Chromatin Changes Associated with Neutrophil Extracellular Trap (NET) Formation Reflect Environment

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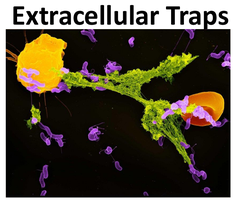
Introduction

- Sepsis is one of the leading causes of death globally and is characterized by a dysregulated immune response
- Neutrophils are in important part of the innate immune system, responsible to sensing the environment and detecting pathogens
- Neutrophils release decondensed chromatin (NETs) to trap pathogens

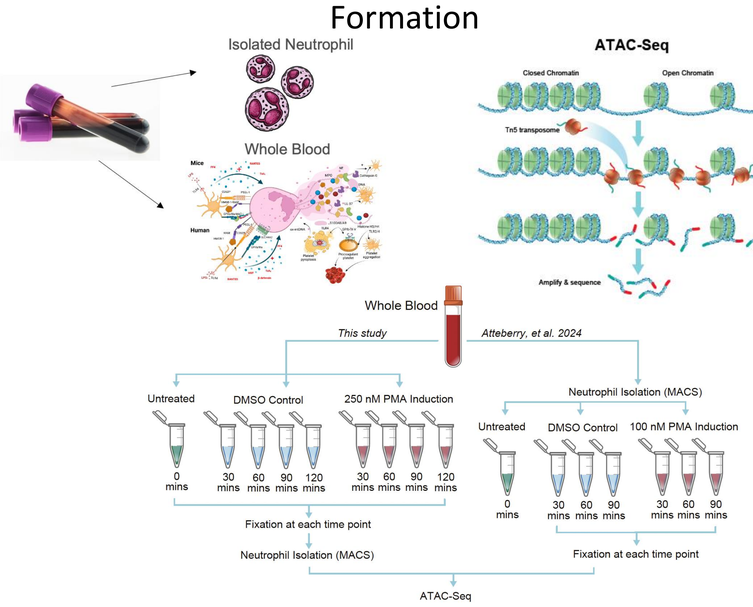
Neutrophil Extracellular Traps



A neutrophil granulocyte (yellow) has ejected a NET (green) to capture bacteria (purple). A red blood cell (orange) is also trapped in the NET. © Volker Brinkmann / Max Planck Institute for Infection Biology

Methods

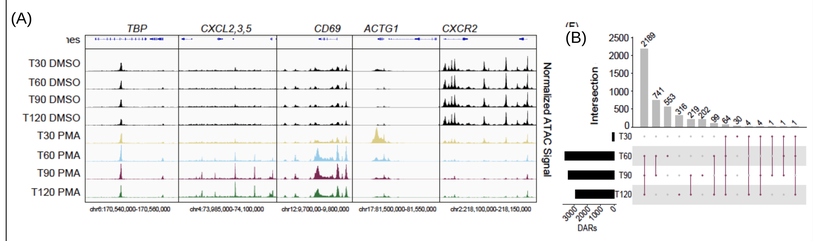
Studying Chromatin Accessibility Changes in Whole Blood and Isolated Neutrophils Undergoing NET Formation



Neutrophils were isolated from whole blood before or after PMA induced NET activation using the MACSexpress Whole Blood Neutrophil Isolation Kit for humans (Miltenyi Biotec #130-104-434).

Results

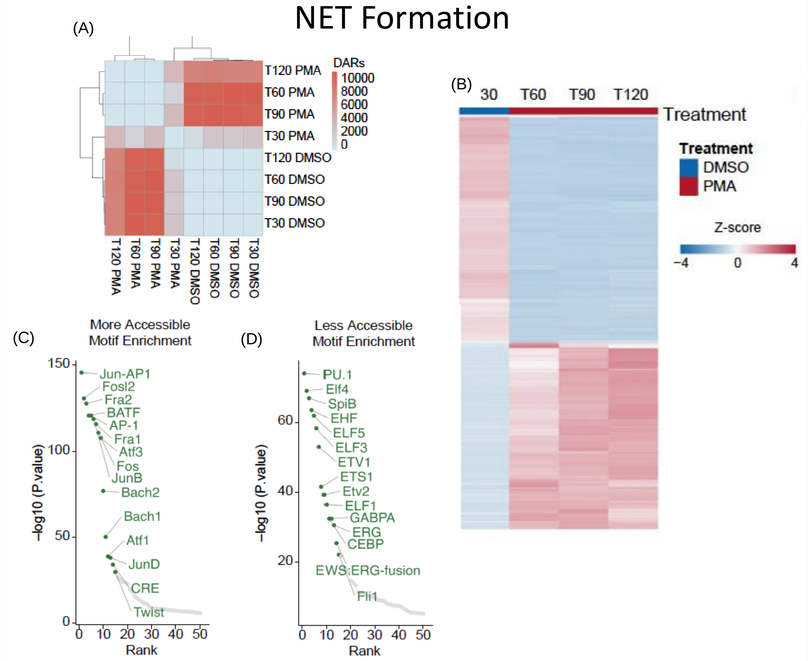
PMA Stimulation Drives a Stable Chromatin Response in Whole Blood Fixed Neutrophils



(A) Merged donor tracks for DMSO treated samples at T30, T60, T90, and T120 minutes (black), T30 PMA (gold), T60 PMA (blue), T90 PMA (purple), T120 PMA (green) at various loci. Accessibility at housekeeping gene TBP, increased accessibility at CXCL2, 3, 5 (T60-T120), bimodal response at ACTG1, and decreased accessibility at CXCR2. **(B)** UpSetR plot of the differentially accessible regions (DARs) at each timepoint versus T30 DMSO (T30, 60, 90, 120 PMA).

Results

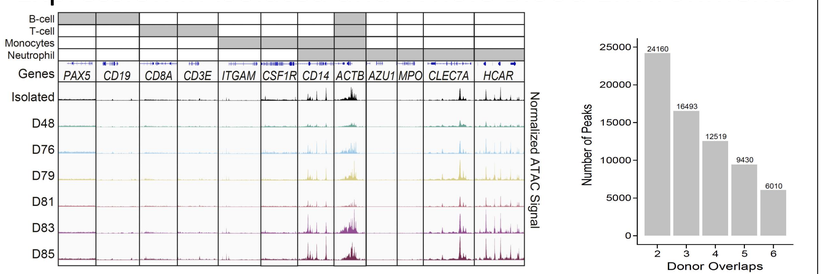
Chromatin Accessibility Changes During PMA Induced NET Formation



(A) Heatmap showing the total number of differential accessible regions (DARs) between all pairwise comparisons (each timepoint for each treatment condition) (DESeq2 p.adj > 0.01 and log2(fold change) less than-1.5 or greater than 1.5). **(B)** Z-score normalized count heatmap of the top 1,000 DARs sorted on DESeq2 p.adj value for T30 DMSO versus PMA at T60, T90, and T120. Treatment is indicated by DMSO (blue) and PMA (red). Columns are organized by treatment and time. Rows are hierarchically clustered. **(C)** HOMER motifs within DARs that gain accessibility in T60-T1120 PMA compared to T30 DMSO are plotted by by-log10(p.value) on the y-axis and the HOMER rank on the x-axis. The top 50 known motifs were graphed, and the top 15 known motifs based on p.value are annotated in green. **(D)** Similar to (E), but DARs that have more accessibility in T30 DMSO compared to T60-T120 PMA are shown.

Results

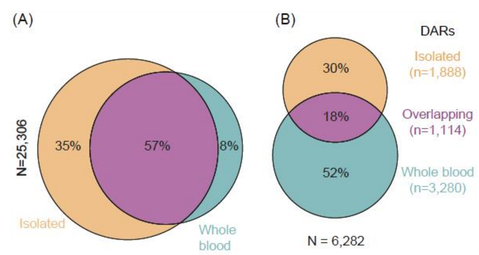
Chromatin Structure Reflects Neutrophil Gene Expression in Isolated and Whole Blood Environments



Merged replicate tracks of untreated healthy donors. Neutrophils isolated prior to fixation (top) and whole blood fixed prior to isolation (D##) are shown below. Grey bars indicate loci specific to different immune cells: B-cell (*PAX5* and *CD19*), T-cell (*CD8A* and *CD3E*), monocytes (*ITGAM*, *CSF1R*, and *CD14*), and neutrophils (accessible regions – *CD14, CLEC7A*, and *HCAR*; inaccessible regions – *AZU1* and *MPO*). The number of peaks that are shared across a given number of n=6 donors.

Results

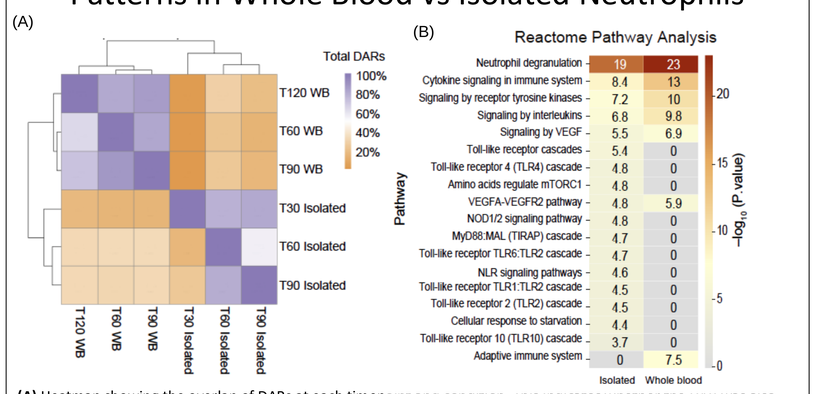
Comparison of Chromatin Accessibility Changes Between Isolated and Whole Blood Fixed Neutrophils



(A) Venn Diagram showing overlaps between consensus peaks of untreated neutrophils (Isolated – orange; Whole blood – teal; Overlapping - purple) (minimum 50% of donors, n=6). Total peaks were 25,306, with percentages for unique and overlapping peaks indicated. **(B)** Venn Diagram of the DARs (DESeq2 q < 0.01) from Isolated (orange), Whole blood (teal), and Overlapping (purple).

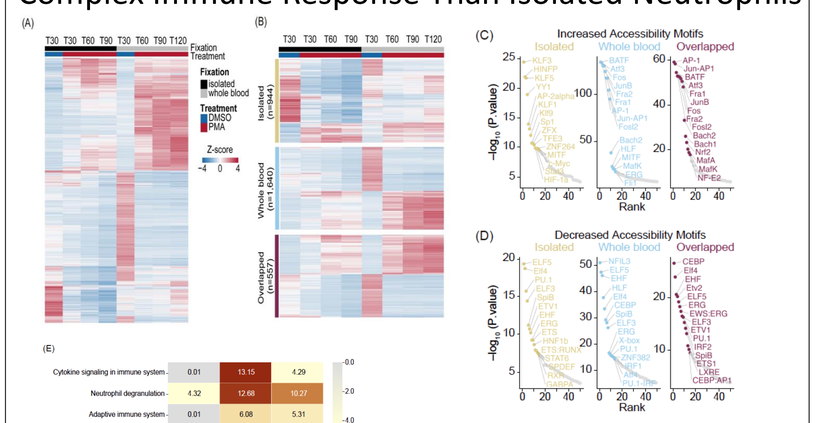
Results

NET Induction Drives Differential Chromatin Accessibility Patterns in Whole Blood vs Isolated Neutrophils



(A) Heatmap showing the overlap of DARs at each timepoint and condition. This indicates whether the DAR was also found at other timepoints between Isolated and whole blood (WB). The diagonal represents 100% of DARs called at each row, and subsequent values within the row represent the percentage of those DARs found. **(B)** Reactome pathway analysis of DAR following PMA induction in isolated neutrophils and whole blood

Whole Blood PMA Induction Leads Reflects More Complex Immune Response Than Isolated Neutrophils



(A) Heatmap of Z-score normalized count for the top DARs (from Isolated, whole blood, and overlapped (n=6,282). T30 DMSO versus PMA at T30 (Isolated - black), T60, T90, and T120 (Whole blood - grey). Treatment is indicated by DMSO (blue) and PMA (red). Columns are organized by treatment and time. Rows were hierarchically clustered and represent different peaks.

(B) Heatmap of Z-score normalized count of the top half DARs in each of the categories sorted on DESeq2 p.adj: Isolated (yellow), Whole blood (blue), and Overlapped (purple). Treatment is indicated by DMSO (blue), and PMA (red) and fixation is indicated by Isolated (black) and Whole blood (grey). Rows were hierarchically clustered. **(C, D)** HOMER motifs graphed by -log10(p.value) on the y-axis and HOMER rank on the x-axis for DARs which gained or lost accessibility in Isolated (yellow), Whole blood (blue), and Overlapped (purple). The top 50 known motifs are graphed, and the top 15 known motifs are annotated. **(E)** Reactome Pathway heatmap showing the -Log10(P.value) for each pathway: Isolated, Whole blood, and Overlapped DARs.

Summary & Conclusions

- Neutrophil chromatin structure is stable across donors and be assessed using whole blood fixed ATAC
- PMA stimulation drives a dynamic chromatin response over time compared to DMSO Controls
- PMA-Induced chromatin accessibility changes in whole blood and isolated neutrophils are similar but distinct
- PMA induction leads to a more complex immune response in whole blood compared to isolated neutrophils

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