Activators, therapeutics and immunity-related aspects of thrombosis

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The articles contained in this Theme Issue “GTH – state of the art” describe major new developments in the field of thrombosis and haemostasis and are based on lectures presented at the meeting of the Society for Research on Thrombosis and Haemostasis (GTH) held in 2013 in Munich, Germany. Currently, we are witnessing considerable dynamics in this area of research which goes along with an increased awareness of the major impact of thrombosis on worldwide morbidity and mortality. Such innovational progress is driven by technical advances such as for example new intravitral imaging techniques allowing monitoring of the complexity of thrombus formation in vivo in unprecedented detail as well as by experimentally founded new concepts on the mechanisms triggering thrombosis. Notably, in recent years several new molecular regulators of thrombosis have been identified such as extracellular nucleic acids, polyphosphates and protein disulphide isomerase as well as supramolecular particles including extracellular nucleosomes/neutrophil extracellular traps (NETs).

The articles by Geddings and Mackman (1) and by Langer and Ruf (2) summarise the molecular mechanisms underlying the procoagulant functions of these new activators of thrombosis. Extracellular RNA initiates coagulation by activating proteins supporting the intrinsic pathway of coagulation (3). Extracellular DNA, as part of extracellular nucleosomes, can stimulate the tissue factor (TF)-triggered pathway of blood coagulation via degradation of the TF antagonist tissue factor pathway inhibitor (TFPI; 4) and most likely also via the factor XII-initiated contact pathway (5). A separate avenue of research indicates a prominent role for another group of polyanions for the growth of intravascular thrombi, polyphosphates, which can stimulate coagulation by activation of factor XII (6, 7). Protein disulphide isomerase (PDI), apart from its role as activator of platelets (8), can mediate the first step of the entire coagulation process, the activation of TF, which enables efficient activation of blood coagulation via binding to factor VII/VIIa (9). A model for this initiation step is presented that takes into account, apart from PDI, additional activators of TF-initiated blood coagulation, such as phosphatidylinerine exposed on the outer leaflet of the cell membrane and complement C5b-7 complex (2).

Different molecular pathways enable extracellular nucleosomes/NETs to act as major prothrombotic particles (1). By mediating prothrombotic effects via the release of NETs innate immune cells such as neutrophils and monocytes acquire a central position in the development of thrombosis. This holds in particular for venous thromboembolism and microvascular thrombosis (4, 5, 10). Together these and other findings enable a new perspective on the interaction between blood coagulation and innate immunity/inflammation. Indeed, thrombosis under certain conditions can represent a biological mechanism of innate immunity termed immunothrombosis (11). Thus, coagulation and inflammatory mechanisms are not only closely connected, but intravascular coagulation, together with platelets, innate cells and extracellular nucleosomes, can promote thrombosis to directly enforce antimicrobial defense in microvessels. On the basis of these findings several previous findings can be rationalised such as for example the observations of functionally active TF in blood and, more in general, the evolution of molecules and supramolecular particles with prothrombotic functions that are without major effects on haemostasis.

The role of von Willebrand factor (vWF) for haemostasis and thrombosis has been elucidated in considerable detail and its role during infections is increasingly appreciated. Huck et al. discuss the biological and pathological role of vWF largely from a biophysical perspective (12). They summarise different steps in the lifecycle of vWF, starting with its release from endothelial cells, followed by binding and aggregate formation of vWF monomers and, finally, its degradation by ADAMTS-13. All of these steps are shown to be markedly influenced by the local shear flow and the physicochemical characteristics of the microenvironment. Thereby this review provides evidence how biophysical insights, in addition to the identification of molecular mediators and of functional pathways, can substantially enhance our knowledge about physiological and pathophysiological mechanisms.

A central target for antithrombotic interventions is thrombin, the core protease of the coagulation system. Based on the crystal structure of thrombin, either alone or in complex with inhibitors, Huntington describes similarities in the actions of natural inhibitors of thrombin such as those contained in the saliva of haematophagous animals (blood suckers) and in those used by the four serpins that act as endogenous thrombin inhibitors and include for example antithrombin and protein C inhibitor (13). All these thrombin inhibitors indeed first interact with the two exozites in thrombin from which they are subsequently directed to the active site cleft (13). This eminently involves the partial unfolding of thrombin which is induced by both the endogenous and natural inhibitors.

The elucidation of the crystal structures of the coagulation factors thrombin and Xa has been of critical importance for the de-
development of the direct oral anticoagulants described in the review by Schulman (14). So far, the most important application for these thrombin and factor Xa antagonists are stroke prevention consequent to atrial fibrillation and venous thromboembolism. These two types of thrombosis are characterised by fibrin-rich thrombi and are therefore most susceptible to anticoagulants. Nonetheless, new trials are exploring the possibility that the oral anticoagulants may also be instrumental in complementing anti-platelet drugs as inhibitors of arterial thrombosis. Undoubtedly, the new oral anticoagulants are superior over vitamin K antagonists in terms of a reduced requirement for diagnostic check-ups and dose adjustments. Overall, Schulman advocates for a careful identification of subsets of patients that may profit from either the new oral anticoagulants or from classical vitamin K antagonisation.

Endogenous anticoagulants in particular activated protein C (aPC) may extend the repertoire of anticoagulant molecules to chronic diseases (15), a group of diseases that so far have been less important targets for anticoagulants. Bock et al. review the effects of in particular cytoprotective aPC variants that induce less bleeding complications than native aPC. These aPC variants may become valuable protectors of cell integrity during chronic diseases such as diabetes and its complications as well as neurodegenerative pathologies. Their ability to affect intracellular signalling pathways in a cell- and tissue-specific way can be potentially of substantial benefit as it may allow long-term cell-type directed therapies.

Antithrombotic therapies could advance by a further step if new substances can be developed that inhibit thrombosis without blocking haemostasis and that thus lack bleeding complications. So far, all anticoagulants and anti-platelet drugs targeting thrombotic diseases suffer from this drawback. Based on their functions as mediators of physiological forms of thrombosis, the identification of molecules that are most likely exclusively or predominantly involved in thrombosis should pave the way for the development of such therapeutics. Moreover, the recently obtained new insights into the pathogenesis of thrombotic diseases need to be accompanied by an increased awareness of the importance of the role of thrombosis in diseases that so far have not been associated with this pathology. For example under all conditions of increased blood D-dimer levels, which are frequently observed in the clinical practice (16), the involvement of thrombotic vessel occlusions in the microcirculation should be considered. Often such increases in systemic parameters of blood coagulation remain unexplained especially when they cannot be related to well-established thromboembolic disorders in large blood vessels.

**Conflicts of interest**

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**References**