To the editor:

Tissue-factor–endowed leukocytes do cause thrombosis

In a series of complex and careful experiments involving wild-type mice and mice with low tissue factor (ITF), Day and colleagues conclude that mice with ITF subjected to artificial vascular lesions had decreased incidence of thrombosis when compared with wild-type mice. Using bone marrow transplanted from ITF mice into wild-type mice, the authors observe no significant differences in thrombosis time or thrombus mass between these groups, and conclude that the role of leukocytes may be secondary.

Perhaps this is so in mice. Unstimulated leukocytes in rabbits, humans, and other species have very low tissue-factor activity (TFA). However, in vivo endotoxin-induced leukocytes from one rabbit appeared to have TFA sufficient to cause thrombosis in an intact recipient rabbit. Indeed, intravenous infusion of such leukocytes at a rapid rate caused nearly instant death, with a solid thrombus extending from the superior vena cava through the right heart to the pulmonary artery. Slower infusions caused thrombosis in the pulmonary artery branches. Furthermore, slow infusion of TFA-endowed leukocytes into the aorta caused thrombosis in all major organs, with infarctions in liver and spleen; stomach rupture; and myocardial and renal infarctions. These findings indicate that TFA-endowed leukocytes from one rabbit could induce widespread and severe arterial and venous thrombosis. Similar findings were obtained in dogs. More recently, Himber et al demonstrated venous thrombosis in rabbits whose leukocytes and platelets stained for tissue factor (TF). The thrombosis was impeded by an anti–rabbit TF antibody.

Many tissues may have higher TFA than activated leukocytes, but this may not be relevant if they are not in contact with blood. Although endotoxin was the first reported inducer of leukocyte TFA, it is neither the sole nor the most powerful inducer. For example, platelets may play a considerable role in TFA enhancement. The effect has been recently attributed to platelet-derived P-selectin. Therefore, comparing TFA of homogenized carotid arteries with endotoxin-induced leukocytes in vitro may not be appropriate.

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Response:

Role of leukocyte- versus vascular-wall–derived tissue factor in murine thrombosis

Our transplant experiments did not support a role for bone marrow–derived tissue factor (TF) in macrovascular thrombosis under basal conditions. We found extremely low levels of procoagulant activity in blood, while TF activity in wild-type arteries vastly exceeded that of low-TF arteries. We concluded that vascular-wall TF drives thrombosis in our models. Oxidative injury denudes the vascular endothelium, allowing TF in the subendothelial matrix and tunica media to trigger thrombosis. Furthermore, oxidative injury activates TF expressed by multiple cell types. We contend that sufficient vascular-wall TF was exposed in our experiments and modulated thrombosis. Since the extent of adventitial disruption in our model is unknown, our in vitro measurements may overestimate the amount of TF that supports thrombosis. Nevertheless, comparing vessel-wall and blood-TF activities provides important insight into the relative contribution of these pools to thrombosis after vascular disruption. While we agree that thrombi shield vascular-wall TF from blood, this dynamic barrier can be dislodged at its leading edge, potentially releasing vessel-wall TF onto the thrombus. Furthermore, a significant portion of circulating TF could originate from injured vascular-wall cells that acutely release TF-positive microparticles. Significant amounts of TF may be released from ruptured atherosclerotic plaques before thrombosis occurs. These are plausible mechanisms by which vascular-wall TF may drive thrombus propagation.

We stated in our paper that there are pitfalls to measuring TF activity in vitro. This was the rationale for our in vivo experiments, our main focus. We compared vessel-wall and blood-TF activities per milligram of protein, not per volume. Despite concentrating blood components, we could not detect unequivocal TF activity in mouse blood. In fact, the procoagulant activity of a 5-mm arterial segment markedly exceeded that of the animal’s entire blood volume, data consistent with those of Butenas et al. Hatchcock and Nemerson are incorrect in implying that photochemical injury is unduly harsh compared with other models. We disagree that the duration of injury in our experiments made interpretation of results difficult. Other animal thrombosis models have used prolonged vascular injury. Nevertheless, we agree that virtually all models have limitations.

An anti-TF antibody attenuated thrombosis in a silicone shunt containing a collagen-coated thread implanted in a rabbit vein. The trauma of implanting the shunt and cannulating vessels could introduce vascular-wall TF into the blood. Even if vascular trauma had no effect, the antibody was not specific for leukocyte-derived TF, and immunostaining does not establish leukocyte-TF function.

REFERENCES

Therefore, although the study by Himber et al supports the hypothesis that circulating TF modulates thrombosis, it does not establish a causal relationship between leukocyte-TF expression and thrombosis.

In response to Niemetz, we did not compare TF activities of homogenized carotid arteries and endotoxin-treated leukocytes. As discussed in our paper, we agree that there may be species variation in the contribution of different TF pools to thrombosis, and that leukocyte-TF expression is enhanced under pathologic conditions, during which it may contribute to thrombosis. Further studies are necessary to test these hypotheses.

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References


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