Letter to Editor response: Endothelial cell tissue factor and coagulation

Thank you for the opportunity to respond to the letter by Drs. Witkowski and Rauch about our editorial [1].

It is very challenging moving from *in vitro* studies with cultured cells to *in vivo* studies that analyze gene expression. In the tissue factor (TF) field it is well accepted that cultured endothelial cells (EC) do not express TF under basal conditions but can be induced to express TF after stimulation with a variety of agonists [2]. Witkowski and Rauch state that the induction of TF in culture ECs "makes it likely that TF derived from ECs contributes to coagulation under pathological conditions" [3]. However, the models they present in support of a role for EC TF in coagulation are not selective for TF. For instance, overexpression of an NFκB inhibitor or EC-specific knock-out of miR-126 will affect many genes in the endothelium [4,5]. Interestingly, miR-126 also regulates TF expression in monocytes [6]. Furthermore, blood vessels are surrounded by pericytes, smooth muscle cells and adventitial fibroblasts, all of which express TF. Therefore, it is very difficult to distinguish the contribution of TF expression induced in the endothelium from that exposed on perivascular cells due to disruption of the endothelial barrier.

We generated $TF^{fl/fl}$,$Tie2^{Cre}$ mice that delete the TF gene in ECs and hematopoietic cells. To specifically investigate the role of EC TF in the activation of coagulation in different diseases, we transplant wild-type bone marrow into $TF^{fl/fl}$,$Tie2^{Cre}$ mice to restore TF expression in hematopoietic cells. We use levels of thrombin-antithrombin (TAT) complexes as a marker of activation of coagulation. Witkowski and Rauch conclude from one of our studies using an endotoxemia model that "EC-derived TF plays no role in activation of coagulation" [3]. This is not correct. We were very careful to state that "deletion of the TF gene in ECs alone had no effect on LPS induced plasma TAT levels" since we cannot exclude a minor role of EC TF [7]. Indeed, we agree with Witkowski and Rauch that "the assays used in murine models may not be sensitive to quantify the procoagulant effects of ECs" [3].

In conclusion, it is possible that EC TF contributes to the activation of coagulation under some circumstances, such as under hypoxic conditions in the valve pockets of large veins. However, it is important to carefully distinguish a role of EC TF from other cellular sources of TF, such as monocytes and vessel wall cells, as well as TF-positive extracellular vesicles, before concluding a critical role of EC TF in thrombosis.

Conflict of interest

None.

References

[1] Antoniak S, Mackman N. Editorial commentary: tissue factor expression by the endothelium: coagulation or inflammation? Trends Cardiovasc Med 2016;26(4):304–305.
[2] Bode M, Mackman N. Regulation of tissue factor gene expression in monocytes and endothelial cells: thromboxane A2 as a new player. Vasc Pharmacol 2014;62(2):57–62.
[3] Witkowski M, Rauch U. Letter to the Editor: tissue Factor of endothelial origin: just another brick in the wall? Trends Cardiovasc Med 2016.
[4] Song D, Ye X, Xu H, Liu SF. Activation of endothelial intrinsic NF-{kappa}B pathway impairs protein C anticoagulation mechanism and promotes coagulation in endotoxemic mice. Blood 2009;114(12):2521–2529.
[5] Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev cell 2008;15(2):261–271.
[6] Witkowski M, Weithauser A, Tabaraie T, Steffens D, Krankel N, Witkowski M, et al. Micro-RNA-126 Reduces the Blood Thrombogenicity in Diabetes Mellitus via Targeting of Tissue Factor. Arteriosclerosis, thrombosis, and vascular biology 2016;36(6):1263–1271.
[7] Pawlinski R, Wang JG, Owens 3rd AP, Williams J, Antoniak S, Tencati M, et al. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. Blood 2010;116(5):806–814.

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