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Investigating Eryptotic Triggers as Possible Mechanisms for Sepsis-Induced RedBlood Cell Dysfunction

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In sepsis, a number of red blood cell (RBC) defects have been (individually) described: altered oxygen affinity, membrane deformability, enhanced aggregation/adhesion, and dysregulated RBC-based nitric oxide processing. We suggest that these defects comprise a class of organ failure, which we term sepsis-induced RBC dysfunction (SiRD). We propose that SiRD disables oxygen transport to tissues, limiting vital organ respiration, even when oxygen supply is sufficient.

We used a murine cecal ligation and puncture (CLP) sepsis model to explore eryptotic mechanism(s) as potential causes of SiRD initiation. Mice underwent sham (G_{cont}), mild CLP (G_{mild}), or severe CLP (G_{sev}). Day one RBCs were collected from each group and analyzed for: (1) intracellular Ca^{2+} accumulation +/- calcium ionophore (Fluo3-based flow cytometry), (2) phosphatidylserine exposure (annexin V binding - flow cytometry), (3) caspase 3 and μ calpain activation (western blot), and (4) cdB3 proteolysis (western blot).

RBCs from septic mice demonstrated: (1) Increased baseline intracellular Ca^{2+} (G_{cont}: 100% ± 0%, G_{sev}: 133.0% ± 26.9%; $p < 0.05$), (2) Increased sensitivity to calcium ionophore, ionomycin (G_{cont}: 100% ± 0%, G_{sev}: 1826.9% ± 458.2%; $p < 0.05$), (3) Evidence of eryptotic mechanisms: calcium positive cells (G_{cont}: 22.4% ± 12.8%, G_{sev}: 30.4% ± 17.8%; $p < 0.05$) and phosphatidylserine exposure (G_{cont}: 3.9% ± 1.8%, G_{sev}: 6.9% ± 5.5%; $p = 0.12$). (4) Increased caspase 3 activation (G_{cont}: 0.12 ± 0.02, G_{sev}: 0.15 ± 0.003; $p < 0.05$), (5) Increased μ calpain activation (G_{cont}: 1.0 ± 0, G_{sev}: 1.14 ± 0.2; $p < 0.05$), and (6) Increased proteolysis of cdB3 (G_{cont}: 1.0 ± 0, G_{sev}: 0.77 ± 0.1; $p < 0.05$).

Septic RBCs are unable to regulate intracellular calcium, leading to calcium-dependent protease activation and cdB3 proteolysis. We propose that this cdB3 breakdown impairs RBC metabolic control, resulting in EMP activation, limited glucose-6-phosphate availability, and HMP flux constraint. This leads to antioxidant system failure and injury to proteins/lipids key to oxygen delivery homeostasis.