PB0878

Physiologically Relevant NETosis Induction Reveals Distinct Triggers and Chromatin Changes

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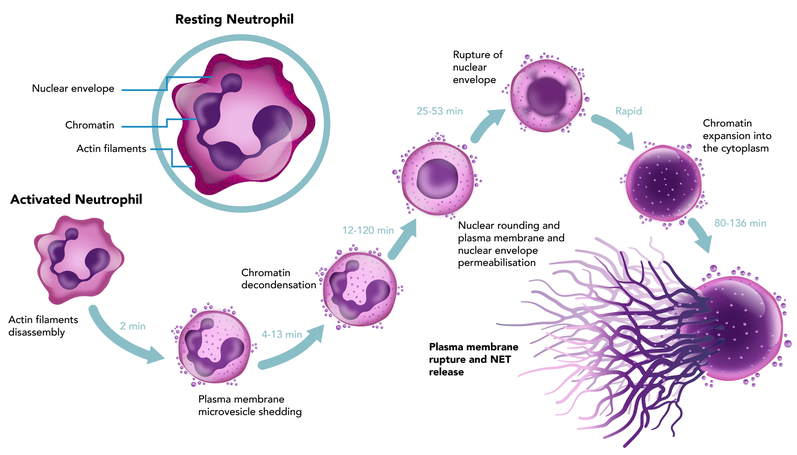
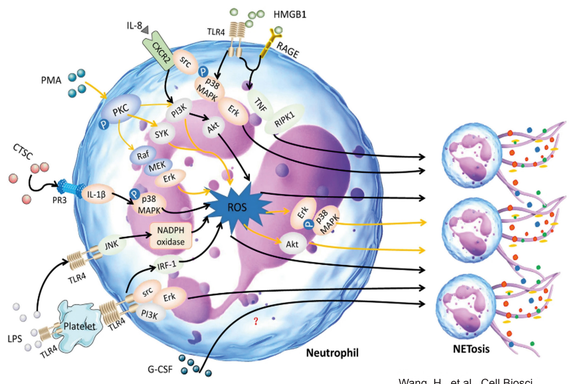
INTRODUCTION

- Neutrophils undergo NETosis in response to pathogen detection
- NETs are a critical part of the innate immune response however NETosis can be pathogenic when not properly controlled
- Dramatic chromatin re-organization and de-condensation precede NET release



AIM

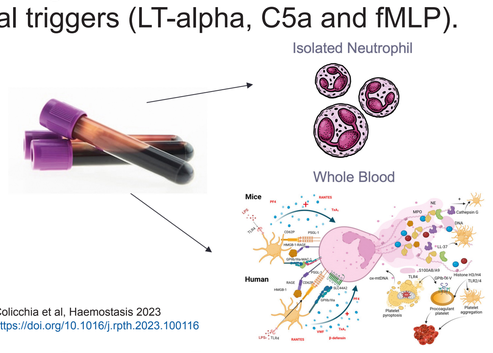
- Compare the ability of a variety of NETosis inducing stimuli to activate NETs in both whole blood and isolated neutrophils
- Determine whether NETosis associated chromatin de-condensation is organized
- Identify chromatin accessibility pattern similarities and differences across multiple NETosis induction environments



Wang, H., et al., Cell Biosci
https://doi.org/10.1186/s13578-021-00708-z

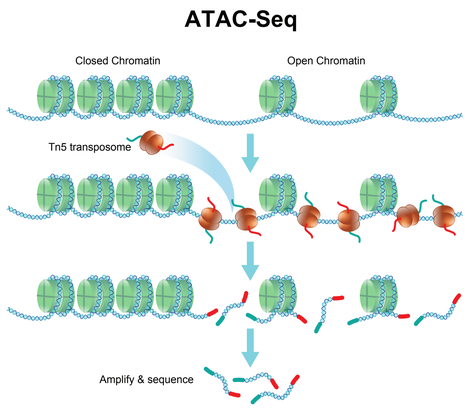
METHOD

- NETosis was induced using PMA, LPS, Calcium Ionophore and a cocktail of natural triggers (LT-alpha, C5a and fMLP).
- NETosis was induced in whole blood and in isolated neutrophils to identify the chromatin changes that occur following direct and indirect stimulation.

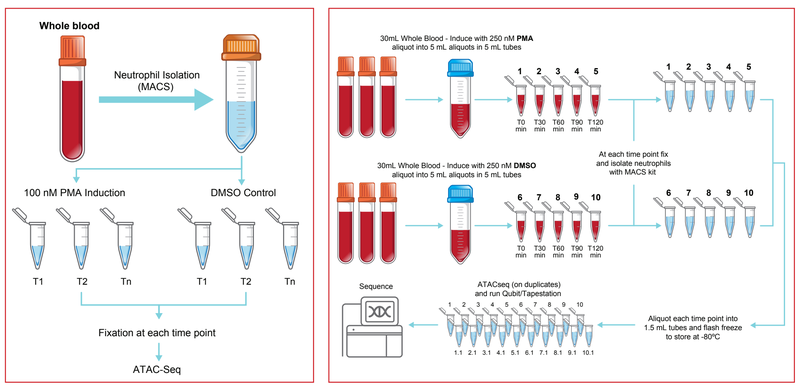


Colicchia et al, Haemostasis 2023
https://doi.org/10.1016/j.rpth.2023.100116

Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) profiles open chromatin regions using transposase mediated insertion of Next-Generation sequencing adaptors



ATAC-seq was performed following induction in isolated neutrophils or in whole blood following by fixation and neutrophil isolation



RESULTS

Different stimuli are required to induce NETs in isolated neutrophils compared to whole blood

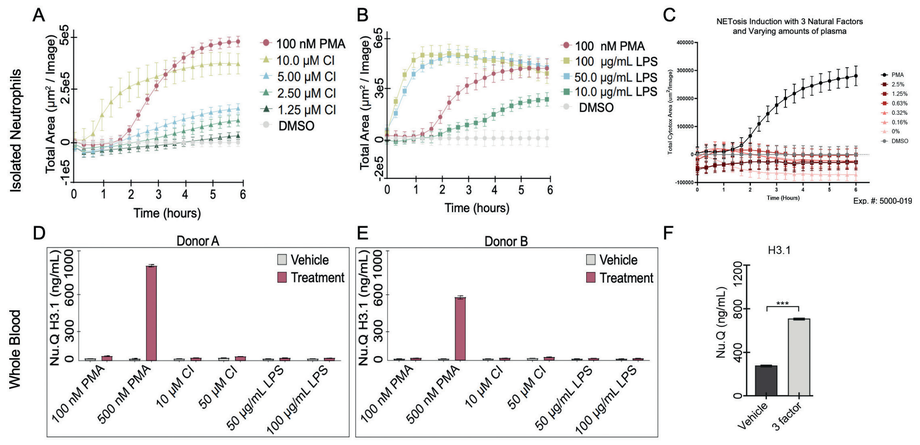


Figure 1: Isolated neutrophils were treated with various concentrations of Calcium Ionophore (CI) (▲) and compared to DMSO (grey ·) and PMA (red ·) (A) and with various concentrations of lipopolysaccharide (LPS) (■) and compared to DMSO (grey ·) and PMA (red ·) (B) . (C) Isolated neutrophils were treated with LT-α, C5a, and fMLP alone and in the presence of various concentrations of plasma. (D & E) Whole blood from two donors were treated with low or high doses of PMA, CI, or LPS and H3.1 Nu.Q® was measured after 4 hours. (F) Whole blood was Plasma was treated with vehicle or a pool of LT-α, C5a, and fMLP and H3.1 Nu.Q® was measured (*** indicates $p < 0.001$).

Chromatin Organization is Consistent with Neutrophils

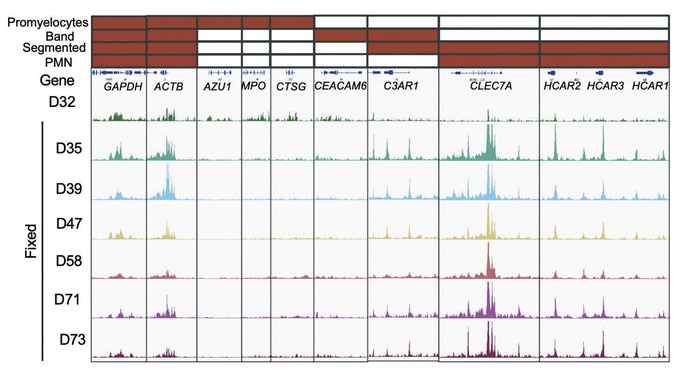


Figure 2: IGV Tracks with healthy donors (n=7), where 1 donor (D32) used standard ATAC-Seq and 6 donors (D35, D39, D47, D58, D71, and D72) used fixed ATAC-Seq. The top bars (red) indicate at which stage of neutrophil development (promyelocytes, band neutrophils (band), segmented, or polymorphonuclear neutrophils (PMNs)) the chromatin would be expected to be open based on H3K4me3 ChIP-Seq (Fridlich, et al. Cell Reports Medicine, 2023). For donor D32 ATAC-seq was performed on unfixed chromatin while the other samples were generated using fixed ATAC-seq.

The chromatin de-condensation that occurs during NETosis is organized

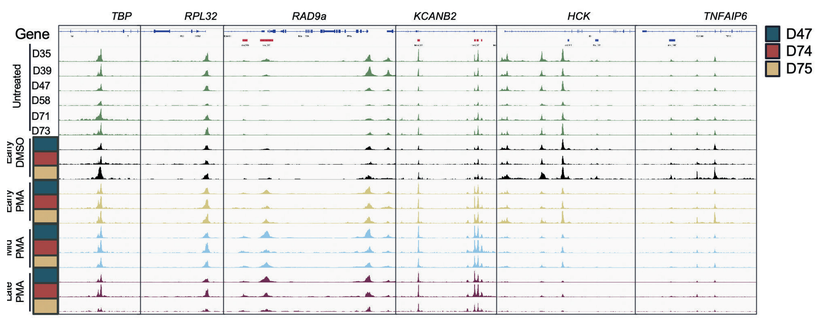


Figure 3: IGV Tracks with untreated healthy donors (green tracks) and PMA or DMSO treated donors at Early (30 min – yellow tracks), Mid (60 min – blue tracks), or Late (90 min – purple tracks). Treated donors D47 (left panel - blue), D74 (left panel - red), D75 (left panel - yellow) are shown at known housekeeping genes: *TBP* and *RPL32*. Examples of gained peaks are shown at the *RAD9a* and *KCANB2* loci. Decreased peak examples are shown at the *HCK* and *TNFAIP6* loci.

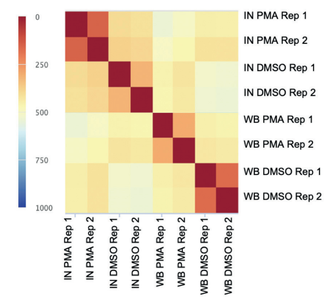


Figure 4: Unsupervised clustering shows similarities in replicates and that isolated neutrophils (IN) and whole blood (WB) samples cluster together followed by treatment condition. Note that separate donors were used for IN and WB which could affect the results.

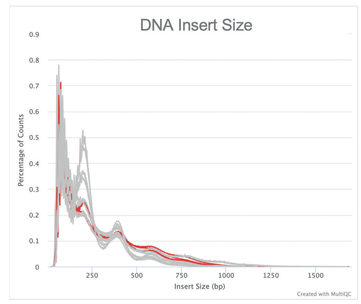
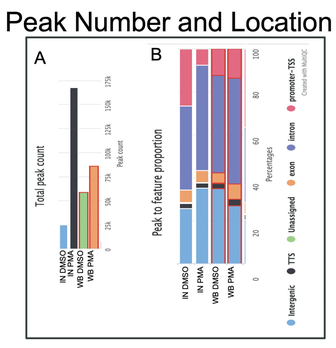


Figure 5:DNA fragment distribution in DMSO (red) and PMA (gray) treated samples. Increased accessibility is seen with PMA treatment as indicated by an increased percentage of reads at the smaller DNA lengths.

Overall chromatin accessibility increases with PMA induction in whole blood and isolated neutrophils with some differential accessibility patterns based on activation environment



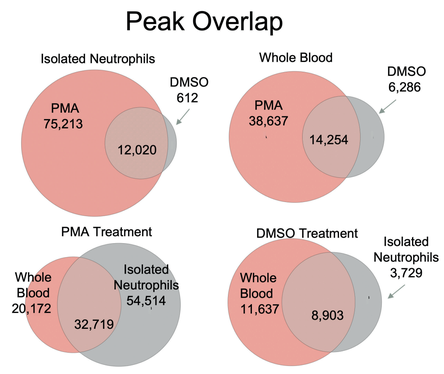


Figure 6: (A) The number of peaks, indicating chromatin accessibility, increased with PMA in both whole blood (WB) and isolated neutrophils (IN) with IN having greater accessibility. Peak distribution across the genome was generally similar with greater differences following NETosis induction in IN compared WB

Figure 7: Venn diagrams indicate peak overlap across conditions. PMA treatment resulted in increased accessibility in both isolated neutrophils (IN) and whole blood (WB) with IN having greater accessibility. There are both shared and unique peaks following PMA and DMSO treatment in WB and IN. Note that separate donors were used for IN and WB which could affect the results.

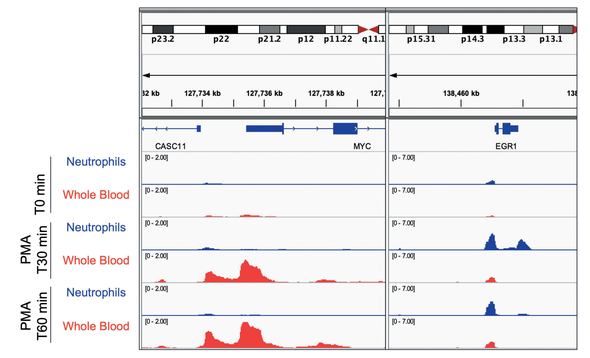


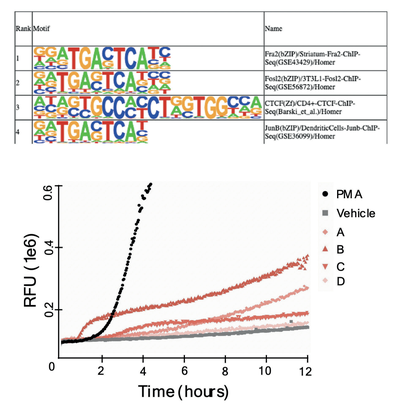
Figure 8: Distinct chromatin accessibility patterns can be seen following NETosis induction in IN and WB: increased accessibility at the MYC promoter when NETosis is induced in whole blood (left) and at the EGR1 promoter in isolated neutrophils (right).

CONCLUSIONS

- Different stimuli are required to induce NETs in isolated neutrophils compared to whole blood
- Chromatin de-condensation occurring during NETosis is organized
- Chromatin accessibility increases with PMA induction in whole blood and isolated neutrophils
- Differentially accessible chromatin patterns are present depending on whether NETosis occurs in isolated Neutrophils or intact whole blood
- Understanding the chromatin de-condensation underlying NETosis could lead to new biomarkers of NETosis sub-types and clinical intervention

FUTURE DIRECTIONS

- Further characterize differential chromatic accessibility patterns (DNA binding motifs)
- Compare isolated neutrophils and whole blood from the same donor
- Correlate accessibility patterns with transcriptomic changes
- Assess chromatin accessibility across a variety of NETosis induction protocols



ACKNOWLEDGEMENTS

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