The Pathway of Exocytosis in Human Platelets

To the Editor:

In a report titled “Regulated Secretion in Platelets: Identification of Elements of the Platelet Exocytosis Machinery,” recently published by Lemons et al in Blood, the authors have investigated the molecular events involved in membrane fusion in platelets. Therein, the function of molecules mediating the secretory events of the exocytosis in platelets was attributed to membranes of structures that might be involved in this process. There are no doubts or any objection to the biochemical results of the article. On the other hand, the investigators discuss their original findings on a critical morphological basis. In the introduction they wrote, “In one model of platelet activation,2,3 these stimuli (collagen, thrombin, and ADP) trigger morphological changes in the platelet resulting in the apparent movement of the secretory granules to the center of the cell and their subsequent fusion with the surface connected canalicular system (SCCS).” The disadvantage of this model is that the fusion of membranes of secretory organelles with the membranes of the SCCS has never been demonstrated.4,5 In contrast, it was clearly shown in studies, using rapid freezing with a time resolution in the range of milliseconds to capture fusion events,6,7 that the secretory organelles (α-granules8,9 and dense core bodies10,11) fuse with the plasma membrane when the platelets were stimulated before. The fusion of secretory organelles with the plasma membrane in human platelets is a phenomenon seen in secretory cells in general.12,13 From morphological observations6,8 and morphometrical measurements12,13 it was concluded that the fate of the SCCS during stimulation is to become evaginated within seconds to allow the surface enlargement necessary for formation of pseudopodia or spreading. Fully stimulated platelets do not show SCCS but do show the membranes of degranulating organelles.8,10,12 As a consequence, thrombin-stimulated gray platelets, which lack α-granules, do not contain such membrane convolutes.14

Furthermore, the investigators wrote that platelet granule membranes do not appear to be docked, in comparison with synaptic vesicles. Interestingly, α-granules and also dense core granules dock before or at the beginning of stimulation to the plasma membrane. In activated platelets after organelle centralization by the action of the contractile cytoskeleton, the membranes of both types of secretory organelles show docking.8,10 Docking, also named apposition, is longer-lasting than membrane fusion and stable enough to be demonstrable with chemical fixation.8,11,14,15,16 Granules in apposition with the plasma membrane maintain this position during subsequent shape change and internal contraction. These reactions progress in the time range of seconds. An investigation using the atomic force microscope on living platelets gave support to this view of platelet exocytosis.17 Secretory organelles that are apposed to the surface membrane (and arrested there) fuse and induce sequential fusion and the formation of compound granules. The constriction of the contractile cytoskeleton in platelets moves (mobile) platelet organelles to the platelet center and into contact with each other, supporting further apposition and leading to formation of compound granules.6,11 It should be noted that the formation of compound granules during platelet secretion was already suggested from immunocytochemical and morphological investigations by Ginsberg et al in 1980.18

From their results, Lemons et al suggested that the molecular mechanism working in platelets for exocytosis is similar to that one described in other cells, most notably neurosecretory cells. I would like to add the suggestion that the compound exocytosis of platelets corresponds to that one of other secretory cells, most likely mast cells.19,20 The attribution of regulatory molecules with distinct functions in exocytosis to certain membranes should reflect the described secretory pathway in platelets.

Eberhard Morgenstern
Department of Medical Biology
Saarland University
Hombury/Saar, Germany

REFERENCES

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Response

The letter by Dr Morgenstern has highlighted the controversy regarding the nature of the intracellular events leading to platelet exocytosis. It has been well established that, subsequent to stimulation, human platelets undergo a series of morphological rearrangements that include the formation of pseudopodia and the centralization of secretory granules. This process culminates in the release of granule contents into the extra-platelet space, but the route of that delivery is ambiguous. It is unclear whether granules fuse directly to invaginations of the plasma membrane called the surface-connected canalicular system (SCCS) or whether, after compound fusion of the granules, the resulting organelle fuses directly with the plasma membrane. Morphological experiments supporting both models have been presented, yet neither has been definitively proven. As stated in a commentary on one of these models, until there is a marker to unequivocally distinguish SCCS from other plasma membrane domains, it will be difficult to resolve this debate using morphological techniques.

Our long-term goal is to determine the molecular mechanisms involved in platelet exocytosis. The SNARE hypothesis offers a framework for this endeavor, and neurotransmission has served as an excellent paradigm, because many of the proteins involved in that regulated exocytosis event have been identified and characterized. In Lemons et al., we demonstrated that platelets contain the general and specific elements of the membrane fusion machinery predicted from the SNARE hypothesis. This justifies our use of neurotransmission as a paradigm in the study of platelet exocytosis. Presently, we are continuing the search for additional proteins that facilitate the various events of platelet exocytosis (and perhaps endocytosis; Bernstein and Whiteheart, manuscript submitted). Subsequent experiments will focus on the specific function(s) and the intracellular localization of these proteins. It is hoped that these experiments will yield a more detailed picture of the molecular events that lead to the platelet release reaction and will ultimately resolve the conflict mentioned above.

Sidney W. Whiteheart
Department of Biochemistry
University of Kentucky College of Medicine
Lexington, KY

REFERENCES
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Eberhard Morgenstern