# Intrinsic Pathway of Coagulation Offers New Targets to Reduce Thrombosis

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The intrinsic pathway of coagulation, also called the contact activation pathway (CAP), represents an arena for potential new therapeutic targets to prevent thrombosis yet maintain hemostasis. Unlike treatments that target steps in the extrinsic, or tissue factor, pathway, the risk of bleeding is low or absent when targeting the CAP. The natural triggers of the CAP are less well understood, but work reviewed in this session has increased the understanding of its mechanisms, the role of polyphosphate (polyP) and immunothrombosis, and the possibility of reducing or eliminating medical device–induced thrombosis (MDIT).

## MEDICAL DEVICE–INDUCED THROMBOSIS

Thrombosis is a common and expensive complication of implanted medical devices that can lead to device failure, thromboembolic complications, and stroke. MDIT is driven by activation of the CAP, and inhibitors of factor XII (FXII) and FXI, which play a key role in this activation, show promise for prevention, according to Jeffrey I. Weitz, MD, McMaster University, Hamilton, Ontario, Canada. Other efforts to reduce MDIT are focused on bioengineering to modify the surface of the devices to reduce their capacity to activate the CAP.

In response to the placement of a medical device, there is rapid protein adsorption and platelet adhesion, activation and aggregation, and simultaneous surface-induced activation of coagulation via the CAP that results in thrombin generation and fibrin formation [Jaffer I et al. *J Thromb Haemost.* 2015]. Thrombin amplifies platelet activation, and the fibrin strands tie the platelet aggregates together to form a platelet-rich thrombus on the surface of the device. Activation of the CAP also leads to complement activation, a process that contributes to device-associated inflammation. Fibrinogen and albumin, which are the first proteins to adsorb to the surface, are replaced by members of the CAP, including FXII, and high-molecular-weight kininogen and kallikrein. There are multiple steps at which amplification of the CAP can occur to further increase thrombin generation and clotting on the medical device.

FXII and FXI were shown to be critical factors in catheter thrombosis in a rabbit model, because their knockdown using specific antisense oligonucleotides prolonged the time to catheter occlusion by 2-fold. In contrast, FVII knockdown had no effect [Yau JW et al. *Blood*. 2014].

Lifelong treatment with a vitamin K antagonist is required for patients with a mechanical heart valve to prevent thromboembolic complications. Valve leaflets accelerate and enhance thrombin generation, which is mediated by FXIIa. However, corn trypsin inhibitor (CTI), a potent and specific inhibitor of FXIIa, markedly attenuated valve leaflet–induced thrombin generation in animal studies.

Dr Weitz and colleagues are studying the role of FXIIa in MDIT. They showed in laboratory work that CTI attenuated catheter-induced clotting in a dose-dependent manner [Yau JW et al. *Blood*. 2011] and that CTI coating on catheters significantly ($P<.05$) prolonged the time to catheter-induced clotting, compared with no catheter or coating with ovalbumin [Yau JW et al. *Acta Biomater*. 2012]. Confirmation that CTI-coated catheters were less thrombogenic was obtained in a rabbit model, suggesting that FXIIa is the driving force in catheter-induced thrombosis.

Other work showed that heparin inhibits FXIIa, FXIa, FXa, and thrombin, thereby blocking the intrinsic pathway at its initiation and attenuating catheter-induced clotting; in contrast, fondaparinux, which only inhibits FXa, had no effect on catheter-induced clotting [Yau JW et al. *Blood*. 2011]. Warfarin was shown to suppress valve leaflet–induced thrombin generation, but dabigatran was less effective than warfarin at preventing stroke in patients with mechanical valves [Eikelboom JW et al. *N Engl J Med.* 2014]. An FXIIa-directed antibody inhibited thrombosis in a

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rabbit extracorporeal membrane oxygenation system as effectively as heparin, but without inducing bleeding [Larsson M et al. *Sci Transl Med*. 2014], and an FXI-directed antibody reduced thrombosis in another animal model [Tucker EI et al. *Blood*. 2009]. In a baboon arteriovenous shunt model, FXI knockdown with an antisense oligonucleotide reduced platelet deposition without prolonging the bleeding time [Crosby JR et al. *Arterioscler Thromb Vasc Biol*. 2014].

## IMMUNOTHROMBOSIS

Immunothrombosis is a new concept of intravascular antimicrobial defense to prevent infection that involves local formation of thrombi in microvessels supported by fibrin generation and the recruitment of immune cells and platelets [Engelmann B, Massberg S. *Nat Rev Immunol*. 2013]. Extracellular DNA and histones are a double-edged sword in this setting, stated Patricia Liaw, PhD, McMaster University, Hamilton, Ontario, Canada, because they exert effects that are procoagulant, proinflammatory, and antifibrinolytic. In some settings, the effects can be beneficial, but in others, they can contribute to the pathophysiology of immunothrombosis. Histones are released from dying cells and activated immune cells, including neutrophils.

In work reviewed by Dr Liaw, DNA was shown to activate coagulation and suppress fibrinolysis. Histones were shown to impair the protein C anticoagulation pathway and activate inflammation, cell death pathways, and platelets. The net effect, she stated, is a hypercoagulant and antifibrinolytic phenotype. Potential avenues for therapeutic targets in immunothrombosis are neutrophils, DNA, and histones.

## POLYPHOSPHATES IN THE CONTACT ACTIVATION PATHWAY

Along with extracellular DNA, polyP has also been identified as a trigger of the CAP in the past decade, stated James Morrissey, PhD, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA [Morrissey JH et al. *Blood*. 2012]. PolyP is a structure of linear polymers of inorganic phosphate connected by high-energy phosphoanhydride bonds.

PolyP acts at 4 points in the clotting cascade. In addition to being a potent trigger of CAP, it accelerates FV activation, enhances fibrin clot structure with thicker fibrils that are more resistant to fibrinolysis, and promotes FXI back-activation by thrombin.

Tissue factor pathway inhibitor is thought to be a major endogenous clotting inhibitor, but polyP was shown to abolish its effect on clotting [Wood JP et al. *Proc Natl Acad Sci USA*. 2013]. PolyP is a procoagulant that reverses the effect of several anticoagulant drugs (heparin, enoxaparin, rivaroxaban, argatroban), and shortens clotting times of hemophilic plasma and in patients taking warfarin. PolyP also targets the conversion of FV to FVa, which is a rate-limiting step in blood clotting.

Long-chain polyP were shown to be present in infectious microorganisms and to be an extremely potent trigger of CAP. PolyP that is platelet-sized accelerates FV activation and promotes FXI back-activation by thrombin. Thus, FXII and FXIIa may be better targets for polyP because inhibition does not trigger bleeding and may protect against thrombosis.

Nontoxic universal heparin reversal agents were shown to abolish the function of polyP [Travers RJ et al. *Blood*. 2014]. PolyP was shown to suppress the complement cascade and thus has some anti-inflammatory effects.

PolyP is a target for novel antithrombotic drugs and likely has undiscovered roles in hemostasis, thrombosis, and inflammation. The new understanding of the role of polyP and DNA and histones in immunothrombosis suggests the need for a new schema of the CAP that includes these and other new mediators.