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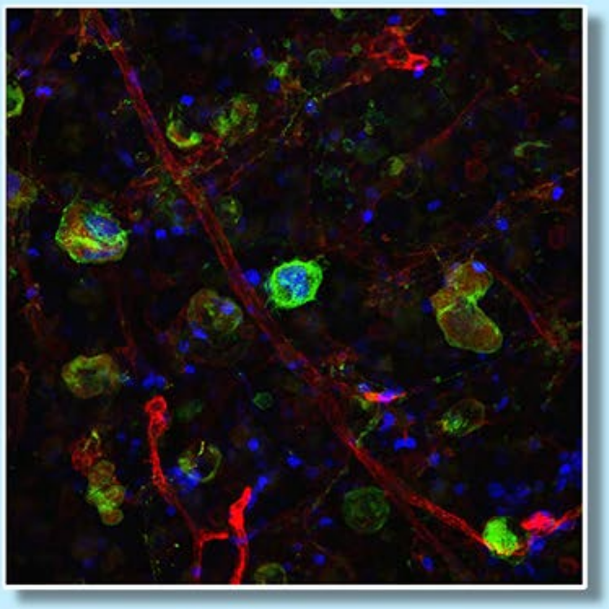

Platelets being produced in bone marrow
Red: blood vessels, blue: nuclei, large green: megakaryocytes, small green: platelets

Flow Cytometric Quantification of RASA3 and CalDAG-GEFI to Determine Platelet Reactivity in Health and Disease

Platelets play a critical role in the body's response to vascular injury (hemostasis), but they also contribute significantly to blood coagulation (thrombosis) associated with cardiovascular disease. The small GTPase RAP1 in platelets is an essential component of the thrombotic pathway as it controls the adhesive state of platelets in circulation and at sites of vascular injury. The guanine nucleotide exchange factor, CalDAG-GEFI, and the GTPase-activating protein, RASA3, are key regulators of RAP1 and balance its activation state. Mice that are deficient in CalDAG-GEFI bleed due to impaired platelet activation and adhesion. Mice lacking RASA3 are thrombocytopenic (low platelet count) due to premature platelet activation and clearance. We hypothesized that interindividual variability in the antagonistic balance between CalDAG-GEFI and RASA3 may predict the risk for thrombosis or thrombocytopenia-induced bleeding. This study aimed to establish an assay for the rapid quantification of CalDAG-GEFI and RASA3 expression in platelets. We performed flow cytometric quantification of intracellular immunofluorescence signals to measure CalDAG-GEFI and RASA3 expression in mouse platelets that were wild-type, heterozygous, or null for RASA3 or CalDAG-GEFI. In parallel, we quantified CalDAG-GEFI and RASA3 expression in these platelets by Western blotting, which can be used to assay protein levels as well but is time-consuming and not currently viable as a clinical test. Our results validate flow cytometry as a powerful technique to quantify RASA3 and CalDAG-GEFI expression in mouse platelets, as results obtained by flow cytometry strongly correlated with those obtained by Western blotting. These results indicate the potential viability of flow cytometry as a rapid clinical tool to identify the biochemical basis of platelet-mediated bleeding disorders.