inside blood

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HEMOSTASIS

Comment on Chen et al, page 1344

Cell surface–targeted anticoagulation in systemic infection and inflammation

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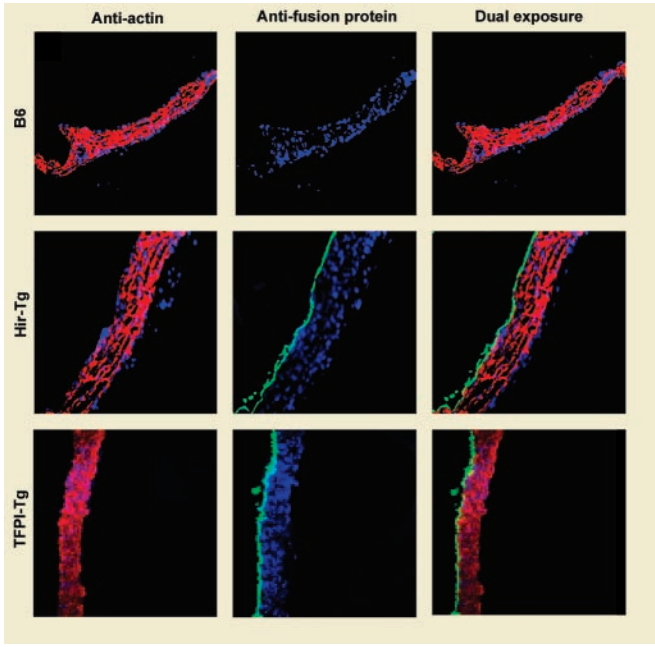
Coagulation predominantly occurs at the surface of cells, yet our anticoagulants mostly are directed at fluid-phase proteins. Experiments in transgene mice published in this issue of *Blood* demonstrate that surface-targeted anticoagulation may indeed provide a potent antithrombotic effect.



Immunohistology of mouse aortas using antifusion protein mAb (antihirudin for Hir-Tg and antihuman TFPI for TFPI-Tg). See the complete figure in the article beginning on page 1344.

Severe infection and the ensuing systemic inflammatory response may lead to organ failure and is associated with substantial mortality. In recent years, the pathogenetic mechanisms involved in this inflammatory response and in the activation of downstream processes have been dissected, and we may now have a reasonably accurate perception of the various factors that play a role in this condition. Systemic activation of the coagulation system is one consequence of the inflammatory response and has been put forward as an important factor in the pathogenesis of organ dysfunction, presumably by the resulting formation of microvascular thrombosis in combination with a further proinflammatory effect by activated coagulation factors.[1,2] In the inflammation-induced activation of coagulation, several factors play a key role. Tissue factor is the principal initiator of coagulation activation, as it is expressed on activated mononuclear cells and potentially on endothelial cells. Tissue factor–dependent generation of thrombin is another pivotal event, since this will not only lead to fibrinogen-to-fibrin conversion but will also activate platelets and bind to thrombomodulin, which will then convert protein C to activated protein C. Interestingly, both tissue factor and thrombin also have a principal role in coagulation-induced inflammation, mediated by so-called protease-activated receptors that will modulate the proinflammatory response. Targeting tissue factor or thrombin therefore seems a rational approach in improving the outcome of severe infection and inflammation, and indeed, animal studies show beneficial effects of this type of intervention. Clinical studies demonstrate that interference in the coagulation system, for example by administration of activated protein C, may improve the outcome of patients with severe sepsis, although other interventions in the coagulation system were less effective.

Most of what we have learned from the function of coagulation in vivo comes from observations done in blood from experimental animals or human subjects. We may therefore tend to forget that activation of coagulation is predominantly occurring at the surface of cells, such as endothelial cells, mononuclear cells, or activated platelets. Hence, specific targeting of coagulation factors or pathways at the surface of these cells may enhance the antithrombotic (and potentially the anti-inflammatory) efficacy of these interventions and may cause fewer unwanted systemic side effects such as bleeding. Indeed, most fluid-phase interventions in the coagulation system that have been studied in patients with sepsis caused an increase in the incidence of bleeding.

In this issue of *Blood*, Chen and colleagues report on their generation of transgenic mice expressing potent anticoagulant proteins, which are directed at thrombin (by hirudin) or tissue factor (by tissue factor pathway inhibitor [TFPI]). Interestingly, the genes of these anticoagulants have been fused with CD4 and P-selectin gene fragments and have been put under control of a CD31 promoter, which tethers them to the surface of secretory granules of endothelial cells, monocytes, and platelets. Hence, the expression of these anticoagulant agents is limited to cells that play a role in the activation of coagulation and will occur only if cells are activated. Upon challenge with intravenous endotoxin, the mice were shown

to be free of widespread intravascular fibrin deposition and had no signs of a consumption coagulopathy, whereas nonchallenged mice had normal bleeding times. In these elegant proof-of-principle experiments it is demonstrated that targeted delivery of anticoagulant agents at the surface of activated cells (ie, at the site of coagulation activation) may be a worthwhile approach to pursue. Additional bone marrow reconstitution experiments in this article show that endothelial cells in particular may be the pivotal target for this local treatment.

A detailed analysis of the function of coagulation in vivo has in recent years led to the development of new, potent, and highly specific antithrombotic agents. The experiments of Chen et al indicate that targeting the cell surface may well be the next step in further improving anticoagulant treatment. ■

REFERENCES

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2. Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation. 2004;109:2698-2704.

IMMUNOBIOLOGY

Comment on Majstoravich et al, page 1396

The core of the lymphocyte microvilli–WASp issue

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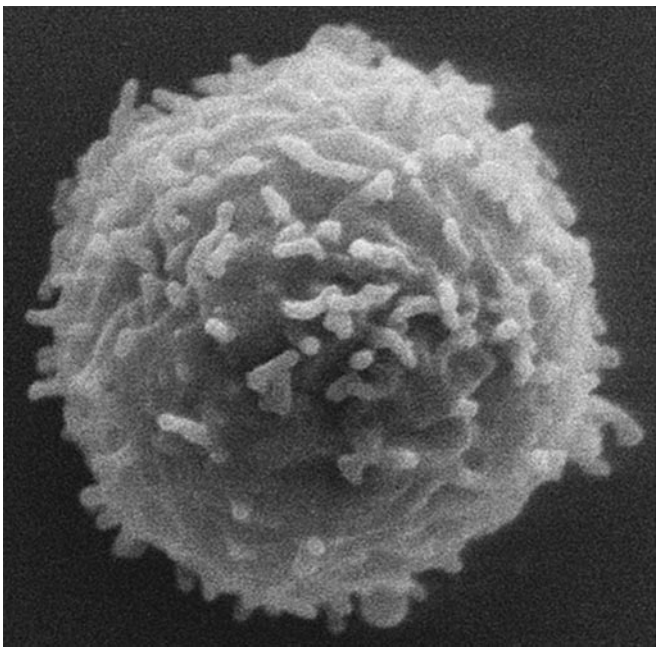
Majstoravich and colleagues have determined that the short microvilli of lymphocytes, although uniform in length and density, are highly dynamic actin filament bundle–containing structures that do not depend upon WASp for their morphology.

Lymphocytes are known to have short, actin filament–rich, microvillus-like projections, and these structures have been implicated in processes of key importance, such as the initial phase of rolling during extravasation and virus recognition. But, primarily because these structures are so short (~ 0.3 μm), relatively little is known about their internal organization and dynamics. An issue of central importance is the role of Wiskott-Aldrich syndrome protein (WASp), the archetype of a family of actin nucleation–promoting factors that stimulate actin-related protein 2/3 (Arp2/3) complex–mediated actin polymerization. WASp is expressed preferentially in hematopoietic cells and is mutated in patients with Wiskott-Aldrich syndrome (WAS), which is characterized by immunodeficiency, thrombocytopenia, and eczema.



Lymphocytes from WASp knock-out mice have normal microvilli. See the complete figure in the article beginning on page 1396.

Of the classes of cell surface specialization assembled using an actin filament–based scaffold, 2 major ones, lamellipodia and filopodia, are believed to involve WASp family members directly or indirectly. The branched actin filament network of lamellipodia uses WASp family members to activate the Arp2/3 complex to form new branches.[1] This lamellipodial actin network can, in turn, be rearranged, cross-linked, and elongated to form the parallel actin bundle scaffold of filopodia.[2] In contrast, remarkably little is known about how the parallel actin bundle scaffolds of microvilli are established and maintained.[3] Compared with lamellipodia and filopodia, microvilli may seem more stable, but recent studies indicate that the actin filaments in the parallel actin bundle at the core of epithelial cell microvilli turn over rapidly through actin treadmilling.[3]

Majstoravich and colleagues used scanning electron microscopy to examine lymphocytes isolated from mice and humans and the larger cells of a transformed pre-B-lymphoma line, and they found a remarkable conservation in microvillar length and density. In addition, using transmission electron microscopy, they observed what appeared to be a parallel actin bundle at the core. Importantly, when the authors exposed the lymphocytes to the actin monomer–sequestering drug Latrunculin A, they observed a rapid and reversible shrinkage of these projections. This behavior was consistent with the existence of actin treadmilling in the filaments of the core actin bundle at a rate of about 1 to 2 actin monomers per second, which is remarkably similar to the treadmilling rate measured in the brush-border microvilli of a kidney epithelial cell line.[3] Finally, these authors found that lymphocytes isolated from WASp knockout mice or human WAS patients of 3 different genotypes showed minimal defects in microvillar length or density, suggesting that wild-type WASp was not required for expression of this baseline microvillar morphology. Although at first glance this result appears to contrast with earlier reports of microvillar abnormalities on lymphocytes from WAS patients[4] and WASp-deficient mice,[5] these earlier studies examined cells under conditions likely to favor lymphocyte activation. This raises the intriguing possibility that there are multiple mechanisms for microvillus formation in lymphocytes and suggests that a WASp-dependent pathway becomes dominant under conditions of activation. ■