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A Munc in the platelet granule works

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The membrane fusion regulator Munc13-4 facilitates calcium–stimulated release of cytolytic and inflammatory mediators from lymphocytes and granulocytes. In this issue of Blood, Ren et al reveal a similar requirement for Munc13-4 in secretion from activated platelets and provide new insights into a human genetic disease.1

Munc13-4 stimulates fusion of platelet granules with the plasma membrane to release granule contents. (Top) Presecretory phase in which secretory lysosomes, platelet α-granules or dense granules, or other LRO is “docked” at the plasma membrane. Fusion requires engagement of a vSNARE (red) on the granule membrane with an ISNARE complex (green) on the plasma membrane. Munc13-4 (orange) stimulates this engagement in a calcium-dependent manner. Upon cell activation and calcium influx (bottom), the membranes fuse, releasing the contents of the granule to the extracellular space. Highlighted are examples of LRO cargoes that are known to be regulated by Munc13-4.

When platelets are stimulated, they release a variety of soluble mediators from 3 different types of intracellular storage compartments: α-granules, dense granules, and lysosomes. To release their contents to the extracellular space, the membrane of each compartment must fuse with the plasma membrane, a process that is driven by cognate interactions between soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) family proteins. Formation of a 4-helix bundle by cytoplasmic coiled-coil domains of a SNARE complex on the plasma membrane and a vSNARE on the granule membrane liberates the energy required to drive membrane fusion.2 Similar SNARE–dependent fusion events mediate intracellular membrane trafficking between all secretory and endosomal compartments, but granule release must be more tightly regulated so that it only occurs after calcium influx induced by platelet activation. The SNAREs themselves are not calcium–responsive; rather, regulatory proteins must ensure that granules accumulate at plasma membrane fusion sites and that appropriate SNARE complexes form at the right time. Identifying these regulatory proteins is of paramount importance both for understanding how to manipulate platelet granule release for therapeutic means and for identifying genetic abnormalities that might underlie bleeding disorders. Ren et al identified Munc13-4 as one such SNARE regulator in platelets.1

Munc13-4 is a member of a small family of proteins that regulate SNARE–dependent fusion at the plasma membrane. The gene encoding Munc13-4 is mutated in familial hemophagocytic lymphohistiocytosis type 3 (FHL3), a disease in which patients suffer from uncontrolled inflammation, lymphoproliferation, and neuronal abnormalities. The hematologic symptoms of FHL3 reflect the failure of several hematopoietic cell types to secrete contents from storage compartments—referred to collectively as lysosome-related organelles (LROs)—in response to agonists. The affected LROs include cytolytic granules of cytotoxic T cells and natural killer cells,3,4 azurophilic granules of basophils and mast cells,4 and secondary and tertiary granules of neutrophils;5 lethality in FHL3 reflects the loss of Munc13-4 function in cytolytic granule release and consequently of cytotoxic T and natural killer cell function. Cytolytic granules in Munc13-4–deficient T cells accumulate properly at the plasma membrane but do not release their contents,1 suggesting that Munc13-4 participates in activating calcium-induced LRO fusion with the plasma membrane. Munc13 family members bind in vitro to tSNAREs (eg, see Guan et al6), suggesting that they prime fusion by either facilitating formation of 4-helix SNARE bundles or by “tethering” LROs to the plasma membrane to allow SNARE complexes to form (see figure).

Ren et al built upon earlier findings by Shirakawa and colleagues3 that platelets express Munc13-4 and that addition of recombinant Munc13-4 to permeabilized platelets enhances calcium–induced release of a dense granule cargo, serotonin. To test whether Munc13-4 is required for platelet granule release, Ren et al turned to the Unc13dJinx mouse model of FHL3, in which the Unc13d gene encoding Munc13-4 is inactivated. The key finding is that after stimulation with thrombin, Unc13dJinx Munc13-4–deficient platelets secrete dramatically less platelet factor-4 (from α-granules) and beta-hexosaminidase (from lysosomes) than wild-type platelets and fail completely to secrete serotonin (from dense granules). This secretion defect correlated with 2 signs that granules failed to fuse with the plasma membrane—a loss of thrombin–stimulated surface expression of α-granule and lysosomal membrane proteins, and retention of intracellular granules by electron microscopy—but was not a consequence of ineffective signaling, because Unc13dJinx platelets responded to thrombin stimulation appropriately by calcium influx, tyrosine phosphorylation, and integrin activation. Using a beautiful in vitro reconstitution system, addition of recombinant full-length Munc13-4 to permeabilized Unc13dJinx platelets fully restored secretion from all 3 granule types. A truncated

form of Munc13-4—lacking a C-terminal calcium-binding C2 domain—not only failed to rescue Unc13d<sup>11001</sup> platelets in this assay, but also acted as a dominant-negative inhibitor of secretion when added to wild-type platelets. This suggests not only that Munc13-4 function requires the C2 domain, but also that other domains—perhaps the 2 MUN domains, which in Munc13-1 bind SNARE proteins<sup>3</sup>—must interact with relevant binding partners. Finally, and perhaps most importantly, the secretion defect was physiologically significant, resulting in decreased agonist-stimulated platelet aggregation and substantially prolonged bleeding times in the Unc13d<sup>11001</sup> mice relative to wild-type mice.

The elegant findings of Ren et al are significant in several ways. First, given that α-granules and dense granules are considered LROs<sup>4</sup>, the data prove that Munc13-4 functions in a similar way in platelets as in other hematopoietic cells, controlling agonist-stimulated release of contents from lysosomes and/or LROs. Defining the mechanism of Munc13-4 activity in SNARE-dependent fusion is an important next step, and platelets might serve as an excellent model system in which to do so. For example, the platelet’s small size and content of very few (3-8) dense granules which can be labeled fluorescently provides a huge advantage in live cell-imaging analysis of agonist-stimulated LRO secretion over other larger, round hematopoietic cell types with many LROs.

Second, whereas neutrophils harbor several LRO types and their release is differentially regulated by Munc13-4<sup>5</sup>,<sup>7</sup> in platelets Munc13-4 seems to control the fusion of all LRO types and lysosomes. This is unexpected given recent data suggesting that α-granule contents can be differentially released,<sup>10</sup> and suggests that all platelet granules use a single mechanism for agonist-dependent fusion. It is noteworthy, however, that a minimal level of thrombin-induced cargo release from lysosomes and α-granules could be detected from Unc13d<sup>11001</sup> platelets.<sup>1</sup> Perhaps other agonists might selectively activate this granule pool for fusion, and they might be less dependent on Munc13-4 activity.

Finally, the data explain clinical reports of abnormal hemorrhage in FHL patients, even after pharmacologic treatment of the inflammatory disease (eg, see Celkan et al<sup>11</sup> and Ueda et al<sup>12</sup>). Clearly, control of excessive bleeding will need to be considered during treatment options for those FHL3 patients who do not receive bone marrow transplantation, and hemorrhage might now be considered as a useful parameter during differential diagnosis for FHL subtypes.

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REFERENCES
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