INDUCTION OF NEUTROPHIL EXTRACELLULAR TRAP (NET) FORMATION BY STORED PLATELETS

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Neutrophil Extracellular Traps (NETs) are networks of DNA, histones, granule enzymes, and antimicrobial proteins that are formed by decondensed chromatin and released by activated neutrophils. Acting like a sieve in the blood, these DNA complexes both capture and kill extracellular bacteria and pathogens. Activated platelets have been shown to induce NET formation in transfusion related acute lung injury (TRALI). Platelets are stored for up to 5 days prior to transfusion. During storage, platelets accumulate Uric Acid, which is a known inducer of NET formation. I predicted the NET inducing activity of stored platelets would increase over storage time. I also predicted that the NET inducing activity would be concentrated in non-cellular fraction (supernatant), and the supplementation with Uric Acid or depletion of Uric Acid would intensify or decrease NET formation, respectively.

To begin to test these predictions, neutrophils isolated from healthy donors were incubated with platelets, plasma, microparticles, and storage solution from Day 1 and Day 5 stored platelet bags. As a positive control, I incubated the neutrophils with LPS, which induces vigorous NET formation. As a negative control, unstimulated neutrophils were used. After incubation, NETs were visualized by staining for extruded DNA using confocal microscopy techniques. In these experiments, supernatant from Day 1 and Day 5 stored platelets was incubated at a 1:1000 dilution with neutrophils. Confocal microscopy indicated that Day 5 supernatant produced more NETs than Day 1 supernatant. This correlated with the hypothesis that increased storage time induces more NET formation. However, in contrast to the interpretation of imaging experiments, quantitative analysis of NETs indicated that Day 1 and Day 5 supernatant similarly induced NET formation.

Going forward, I will explore whether the supplementation of purified Uric acid into storage bags will promote more robust NET formation, and whether depletion of Uric Acid production will reduce NET formation. From these experiments, I will be able determine the extent that Uric acid contributes to the formation of NETs in stored platelets. The results of these experiments have a direct clinical application in devising new strategies to minimize inflammation and adverse events such as TRALI caused by platelet transfusions.
Negative Control
Confocal imaging of neutrophil extracellular traps induced by LPS compared to unstimulated neutrophils. White is nuclear DNA and red is extracellular DNA. Shown is a representative image from 4 independent experiments.

LPS Control

Day 1 Platelet Plasma
Confocal imaging neutrophil extracellular trap induced by Day 1 and Day 5 platelet plasma. White is nuclear DNA and red is extracellular DNA. Shown is a representative image from 4 independent experiments.

Day 5 Platelet Plasma