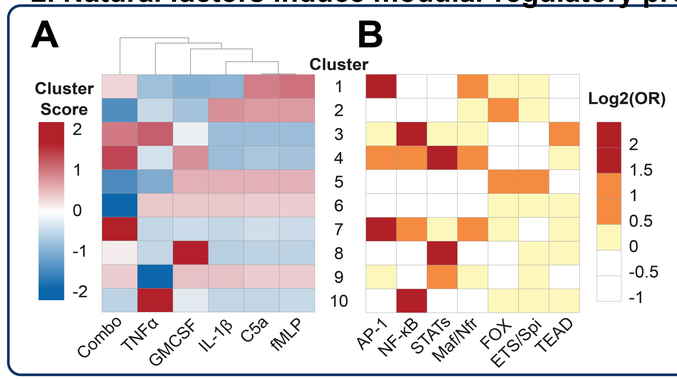


(A) Variance partitioning showed that PMA and Combo explained the largest fraction of chromatin accessibility variation, while individual natural factors (NFs) were more strongly influenced by donor-to-donor variability.

(B) Hierarchical clustering of PMA and Combo DARs showed broad remodeling in both conditions, but with distinct accessibility patterns

Takeaway: PMA and Combo produced the strongest responses, but PMA did not fully recapitulate the Combo response.

2. Natural factors induce modular regulatory programs

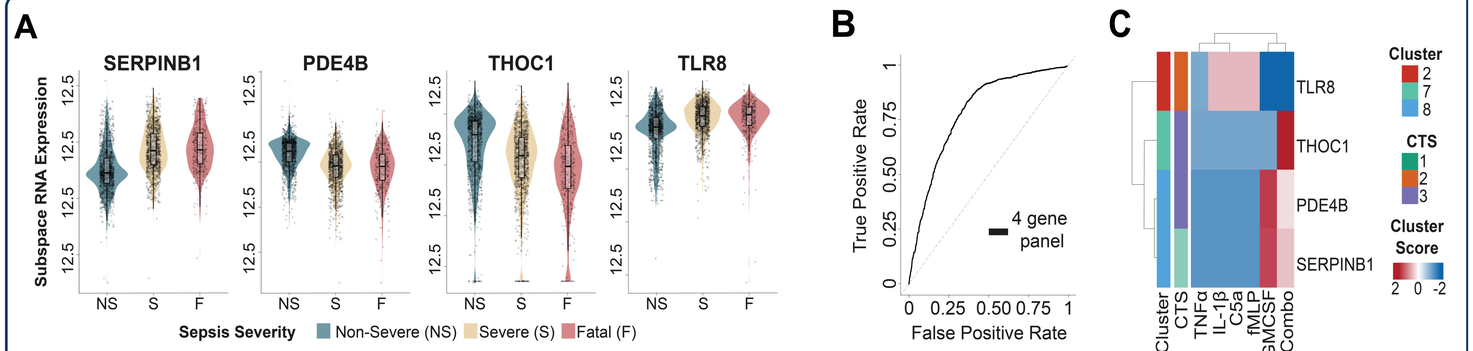


(A) DARs were clustered across Combo and individual NF conditions, revealing modules with distinct stimulus associated accessibility patterns.

(B) Motif enrichment linked the modules to inflammatory transcription factor families, including AP-1 NF-κB, STAT, and CEBP-related programs.

Takeaway: Physiological inflammatory stimulation engages distinct neutrophil regulatory programs rather than a single shared activation state.

3. NF-linked promoter programs map to sepsis transcriptional states



(A) Promoter linked genes from NF responsive accessibility modules showed expression differences across non-severe, severe, and fatal sepsis groupings from the SUBSPACE cohort.

(B) The four gene ROC panel from (3A) showed an AUC of 0.79 suggesting cooperativity of the genes in disease progression

(C) Genes were mapped back to the NF stimulation and chromatin response highlighting cluster and clinical transcription states.

Takeaway: NF induced neutrophil chromatin responses correspond to clinically observed sepsis transcription programs.

CONCLUSIONS

- Whole-blood stimulation enables chromatin accessibility profiling of neutrophils in a physiologically relevant inflammatory context.
- PMA and Combo inflammatory stimulation induced strong but distinct accessibility programs, supporting the need for more physiological stimulation models.
- Individual natural factors (NFs) engage modular regulatory programs linked to AP-1, NF-κB, STAT, and CEBP-related transcription factor activity.
- NF-linked promoter accessibility programs correspond to sepsis-associated transcriptional states and severity-linked gene expression patterns.

Overall: This approach links cytokine-driven neutrophil regulation to clinically observed inflammatory heterogeneity in sepsis.

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