Editor’s choice articles 2008–2009

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Modification of coagulation and fibrinolysis factors

Patients with severe haemophilia A or B are at high risk of recurrent bleeding and require treatment with procoagulant factors, such as factor VIIa or factor IX. Unfortunately, the half-life of the recombinant forms of these proteins is rather short (2–3 hours). The generation of genetically fused constructs with albumin, as reported by Weimer et al. (1) and Metzner et al. (2), not only prolongs the half-life of the coagulation proteins in animal models, but they also express biological activity comparable to the unmodified counterparts or to proteins chemically modified by poly-ethylene-glycol (3). These data are promising for future therapies, making long-living natural drugs available for prevention of bleeding risks in haemophilia patients.

Under conditions of severe sepsis, for which activated protein C (APC) has been approved as a therapeutic agent, reactive oxygen species can lead to protein modifications. As reported by Nalian and Lakhaiev (4), oxidation of APC in vitro leads to methionine modification, thereby altering the conformation and molecular architecture of the protease and its active site. Understanding the structural changes that occur upon oxidation of APC may result in the generation of protected proteins with increased functional stability upon oxidative stress conditions.

The cellular quality control during protein biosynthesis is greatly dependent on the ubiquitin/proteasome system. Using mutants of protein C or plasmin inhibitor as prototype examples, Nishio et al. (5) could show that N-glycosylation facilitated proteasomal degradation, independent of ubiquitination of the protein substrates. Provided the mutated proteins are functionally active, their biological half-life could be prolonged using proteasome inhibitors.

Vascular protection by activated protein C

The anti-inflammatory and barrier-protective functions of activated protein C (APC) were shown by Schuepbach et al. (6) to be mediated by protease-activated receptor-1 (PAR-1) on endothelial cells, documented in mouse models of sepsis and pulmonary leakage in wild-type but not in PAR-1-deficient mice. However, respective human studies are required to document the applicability of these mechanisms in patients. In another study by Bae and Rezaie (7) the pro-inflammatory stimulation of endothelial cells by thrombin or a PAR-1 inducing peptide leading to e.g. expression of adhesion molecules was switched into an anti-inflammatory response when the protein C receptor was bound to its ligand. In this situation as was true in the case with APC, no NFκB-signalling pathways were triggered. Thus, various effects of protein C/APC on endothelial cells appear to protect the vessel wall against pathological injury.

Platelet interactions in coronary disease and obesity

It has recently been appreciated that platelets, newly released from the bone marrow, display a higher RNA content and haemostatic activity than mature platelets, and that this immature fraction reflecting platelet production and turnover can be reliably quantified by flow cytometry. A study by Grove et al. (8) revealed that the fraction of immature platelets is increased in acute coronary syndromes, especially in the acute phase of ST-elevation myocardial infarction. This increase was particularly notable in active smokers and diabetic patients and, via an enhanced haemostatic potential, this fraction may contribute to ongoing coronary thrombus formation.

Conversely, the quest for the optimal antithrombotic therapy to prevent ischaemic events after percutaneous coronary intervention (PCI) and stenting to treat coronary disease continues. Busch et al. (9) addressed the influence of anticoagulant agents on platelet and leukocyte activation in patients undergoing PCI, demonstrating that platelet surface expression of PAC-1, P-selectin and glycoprotein (GP) Ibα but also CD11b expression on CD14+ monocytes was reduced in patients receiving bivalirudin as compared to heparin. These data imply that bivalirudin may reduce platelet and monocyte activation in patients undergoing PCI and thereby limit peri-interventional thrombotic complications.

The Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) has recently been identified as an immunoglobulin-like molecule expressed on platelets. Besides its function in regulating the synthesis of matrix metalloproteases, a study by Seizer et al. (10) unveils that EMMPRIN serves as an adhesive ligand for platelet GPVI. Specific binding of EMMPRIN to GPVI was demonstrated using a modified ELISA and surface plasmon resonance technology, yielding a remarkable dissociation constant of 88 nM. The interaction of EMMPRIN with its platelet receptor GPVI is functionally important in mediating platelet rolling.

Clinical studies have implicated elevated leptin levels as an independent cardiovascular risk factor, but little is known about
the effects of leptin on platelets in the setting of obesity. A study by Dellas et al. (11) examined the effects of leptin on platelet aggregation in morbidly obese subjects. The aggregatory response to adenosine diphosphate (ADP) was significantly increased in platelets from obese compared to lean donors. Plasma leptin levels but not body mass index correlated with stronger platelet aggregation to ADP, and preincubation with leptin promoted ADP-induced platelet aggregation. In conclusion, this indicates that platelets from obese donors show increased aggregatory response to ADP, that this might partly be due to increased circulating leptin levels, and may contribute to increased thrombotic risk in obesity.

Lastly, the topic of antiplatelet therapy resistance continues to remain under close scrutiny (12). The arguments continue whether aspirin resistance is a reflection of compliance, or whether „aspirin nonresponsiveness” is a major entity. If the latter is so, clearer definition of this entity is needed. New antiplatelet drugs may overcome this issue, and this journal has published interesting viewpoints on the new agents, such as prasugrel (13).

C-reactive protein and complement activation in vascular disease

Mural thrombus formation following atherosclerotic plaque rupture is the main event compromising blood flow in acute myocardial infarction. A study by Distelmaier et al. (14) compared proteomic profiles of plasma derived from sites of thrombus formation of patients with myocardial infarction and identified a localised activation of the complement system, with accumulation of C-reactive protein (CRP) and the downstream complement effectors C3a and C5a. In vivo, neutrophil accumulation at the site of thrombus formation paralleled the delay from symptom onset to intervention and correlated with C5a and infarct size. This represents direct evidence for local complement activation in acute coronary thrombi, which may contribute to the vascular occlusion process in acute myocardial infarction.

The association between increased CRP levels and future cardiovascular events is well established, but it remained unclear and equivocal whether CRP beyond this clinical observation can actively promote the development of atherosclerosis. A study by Torzewski et al. (15) used mice deficient in low-density lipoprotein receptor (LDLR-/−) crossbred with CRP transgenic animals expressing the human CRP pentraxin (huCRP) for feeding a Western-type diet and analysis of atherosclerotic lesion development. Remarkably, significant differences of lesion size or lesion composition could not be detected between huCRP-positive and huCRP-negative LDLR-/− mice, supporting the notion that CRP does not play a causative pathogenetic role in atherogenesis.

LDL can be enzymatically modified to yield a derivative (E-LDL), which binds CRP, activates complement, and is rapidly taken up by monocytes/macrophages. Apoprotein J or clusterin is a multifunctional glycoprotein with cytoprotective and anti-inflammatory properties. Another study by Schwarz et al. (16) shows that ApoJ can be detected in early atherosclerotic lesions in co-localisation with E-LDL, binds to E-LDL and reduces its cytotoxicity. In atherosclerotic lesions, ApoJ may thus exert protective functions by its capacity to inactivate C5b-9 complement membrane attack complexes.

The role of inflammation in the causation of venous thromboembolism (VTE) is uncertain. A prospective, population-based study by Folsom et al. (17) in 10,505 participants of the Atherosclerosis Risk in Communities (ARIC) Study indicates that elevated CRP is independently associated with increased risk of VTE.

Endothelial cell functions in vascular disease

Endothelial progenitor cells (EPC) or early-outgrowth cells are believed to be involved in cardiovascular healing processes and can be found in resolving venous thrombi. A study by Disanto et al. (18) investigated whether the therapeutic administration of EPC might enhance the resolution of venous thrombi in 28 athymic nude rats. Indeed, EPC transplantation significantly increased thrombus neovascularisation and intrathrombus blood flow accompanied by macrophage recruitment into resolving thrombi. This might be of clinical value to facilitate venous thrombus resolution when other options have failed.

Angiopoietin (Ang)-2 (derived from endothelial cells) antagonises effects of Ang-1 on blood vessel maturation and cooperates with vascular endothelial growth factor (VEGF) to induce neovascularisation. A study by Feng et al. (19) demonstrates that mice deficient in Ang-2 fail to form a proper vascular network in the retina, as evident by reduced large vessel numbers and defects in the superficial arteriolar periphery and deep capillary network. Hypoxia in the retinal periphery induced VEGF upregulation and active endothelial cell proliferation. Together, these data suggest that Ang-2 has an important function in the spatial configuration of the three-dimensional retinal vasculature, while prolonged VEGF activity results in a model of persistent proliferative retinopathy.

Circulating endothelial cells (CEC) detected by the Cell-Search™ assay (CD146+CD105+ CD45− nuclear cells) are thought to derive from damaged vasculature. Because CD105 is strongly expressed by endothelial cells from malignant vascularity, it was unclear whether this assay is suitable to determine CEC in non-malignant diseases. A study by Strijbos et al. (20) shows that numbers of CEC significantly increased after venesection and cannulation and non-intact, possibly apoptotic CEC occurred after administration of tumor necrosis factor-α (TNF). This indicates that the Cell-Search™ assay can detect CEC originating from damaged normal vasculature, indicating a value in non-malignant disorders characterised by vascular damage.

Microparticles and transmission of molecules

The mechanisms underlying the progression of aortic valve stenosis remain unknown but may involve shear stress and in-
flammation. Diehl et al. (21) analysed circulating microparticles (MP) in patients with aortic valve stenosis and found increased numbers of platelet- and leukocyte-derived MP, as well as monocyte activation and platelet-MP-monocyte conjugates correlating with valvular shear stress and the number of endothelial MP. In conjunction, this indicates that aortic valve stenosis is accompanied by increased levels of MP and that shear stress can induce MP formation and inflammatory endothelial cell activation to sustain the disease process.

As reported by Ray et al. (22) for the first time, expelled platelet MP contain the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) together with retinoic X receptor, the complex retaining DNA-binding ability. Upon translocation and internalisation by leukocytes, these cells acquire PPAR-γ responsiveness. Thus, platelet MP may spread transcription factors and thereby influence gene expression of vascular (wall) cells in conjunction with inflammation or metabolic syndrome.

A substantial amount of cell-associated prion protein (PrPc) in blood resides in platelets, and their activation leads to up-regulation of PrPc on the platelet surface and the release of exosomes and MP. Brouckova and Holada (23) reported on the association of PrPc with cytoskeleton-linked platelet membrane domains (lipid rafts) that would favour release of MP and interaction of PrPc with variant Creutzfeldt-Jakob disease prions. Whether these new relations may explain the transmissibility of the neurological disease remains to be analysed.

Atrial fibrillation and VTE

Atrial fibrillation (AF) is the commonest cardiac arrhythmia and is associated with a significant risk of stroke and thromboembolism – irrespective of whether the rhythm is persistent or paroxysmal (24). However, the risk of stroke and thromboembolism in AF is not homogeneous, and an important systematic review of stroke risk factors, risk stratification schemes and cost effectiveness data, as part of the United Kingdom National Institute for Health and Clinical Excellence (NICE) was published in this journal (25). This work, and others have been used to inform stroke risk stratification schema, but many analyses comparing various published schemata have been performed. These data suggest that risk schema have modest predictive value for high risk subjects (26), given new oral anticoagulants becoming available as alternatives to the vitamin K antagonists, we should perhaps focus on new schema that are better at identifying “low risk” subjects.

Genetic disposition, cardiovascular disease and thrombosis

Continued interest into the genetic predisposition to acute myocardial infarction continues. A paper by Rallidis et al. (27) examined the Factor XIII Val34Leu polymorphism and the risk of myocardial infarction (MI) under the age of 36 years, given the limited and controversial data regarding the impact of this polymorphism in the pathogenesis of premature MI. The authors found that FXIII Val34Leu polymorphism had a protective effect against the development of MI.

In the field of VTE, what is predictive of risk and recurrences? Many clinical risk factors have been identified; Ay et al. (28) reported how P-selectin gene haplotypes modulate soluble P-selectin concentrations (an index of platelet activation) and contribute to the risk of VTE. They assessed the effect of four single nucleotide polymorphisms (SNP) (one in the promoter region [-1213C>G] and three in the coding region [S290N, 1087G>A; D562N, 1902G>A; T713P, 2363A>C]) as well as the calculated haplotypes in the P-selectin gene on soluble P-selectin concentrations and VTE risk. They found that such haplotypes modulate plasma concentrations of soluble P-selectin and affect the risk of recurrent VTE.

Certainly, a gene-based default is only one of multiple reasons of causality leading to phenotype alterations. In a report from an intensive care setting, Levine et al. (29) raise awareness of severe sepsis as a cause of thromboembolism, both venous and arterial. The risk of long-distance air travel has always gained much media attention as a risk factor for VTE, and many causes have been suggested, including dehydration. In an elegant study, Schreijer et al. (30) extend the information in this area by showing that fluid loss does not really add much to coagulation activation during air travel.

Pharmacology of vitamin K antagonists and bleeding risk

The chronic treatment of thrombosis has centred on the vitamin K antagonists, and much attention has been directed towards the pharmacogenetics of such oral anticoagulants. In an important study, Meckley et al. (31) showed that genetic variation in the vitamin K-epoxide reductase VKORC1 appears to have a different influence than the cytochrome P450-enzyme CYP2C9 on anticoagulation-related outcomes such as bleeding events and time in therapeutic range. Nonetheless, they found that this difference may be due, in part, to pharmacokinetics factors (e.g. drug half-life), which are influenced primarily by CYP2C9. However, attention to bleeding risk is highly relevant in the management of treatment with oral anticoagulants. Palareti and Cosmi (32) provide a comprehensive overview on who is at risk, and how best to identify patients at risk of bleeding on oral anticoagulants. Clearly, some clinical risk factors will be recurrent feature, and (for example) Trujillo-Santos et al. (33) nicely demonstrated this for cancer patients with VTE.

References