that although treatment of Gp1b−/− platelets with neuraminidase caused significant desialylation, desialylated Gp1b−/− platelets failed to rescue impaired hepatic TPO production both in vivo and in vitro. The data support the notion that GPIbα, independent of other receptors and platelet desialylation, is a prerequisite for hepatic TPO production. Additional evidence in support of this notion was provided by recapitulating impaired hepatic TPO production in IL-4Rx/GPIbα transgenic mice, which lack the extracellular GPIbα domain, as well as with antibodies targeting extracellular portions of GPIbα. These results demonstrated that the N-terminus of GPIbα is required for platelet-mediated hepatic TPO generation.

Platelet GPIbα is abundantly decorated with sialic acid moieties, accounting for perhaps 70% to 80% of the total sialic acid on the platelet surface. However, unlike human GPIbα, the murine GPIbα amino acid sequence lacks any N-glycosylation consensus sequences. Grewal et al demonstrated that even in mice lacking GPIbα, neuraminidase treatment leads to efficient platelet clearance, albeit at a slower rate than in control mice. The data suggest that the glycans on GPIbα are necessary to set off a rapid rate of AMR-dependent clearance, but the exposed galactose moieties on other platelet glycoproteins present counterreceptors for the AMR or other asialoglycoprotein receptors.

The AMR presents a complex receptor that prefers complex N-linked glycans that are clearly absent from mouse platelets on GPIbα. Hence, other binding partners could be involved in AMR-mediated platelet clearance. For example, platelet bound von Willebrand factor, the ultimate GPIbα ligand, which expresses multiple N- and O-linked glycans in human and mice, could clearly contribute to the AMR-platelet recognitions. It is unclear whether asialylated GPIbα O-linked glycans contribute to AMR-mediated platelet clearance. A recent study demonstrated that hepatic AMR promotes preferential adherence to and phagocytosis of asialylated and/or platelets that lack O-glycans, that is, platelets from hematopoietic cell conditional C1galt−/− mice, by Kupffer cells through the C-type lectin receptor CLEC4F. These findings provide further insight into an essential role for core 1 O-glycosylation of platelets in their clearance in the liver. It remains to be determined if removal of sialic acid moieties (which come in different flavors) from O-linked glycans play a role in AMR-platelet recognition. These intriguing observations require further investigation to fully understand the contribution of the published phenomena to platelet clearance and TPO production.

Despite the limitations of the here published findings, the data reveal a novel nonredundant regulatory role of platelets in hepatic TPO homeostasis, which further our understanding of TPO regulation and may have important implications in diseases related to GPIbα such as Bernard-Soulier syndrome and auto- and alloimmune-mediated thrombocytopenias.

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

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Magoulas et al, page 658

Endosome trafficking: blood and more

Gary M. Kupfer | Yale University School of Medicine

In the day-to-day practice of hematology, we are often asked to comment on seemingly innocuous findings such as a low neutrophil count. With the evolution of the modern genetic age, we have gained the ability to provide explanations for pathophysiological findings, thus adding to the base of knowledge regarding hematopoiesis. In this issue of Blood, Magoulas et al add to our knowledge with their thorough analysis of a rare consanguineous family, proving again the utility of studying rare disease for the benefit of wider biologic knowledge.1 We have thus gained a deeper understanding of myelofibrosis (MF), a disease found mostly in older people, by studying it in the young.

MF is generally a variant of myeloproliferative neoplasms that affects mainly those older than 50 years and is associated primarily with mutations in genes related to myeloproliferative neoplasms such as JAK2, MPL, and CALR. Approximately 10% of MF cases have no definable genetic cause. In pediatrics, MF is an extremely rare disease, and cases typically culminate in leukemia.2 The case presented by Magoulas et al is marked by the presence of MF in the context of multiple congenital abnormalities, including severe neurocognitive dysfunction.

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This serves as a reminder that although the most appreciable part of the phenotype is hematologic, the defective protein function involved plays a role within many tissues throughout the body; indeed, before the Magoulas et al letter was published, reports showed that RBSN mutations had a relationship with severe physical and neurocognitive abnormalities.4

Haploinsufficiency is suggested as a cause of other blood cell abnormalities in RBSN carriers in the family presented by Magoulas et al. The unaffected child and both parents exhibited blood count abnormalities, and those of us who are hematologists can easily envision encountering innocuous cases and cases with milder manifestations many times throughout our careers. This is a reminder that family history can provide important clues to understanding the etiology of the abnormality being evaluated and also that rare disorders are not always so rare.

The putative binding partner of rabenosyn (RBSN) is VPS45, in which mutations have been shown to be a cause of MF, severe congenital neutropenia, and severe infection.5-8 Studies of VPS45 have solidified the role of the entire protein complex in protein trafficking via endosomes (see figure). In granulocytes, such protein transport entails the proper localization of granules that bind and cause the death of engulfed bacteria. Such a mechanism can be extrapolated to hematopoietic cells as in the regulation of proinflammatory proteins in megakaryocytes implicated in the primary formation of MF. As a reminder that these proteins are systemic, patients with MF exhibit profound neurocognitive developmental defects.

In sum, it should not be surprising that RBSN mutations result in primary MF, given the previous findings from the analysis of VPS45. Epistasis of RBSN is confirmed by the phenocopy of its mutant phenotype being so consistently similar to that of VPS45. Such established biochemistry can enable the rapid confirmation of observed DNA sequencing data without further validation.

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

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