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## Platelet RNA chips dip into thrombocytosis

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protein precursor, which contains a Kunitz-type protease inhibitor domain.<sup>4,5</sup> Whereas PN-1 is a potent and specific inhibitor of thrombin, PN-2 operates by an entirely different mechanism, characteristic of the kunitins, to serve as a potent ( $K_i \sim 500\text{pM}$ ) and highly specific inhibitor of the unique, homodimeric coagulation proteinase, factor XIa (FXIa). Both PN-2 and PN-1 are present in plasma at concentrations far too low to inhibit their cognate proteinases, but are secreted from platelet  $\alpha$ -granules to achieve high concentrations ( $\sim 30\text{nM}$  in the case of PN-2) in the surrounding plasma. This is sufficient to inhibit enzymes at the initiation (FXIa) and termination (thrombin) of the consolidation pathway of coagulation, thereby preventing the propagation of intravascular coagulation beyond the nidus of the platelet hemostatic thrombus.

Another important inhibitory mechanism relevant to the observations of Boulaftali et al<sup>1</sup> involves another Kunitz-type inhibitor, tissue factor pathway inhibitor (TFPI), which regulates the initiation of blood coagulation at sites of vascular injury.<sup>6</sup> In contrast to PN-1 and PN-2, however, TFPI is present in human plasma at high enough concentrations to inhibit FVIIa and FXa and the generation of thrombin. Once sufficient quantities of FXa have been formed to produce thrombin at the low concentrations required to activate platelets, FXI, FVIII, and FV, the consolidation pathway of blood clotting produces thrombin in sufficient quantities to convert fibrinogen to fibrin and form a hemostatic thrombus.<sup>6</sup> Moreover, platelets contain approximately 10% of the TFPI in blood and can release sufficient TFPI to increase the concentration roughly 3-fold to further inhibit the TF pathway.<sup>6</sup>

These new findings,<sup>1</sup> interpreted in the context of our current knowledge of procoagulant and anticoagulant mechanisms mediated by activated platelets, emphasize the importance of the yin and yang of platelets in blood coagulation. Defects in the procoagulant contributions of platelets to the assembly of coagulation complexes (yang) results in hemorrhagic complications, whereas defects in the anticoagulant mechanisms (yin) produce serious thrombotic consequences that account for the vast majority of premature deaths in Western societies.

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

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## ● ● ● PLATELETS & THROMBOPOIESIS

Comment on Gnatenko et al, page 7

# Platelet RNA chips dip into thrombocytosis

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In this issue of *Blood*, Gnatenko and colleagues have conducted studies to determine whether platelet RNA profiling can predict causes of thrombocytosis.<sup>1</sup> 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.

**T**hrombocytosis is a relatively common hematologic abnormality and is associated with iron deficiency, malignancy, and chronic inflammatory processes. Chronic myeloproliferative disorders (polycythemia vera [PV], essential thrombocythemia [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.<sup>2</sup> 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.<sup>3</sup> 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," 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*, *SRP72*, *C20orf103*, and *CRYM*) 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

differential platelet RNA expression in clinical hemorrhagic or thrombotic phenotypes, including complex phenotypes such as sickle cell disease or cardiovascular disease.<sup>4-7</sup> Platelet RNA profiling of ET has particular appeal considering that the transcripts are derived from the tissue of primary clinical and pathophysiologic interest. The success of these genomic approaches is critically dependent on the precision of the patient phenotyping, generally large numbers of subjects, and appropriate bioinformatic and statistical analyses. Regarding the precision of phenotyping, there is no “gold standard” for the diagnosis of ET, such that misclassification (other MPDs may masquerade as ET) would undermine any genetic association. Other variables—such as age, sex, and platelet-lowering therapies—could also impact on megakaryocyte/platelet gene expression. Although larger numbers of patients would be needed to replicate the results of the current work, this study, nevertheless, lays the foundation for the use of RNA expression profiling in identifying genes associated with specific platelet phenotypes. Of particular interest is the “platelet chip” generated by the authors, which could help to move this field forward, although more details on the selection of the included genes are needed. Validation of such a chip would be very helpful in overcoming the known difficulties of preparing leukocyte-free platelet preparations. Extending RNA expression profiling to other platelet phenotypes may also permit identification of genes involved in the interindividual variation in platelet reactivity and other platelet-dependent disorders of bleeding and clotting. Last, there is a great need for accurate predictors of thrombotic and hemorrhagic risk in ET. It is hoped that genomic approaches such as those used by Gnatenko et al will be fruitful in this respect, but this will likely require consortia with large numbers of patients.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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## ● ● ● TRANSPLANTATION

Comment on Markey et al, page 122

# A role for lymphotoxin in GVHD and GVL

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In this issue of *Blood*, Markey and colleagues reveal a key role for the often forgotten TNF family member, lymphotoxin, in graft-versus-host responses following allogeneic hematopoietic stem cell transplantation.<sup>1</sup>

**A**llogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many patients with hematologic malignancies.<sup>2,3</sup> In addition to delivering effective anticancer treatment, the therapeutic potential of allogeneic HSCT relies on potent graft-versus-tumor (GVT) effects, which eradicate residual malignant cells via immunologic mechanisms. Unfortunately, GVT activity is closely associated with acute graft-versus-host disease (GVHD), the most frequent and serious complication of allogeneic HSCT. The pathophysiology of acute GVHD is complex. Experimental and clinical data support the hypothesis that immune dysregulation that occurs during GVHD evolves in 3 distinct phases.<sup>4</sup> In phase 1, chemotherapy and irradiation included in HSCT conditioning regimens contribute to diffuse, nonspecific damage of host tissues and the secretion of soluble, immunomodulatory proteins. The resultant proinflammatory milieu optimizes the allo-stimulatory capacity of host APCs and enhances chemokine release that is responsible in part for recruiting donor T cells into host target organs. In phase 2, host APCs present alloantigens to T cells infused with the stem cell graft resulting in donor T-cell activation and clonal expansion. Phase 3 involves both cellular and soluble effectors and culminates in target organ damage and dysfunction characteristic of GVHD. This is also beneficial when the targets are residual host malignant cells.

Standard therapies to prevent or treat GVHD are suboptimal and may predispose to opportunistic infections and relapse. Thus,

the development of novel strategies that reduce GVHD, preserve GVT, facilitate engraftment and immune reconstitution, and enhance survival after allogeneic HSCT remains the most significant challenge facing HSCT investigators and the patients we serve. While simplistic, the aforementioned hypothesis uncovers novel opportunities to control immune dysregulation that is responsible for GVHD. To this end, experimental data have revealed that TNF $\alpha$  is a key contributor to each phase of GVHD,<sup>5</sup> and TNF inhibitors have shown activity in clinical trials for GVHD.<sup>6</sup>

TNF $\alpha$  signals through the TNF receptors (TNFRs). These receptors have wide tissue distribution and also bind other related soluble ligands. In this issue of *Blood*, Markey and colleagues examine the heretofore unstudied role of the often forgotten member of the TNF superfamily, lymphotoxin (LT $\alpha$ 3), in acute GVH reactions following allogeneic HSCT. Lymphotoxin (formerly referred to as TNF- $\beta$ ) was originally identified in 1968 as a soluble cytolytic factor produced by lymphocytes,<sup>7</sup> that also bind TNFRs. Like TNF $\alpha$ , LT $\alpha$ 3 promotes apoptosis and proliferation of T cells and contributes to a variety of inflammatory responses.<sup>8</sup> However, a paucity of data exists regarding the precise role of LT $\alpha$ 3 in various clinical disease states. Results generated by Markey and colleagues using established, preclinical GVHD models show that LT $\alpha$ 3 contributes to the development of GVHD and GVL activity. In a series of elegant and well-planned experiments, the authors demonstrate that naive and alloreactive