of a model to evaluate platelet granule trafficking. Granule trafficking is notoriously difficult to study in megakaryocytes. The fact that these studies were performed in MEG-01 cells, a megakaryoblastic leukemia cell line, will raise questions of their validity in bona fide megakaryocytes. The authors anticipate this concern and, to their credit, take great pains to compare the MEG-01 cells with megakaryocytes. They show that morphologically, dense granule formation in MEG-01 cells resembles that of immature megakaryocytes. They recapitulate key aspects of their model in megakaryocytes. Thus, there is good reason to believe that the MEG-01 model is highly relevant to megakaryocytes.

A manipulable megakaryocytoid granule trafficking model could help answer several recalcitrant questions in platelet granule biology. The majority of known genetic defects of granule formation result in either loss of dense granules or vice versa. This observation implies marked early divergence of dense granule and α-granule synthetic pathways. However, both dense granules and α-granules appear to derive from an MVB/late endosomal compartment. The MEG-01 model could help resolve this issue, demonstrating how factors involved in dense granule synthesis (BLOC proteins, specific RabS, VSP33a) and factors involved in α-granule synthesis (NBEAL2, VSP33b) enable granules to segregate from one another and mature along different pathways. The MEG-01 model will be useful in identifying the role of each of these proteins and ordering them in a pathway. Applying this model to α-granules could also help resolve the controversy of whether α-granules represent a single homogenous population or rather a heterogeneous population of granules carrying distinct cargos. By overcoming a major roadblock in our ability to map the pathways involved in granule trafficking, Ambrosio et al open new avenues into the study of platelet granule biogenesis.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

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**THROMBOSIS & HEMOSTASIS**

Comment on Langhauser et al, page 4082

**Contact with stroke**

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In this issue of Blood, Langhauser and colleagues report that kininogen knockout mice (KNG−/−) are protected from stroke induced by transient mechanical occlusion of the middle artery (MCAO). KNG−/− mice develop smaller infarcts, less neurologic impairment, and exhibit lower mortality than wild-type (WT) mice studied at 24 hours and beyond. Benefit was retained in elderly mice and was conferred through reduction in microvascular thrombosis, preservation of blood-brain-barrier function, and attenuation of inflammation. These are important findings because they provide insight into the pathophysiology of stroke and identify potential novel targets for intervention.

Ischemic stroke remains the one of the most common causes of mortality and protracted morbidity. It is estimated that approximately 750 000 new patients a year in the United States are affected. Notwithstanding extensive investigation, thrombolysis by tissue–type plasminogen activator (tPA) remains the sole US Food and Drug Administration–approved
intervention. However, only 3% to 6% of patients with ischemic stroke receive tPA because of delays in medical care, lesion size, and evidence of hemorrhagic transformation that make the risk of intracerebral hemorrhage prohibitive. Moreover, even patients who respond to tPA may be left with significant neurologic defects and risk for recurrent stroke. Thus, there is a clear need for new, more effective, and safer treatments.

This study by Langhauser and colleagues builds on emerging literature, which indicates that components of the contact factor pathway of coagulation, including factor XII, prekallikrein and KNG, the proteolytic product bradykinin (BK), and the BK 2 receptor, are involved in the response to vascular injury, but have less prominent roles in hemostasis. It has been posited that activation of the contact factor pathway in vivo requires higher concentrations of thrombin, found only in pathologic conditions, than does activation of the intrinsic pathway. On the other hand, inflammation and tissue injury expose activators of the contact factor pathway found in the extracellular matrix and others released by cell activation or (peri)vascular inflammation, and changes in cellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med. 2016;18(8):887-896.

This study also highlights the concept that the pathophysiology of stroke and the sequelae of treatment in this mouse model, as in humans, extend beyond intravascular thrombosis. Neuroprotection after transient MCAO simulates human ischemic stroke treated successfully with tPA. The finding that BK restored deleterious outcomes in KNG Δ/Δ animals and the absence of significant differences in cortical blood flow immediately after reperfusion speaks to the potential contribution of nonhemostatic pathways, including alterations in vascular contractility. Within minutes of MCAO, cerebrovascular autoregulation, which matches blood flow to local metabolic needs, is lost, reducing blood flow to ischemic neurons and the penumbra, while diverting flow to surrounding healthy tissue. Local release of glutamate and plasminogen activators from ischemic tissue causes intense activation of N-methyl-d-aspartate and the low-density lipoprotein receptors, which exacerbates loss of adaptive cerebral vasoregulation and leads to neuronal apoptosis. Improved outcomes are seen in models of thrombotic and mechanical occlusion when the deleterious effects of plasminogen activators on vascular contractility are blocked without affecting plasminogen activation. Such approaches might help dissociate the deleterious effects of plasminogen activators from their salutary effects on fibrinolysis.

This study by Langauer and colleagues also points to the need to better delineate the contribution of inflammation to vascular damage and thrombosis. Generation of cytokines, up-regulation of leukocyte adhesion molecules, leukocyte infiltration and generation of metalloproteinases, reactive oxygen species, and lipid peroxidation, among diverse other factors, enhance ischemic and reperfusion injury and promote clot stability and progression. Attenuating the inflammatory response with activated protein C, cytokine antagonists and inhibitors of cell adhesion have been effective in this model, although the clinical benefits remain modest or uncertain, highlighting the complexity of human stroke.

To the extent that thrombosis is superimposed on vascular injury caused by ischemia, (peri)vascular inflammation, and changes in contractility, among many other factors, it may be possible to intervene proximal to the onset of coagulation, breaking the so far inexorable link between the benefits and risks of antithrombotic therapy. Timing here may be critical. Understanding the most proximal events and the sequence of injury will help to delineate both the targets and the windows of opportunity for preventing vascular and parenchymal damage. Thus, this important study by Langhauser and colleagues should prompt further investigation into the diverse activities of KNG and the other members of this pathway and its implications for novel approaches to mitigate thrombotic occlusion and reperfusion injury.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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