Comment on Boulaftali et al, page 97

Platelets: yin and yang

Peter N. Walsh  TEMPLE UNIVERSITY

In this issue of Blood, Boulaftali and colleagues have made the important and novel observation that deficiency of the serine protease inhibitor (serpin), PN-1, in platelets results in a prothrombotic state, supporting the role of platelet PN-1 in thrombosis.1

Specifically, Boulaftali et al have initially confirmed previously published studies by Gronke et al indicating that protease nexin-1 (PN-1) is expressed by platelets.2 In studies on human platelets from healthy donors and from patients with the gray platelet syndrome (α-granular deficiency), the authors demonstrate that PN-1 is stored in platelet α-granules, from which it is secreted into plasma and bound to the platelet membrane. There, it inhibits thrombin and urokinase-type plasminogen activator (uPA) and reduces thrombin generation by tissue factor (TF). Platelets from PN-1–deficient mice demonstrate increased sensitivity to thrombin in P-selectin expression and platelet aggregation. Thrombus formation, induced ex vivo by collagen under blood flow conditions and in vivo by ferric chloride–induced injury, was significantly increased in PN-1–deficient mice, demonstrating the antithrombotic properties of platelet PN-1. These studies demonstrate that platelet PN-1 has important antithrombotic properties that have heretofore gone unrecognized.

It has been amply demonstrated that, in addition to playing a part in primary hemostasis by adhering to sites of vascular injury and aggregating to form the initial platelet plug, activated platelets have an essential role in blood coagulation by exposing receptors and assembling active enzymatic complexes for virtually all the coagulation proteins of the contact and consolidation pathways of the clotting cascade3 (see figure). What is less well appreciated is the regulatory role played by activated platelets in the secretion of coagulation inhibitors that limit the generation and activity of thrombin, localize active coagulation complexes to the platelet surface, and prevent uncontrolled intravascular thrombotic processes (see figure). Thus, for example, in addition to the serpin PN-1, another potent inhibitor stored in and secreted from platelet α-granules is the Kunitz-type inhibitor (kunin), protease nexin-2 (PN-2). PN-2 is otherwise known as the Alzheimer β-amyloid...
protein precursor, which contains a Kunitz-type protease inhibitor domain.\(^4\)\(^5\) Whereas PN-1 is a potent and specific inhibitor of thrombin, PN-2 operates by an entirely different mechanism, characteristic of the kunitins, to thrombin, PN-2. In contrast to PN-1 and the vast majority of premature deaths in Western societies.

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

REFERENCES


Platelet RNA chips dip into thrombocytosis

Srikanth Nagalla and Paul F. Bray THOMAS JEFFERSON UNIVERSITY

In this issue of Blood, Gnatenko and colleagues have conducted studies to determine whether platelet RNA profiling can predict causes of thrombocytosis.\(^1\) The authors show that expression levels of only 4 platelet transcripts are able to predict JAK2 V617F-negative ET in more than 85% of samples.

Thrombocytosis is a relatively common hematologic abnormality and is associated with iron deficiency, malignancy, and chronic inflammatory processes. Chronic myeloproliferative disorders (polycythemia vera [PV], essential thrombocytosis [ET] and primary myelofibrosis [PMF]) are important albeit less common causes. Hematologists are often consulted to exclude myeloproliferative disease (MPD) as a cause of high platelet counts. Most often, the clinical picture does not present difficulties distinguishing MPD from reactive thrombocytosis (RT). Among the MPDs with thrombocytosis, ET is typically considered after PV and PMF have been excluded. The somatic mutation V617F in the Janus kinase 2 gene (JAK2) distinguishes MPD from RT, but only half of patients with ET express the JAK2 V617F mutation.\(^2\) Thus, biomarkers specific for ET patients who are JAK2 V617F-negative would have diagnostic value and possibly provide mechanistic insights.

The authors’ earlier work has shown that platelet gene expression can distinguish ET from healthy persons.\(^3\) In the current study, 126 subjects were recruited: 48 healthy controls, 40 ET patients, and 38 RT patients. The investigators have fabricated a novel “platelet gene chip,”\(^7\) which contains probes for 432 mRNAs. Most of these transcripts are reportedly expressed exclusively or predominantly in platelets; a small subset is expressed predominantly in leukocytes, permitting assessment of leukocyte contamination. Using an initial cohort of subjects as a “training set,” sophisticated statistical analyses identified 11 transcripts, the expression of which effectively distinguished the 3 study groups. One hundred percent of the RT subjects and 87.5% of the ET patients were classified correctly using the 11 transcripts measured by the microarray analysis. Importantly, these findings were validated by qRT-PCR in 10 randomly selected subjects from each of the ET and RT cohorts. To validate further this set of biomarkers, the 11 genes were used to predict the phenotype in 31 additional subjects with thrombocytosis. Using platelet RNA, qRT-PCR was able to correctly classify 87% of these subjects. The authors provide evidence that expression levels of only 4 transcripts (HIST1H1A, SRRP72, C20orf103, and CRYH) were able to predict JAK2 V617F-negative ET patients in more than 85% of samples.

RNA expression profiling has been used in a variety of diseases for the purpose of identifying novel genes involved in the pathophysiology, as well as for diagnostic and prognostic purposes. Only a few studies have considered...
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