ARTERIAL THROMBOSIS

ATHEROTHROMBOSIS, TISSUE FACTOR AND PLATELETS: AN UPDATE

M. Camera

Dept. Pharmacological Sciences, University of Milan, and Centro Cardiologico Monzino IRCCS, Milan, Italy

Tissue Factor (TF) is the main cellular initiator of blood coagulation, and it is also currently considered the protein that links proinflammatory and prothrombotic mechanisms in the progression of atherosclerosis. Platelets play a key role in atherothrombosis and activated platelets and circulating platelet-leukocyte aggregates (PLA), a sensitive marker of *in vivo* platelet activation, plaque instability, ongoing vascular thrombosis, and inflammation, have been found to be significantly higher in patients with unstable angina (ACS) than in those with stable angina (SA). We have previously shown that platelets from healthy subjects express TF on activation. Recently we have provided the evidence that ACS patients have a significantly higher amount of TF-positive platelets than SA or Controls and significantly higher thrombin generation capacity. TF mRNA expression in platelets is also significantly higher in ACS patients than in SA or Controls. In ACS patients the greater expression of TF in platelets and PLA strengthens the link between platelet activation, blood coagulation, and thrombus formation and may further contribute to the hypercoagulability associated with the disease. TF is also ascribed a non-haemostatic function in cell migration and proliferation, suggesting a role of TF not only in thrombosis but also in atherosclerosis development. Polymorphisms in the TF gene promoter have been shown to modulate the expression of TF, and thereby potentially also its involvement in atherosclerosis and individual predisposition to atherosclerotic disease. We have recently investigated the associations between TF promoter polymorphisms and carotid intima-media thickness (IMT), a well-established surrogate marker of atherosclerotic disease. To this end, the TF A-603G polymorphism was analysed in 316 subjects enrolled in a primary and secondary cardiovascular risk prevention programme, with measurements of carotid IMT by B-mode ultrasound. A significant association between TF promoter genotype and carotid IMT was observed, perhaps mediated via alterations of TF expression levels in the circulation or within the carotid vessel wall. These findings support the hypothesis that TF plays a role in the atherosclerotic process, beyond its well-known role in haemostasis and thrombosis, thus further implicating TF not only in thrombotic complications of atherosclerotic disease, but also in plaque progression.

OXIDATIVE STRESS AND ATHEROSCLEROSIS

F. Violi, P. Pignatelli

University of Rome, La Sapienza, IV Clinic of Internal Medicine, Rome, Italy

There is compelling evidence that enhanced oxidative stress is detectable in patients with classic risk factors for atherosclerosis, but its impact in the context of atherosclerosis progression is still unclear. This uncertainty is due to the lack of a clear prospective study indicating that markers of oxidative stress, such as blood lipid peroxides or urinary F_2- isoprostanes, are of some value for predicting the progression of atherosclerosis. even if some evidence suggest that antibodies against oxidized LDL may be useful. Conversely, epidemiologic studies seem to indicate that low antioxidant status increases the risk for CVD. Clinical characteristics of patients with low antioxidant status have not been defined and should be studied in the future. So far, clinical trials with antioxidants have included patients without evaluating either oxidative stress or antioxidant status. Such indiscriminate enrollment could account for the negative results of antioxidant trials recently emphasized by meta-analysis. Moreover, as recently discussed, the antioxidants might be effective in inhibiting the initial stages of human atherosclerosis and yet be ineffective or much less effective in reducing plaque instability and rupture. If this was the case, it might be necessary to find some way to assess early stages of lesion development (perhaps by high-resolution ultrasound or magnetic resonance imaging) rather than relying on the usual late clinical endpoints. Of course, if the development of early lesions was successfully inhibited, there should eventually be a decrease in the frequency of clinical events, but in that case, the trials might need to extend beyond the conventional 5 years. Another issue that deserves further attention is the choice of appropriate antioxidant treatment. So far, several mechanisms, including enzymatic and non-enzymatic oxidation of LDL, have been proposed, but the exact process leading to LDL accumulation within the vessel wall is still unclear. This fact creates uncertainty in the type of antioxidants that could be relevant for inhibiting atherosclerotic progress. Thus, future trials with antioxidants should not be discouraged; conversely, better identification of criteria identifying potential candidates for antioxidant treatment, together with the choice of an adequate daily regimen of antioxidants, should be studied.

LYMPHOCYTE ACTIVATION AND CORONARY INSTABILITY

F. Crea

Institute of Cardiology, Catholic University, Rome, Italy

The transition from stable to unstable coronary artery disease is not predicted by the severity of coronary stenoses nor by the extent of coronary atherosclerosis and is due to yet unknown inflammatory stimuli causing occlusive or subocclusive thrombus formation. Indeed, the sudden activation of inflammatory cells in the culprit stenosis results in the release of cytokines which have the potential to cause tissue factor expression, plaque fissuring and vasoconstriction, followed by thrombus formation. Although the triggers of inflammation are probably multiple and still largely unknown, several studies have recently shown that dysregulation of T cell repertoire, characterized by expansion of cytolytic CD28null T cells

and of Th17 cells and by impairment of regulatory $CD25^{+}foxp3^{+}$ T cells plays a key pathogenetic role. It is well established that a potent antithrombotic treatment and an invasive approach frequently results in prompt remission of symptoms. Yet, among patients with persistent dysregulation of T cell repertoire the recurrence rate of coronary instability remains high, in spite of the best currently available treatment. Modulation of the aberrant T cell response might become a new therapeutic target.

EARLY SIMULTANEOUS ACTIVATION AND CROSS-TALK OF PLATELETS AND LEUKOCYTES IN THE VERY EARLY PHASE OF ACUTE MYOCARDIAL INFARCTION

N. Maugeri, A. Maseri

Cardio-Thoracic and Vascular Department, University Vita-Salute San Raffaele, Milano, Italy

The identification of the mechanisms of development of acute coronary syndromes could suggest novel diagnostic and therapeutic targets aimed at quenching occasional or recurrent thrombogenic stimuli and/or the enhanced thrombogenic response, rather than at reducing the physiological haemostatic repair. Platelet thrombi grow from the progressive deposition of a variable fraction of those platelets actually passing through the culprit artery, and such fraction and its stable aggregation may depend on the local microenvironment. The major role of platelet activation in acute coronary syndromes is also indicated by the increased platelet-derived thromboxane urinary metabolites excretion, and by the established benefit of anti-platelet therapy for the secondary prevention of acute coronary events. However, acute coronary syndromes the recurrence of ischemic events is reduced, but not abolished, by drugs which inhibit platelet activation-amplification signals. Indeed activated platelets, not only play a major role in thrombus formation but, through heterotypic interaction with leukocytes produce inflammatory responses, which may contribute to local platelet adhesion, aggregation and stabilization of coronary thrombi. Several molecular events of the functional cross-talk between platelets and different classes of leukocytes have been identified by *in vitro* and animal models. In acute coronary syndromes as well as in acute cerebrovascular events platelet activation can result in the formation of heterotypic platelet aggregates with circulating leukocytes, a condition typically observed in inflammatory states. The possibility that occasional inflammatory mechanisms might contribute to the sudden transition from stable to unstable phases of coronary atherosclerotic disease is suggested by the transient elevation of circulating soluble and cellular inflammatory markers, commonly observed in acute coronary syndromes compared to stable coronary atherosclerosis and to severe, chronic peripheral artery disease. Our recent findings demonstrate a simultaneous, transient burst of activation of circulating platelets and leukocytes, as well as an increase of their heterotypic aggregates, in the very early hours of acute myocardial infarction when troponin levels were still very low. All markers of neutrophil activation showed a close correlation with the percentage of platelets expressing P-selectin, but not with the initial or peak troponin I levels. In the samples obtained 24-48 hours after admission neutrophils activation, platelet-neutrophil aggregates and P-selectin expressing platelets were significantly reduced towards to normal range whereas surface makers of activation on monocytes and monocytes-platelet aggregates and IL-6 serum levels remained unchanged and troponin levels increased. The findings were not influenced by aspirin treatment. Our study confirms for the first time, in a relevant clinical setting, many cellular and molecular events of the process of platelet-leukocyte interaction so far described only in experimental models. The intense platelet-leukocyte cross talk observed in acute infarction, unaffected by aspirin, could suggest diagnostic test in acute coronary instability as well as of novel therapeutic strategies.

ARTERIAL AND VENOUS THROMBOSIS: IS THERE A LINK?

P. Prandoni

Department of Medical and Surgical Sciences, Thromboembolism Unit, University Hospital of Padova, Italy

An increasing body of evidence suggests the likelihood of a link between arterial and venous disease. According to the results of recent studies, atherosclerosis and venous thromboembolism (VTE) share common risk factors, including age, obesity, diabetes mellitus, and metabolic syndrome. Atherosclerosis has the potential to promote the development of thrombotic disorders in the venous system. Another scenario assumes that the two clinical conditions are simultaneously triggered by biological stimuli responsible for activating coagulation and inflammatory pathways in both the arterial and the venous system. Several recent studies have consistently shown that patients with VTE of unknown origin are at a higher risk of cardiovascular diseases, including atherosclerotic complications, than patients with secondary VTE and matched control individuals. Future studies are needed to clarify the nature of this association, to assess its extent, and to evaluate its implications for clinical practice.