Effect of Red Blood Cell Lysis on NET Formation From Primary Human Neutrophils Following Addition of Physiologically Relevant Stimuli

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INTRODUCTION

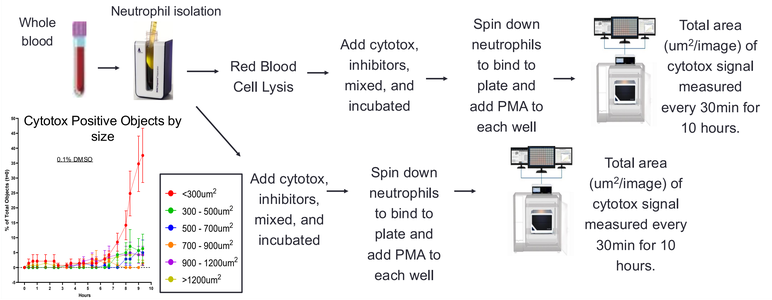
Neutrophils are an important part of the innate immune system, responding to infection in part by releasing chromatin as Neutrophil Extracellular Traps (NETs) to trap pathogens and remove them from circulation. However, when NET formation becomes dysregulated it can be pathophysiological resulting in immunothrombosis. Thus, understanding NET formation and the underlying regulatory mechanisms are important for treating patients with NETs related diseases. Neutrophils are coated with cell surface receptors to sense the extracellular environment and thus isolation and the specific methods employed can impact cellular behavior and response to stimuli. Synthetic factors like PMA can bypass normal signaling and regulatory pathways masking these methodological biases, but they do not faithfully recapitulate clinically relevant NET formation and as a result models using naturally occurring NET inducing factors is critical.

AIM

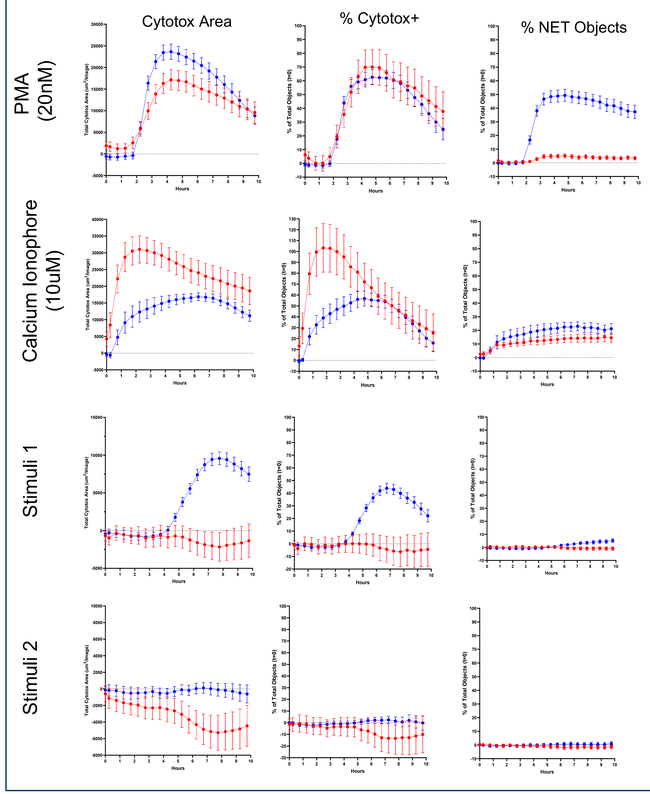
We aimed to understand the impact of neutrophil isolation methods, including the lysis of red blood cells (RBCs), on NET formation from human primary neutrophils in response to synthetic and physiological relevant factors.

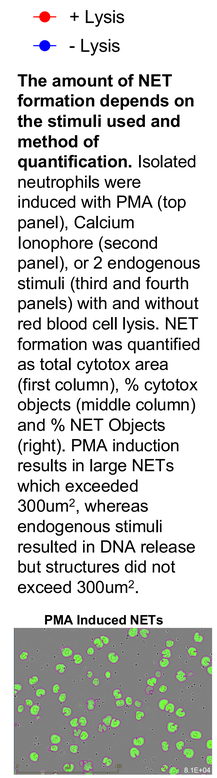
METHOD

- Whole blood is collected from self-declared healthy donors and processed within 1 hour
- Neutrophils were isolated using the MACSxpress® Whole Blood Neutrophil Isolation Kit from Miltenyi
- Red blood cell lysis was preformed by incubating in a hypotonic lysis buffer followed by centrifugation
- Real-time data was collected on the Incucyte Live Cell Analysis System
  Total Cytotox area (um^2): Total area that is positive for cytotox green.
- % Cytotox positive: percentage of objects that are cytotox positive vs t=0.
- % NET Releasing: percentage of objects that are cytotox positive and > 300um^2 vs t=0.



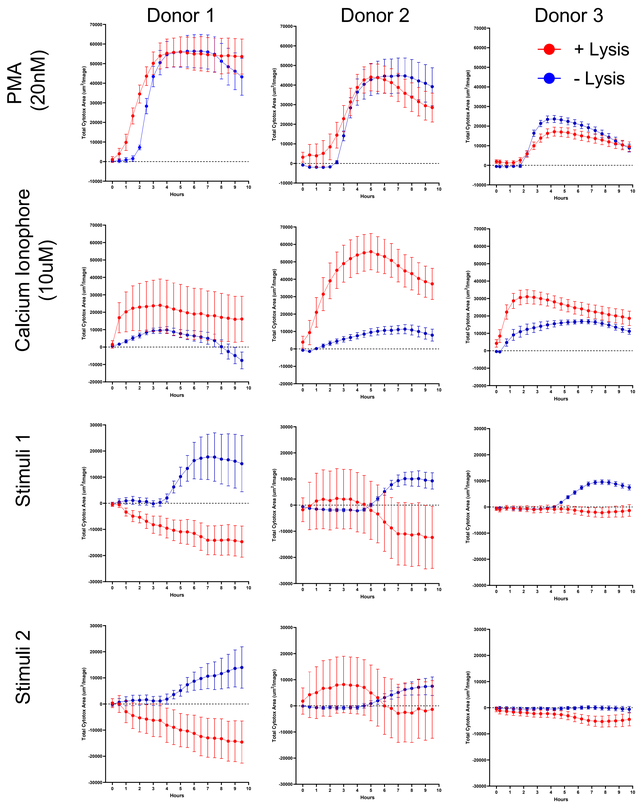
RESULTS





The amount of NET formation depends on the stimuli used and method of quantification. Isolated neutrophils were induced with PMA (top panel), Calcium Ionophore (second panel), or 2 endogenous stimuli (third and fourth panels) with and without red blood cell lysis. NET formation was quantified as total cytotox area (first column), % cytotox objects (middle column) and % NET Objects (right). PMA induction results in large NETs which exceeded 300um^2, whereas endogenous stimuli resulted in DNA release but structures did not exceed 300um^2.

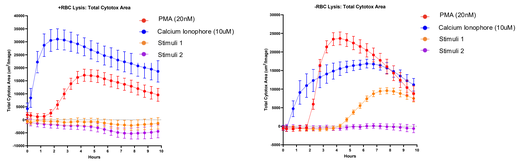
PMA Induced NETs



Red blood cell lysis has minimal effect on PMA induced NETs but impacts NET induction following treatment with Calcium Ionophore and endogenous stimuli. Isolated neutrophils were induced with PMA (top panel), Calcium Ionophore (second panel), or 2 endogenous stimuli (third and fourth panels) with and without red blood cell lysis. NET formation was quantified as total cytotox area. Across conditions PMA consistently induced NET formation across donors, whereas there was more variability in Calcium Ionophore and stimuli and stimuli 2 was consistently unable to induce NET formation, despite reported roles in neutrophil biology.

CONCLUSIONS

- Neutrophil isolation procedures can impact responsiveness of neutrophils to NET inducing stimuli
- Lysing red blood cells can prevent NET formation from some stimuli and alter the time course of other inducing molecules
- Red blood cell lysis has minimal impact on PMA induced NET formation



FUTURE DIRECTIONS

What is the mechanism underlying the impact of red blood cell lysis?

Does RBC lysis affect other aspects of NET formation in addition to DNA release?

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