addition to direct pathogenic effects of antibodies on megakaryocytes, there is evidence that cell-mediated cytotoxicity, complement activation and the generation of proinflammatory cytokines may contribute to megakaryocyte dysfunction in ITP. Thus, ex vivo studies on megakaryocytes may only provide part of the answer into the complicated mechanisms underlying megakaryocyte dysfunction in the bone marrow. Equally relevant is how TPO mimetics counteract the effect of autoantibodies on platelet production. Binding of TPO to the megakaryocyte receptor c-Mpl improves megakaryocyte survival and differentiation through well defined signaling pathways, and it is conceivable that these processes help limit the deleterious effects of autoantibodies on megakaryocyte platelet production. Unraveling these mechanisms may identify additional approaches to overcome ineffective thrombopoiesis in individuals with ITP.

On the clinical side, a rational approach for the treatment of ITP is a key development, particularly for patients who respond poorly to conventional therapies. It will be important to establish whether the use of TPO-R agonists in combination with agents that minimize platelet removal, such as the monoclonal antibody to CD20, rituximab, will provide additional clinical benefit. Although early indications suggest that these new therapeutics are well tolerated, stimulation of c-Mpl may potentiate platelet activation and trigger thrombosis, a particular concern for older patients with co-existent vascular disease. In contrast, worsening thrombocytopenia and severe bleeding has been described in some individuals who have ceased using TPO mimetics, possibly related to chronic suppression of endogenous TPO. Chronic therapy with TPO-R agonists may also lead to increased bone marrow reticulin, an incidental finding reported in at least two individuals treated with romiplostim.

Finally, potential stimulation of TPO-Rs on leukemic stem cells in individuals with thrombocytopenia and an underlying bone marrow disease, such as myelodysplastic syndrome, remains a lingering concern.

The emergence of TPO-R agonists will probably enhance our understanding of disease in other disorders associated with thrombocytopenia. For example, eltrombopag has already shown clinical benefit in subjects with thrombocytopenia secondary to hepatitis C–associated liver disease. Hopefully, we have only witnessed the tip of the iceberg in terms of the possible clinical benefits of TPO-R agonists. Stay tuned for more surprises.

Mark L. Kahn

Acute bacterial infection leading to sepsis affects over half a million individuals annually in the United States. Available therapies have been primarily supportive, and mortality rates still hover at 30–50%.

More than 100 years ago, German clinicians noted that severe infection could lead to a coagulation syndrome disseminated throughout the vessels of the body that coincided with rapid death—but how infection and coagulation are linked and the role of coagulation in lethal inflammatory states like sepsis has been difficult to unravel.

Historically, studies of sepsis and coagulation have focused on the endothelial cells that line blood vessels. Endothelial cells normally prevent the formation of blood clots, but they seem to lose this vital protective function in septic states. Dramatic support for such an endothelial mechanism—and proof of the principle that the coagulation pathway is a therapeutic target in sepsis—was provided by a landmark study in which individuals with sepsis were treated with a recombinant version of activated protein C (APC), a natural anticoagulant generated on the endothelial cell surface. APC reduced mortality, and this is now a standard therapy for severely ill patients with sepsis. However, how APC thwarts sepsis and the precise role of the coagulation system in sepsis is unclear.

Members of one family of molecules have stood out as obvious candidates for the missing link between the activation of coagulation enzymes and the generation of cellular inflammatory responses—the protease-activated receptors (PARs). These G protein–coupled receptors are activated by coagulation proteases Mark L. Kahn is at the University of Pennsylvania, 925 BRB II/III, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA.

e-mail: markkahn@med.upenn.edu

BENCHED TO BEDSIDE

Counteracting clotting in sepsis

Mark L. Kahn

Acute bacterial infection leading to sepsis affects over half a million individuals annually in the United States. Available therapies have been primarily supportive, and mortality rates still hover at 30–50%.

More than 100 years ago, German clinicians noted that severe infection could lead to a coagulation syndrome disseminated throughout the vessels of the body that coincided with rapid death—but how infection and coagulation are linked and the role of coagulation in lethal inflammatory states like sepsis has been difficult to unravel.

Historically, studies of sepsis and coagulation have focused on the endothelial cells that line blood vessels. Endothelial cells normally prevent the formation of blood clots, but they seem to lose this vital protective function in septic states. Dramatic support for such an endothelial mechanism—and proof of the principle that the coagulation pathway is a therapeutic target in sepsis—was provided by a landmark study in which individuals with sepsis were treated with a recombinant version of activated protein C (APC), a natural anticoagulant generated on the endothelial cell surface. APC reduced mortality, and this is now a standard therapy for severely ill patients with sepsis. However, how APC thwarts sepsis and the precise role of the coagulation system in sepsis is unclear.

Members of one family of molecules have stood out as obvious candidates for the missing link between the activation of coagulation enzymes and the generation of cellular inflammatory responses—the protease-activated receptors (PARs). These G protein–coupled receptors are activated by coagulation proteases...
such as thrombin\(^4\) and mediate many of the cellular responses in thrombotic states. However, determining the relationship between PARs and inflammatory responses such as sepsis has been complex, highly contextual and decidedly controversial.

Two recent studies\(^3\) provide insight into how PARs may link inflammation and coagulation during sepsis. The results are not straightforward, or even compatible, but they suggest that PAR signaling can both augment and oppose inflammatory responses and provide sorely needed direction for a clearer understanding of this intricate connection.

The discovery that APC could cleave and activate the canonical thrombin receptor PAR1 seemed to provide a new mechanistic insight into the coagulation–inflammation axis\(^3\)—but the weak activation of PAR1 by APC compared to thrombin suggested that the effect of APC-activated PAR1 in vivo might be negligible\(^3\). The role of PARs in sepsis was next tested genetically, and an exhaustive study of the response of mice lacking each of several PAR proteins (PARs 1–4) failed to reveal any change in survival rate in response to lipopolysaccharide (LPS), the component of the bacterial wall responsible for many of the inflammatory responses of sepsis\(^5\). Like many pathways involving coagulation enzymes, these studies have gone full circle, leaving the field more or less where it started.

The two new studies begin to break this deadlock. Kerschen et al.\(^5\) take advantage of a mutant APC protein to ask if the clinical protection conferred by APC is through its anticoagulant effect or its ability to generate signals via receptors such as PAR1. First, using mice deficient in PAR1 or the endothelial protein C receptor (EPCR), a protein C receptor that signals independently of PAR1 and enables protein C to both activate PAR1 and act as an anticoagulant\(^6\), they show that both receptors are necessary for the protective effect of APC. Next they show that signaling through one or both of these receptors is sufficient to confer that protection. To show this, they used a mutant APC with minimal anticoagulant activity that retains its ability to activate PAR1 and EPCR; infusion of this mutant APC shielded mice from the lethal effects of sepsis in a manner identical to the effect of wild-type APC\(^5\).

However, they find that PAR1 deficiency is protective, not sensitizing. This protection is exclusively dose sensitive and is seen only at doses of LPS that kill 90% of injected mice, not at higher or lower doses, perhaps explaining the inability of other groups to detect it.

Whereas Kerschen et al.\(^5\) found the sepsis protection conferred by APC administration to be independent of its effect on thrombin generation, Niessen et al. observed something different. They found that the protection conferred by PAR1 deficiency could be reproduced by an antithrombin agent and reversed by activation of sphingosine-1-phosphate (S1P) signaling, a pathway tightly linked to inflammatory responses\(^10\).

Most interesting is the discovery that the cell type by which PAR1 contributes to inflammatory signals in this system is a circulating cell, the dendritic cell. The dendritic cell responds to thrombin by generating sequential PAR1 and S1P3 signals, resulting in the release or ‘dissemination’ of inflammatory mediators such as interleukin-1β and procoagulant molecules such as tissue factor. Niessen et al.\(^6\) outline how thrombin activation of PAR1 fuels the inflammatory responses associated with sepsis. In contrast, Kerschen et al.\(^5\) find that APC activation of PAR1 dampens the inflammatory responses of sepsis independent of thrombin.

What are the mechanistic and clinical implications of these studies? Clearly they establish the principle that coagulation enzymes can regulate inflammatory responses during sepsis through PARs. They also suggest that PAR signaling can both augment and oppose those responses. Such a paradoxical role for the PARs should perhaps not be surprising, as it mirrors the paradox of thrombin itself, an enzyme that drives both coagulant and anticoagulant pathways\(^11\).

The cell type here seems key. Signals downstream of PAR1/EPCR in nonhematopoietic cells seem essential for the protective effect of APC\(^12\), consistent with a protective role for PAR1 in endothelial cells where PAR1-S1P signaling may tighten endothelial junctions and oppose the vascular leak sepsis\(^13\). Conversely, Niessen et al.\(^6\) suggest that PAR1-S1P signals in hematopoietic cells such as dendritic cells may exacerbate inflammatory responses.

Genetic dissection of the role of the PARs in distinct cell types with mice carrying conditional alleles encoding the PARs and S1P signaling proteins, as well as cellular transplantation studies, should explain these paradoxical findings and provide a map of PAR signaling during this complex process. Perhaps then we will know whether and when to tap the PAR brake or PAR accelerator in people with sepsis.

---

**Figure 1** Opposing cell-specific roles for PAR1 during sepsis. Two recent studies define opposing roles for PAR1 during sepsis that are mediated by distinct cell types. Kerschen et al.\(^5\) find that APC drives signaling by PAR1 and EPCR in endothelial cells that protects against hypotension and coagulopathy. Niessen et al.\(^6\) find that thrombin drives signaling by PAR1 to generate sphingosine-1-phosphate (S1P) and signaling by the S1P receptor-3 (S1P3) in circulating dendritic cells that results in the release of tissue factor (TF) and interleukin-1β (IL-1β), inflammatory mediators that increase vessel permeability.

---

2. Manasse, P. Vinchows Arch. 130, 217 (1882).