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NEIGHBORHOOD WATCH

Megakaryocytes listen for their progeny's progeny during inflammation

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INTRODUCTION

Platelets are the smallest circulating cells in the blood and their primary physiological role is to maintain the integrity of the vasculature and mediate the arrest of bleeding. In addition to hemostasis, however, platelets also can elicit important non-hemostatic immunological functions.[1,2] This is due the platelets' capability to express and secrete a horde of immune molecules that are capable of regulating immune responses.[3] It now appears that platelets may receive these traits from their parent cells, the megakaryocytes (MKs).[3,4] For example, both platelets and MKs express the entire proteasome system, including transporter associated with antigen processing (TAP) molecules.[5] Platelet activation can lead to expression of nascent major histocompatibility complex (MHC) class I molecules, which are capable of presenting antigens to CD8+ T cells.[6] Activated platelets were shown to present malarial peptides to malaria-specific T cells resulting in enhanced immunity against the parasite.[6] These activities have also been shown in MKs: mature MKs were able to uptake exogenous protein antigens and process and present their peptides on MHC class I molecules, which effectively triggered antigen-specific $CD8^+$ T cell activation in vitro and in vivo.[7] Of interest, it was demonstrated that the MKs were able to transfer immunologic MHC class I/peptide complexes to their progeny, the platelets, and this correlated to their ability to present self-antigens and mediate immune thrombocytopenia.[7]

During acute infections and inflammation platelets can be extensively consumed resulting in life-threatening thrombocytopenia. The biological mechanisms responsible for rapid regeneration of circulation platelets under these conditions are incompletely understood. Previously, it was shown that that the hematopoietic stem cell (HSC) compartment contains stem-like MK-committed progenitors that are quiescent during homeostasis but in response to acute inflammation can become activated and undergo maturation resulting in increased platelet production that replenishes the platelet pool.[8] What exactly triggers this rapid hematopoietic switch toward emergency platelet production from MKs has not been thoroughly investigated. Recently, however, French et al have shed some light on this issue by demonstrating that under inflammatory conditions platelet-derived extracellular vesicles (EVs) are able to infiltrate the bone marrow and cause reprogramming of MKs.[9]

Platelets have the striking ability to produce EVs and these have been shown to transport cargo to distant sites and can affect various cell types.[10-12] For example, several inflammatory autoimmune diseases including immune thrombocytopenia (ITP),

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rheumatoid arthritis, and systemic lupus erythematosus are associated with platelet-derived EVs.[13-15] Platelet-derived EVs are small (<1 μm) fragments that appear to be derived from platelets because of their expression of platelet-specific markers such as CD42 and CD41.[16] The exact mechanism of their production is unknown but in ITP most are generated from the platelet destruction process, as a subset of them also co-express monocyte markers such as CD14 and CD80 that are the result of platelet–leukocyte interactions.[13] For example, Boilard et al[14] elegantly demonstrated that platelets may be essential for the progression of inflammatory arthritis. They identified platelet glycoprotein VI and the Fc receptor γ-chain as activators for platelet EV production in patients with rheumatoid arthritis and EV shedding appeared to be stimulated by fibroblast-like cells in the joint cavity.[14] It was also shown that platelet-derived EV-associated interleukin (IL)-1 appeared to be a significant factor in intensifying the inflammation associated with the rheumatoid joint.[14] These studies suggest that not only are platelet-derived molecules, eg, IL-1, important for disease progression but platelet-derived EVs may act as transporters of inflammatory mediators to sites of inflammation and tissue damage. MKs are also able to produce EVs, but unlike platelet-derived EVs, they are far less studied and currently, there is little evidence supporting that they convey a pathogenic role.

French et al hypothesized that in a setting of inflammation, platelet-derived EVs are generated from activated platelets and that these EVs travel between the circulation and the bone marrow compartment where they may interact with MKs and MK-precursor cells. To test this, the authors used mice deficient in the thrombopoietin (TPO) receptor (MPL) and infused them with wild-type (WT) MPL+ donor platelets and then tracked them in vivo. Subsequently, lipopolysaccharide (LPS) was introduced to induce systemic inflammation. Using immunohistochemistry and flow cytometry of the bone marrow, a small but significant MPL+ staining could be observed throughout and quantified. These platelet-like particles were only significantly present under conditions of LPS-induced inflammation and comprised up to 8.3% of all bone marrow events. This indicated a significant infiltration of platelet-like particles into the bone marrow under systemic inflammatory conditions. In addition to LPS-induced inflammation, the authors also confirmed these findings in a setting of autoimmune-induced inflammation triggered by infusion of heat-aggregated immunoglobulin G (IgG) into FcγRIIa-transgenic mice.[17] It was also confirmed that these platelet-derived EVs were present in the human bone marrow. Focusing more on the nature of the platelet-derived EV interaction with bone marrow cells, it was next observed that the EVs bound to bone marrow–resident CD41+ cells in vivo. Further characterization of this interaction using confocal microscopy showed that the platelet-derived EVs interacted with and were taken up by CD41+ MKs in vitro and ex vivo.

An important next question addressed was if these interactions had any potential functional consequences. They hypothesized that the platelet-derived EVs may reprogram hematopoietic cells. To examine this they extracted bone marrow cells from c-MPL-/- mice and co-cultured them with WT-type fluorescent MPL+ platelet-derived EVs, with or without the additional presence of TPO. Interestingly, after 72 hours, they observed an increased presence of large cells only in the platelet-derived EV-treated groups, regardless of the presence of additional TPO. Most notable was that these large cells were fluorescent, suggesting that they had communicated with the bound and internalized EVs. Importantly, these large cells could be further identified to be bona fide MKs (and not aggregates of EVs), indicating that functional reprogramming by platelet-derived EVs may have occurred that stimulated megakaryopoiesis in the bone marrow.

This study importantly uncovers a novel mode of communication between the plasma and the bone marrow compartment under inflammatory conditions, via platelet-derived EVs that target MKs functionally reprogramming to enhance megakaryopoiesis. As with many seminal articles, several new questions have arisen from this work. How do the platelet-derived EVs infiltrate the bone marrow? What signaling is involved in this process that allows the plasma EVs to respond to the emergency signals in the bone marrow? A similar study recently demonstrated that human MK-EVs could also induce de novo platelet production in WT mice.[18] In that study, biodistribution experiments revealed that MK-EVs not only localized to the bone marrow but also to the lungs and liver. The mechanisms driving the homing of MK- and platelet-derived EVs to the bone marrow and other organs will be important to analyze in future studies and whether this is altered by different inflammatory conditions. These insights may also have important implications for delivering therapeutics to the bone marrow under inflammatory thrombocytopenic conditions.

Another important question is how the platelet-derived EVs exactly trigger the functional reprogramming of bone marrow MKs. Platelet- (and MK)-derived EVs can carry a diverse cargo, which is used to convey functional effects. This EV cargo can include diverse cytokines, chemokines, mitochondria, lipids, and transcription factors.[1] It will be imperative to identify the specific EV cargo that is responsible for triggering megakaryopoiesis and platelet production. This cargo could be perhaps be the MPL receptor; however, in the experiments conducted by French and colleagues the effect of restoring megakaryocytes by platelet-derived EVs was not dependent on the presence of TPO in the culture media indicating that the EV cargo is likely another factor independent of the MPL receptor.

In summary, French et al report a novel mechanism in which platelet-derived EVs are able to penetrate the bone marrow compartment and engage with bone marrow cells resulting in functional reprogramming of bone marrow MKs to stimulate megakaryopoiesis. The mechanisms and conditions driving the homing of EVs from plasma to the bone marrow, as well as the identity of the specific cargo carried by the EVs, will need to be analyzed in future studies.

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

RK wrote the original draft JWS provided financial resources and edited the manuscript.

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