SYSTEMIC SCLEROSIS

HMGB1$^+$ platelet microparticles damage the endothelium

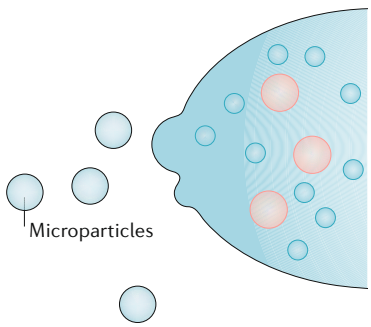
> HMGB1 is already known to activate neutrophils to release neutrophil extracellular traps

New research published in *Science Translational Medicine* confirms a mechanism by which platelet-derived microparticles contribute to vasculopathy in systemic sclerosis (SSc).

SSc, also known as scleroderma, is a debilitating autoimmune disease characterized by collagen deposition that damages and thickens the skin. Another hallmark feature of SSc is endothelial damage. Platelets from patients with the disease are in a constitutively active state and release (partly by microparticles) a variety of vasoactive growth factors as well as the damage-associated molecular pattern (DAMP) high-mobility group box 1 (HMGB1).

"This paper is the third part of a long-standing project," says Norma Maugeri, one of two corresponding authors of the new article. "In the first report, we demonstrated the presence of a high number of platelet-derived microparticles expressing HMGB1 in blood from patients with SSc, and then in the second we demonstrated that HMGB1 expressed on platelet microparticles is biologically active."

HMGB1 is already known to activate neutrophils to release neutrophil extracellular traps (NETs).



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Although NETs are central to the pathogenesis of systemic lupus erythematosus (SLE), less is known regarding neutrophil functions in the pathogenesis of SSc.

In the new study, the researchers used an anti-CD61 antibody to label and subsequently isolate platelet-derived microparticles from 53 healthy donors, 6 patients with SLE (as a disease control) or 57 patients with SSc.

"We are fascinated by the fact that each of the platelets, which do not have a nucleus, contain approximately 1 ng of HMGB1," adds Angelo Manfredi (the other corresponding author) in reference to the SSc-derived platelets and his new data that reconfirm that HMGB1$^+$ platelet-derived microparticles are more abundant in patients with SSc than in the other groups tested.

The researchers also co-cultured the microparticles with neutrophils from healthy donors. In these experiments, the microparticles from the patients with SSc were the strongest at activating fibronolytic activity and autophagic flux, as measured by redistribution of myeloperoxidase to the neutrophil surface and by confocal microscopy of autophagocytic vacuoles and LC3 expression. NET formation was also greatest in neutrophils stimulated by the SSc platelet-derived microparticles.

The researchers show that the bioactivity of these cultured microparticles and their effect on neutrophils is mirrored in the patient data; as in vitro stimulated neutrophils, neutrophils circulating in patients with SSc also seem to be more autophagic than neutrophils from healthy donors.

Importantly, the researchers then injected the various platelet-derived microparticles into immunodeficient NSG mice to see if they could recapitulate the pathology of the human donors.

"The mice displayed some of the characteristic features of SSc, such as damage to the microvasculature," says Maugeri referring to the mice that were injected with SSc microparticles. "And the endothelial damage was followed by increased leukocyte infiltration in the lungs, which is associated with pulmonary fibrosis," adds Manfredi. "More work is needed," he says "We think that blocking the production of platelet-derived microparticles, or the effect of the HMGB1 they carry, could be a therapeutic approach to limit disease progression, or even reduce the clinical manifestations of SSc."

Some evidence for this potential is provided within the new article. The researchers were able to neutralize most of the microparticle-induced mouse pathology by pre-treating the microparticles in vitro with a competitive antagonist of HMGB1 called BoxA.

Although development of a targeted therapeutic might be a long time coming, the researchers hope their new data can inform clinical management of patients with SSc. To this end, Maugeri says, "The next step is to define if HMGB1$^+$ platelet-derived microparticles can be used as a biomarker of disease activity or progression."

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ORIGINAL ARTICLE Maugeri, N. et al. Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis. *Sci. Transl Med.* **10**, eaao3089 (2018)