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.

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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. 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 evolves 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 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 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, lymphotoxin (LTα3), in acute GVHD reactions following allogeneic HSCT. Lymphotoxin (formerly referred to as TNF-β) was originally identified in 1968 as a soluble cytotytic factor produced by lymphocytes, that also bind TNFRs. Like TNFα, LTα3 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 LTα3 in various clinical disease states. Results generated by Markey and colleagues using established, preclinical GVHD models show that LTα3 contributes to the development of GVHD and GVL activity. In a series of elegant and well–planned experiments, the authors demonstrate that naive and allosreactive
CD4+ cells secrete soluble LTα3 following T-cell receptor stimulation. Subsequent studies using genetically modified HSCT donor mice and the administration of a TNFα binding construct confirm that LTα3 of both donor and recipient origin contributes to GVHD-mediated epithelial apoptosis, target organ damage, and mortality to an extent comparable to that observed for TNFα. This effect is attributed to the soluble (as opposed to membrane-bound) protein, is most striking at lower T-cell doses, and is driven primarily by the direct cytopathic capacity of LTα3 via TNFR signaling. Importantly, the protective effects associated with the absence of LTα3 production by donor cells do not influence donor T-cell function, findings that set the stage for critical GVL experiments. Using 2 distinct models, the investigators make the important observation that LTα3 is required for optimal GVL effects exclusively when recipient leukemia cells are susceptible to apoptosis via signaling through the p55 TNFR. The contribution of LTα3 to GVL activity in this context is non-redundant (and equivalent) to that which can be attributed to TNFα alone. These findings have significant translational research implications for treating GVHD and other disorders. TNF inhibition has become an accepted therapeutic option for steroid-refractory acute GVHD, and one recent study suggested that TNF blockade administered in conjunction with steroids may be an effective upfront treatment for GVHD as well. The data reported by Markey et al suggest that these beneficial effects are secondary, at least in part, to combined TNFα and LTα3 neutralization; because LTα3 is also generated during GVHD and uses the same signaling pathways as TNFα, it is reasonable to assume that this molecule is responsible for much of the damage previously attributed to TNFα. Moreover, the additional neutralization of LTα3 by TNFR:Fc molecules (as opposed to specific anti-TNF monoclonal antibodies, which do not cross-react with LTα3) may help to explain why the use of TNFR:Fc for disorders such as GVHD and rheumatoid arthritis may result in different clinical outcomes compared with the blockade of TNFα alone. In sum, preliminary clinical results, in combination with mechanistic preclinical studies reported herein, suggest that combined blockade of TNF and LTα3 may have considerable therapeutic potential for the treatment of GVHD. However, one must proceed with caution in situations in which the underlying malignancy expresses the p55 TNFR; in this scenario, such a therapeutic approach would likely attenuate GVL effects as well.

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