These findings confirm the specificity of IL-18 involvement in arrhythmias and cardiac remodeling in the model of SCD. These results were correlated with clinical findings. SCD patients with lower IL-18 plasma levels had shorter QTc intervals, and the SCD patients with lower *IL18* expression in peripheral blood mononuclear cells had shorter QTc and better 4-year survival rate. However, Gupta et al did not address the effect of IL-18 on oxidative stress and microvascular dysfunction.

Dissecting the role of IL-18 in cardiovascular disease has a twofold importance. On the one hand, anti–IL-18 therapies have a potential in treating cardiovascular diseases, and a trial of the safety and tolerability of an IL-18 antibody has been conducted (#NCT01035645) with early promising results.[10] These results suggest that blocking IL-18 may have therapeutic effects in patients with SCD at risk for cardiac arrhythmias and adverse outcomes. On the other hand, IL-18 has been proposed as a component of anticancer treatment (#NCT00659178), and such use of IL-18 creates a potential risk of cardiac fibrosis and arrhythmias, creating a need for possible mitigating strategies and adjuvant therapies. These results illustrate the important and complex relationship between the immune system and heart disease and the potential of targeting this relationship as novel therapies for heart disease.

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THROMBOSIS AND HEMOSTASIS

Comment on Feinauer et al, page 1219

Hemostasis and cerebral metastases in a model system

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In this issue of *Blood*, Feinauer et al focus on central nervous system (CNS) metastases generated by embolization of tumor cells into the cortical capillary network in a mouse model, and early thrombotic events that might facilitate metastasis.[1]

Metastases into the CNS can significantly impair function and reduce survival. In breast cancer, at diagnosis, 15% to 35% of patients have detectable metastases to the brain. How metastatic tumors are established and invade the CNS is of much importance,[2] however, the acute and early events are poorly understood. Several reports suggest variable responses of tumor metastases to anticoagulation in other model systems.

The cerebral capillary bed is unique in that, in capillaries, the vascular endothelium is separated from astrocyte end-feet, a part of both the brain and microvessels, by the basal lamina matrix.[3] Local and regional collaterals unique to the capillary beds differ between the cortex and the striatum, for instance.

In 1999, Rosenberg and Aird posited organ-specific hemostasis, however, at that time, a unique hemostatic system or response in the CNS was not well defined.[4] Unique to CNS microvessel beds is the perivascular expression of tissue factor (TF) in the astrocyte end-feet.[5] In the nonhuman primate, cerebral capillaries express TF less than larger microvessels.[6] One notion is that, with increased microvessel permeability, local thrombin generated when the plasma interacts with TF could limit hemorrhage in the CNS.

Feinauer et al describe a new model for examining the impact of embolized tumor cells in the brain microvasculature. This mouse model uses an implanted cranial window to expose the vasculature of the cortical surface and 2-photon imaging to a cortical depth of 500 μm, transcardiac embolization of tumor cell lines, and standard imaging techniques to examine the participation of hemostatic elements in a longitudinal descriptive study. The cranial window model is used to define microvessel structure and alterations in other settings.[7] This labor-intensive project demonstrates (i) thrombosis at day 1 at capillary impact sites, (ii) an association of tumor cell extravasation with early activation of hemostasis, (iii) the presence of von Willebrand factor (VWF), fibrin, and platelets at the sites of arrested tumor cells, and (iv) the expression of TF on the surface of Jimt1 cells ex vivo that stimulates thrombin formation, but no direct platelet-tumor cell interactions. Antithrombotic approaches including acetylsalicylic acid/clopidogrel, tinzaparin, or a polyclonal anti-VWF antibody (applied early, but not late) decreased Jimt1 tumor cells in microvessels, whereas early

anticoagulation decreased metastasis size and metastasis count at 4 weeks.

These studies implicate variable activation of hemostasis by the tumor cell lines that influence events after impact in brain capillaries. The method of embolization used here is a convenient mimic for pathological changes associated with brain metastases.[2] Other recent experiments have also implicated components of tumor cell–associated activation of hemostasis (by direct activation, cell expression of TF, and release of thrombogenic microparticles, sometimes involving platelet adherence and activation) or the generation of neutrophil extracellular traps.

Similarities between these observations and microvascular events during experimental focal ischemia in animal models are instructive. In the setting of experimental focal ischemia (as in ischemic stroke), reperfusion of the ischemic bed can result in acute focal "no-reflow," in which microvessel obstructions downstream occur that consist of activated polymorphonuclear leukocytes, fibrin, and/or degranulated platelets,[8] among other contributors. Acute interference with platelet activation and deposition and fibrin formation prevents microvessel obstructions.[9] Hence, any process that can cause local microvessel "activation" or activate platelets or the coagulation system could obstruct the cerebral microvasculature.

The simple evaluation of permeability with fluorescein sodium suggested no effect on the permeability barrier at the site of tumor cell impact.[1] This observation may speak to the limits of optical resolution of the methodology, as local thrombin generation can be a part of capillary impact in this model. In ischemia, the appearance of perivascular fibrin, demonstrated by electron microscopy and other methods, implies increased microvessel permeability.

In ischemia, acute breach of the blood-brain permeability barrier coincides with the appearance of signals associated with angiogenesis, including integrin αvβ3 expression. These events highlight possible, yet-to-be-defined, roles for the microvessel wall and surrounding tissue acutely.

Although this report provides further evidence that alterations in local hemostasis could participate in the acute evolution of CNS metastases, the situation is undoubtedly more complex.

Some of the limitations here include the use of cell lines, species/strain relationships, animal age, effects of the cranial window itself, the focus on superficial cortical events only, optical resolution, and as-yet-unexplored effects of the inflammatory and immune systems in the model. Importantly, changes in cell phenotype and the involvement of integrin-mediated signaling, for instance, hint at the further complexity of the metastatic processes that might occur in the CNS that may be cell or environment dependent.[10]

There are important unanswered questions that will keep the investigators busy. Do events observed in the cortex reflect events in other brain regions? Does locally increased permeability occur? What are the roles of the CNS microvessel endothelium and the neuropil in impact events? How do tumor cells initiate these processes in the CNS, or do they occur outside of the CNS? Do events in the postimpact vasculature involve glial responses? Does the extrusion of cells involve roles for integrin αvβ3 and the initiation of angiogenesis? Do these occur in sanctuary niches in the CNS related to endothelial phenotype?

Could events here represent local ischemia due to microvessel obstruction? Given the similarities between intramicrovascular events in this model and those of focal ischemia, does the brain produce responses to vascular injury that are stereotypic? How do the acute/early events seen here affect the microvessel and how does the microvessel environment facilitate or limit the fate of the tumor cell?

Hence, model systems, such as that described here, may also offer opportunities to further define vascular signaling and phenotypic contributions to the early stages of metastases in the CNS. These may have therapeutic potential.

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