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 kunitz, to serve as a potent (\(K_i \sim 500\text{nM}\)) and highly specific inhibitor of the unique, homodimeric coagulation proteinase, factor Xla (FXla). 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 (FXla) 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\(^1\) involves another Kunitz-type inhibitor, tissue factor pathway inhibitor (TFPI), which regulates the initiation of blood coagulation at sites of vascular injury.\(^6\) 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.\(^6\)

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.\(^6\)

These new findings,\(^1\) 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.

REFERENCES

differential platelet RNA expression in clinical hemorrhagic or thrombotic phenotypes, including complex phenotypes such as sickle cell disease or cardiovascular disease. 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 individual 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.

**REFERENCES**


---

**A role for lymphotixin in GVHD and GVL**

**Kenneth R. Cooke** CASE COMPREHENSIVE CANCER CENTER

In this issue of Blood, Markey and colleagues reveal a key role for the often forgotten TNF family member, lymphotixin, in graft-versus-host responses following allogeneic hematopoietic stem cell transplantation. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many patients with hematologic malignancies. 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 evokes in 3 distinct phases. 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 dys-function 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 TNFα is a key contributor to each phase of GVHD, and TNF inhibitors have shown activity in clinical trials for GVHD. TNFα 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, lymphotixin (LTo3), in acute GVH reactions following allogeneic HSCT. Lymphotixin (formerly referred to as TNF-β) was originally identified in 1968 as a soluble cytolytic factor produced by lymphocytes, that also bind TNFRs. Like TNFα, LTo3 promotes apoptosis and proliferation of T cells and contributes to a variety of inflammatory responses. However, a paucity of data exists regarding the precise role of LTo3 in various clinical disease states. Results generated by Markey and colleagues using established, preclinical GVHD models show that LTo3 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
Platelet RNA chips dip into thrombocytosis

Srikanth Nagalla and Paul F. Bray