Shedding New Light on the Platelet Storage Lesion

Joel S. Bennett

In this issue of ATVB, Chen et al report that a monoclonal antibody whose epitope includes the ADAM17 cleavage site in human platelet glycoprotein Ibα (GPIbα) inhibits GPIbα shedding during platelet storage under blood banking conditions and improves the recovery and survival of platelets in murine platelet transfusion models.

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The lifespan of circulating endogenous platelets is determined by a combination of random platelet removal and a nonrandom aging mechanism after which senescent platelets are removed by the reticuloendothelial system. At least a portion of platelet senescence is regulated by an intrinsic apoptotic pathway that acts as an internal timer. Platelets contain members of the antiapoptotic Bcl-2 family, such as Bcl-xL, as well as the proapoptotic proteins Bax and Bak. The half-life of Bcl-xL in platelets is shorter than that of Bak. Thus, it is likely that as the effect of Bcl-xL declines, a point is reached at which Bak is able to cause platelet apoptosis. How apoptosis causes platelet clearance is unknown. Apoptosis causes phosphotidylserine exposure on cell surfaces, a signal for the phagocytosis of apoptotic cells, but it remains to be established whether phosphotidylserine exposure plays a role in the clearance of senescent platelets.

In contrast to endogenous platelets, transfused platelets seem to be cleared because glycans and proteins on their surface are perturbed during storage before transfusion (Figure). Short-term platelet storage in the cold (ie, hours) results in GPIbα clustering, removal of small amounts of sialic acid from the GPIbα ligand-binding domain, removal of newly exposed galactose residues, predominantly on platelet GPIbα, and platelet clearance via the integrin αMβ2 expressed on hepatic macrophages. However, although galactosylation improved the survival of short-term chilled platelets in mice, transfusing galactosylated platelets refrigerated for 48 hours into humans did not extend their circulation time, implying that other cold-induced lesions had occurred. Sialic acid removal alone causes the rapid clearance of transfused platelets. Moreover, platelet storage for 48 hours in the cold causes desialylation of platelet glycoproteins when the platelets are rewarmed, likely because of cold-induced upregulation of the sialidase Neu-1 on the platelet surface. Then rapid platelet clearance occurs when the platelets are transfused. Sialic acid loss exposes penultimate galactose residues, predominantly on platelet GPIbα, and causes platelet clearance via hepatic asialoglycoprotein (Ashwell-Morell) receptors. Whether these observations are relevant to the clearance of endogenous senescent platelets is not clear because their clearance has been attributed to reticuloendothelial rather than hepatic cells. However, depletion of hepatic and splenic macrophages has little effect on the lifespan of freshly transfused platelets, suggesting that desialylation as platelets age could play a role in the recognition of senescent platelets.

Proteolysis of GPIbα by the membrane-associated metallopeptinase ADAM17 occurs continuously as platelets circulate, releasing the 130-kDa N-terminal ectodomain fragment glyco-calcin into the plasma. GPIbα ectodomain shedding also occurs after platelet stimulation by agonists and when platelet alpha-granules are exposed to W7, a compound that sequesters calmodulin, to CCCP, a drug that damages mitochondria and induces apoptosis, and to the protein kinase C activator phorbol myristate acetate. Mouse platelets stored in vitro shed substantial amounts of GPIbα and are rapidly cleared when reinfused. Because platelet recovery and survival are improved when ADAM17 activity is inhibited, Chen et al postulated that blocking GPIbα shedding could inhibit the clearance of stored platelets. Previously, they had produced a monoclonal antibody 5G6 that recognizes the ADAM17 cleavage site between residues Gly464 and Val465 in human GPIbα, thereby inhibiting GPIbα ectodomain shedding. Here, they formally tested their hypothesis by comparing the recovery and survival of platelets stored at room temperature in the presence or absence of 5G6 Fab fragments. Because 5G6 only binds to human GPIbα, they measured its effect using human platelets transfused into SCID (severe combined immunodeficiency) mice or in transgenic mice whose platelets expressed human rather than mouse GPIbα. In SCID mice, 5G6 Fab improved platelet recovery and lifespan when platelets were stored in vitro for 8 days, whereas recovery and lifespan were the same as controls when platelets were stored for 4 days. In the transgenic model, storage with 5G6 Fab improved platelet recovery, but did so without affecting platelet lifespan. Importantly, 5G6 Fab did not exacerbate the impaired platelet responses to ristocetin, ADP, and collagen that result from in vitro storage, and it preserved the ability of stored platelets to shorten the prolonged bleeding times of mice in whom the ectodomain of GPIbα was replaced with the ectodomain of the IL4 receptor.

Based on these results, it is likely that inhibiting GPIbα shedding may be a useful way to optimize platelet storage conditions. Nonetheless, the results raise interesting questions about the accelerated clearance of stored platelets after transfusion. First, GPIbα ectodomain shedding removes the glycans recognized by αMβ2 and the asialoglycoprotein receptor. Thus, what receptor is responsible for the clearance of glycolcalcin-depleted
None.

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References


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