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## Platelet tissue factor comes of age

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termed a major molecular response<sup>1</sup> or who have had raised BCR/ABL transcript levels with serial monitoring. The cytogenetic relapse rate remains extremely low in patients in CCyR, and we are only beginning to learn how to react to these more sensitive measurements of residual disease.<sup>2</sup> Before succumbing to the new disorder of “PCRitis,” it is important to remember that the treatment of chronic phase is a marathon, not a sprint. Currently, there is inadequate standardization of results of PCR assays among different laboratories in the United States, and fluctuation in values is common. If increasing transcript values are confirmed repeatedly in patients still in CCyR, an initial trial with a dose increase to 800 mg imatinib mesylate is reasonable, switching to

dasatinib if no response is noted. If BCR/ABL mutations known to be resistant to the available tyrosine kinase inhibitors are detected, allogeneic transplantation should also be considered if there is cytogenetic relapse.

*Conflict-of-interest disclosure: The author has received grant support for clinical trials and has served on advisory boards for Novartis and Bristol, Myers, Squibb.* ■

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## ● ● ● HEMOSTASIS

Comment on Panes et al, page 5242

# Platelet tissue factor comes of age

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The origin of blood-borne tissue factor (TF) is a highly debated topic that is filled with controversy. In this issue of *Blood*, Panes and colleagues report that activated human platelets synthesize functional TF.

**E**xpression of TF around blood vessels is thought to initiate clotting after vessel injury. However, in 1999, Giesen et al<sup>1</sup> demonstrated that TF was present in blood, and that this so called blood-borne TF contributed to thrombus formation *ex vivo*. TF was found on microparticles (MPs), which are small membrane fragments derived from activated or apoptotic cells. Positive staining was also observed on platelets and was presumably due to the adsorption of TF-positive MPs. This bred a popular concept that TF present in blood was derived from MPs. Indeed, P-selectin glycoprotein-1 expressed by leukocyte-derived MPs can dock to P-selectin present on the surface of activated platelets.<sup>2</sup>

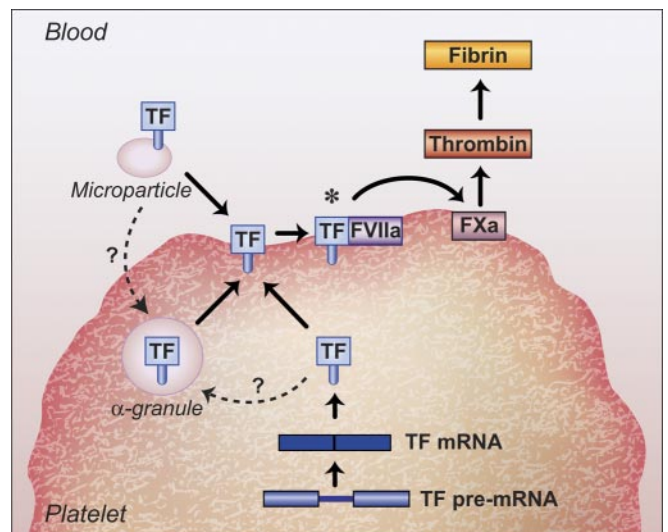
Whether or not platelets intrinsically express TF has been controversial. In 2001, Zillman et al<sup>3</sup> identified TF on the surface of platelets in collagen-stimulated whole blood. This observation was generally confirmed by others, and in a subsequent study, TF protein was localized to platelet  $\alpha$ -granules. This suggested that platelets store TF,

but did not distinguish between TF that was preformed in megakaryocytes and packaged into platelets versus the endocytosis of TF-positive MPs. These studies also did not take into account *de novo* protein synthesis, a mechanism of control used by platelets to generate new proteins.<sup>4</sup>

The studies by Panes and coworkers provide further insight into these issues and demonstrate that activated human platelets synthesize TF. They show that unstimulated platelets express low levels of TF protein, which is enhanced

in response to cellular activation. TF mRNA, the template for protein synthesis, is absent or expressed at low levels in unstimulated platelets. In response to activation, however, platelets from every subject express TF mRNA. The differential expression of TF mRNA in anucleate platelets can be explained by studies from our group demonstrating that resting platelets contain TF pre-mRNA that is spliced into mature mRNA upon platelet activation.<sup>5</sup> One limitation of the study by Panes and colleagues is that it is unclear if resting platelets express low basal levels of TF *in vivo*, or if these levels are due to postisolation activation of the platelets. This is a critical question to resolve, because constitutive versus inducible expression of platelet TF may have distinct functions in the initiation, propagation, and stabilization of a thrombus. It also raises the possibility that TF protein expression patterns in platelets may vary in human disease.

The fact that activated platelets express TF may have important implications for the therapeutic use of recombinant factor VIIa (Novo-Seven, Clayton, NC) in the treatment of patients with bleeding disorders. High-dose recombinant factor VIIa has been proposed to restore hemostasis by binding to activated platelets in a TF-independent manner. However, low levels of TF generated by platelets may play a role in the hemostatic effects of recombinant factor VIIa.



**Regulation of TF in platelets.** TF on the surface of activated platelets may be derived from (i) binding of TF-positive MPs, (ii) TF stored in  $\alpha$ -granules, and (iii) splicing of TF pre-mRNA and translation of the mature mRNA into protein. Once at the cell surface, TF may require activation to reveal its procoagulant activity (\*). The dashed lines with question marks identify possible sources of  $\alpha$ -granular TF in platelets that require further investigation.

Further studies are also required to sort out the physiological relevance of platelet-derived TF and the mechanism by which TF is activated on the surface of the platelets. Answers to these types of questions will go a long way in determining whether platelet-derived TF truly comes of age.

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

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components of the tumor that can express MHC class II molecules include myeloid cells and activated endothelial cells (Figure). The tumor-rejecting mechanisms of CD4 could involve destruction of tumor blood vessels with cytokines and proapoptosis ligand/receptor pairs. Besides, CD4 Th1 cells are known to activate macrophages and therefore could turn pro-tumor stromal myeloid cells into tumor-destructive activated macrophages. Interestingly, the authors find a key role for host NK cells in tumor rejection. These mechanisms must be further explored to understand this CD4–NK duo, which might involve antiangiogenesis and tumor blood vessel destruction in addition to direct tumor cytotoxicity (Figure).

The reason for the inferiority of CD8 T cells is less clear. Perez-Diez and colleagues report that in vivo killing activity of adoptively transferred CD8 killers remained normal in tumor-bearing females, but tumors progressed regardless of proper presentation of antigen. Mysteriously, the problem is not lack of early CTL arrival and infiltration into the tumor tissue. The findings of Perez-Diez and colleagues are extremely provocative, yet more models of CD8 TCR transgenic T cells should be analyzed in this context.

## IMMUNOBIOLOGY

Comment on Perez-Diez et al, page 5346

# Antitumor T-cell wars: do CD4s outwit CD8s?

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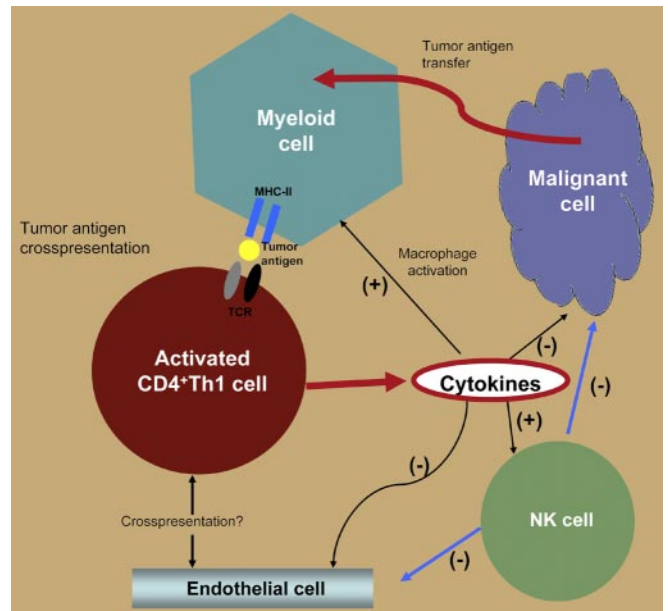
In this issue of *Blood*, Perez-Diez and colleagues report the findings of a series of side-by-side comparisons on the ability of monoclonal CD4<sup>+</sup> and CD8<sup>+</sup> T cells to reject transplanted tumors expressing the cognate antigen. Their model focuses on tumors expressing male-exclusive minor histocompatibility antigens and female T-cell receptor (TCR) transgenic mice, whose only TCRs are specific for male antigens presented either by MHC class I or class II molecules.

In vitro exposure of tumor cells to activated antigen-specific CD8<sup>+</sup> T cells leads to tumor-cell death, while CD4<sup>+</sup> cells do not kill malignant cells in vitro. In sharp contrast, the results of in vivo experiments by Perez-Diez and colleagues come as a surprise, revealing that CD4<sup>+</sup> T cells reject tumors while CD8<sup>+</sup> T cells fail to do so. This unexpected result was found both upon adoptive transfer experiments and when transplanting tumors expressing male-exclusive minor histocompatibility antigen to the TCR-transgenic female hosts.

Intriguingly, major histocompatibility complex II (MHC II) expression on tumor cells is dispensable for rejection, whereas it is critical in the host mouse. Therefore, one has to assume that male antigens from the tumor are cross-presented by MHC class II molecules on stromal cells, leading to recognition by primed specific CD4<sup>+</sup> T cells (Figure). This suggests important consequences for the immunotherapy of malignant diseases. First, CD4<sup>+</sup> T cells have probably been seriously underestimated in their potential for adoptive

therapy. Second, CD4-recognizable tumor antigens should be incorporated in antitumor vaccines, not only because they provide help for CTLs, but also because of the CD8-independent antitumor activity of CD4s. For instance, attention must be paid in allogeneic bone marrow transplantation to donor CD4 T cells recognizing minor histocompatibility or tumor antigens.

The mechanisms of the antitumor effect of CD4<sup>+</sup> T cells against solid tumors have not been fully addressed by Perez-Diez and colleagues. The stromal



**Indirect antitumor mechanisms that can be mediated by activated CD4<sup>+</sup> T cells. Rejection implies crosspresentation of tumor antigens by MHC class II molecules on intratumor myeloid cells and maybe on endothelial cells in the tumor stroma. T-cell cytokines (IFN- $\gamma$ , TNF  $\alpha/\beta$ , GM-CSF) display deleterious effects on tumor vascularization and activate both macrophages and NK cells to become tumoricidal.**