by a single point mutation in the FV gene (FVLeiden) that not only renders FVa less susceptible to the proteolytic inactivation by APC but also impairs the anticoagulant properties of FV. The presentation will give a description of the dualistic character of FV, a discussion about structure-function relationships of FV, and the details of a recently created model of the prothrombinase complex.

THROMBIN SIGNALING IN HEMOSTASIS AND THROMBOSIS

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Thrombin is a critical effector of hemostasis and thrombosis. In addition to mediating fibrin formation, thrombin activates platelets and other cells via G protein-coupled protease-activated receptors (PARs). PARs are necessary for activation of human and mouse platelets by thrombin, and recent studies in knockout mouse models suggest that PARs and fibrin formation can together account for the importance of thrombin in hemostasis. Studies in a variety of mouse models of thrombosis also suggest that thrombin signaling via PARs is particularly important in settings in which propagation of a thrombus is required and less important for the formation of extramural and juxtamural thrombi. These results raise a number of interesting questions. What accounts for initial platelet activation in juxtamural thrombi in the absence of thrombin signaling? How is the thrombin that normally activates platelets at a distance from the vessel wall generated? How does thrombin activation of platelets interact with thrombin generation and fibrin formation? These and other questions will be discussed. In addition to activating platelets, PARs trigger responses to coagulation proteases that are potentially both proinflammatory and cytoprotective in endothelial cells and other cell types. Thus, it has been tempting to hypothesize that PARs may help orchestrate inflammatory and/or cytoprotective responses in the setting of disseminated intravascular coagulation in endotoxemia. Studies that attempt to define the roles of PARs in a mouse model of endotoxemia will be presented.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS - IV

PLATELETS AND ATHEROTHROMBOSIS: NEW MOLECULAR INSIGHTS

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It has been known for many years that platelets play an important role in hemostasis and thrombosis. Recently, however, it has become evident that platelets also have relevant functions in inflammation. Thrombosis and inflammation are intrinsically linked processes sharing several key molecular mechanisms and in this contest platelets represent an important linkage between inflammation, thrombosis and atherogenesis. Although it was previously thought that platelets were relatively inactive cells, it is now known that this is not the case. Platelets secrete and express a large number of substances that are crucial mediators of both coagulation and inflammation and, as recently recognized, they synthesize proteins from constitutive mRNAs such as IL-1β, Bcl-3, PAI-1 and also Tissue Factor (TF). TF is the key initiator of the blood coagulation cascade; it binds factor VIIa resulting in activation of factor IX and factor X, ultimately leading to fibrin formation. Over the last decade it has become clear that in addition to its well established role in coagulation, TF participates in other cellular processes including inflammation and is involved in the pathogenesis of cardiovascular diseases such as acute coronary syndromes. It is worth mentioning that functionally active platelet-associated TF is significantly higher in unstable angina compared to stable angina patients. Recently, an alternative spliced form of TF (asTF) has been discovered, which is soluble, circulates in the blood and exhibits procoagulant activity. A consistent expression of asTF mRNA levels has been observed in linfomonocytes from patients with acute coronary syndromes compared to controls. These data provide evidence that different TF mRNA isoforms present in linfomonocytes and platelets of ACS patients can contribute to the hypercoagulability associated with the disease. In view of the inflammatory and thrombotic nature of cardiovascular complications, interruption of the coagulation-inflammation cycle is of great interest in cardioprotection.

ENDOTHELIAL PROGENITOR CELLS FOR VASCULAR REPAIR AND TUMOR ANGIOGENESIS

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Evidence supports the existence of a common hematopoietic and endothelial stem cell (hemangioblast) during embryonic development, and suggests that cells with hemangioblast activity perisist into adult life in humans. Asahara *et al.* first hypothesized that endothelial progenitor cells (EPCs) contribute to vessel production. EPCs can be assessed by their immunophenotype or capability to form endothelial colonies *in vitro* (CFU-End). Immunophenotype identification of EPCs relies on co-expression of specific cell-surface proteins, in particular endothelial markers, including CD34 and VEGFR-2 (KDR), and the human stem cell marker CD133. Lower numbers of EPCs were reported in patients with coronary artery disease compared to healthy controls and in men free of a history of clear atherosclerosis an

inverse correlation between EPCs and the Framingham risk factor score was reported. Augmentation of EPC number and/or function may be a useful therapy. Many reasons drive to guess that the study of EPCs in patients with cancer could be of biological and clinical relevance. The preliminary results strengthen the idea that mobilization of EPCs from the bone marrow is a phenomenon that occurs during the early, premetastatic phase of cancer and open a number of new perspectives in the role of cancer angiogenesis and disease progression. In 110 patients with MMM we measured a median percentage of CD34+CD133+VEGFR-2+ cells of 0.26%, which was significantly higher (p=0.04) than in both healthy controls (median, 0%), and in patients with other Ph- chronic myeloproliferative disorders (median, 0.1%). We found that those patients with a diagnosis of *prefibrotic myelofibrosis* had a higher level of EPCs than those with a diagnosis of MMM in a fibrotic stage (median, 2.1% vs. 1.05%; p=0.05). Patients with higher numbers of CD34+CD133+VEGFR-2+ cells had a significantly lower frequency of circulating immature myeloid cells and blasts (R=-0.49; p=0.005), and lower numbers of circulating CD45+CD34+ HPCs (R=-0.72; p<0.001).

CIRCULATING ENDOTHELIAL CELLS AND ENDOTHELIAL PROGENITOR CELLS: TWO SIDES OF THE SAME COIN, OR TWO DIFFERENT COINS?

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Circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs) are two populations of recently discovered endothelioid cells present in the blood. The former are believed to arise from the blood vessel intima, the latter from the bone marrow. However, it is becoming clear that these are not in fact homogenous populations (e.g. differing degrees of apoptosis, necrosis and viability, differing expression of monocyte and immaturity markers) but do in fact represent more than one species of endotheliod cell. Thus whilst originally defined by different criteria (e.g. CD146 on CECs by immunobeads, CD34 on EPCs by flow cytometry) and the perception of independence, there is also growing evidence of some degree of commonality, i.e. some cells co-expressing CD146 and CD34. Furthermore, relationships between these two cells types and, for example, plasma and physiological indicators of vascular damage, and the risk factors for atherosclerosis, suggest a potential role for these cells in the pathophysiology of this disease, possibly as markers. Despite some confusion, increased numbers of CECs continue to be seen as evidence of severe vascular insult and thus may have some value for vascular biologists. Conversely, EPCs (and bone marrow derived progenitor [stem?] cells) are taken to have therapeutic value in treating cardiovascular diseases such as myocardial infarction. Thus, one view is of some degree of shared ancestry that may have implications for pathophysiology and cell biology. However, based on these uncertainties, most of the evidence is that, despite some commonality, CECs and EPCs are indeed two different cells.

FIBRINOLYSIS AND THROMBOSIS

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While it is widely recognized that enhanced fibrinolytic mechanisms can cause bleeding, the causal relationship between defective fibrinolysis and development of venous or arterial thrombosis has been much more difficult to demonstrate. In earlier studies, attempts to associate fibrinolytic changes with thrombosis met with failure, most likely because fibrinolytic assays were poorly specific, relatively insensitive in detecting hypofibrinolysis and lacked standardization. The identification and purification of the main components of the fibrinolytic system, particularly of tissue type plasminogen activator (t-PA) and of its inhibitor (PAI-1), allowed in later studies to quantitate plasma levels of these proteins by specific immunochemical and functional assays. Using these methods, several investigators reported a defective synthesis/release of t-PA and/or an increase in PAI-1 in patients with a history of venous thrombosis. However, evidence of a relation between these abnormalities, particularly elevated PAI-1 levels, and the risk of a first episode or recurrence of venous thromboembolism is highly conflicting. Likewise, inconsistent data have been published on the association of the PAI-1 polymorphisms affecting plasma concentration of the protein, particularly the 4G/5G, with venous thrombosis. Therefore, in this setting, the predictive value of conventional fibrinolytic changes remains uncertain. As regards arterial thrombosis, numerous, but not all studies have shown that increased plasma levels of PAI-1 (antigen or activity) and/or t-PA antigen can predict future occlusive vascular events in apparently healthy populations, including postmenopausal women, and, more so, in patients with a history of myocardial infarction or angina pectoris or acute ischemic stroke. Elevated t-PA, likely resulting from a diffuse inflammatory damage to the vessel wall, appears to be a better predictor of ischemic vascular events than increased PAI-1, as indicated by the observation that its predictive strength is either unchanged or reduced but still significant after correction for a number of confounding variables (particularly those indicative of insulin resistance), whereas that of PAI-1 is completely lost. As in the case of venous thrombosis, studies on the association of PAI-1 or t-PA polymorphisms with coronary artery disease (CAD) gave conflicting results. In the last decade evidence has accumulated showing a close interplay between coagulation and fibrinolysis. The molecular link between the two processes is represented by a procarboxypeptidase, named TAFI (thrombin activatable fibrinolysis inhibitor), which, once activated by thrombin, removes the plasminogen binding sites exposed onto the fibrin surface thereby inhibiting t-PA-catalyzed plasmin formation. The potential relevance of TAFI as a modulator of fibrinolysis *in vivo* is inferred from animal studies showing that the inhibition of TAFI activation or TAFIa activity enhances both spontaneous and t-PA induced thrombolysis. TAFI is synthesised by the liver and circulates in blood at a concentration far below its Km for activation by thrombin or other enzymes. This implies that the generation of TAFIa will depend on the concentration of both the enzyme (i.e. thrombin) and the substrate (e.g. TAFI). Evidence suggesting a TAFI-mediated inhibition of fibrinolysis related to an increase in thrombin generation has been obtained in carriers of the FV Leiden mutation (homozygotes) and in carriers of the prothrombin G20210A mutation. In recent years several clinical studies have addressed the possible relevance

of TAFI level as well as of TAFI polymorphism in relation to thrombotic diseases. In a number of case-control studies, high TAFI levels were found to be associated with CAD and ischemic stroke and to increase the risk for restenosis after percutaneous coronary intervention. In other studies, however, the occurrence of high TAFI levels was reported to protect against myocardial infarction and coronary death and to be associated with a more favourable clinical outcome in patients with unstable angina. This discrepancy might partly be explained by methodological artefacts in TAFI antigen assay due to a variable antibody reactivity towards TAFI isoforms. As a matter of fact, in a large prospective study, the inverse association between TAFI concentration and CAD could not be confirmed when the patients were re-evaluated using an ELISA insensitive to TAFI isoforms. Inconsistent results were also obtained in studies that evaluated the TAFI gene polymorphism. As regards venous thrombosis, the available clinical studies suggest that TAFI level is a mild risk factor for both the first thrombotic event and recurrence. Interestingly, using a global fibrinolytic assay, originally developed for evaluating TAFI function, a reduced plasma fibrinolytic activity was found to be a risk factor for venous thrombosis. Taken altogether, the epidemiological studies so far available, while suggesting a relationship between impaired fibrinolysis and thrombosis, are still insufficient to define the exact pathogenetic role and the predictive value of the fibrinolytic alterations. Moreover, it should be kept in mind that basal systemic levels of fibrinolytic components do not reflect the local fibrinolytic balance and that studies evaluating the regulation of fibrinolytic activity at organ level are likely to be of greater pathophysiological relevance.

COAGULATION ACROSS DISCIPLINES

THE CASE OF CEREBROVASCULAR DISEASE

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According to the World Health Organization, stroke is defined as the acute onset of focal or global neurological deficits, lasting for more than 24 hours. Therefore, within this definition, the ischemic stroke, the hemorrhagic stroke, the subaracnoid haemorrhage, and a small percentage of other types of cerebrovascular events are included. The ischemic strokes, which represent the higher percentage, are further classified according to the TOAST classification in large vessels disease, lacunar disease, stroke from cardioembolic sources, and stroke from other causes, including, within this last group, coagulation defects. Among the causes of hemorrhagic strokes, defects of coagulation per se, or responsible of cerebral venous thrombosis, are listed among the possible causes.

When we consider coagulopathies in cerebrovascular disease there are some issues to be considered:

1) What to test?

According to the level of evidence, coagulation disorders are variably associated to predisposition to thrombosis.

2) Who to test?

If we look at the incidence of coagulation defects as causes of stroke, it varies according to age, arterial vs venous thrombosis, clinical features, and type of defect.

3) When to test?

Functional tests should generally be performed when the patient is not in an active thrombotic state and the coagulation and fibrinolytic factors have stabilized.

4) What to do with the results?

When a coagulation defect is detected, therapy should be tailored to the individual case. While warfarin is commonly used for secondary prevention, efficacy compared with alternative forms of antithrombotic therapies based on randomized controlled trials has generally been lacking.

These issues are relevant in clinical practice both in the diagnostic process and for therapeutic decisions.