Evidence for long-lived tissue-resident macrophage progenitor cells that are resistant to total body irradiation has been reported. Development of methods to engraft the human iPSC-derived JMML-like cells in an optimized immunodeficient mouse model system (see figure) may assist in examining some of these exciting new questions.

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How low can you go?

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In this issue of Blood, Morowski et al use a mouse model of thrombocytopenia to determine the lowest platelet count needed to support thrombosis in a range of thrombosis challenges.1

How many platelets are needed for hemostasis? Are low platelet counts protective from thrombosis? Why do immune thrombocytopenia (ITP) patients have myocardial infarctions (MIs) and strokes? Why do some patients bleed and others do not at comparably low platelet counts? As anyone who has formulated guidelines or is in clinical practice knows, there is considerable empiricism but little actual data available to answer these questions.

The answer: relatively few platelets are necessary for thrombosis. The authors asked the seemingly simple question of how low can the platelet count fall in a mouse before interfering with common models of thrombosis. Thrombosis of small, medium, and large vessels remained intact at platelet counts down to 10% to 30% of normal. Mice ultimately need <2.5% of their normal platelet number to maintain the most basic level of hemostasis.

The authors used antibody-mediated clearance of platelets from circulation to induce thrombocytopenia. The anti-GPIb antibody they used binds specifically to platelets and induces their clearance through FcR-mediated uptake. They calibrated the dose of antibody needed to decrease the platelet count by a specific percentage and then induced different degrees of thrombocytopenia for the thrombosis challenges.

The models of thrombosis spanned the size and flow spectrum, including aortic and carotid artery injury, a small arteriole ischemia-reperfusion model of stroke, and the very low-flow tail-snip bleeding time. Aortic thrombosis remained intact at platelet counts down to 30% of normal. As the vessels tested became smaller, the platelet count needed for thrombosis decreased. The carotid artery injury model continued to thrombose normally down to a platelet count of 20% normal. A model of ischemia-reperfusion stroke injury that is known to be platelet dependent continued to thrombose normally down to platelet counts of only 10% of normal. Finally, the tail-snip bleeding time, roughly equivalent to the human bleeding time, remained normal until platelet counts fell to >97% below normal.

Although there are numerous factors contributing to thrombosis, it is notable that as shear stress increased from aorta to carotid to arteriole, the efficacy of the platelets to form thrombi increased as well (ie, fewer platelets or lower platelet density was required). In the rheological model of blood vessel flow, laminar flow with high shear rate pushes platelets into a marginal zone next to the endothelium, whereas red cells populate the center of the vessel.2 In the current study, higher shear flow appeared to correlate with more efficient thrombus formation. Factors in addition to the platelets themselves appeared to play a larger role in the bigger arteries. Thus, the “flavor” of the thrombus may change depending on the size and flow characteristics of the blood vessel. These phenomena could potentially be tested in some of the newly developed ex vivo flow systems using endothelialized microfluidics devices.

Another interesting phenomenon was the appearance of a population of young platelets after destruction of the majority of circulating platelets. The authors showed that this population did not alter the overall platelet function. However, if at low platelet counts platelet function trumps number (as Karpatkin showed many years ago),3 these young platelets may play a disproportionate role in maintaining hemostasis. An important factor that may impact this model is the likely large release of highly thrombogenic platelet microparticles on immune platelet destruction.4 The authors did use a second immune model but did not explore thrombocytopenia induced by other means, ie, hypoplasia, in which the level of microparticles would be expected to be far less. These issues become important when comparing bleeding and clotting tendencies in ITP patients, who have elevated numbers of young platelets (and microparticles), with chemotherapy patients, who do not. At least 1 study supports the notion that ITP patients bleed less than chemotherapy patients with the same platelet counts presumably because of the increased function of young platelets.
What are the corresponding thrombosis models in humans? Empirically, overall hemostasis requires very few platelets. In humans receiving chemotherapy before the availability of platelet transfusions, spontaneous bleeding such as intracranial hemorrhage and gastrointestinal bleeding did not occur until platelets were \(<5000/\mu\text{L}\).\(^5\) A certain number of platelets, \(\sim7000\) to \(10\,000/\mu\text{L}\), are needed just to maintain vascular integrity in humans.\(^6\) ITP patients generally do not spontaneously bleed beyond the skin even with platelet counts \(<10\,000/\mu\text{L}\). What is less clear is how many platelets are required to have a pathologic clot resulting in a stroke or heart attack. Clinicians are often faced with the challenge of managing a patient on anticoagulation who is also thrombocytopenic: at what (if any) platelet count does the risk of thrombosis recede?

Taking \(300\,000/\mu\text{L}\) as the average platelet count, the corresponding platelet counts of \(30\%\), \(20\%\), and \(2.5\%\) are \(90\,000/\mu\text{L}\), \(60\,000/\mu\text{L}\), and \(\approx10\,000/\mu\text{L}\). These numbers are in remarkably good agreement with general platelet transfusion guidelines. Platelets are generally recommended to be \(80\,000\) to \(100\,000/\mu\text{L}\) for major surgery (think aorta). Anticoagulation is generally held for platelet counts \(<50\,000/\mu\text{L}\) (think carotid and ischemia-reperfusion). Meanwhile, chemotherapy patients are generally transfused and children with ITP treated to keep platelets \(>10\,000/\mu\text{L}\).

The studies reported here in mice correlate nicely with clinical observations in humans, and interesting physiological implications can be drawn from these observations. For example, this model implies that bleeding phenotypes in the inherited thrombocytopenias, especially Wiskott Aldrich, are due to the functional defects of the platelets beyond the low numbers, similar to the effects of uremia or in ITP following nonsteroidal anti-inflammatory drug ingestion. Although development of animal models of thrombosis has had limited success thus far, with humanized mice important to this effort, the findings reported here encourage us to believe that this model will be useful to better understand factors affecting the balance of thrombosis and hemostasis: inflammation,\(^7\) microparticles, flow, and newborn platelets,\(^8\) to name a few. Furthermore, studies at the tipping point of thrombosis may inform the field of anticoagulant and antiplatelet therapies, as well as therapies to improve hemostatic function.

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