Infections and the role of plasma proteins and platelets

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In this Theme Issue of *Thrombosis and Haemostasis*, six contributions by experts in the field of coagulation and immune defence were collected. The articles address the role of the plasmatic coagulation system, fibrinogen and factor XIII at the intersection of coagulation and inflammation, the immune function of platelets, the role of platelets in infection and the formation of neutrophil extracellular traps, platelets in sepsis and platelet immunology in fungal infections.

Recently, it has been shown that platelet-rich plasma (PRP) and platelet-poor plasma (PPP) provide early protection against bacterial infections during surgical interventions. The effect of PRP was higher than that of PPP which presumably was due to the larger quantity of platelets. Platelets are the most abundant cells with immune functions present in the circulation, and they apparently make PRP particularly valuable for tissue healing and microbiocidal activity (1). Platelets are small anucleate cells circulating in the blood. They adhere and aggregate at the site of vascular injury which is critical for efficient haemostasis and initiation of clot formation to seal injured vessels and prevent major blood loss. Activation of the haemostatic system has to occur locally, any systemic activation can cause severe problems including thromboembolism and disseminated intravascular coagulation (DIC) resulting in morbidity and even mortality. The haemostatic system comprises a cascade of enzymatic events of the plasmatic coagulation proteins which has been extensively studied since the early 1960. Yet, the contribution of coagulation proteins to immune defence at a very early stage of microbial invasion has only recently received intensive attention. In their contribution van der Poll and Herwald (2) review the reaction of plasma proteins to an invading pathogen leading to an activation of the extrinsic (specifically tissue factor and tissue factor pathway inhibitor 1) and the intrinsic coagulation system (particularly the contact factors) and the potential elimination of the pathogen. They discuss the generation and function of bradykinin as potent inflammatory mediator and contributor to early defence microcirculatory factors which had been shown previously by Monteiro (3). They also outline modes of action of protease activated receptors (PAR), and they give examples of peptide sequences in the carboxyterminal region of several coagulation proteins that develop antimicrobial activity when proteolytically liberated. A connection between the coagulation system and the innate immune system is discussed on the basis of the shared ancestry of vertebrate coagulation factors with complement proteins. Previously, phylogenetic studies have shown that a simple coagulation system comprising proteins that are structurally related to fibrinogen, and factor XIII existed already in primitive anthropods. Together with the precursors of platelets, the so called haemocytes, these proteins had also anti-microbial features (4, 5). Today we know that during fibrin formation active peptides are released from fibrinogen which influence cellular processes. Sequence regions in the fibrinogen α, β and γ chain and in factor XIII contribute to inflammatory processes. Some of these regions have been identified and their function and the interaction partners, e.g. the complement system, are addressed by B. Hoppe in this theme issue (6). He describes the regulation of fibrinogen synthesis as well as it’s variation during acute phase reaction, and he discusses the fibrin matrix as inflammation mediator. Furthermore, he presents interesting effects of FXIII, such as the regulation of locomotion and phagocytosis.

In the contribution on the immune function of platelets Duerschmied et al. present important clinical and experimental evidence (7). They outline that cessation of antiplatelet therapy could theoretically improve infectious disease outcome, but any evidence for that is currently missing. Antiplatelet therapy has not been linked to an increase in infectious disease prevalence or morbidity. There are no epidemiologic data showing that platelet suppression affects infections. Furthermore, patients with GPIb deficiency in Bernard-Soulier syndrome are not known to suffer from immune defects. Duerschmied et al. also refer to the important property of platelets to newly synthesise mediators such as interleukin (IL)-1β following signal-dependent splicing of pre-mRNA which is present in platelets (8). It is known that platelet activation is associated with the release of chemokines and proinflammatory lipids which induce pleiotropic effects on many tissues and cells, including leukocytes. During thrombosis, the recruitment of leukocytes to activated platelets is considered an important step which not only links thrombosis to inflammatory responses but may also enhance procoagulant state. Platelet-leukocyte interaction involves adhesion molecules, chemokines and chemotaxtract molecules, various proinflammatory lipids and other materials (9). Very recently data have been presented that during systemic infections microvessel-thrombosis can occur and mediate an intravascular innate immune response (immunothrombosis). This form of thrombosis is based on the generation of fibrin in blood vessels and is triggered by neutrophils and their interactions with platelets (10). Activated neutrophils then release their granular contents including antimicrobial peptides and a number of serine and metalloproteinases, and under certain circumstances they form neutrophil extra-
cellular traps (NETS) (11). Immunothrombosis is mediated by neutrophil elastase, tissue factor and coagulation factor XII. While the plasma contact system comprising factor XII, prekallikrein and high-molecular-weight kininogen, triggers the kalikrein-kinin system, the intrinsic pathway of coagulation, the classical complement cascade and the fibrinolytic system, the main route by which infections initiate coagulation seems to be via tissue factor. Also in this Theme Issue, Andrews et al. review NETs and the role of platelets in infection (12). They summarise how platelets orchestrate the involvement of neutrophils at sites of injury and inflammation, how NETs influence platelet function, and how they modulate platelet-driven coagulation. The electrostatically charged NETs limit dispersion of pathogens by entrapping them, but they also trigger intrinsic coagulation. The authors refer to recent studies showing that bacterial DNA may substitute for heparin in the formation of antigenic complexes similar to those known in heparin-induced thrombocytopenia (HIT). This could imply that the rapid immune response observed in HIT may in part be an unfortunate consequence of a misdirected platelet-immune response to bacterial infection. The articles by de Stoppelaar et al. (13) and Speth et al. (14) review the role of platelets in sepsis and discuss platelet immunology in fungal infections. Both contributions make it very clear that platelets are important immune cells and contribute to innate and adaptive immunity. Once again examples are presented that they have relevant functions in inflammation and in immune response (15). Their pro-inflammatory molecules and cytokines (e.g. P-selectin, CD40L, IL-1β, etc.), can support leukocyte trafficking, modulate immunoglobulin class switch, and germinal centre formation. Indeed, an intact platelet-fibrinogen plug can form a surface that allows the recruitment of phagocytizing cells. Platelets express several functional Toll-like receptors (TLRs), such as TLR-2, TLR-4, TLR-9 and the most recently investigated TLR-7 (15). It had been shown that platelet TLR-7 stimulates formation of platelet-neutrophil aggregates which results in internalisation of platelet fragments by neutrophils, platelet-granule release, surface expression of P-selectin and increased platelet-neutrophil adhesion. The activation of platelet TLR-7 represents a link between platelets and the response to RNA viruses. Interestingly, platelet-TLR7 stimulation is independent of thrombosis. In their article de Stoppelaar et al. (13) summarise that inappropriate accumulation and activation of platelets are key events in the development of sepsis-related complications. Along a number of examples it is explained that platelet activation readouts, e.g. of platelet secreted products (platelet factor 4, soluble P-selectin), platelet P-selectin surface expression, platelet-leukocyte complex formation or platelet function (whole blood impedance aggregometry) could serve as biomarkers for early sepsis recognition. In their article the authors also discuss the role of the complement system and of FcyRIIs in bacterial sepsis. They give details on acute kidney and lung injury and show first experimental results and data of clinical studies on the effects of antiplatelet therapy in sepsis. The article by Speth et al. (14) presents current knowledge on the role of platelets in fungal infections. It highlights aspects of interaction between platelets and fungi, specifically candida, aspergillus, and cryptococcus, and the relevance of these processes for the pathogenesis of the fungal infection at different sites. Besides platelet microparticles, molecules present in the granules of platelets e.g. serotonin and the complement system are discussed in regard to the protective role of platelets and the reduction of the fungal load. In addition, possible negative effects of platelets are outlined which could be due to endocytosis of spores by platelets, followed by their compartment through the body and protection of the pathogen from immune attacks. Finally, the article also addresses different aspects of antymycotic therapy in context with platelets.

There is general agreement that haemostatic proteins and blood platelets not only modulate immune functions of leukocytes but also contribute directly to the capture and in some cases even killing of pathogens. Currently, however, it is still unclear if and how these mechanisms can be exploited for patient treatment.