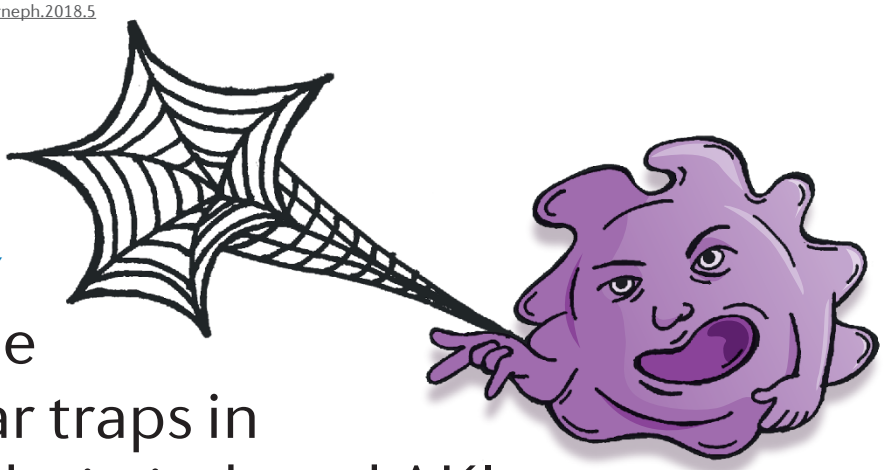
*Nature Reviews Nephrology* | Published online 22 Jan 2018; doi:10.1038/nrneph.2018.5



Carl Conway/Macmillan Publishers Limited

ACUTE KIDNEY INJURY

# Macrophage extracellular traps in rhabdomyolysis-induced AKI

> “Our findings identify METs as a pathogenic mediator of renal damage”

Rhabdomyolysis is a life-threatening condition characterized by the breakdown of skeletal muscle in response to injury. Acute kidney injury (AKI) is a major complication of this disorder, but the molecular mechanisms by which rhabdomyolysis causes AKI are unclear. New research demonstrates that haem-activated platelets from necrotic muscle induce the production of macrophage extracellular traps (METs), which contribute to AKI in the context of rhabdomyolysis. “Our study reports an unanticipated role for METs and platelets as a sensor of myoglobin-derived haem in rhabdomyolysis-induced AKI,” explains Junichi Hirahashi. “To our knowledge this is also the first demonstration that METs can contribute to disease,” he adds.

Extracellular traps (ETs) are a mesh-like network of chromatin fibres containing DNA, histones and granule content that can be extruded from neutrophils, monocytes and macrophages. Neutrophil extracellular traps (NETs) are the best studied of these and are known to contribute to host defence, inflammation and autoimmune disease. The finding that activated platelets promote NET formation and that haem-induced NETs contribute to the pathogenesis of sickle cell disease led Hirahashi and colleagues to hypothesize that haem released during rhabdomyolysis might activate platelets and induce the release of METs to cause kidney injury.

Using a mouse model of rhabdomyolysis induced by intramuscular injection of glycerol, the researchers first confirmed the presence of ETs within damaged renal tubules. The release of ETs involves citrullination of histones by protein-arginine deiminase type 4 (PAD4); pharmacological or genetic inhibition of PAD4 led to reduced levels of ETs and attenuated renal dysfunction in glycerol-treated mice. Depletion of macrophages and platelets, but not neutrophils, reduced extracellular DNA release in these mice, suggesting that macrophages and platelets contribute to rhabdomyolysis-induced ET release and AKI.

Integrin αM (also known as macrophage antigen 1; MAC1) is a β2 integrin expressed on leukocytes that binds platelets via the platelet glycoprotein subunit GPIbα or intercellular adhesion molecule 2 (ICAM2) and regulates platelet-induced NET formation. To assess the contribution of MAC1 to MET formation, Hirahashi and colleagues assessed the effects of muscle injury in *Mac1*$^{-/-}$ mice. Despite a similar level of skeletal muscle destruction to that of wild-type mice, *Mac1*$^{-/-}$ mice were protected against glycerol-induced ET formation and kidney injury, demonstrating that MAC1 contributes to rhabdomyolysis-induced AKI. In vitro, stimulation of macrophages with haem or iron-activated platelets induced the production of reactive oxygen species (ROS) and MET formation, whereas direct stimulation with haem, iron or resting platelets had no effect. Inhibition of MAC1 activity, iron, ROS or PAD activity attenuated the ability of activated platelets to induce MET formation, suggesting that activation of MAC1 by haem-activated platelets triggers the generation of ROS and histone citrullination leading to MET formation. Further studies demonstrated that these actions require cell–cell contact between platelets and macrophages.

To assess the relevance of their findings to humans, Hirahashi and co-workers examined the presence of METs in six patients with rhabdomyolysis. Indices of ETs were higher in the plasma of patients with rhabdomyolysis resulting from a crush injury than in patients with rhabdomyolysis resulting from a burn injury, viral infection or another cause.

Finally, to assess the therapeutic potential of targeting this pathway, the researchers assessed the ability of lactoferrin — a glycoprotein that inhibits NETs — to prevent MET formation. Lactoferrin treatment inhibited MET formation in vitro and attenuated MET formation and renal damage in mice with glycerol-induced rhabdomyolysis. “Many macrophage-dependent inflammatory diseases exist, and we believe that some of these diseases may be MET dependent,” says Hirahashi. “Our findings identify METs as a pathogenic mediator of renal damage and also identify this pathway as a therapeutic target for the prevention of rhabdomyolysis-induced AKI.”

*Susan J. Allison*

**ORIGINAL ARTICLE** Okubo, K. et al. Macrophage extracellular trap formation promoted by platelet activation is a key mediator of rhabdomyolysis-induced acute kidney injury. *Nat. Med.* https://doi.org/10.1038/nm.4462 (2018)