donors (control A, n=100) and age&sex-matched healthy persons (control B, n=100) were enrolled. The severity of bleeding was quantified in different ways - clinically significant or not for group A, according to bleeding frequency, factor requirement and joint score for group B and with exact measurement of intraoperative blood loss plus estimation of postoperative bleeding for group C. The 4G-5G polymorphism was analyzed in a subset. All studies are conducted at Karolinska University Hospital.

Results. Study of group A and control A and B: We defined low PAI-1 activity as <1 U/mL, since values of absorbance of less than 1 U/mL did not differ significantly from the 0-line. The PAI-1 activity increases with body mass index (r-square=0.2, $p<0.001$), as previously described. The proportion of individuals with low PAI-1 activity is 2-3 times higher among females than males in the different groups. Younger patients have slightly lower PAI-1 activity, but that can be ascribed to the effect of exogenous estrogens on the subset of young women. The adjusted odds ratio for low PAI-1 activity among patients compared to matched healthy controls was 3.23 (95% CI, 1.22±8.56, $p=0.019$). There was no interaction with 4G-5G genotypes. No typical pattern of bleeding was seen among patients with PAI-1 deficiency. Study of group B and C: Analysis is hampered by the lower prevalence of low PAI-1 activity among men. Among the patients with transurethral prostatic resection low PAI-1 activity was found in 4 of 62 patients. Three of these four patients had bleeding complications compared to 16 of 58 patients with normal PAI-1 ($p=0.082$) and after adjustment for resection time, resected mass and systolic blood pressure this becomes borderline significant. In none of the populations could we identify a quantitative effect of the PAI-1 levels above 1.0 U/mL on the risk of bleeding. Analysis of group B is still going on.

Conclusions. There appears to be a qualitative effect on the risk of bleeding from PAI-1 activity of less than 1 U/mL. This effect may become more pronounced in certain situations, such as surgery in areas with a high fibrinolytic activity. However, further studies will be needed to define the clinical relavance of this hemostatic defect.

PLATELET PATHOPHYSIOLOGY

BIOLOGICAL AND CLINICAL CONSEQUENCES OF MYH9 MUTATIONS

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Eighteen distinct classes of myosin have been established based on phylogenetic analysis of the motor domain. Class II comprises filament-forming (also named conventional) myosins that are found in muscle and non-muscle cells. Humans express three isoforms of non-muscle class II myosins, termed A, B, and C. Like all conventional myosins, these are hexameric proteins composed of two heavy chains and two pairs of light chains. MYH9 is the gene for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). Myosin IIA is ubiquitously expressed in cells and tissues, where it is thought to play several roles, including cytokinesis, cell motility, and maintenance of cell shape. Heterozygous MYH9 mutations originate a complex disorder named MYH9-related disease (MYH9-RD). At birth, patients presents only macrothrombocytopenia and leukocyte inclusion bodies, but many of them subsequently develop deafness, cataracts and/or a glomerulonephritis leading to end-stage renal failure. Several questions on MYH9-RD are still unanswered: which are the cellular and molecular consequences of MYH9 mutations? Why several cell/tissues expressing NMMHC-IIA are not affected? Why mutations of one gene produce different clinical pictures? Recently, analysis of large case series of patients with different MYH9 mutations and development of cell-animal models for MYH9-RD allowed hypothesizing some answers to these questions. First, it has been suggested that processing of mutant NMMHC-IIA varies in different cell types, in that a dominant negative effect of the mutant allele has been observed in leukocytes, while haploinsufficiency has been demonstrated in megakaryocytes and platelets. Moreover, the study of tissue distribution of myosin II isoforms suggested that cells expressing only II-A manifest the congenital defects, while tissues expressing additional myosin II isoforms show either late onset of abnormalities or no pathological signs. Finally, genotype/phenotype correlation studies suggested that the main determinant of MYH9-RD clinical features is the type of MYH9 mutation. Advances in understanding MYH9-RD pathogenesis are going to improve our clinical approach to patients with MYH9 mutations.

ANIMAL MODELS IN PLATELET PATHOPHYSIOLOGY

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It is commonly recognized that blood platelets play a role in hemostasis and thrombosis but over the last few years increasing evidence has accumulated on a central role of platelets in inflammation and atherogenesis too. Studies on human platelets have several obvious limitations, thus crucial informations on the pathophysiologic role of platelets in disease have been obtained from animal models, and in particular from the manipulation of mice genome.

Recent examples of studies on *in vivo* platelet pathophysiology evaluated by our group are the role of matrix metalloproteinase type 2 (MMP2) in primary hemostasis and thrombosis and the role of platelet P-selectin in lung eosinophil infiltration in allergic asthma. Platelets contain and release, upon activation, MMP2 that in turn potentiates platelet response to

stimuli. We have established a colony of mice deficient in MMP2 and shown that they have defective aggregation, reduced P-selectin expression and impaired adhesion to collagen. *In vivo*, MMP2 knockout mice show an antithrombotic phenotype with impaired collagen+epinephrine-induced platelet pulmonary thromboembolism and reduced femoral artery thrombosis after photochemical injury. $MMP2^{-/-}$ mice had a hemorrhagic phenotype, with prolongation of the bleeding time which was shortenend by the i.v. injection of human Pro-MMP2. In particular, platelet-derived MMP2 contributed crucially to *in vivo* hemostasis, as established by platelet cross transfusion- and bone marrow cross transplatantion-experiments between $MMP2^{-/-}$ and WT mice. In a model of pulmonary allergic inflammation, we have assessed whether surface-expressed P-selectin on platelets is involved in pulmonary leukocyte recruitment. Mice immunised with aerosolised OVA were selectively depleted of platelets and their platelet count was then restored by the transfusion of resting washed platelets or washed platelets from P-selectin knockout mice or of activated platelets pretreated with an anti P-selectin antibody. Bronchoalveolar lavages were then performed and eosinophil recruitment in lungs assessed. Platelet depletion by >90% significantly inhibited eosinophils recruitment. Transfusion of stimulated-fixed platelets, expressing P-selectin, restored eosinophil recruitment whilst platelets incubated with an anti P-selectin antibody or P-selectin knockout platelets failed to restore eosinophilia. These data demonstrate that P-selectin expression on activated platelets is required for pulmonary eosinophil recruitment in murine allergic inflammation.

In conclusion, animal models, and especially murine knockout models, have greatly contributed to gain deeper insights in platelet pathophysiology *in vivo*.

PLATELET-LEUKOCYTE INTERACTION: A LINK BETWEEN THROMBOSIS AND INFLAMMATION

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Recent studies on the pathogenesis of thrombosis point towards the crucial role of inflammation during thrombogenesis, particularly of inflammatory cells such as leukocytes and their interaction with platelets and vascular cells.

Epidemiological evidence suggests positive correlation between the number of leukocytes, in particular polymorphonuclear (PMN), and the risk of ischemic vascular disease. Indeed, a higher PMN count was associated with an increased risk of acute myocardial infarction and its recurrence or transient ischemic attacks. Further studies reported increased *ex vivo* functional responsiveness as well as *in vivo* PMN activation and platelet-leukocyte interaction in different clinical manifestations of ischemic heart disease and suggested an active role of these cells in the progression of vascular occlusion.

Platelet-leukocyte aggregates have been observed in peripheral blood from patients with unstable angina, myocardial infarction and other acute coronary syndromes, or mechanical heart valve replacement. They have also been considered a predictive index of acute reocclusion following percutaneous coronary surgery.

Platelet-leukocyte aggregates have also been found in patients with cancer and positively correlated with thrombotic events.

PMN leukocytes may be activated by adhesive molecules of the selectin family, primarily by P-selectin expressed on platelets. The interaction of P-selectin with P-Selectin Glycoprotein Ligand-1 (PSGL-1), its counter-receptor on PMN and the consequent adhesion of platelets to PMNs results in the activation of leukocytes and the formation of stable platelet-leukocyte aggregates.

Several antithrombotic/ antiplatelet drugs have been evaluated as possible blocker of mixed cell aggregate formation. For example blocking the glycoprotein IIb/IIIa receptor by abcximab and tirofiban in acute myocardial infarction only partially prevented the formation of platelet-leukocyte aggregates and the surface expression of Mac-1.

Heparin modulates platelet-PMN interaction via several mechanisms: interference with P- and L-selectin-dependent cell adhesion, or prevention of platelet activation induced by proteases released from PMN. Moreover, heparin and other glycosaminoglycans may inhibit the release of lysosomal enzymes and production of superoxide by activated PMNs.

ASPIRIN RESISTANCE: IS THIS TERM MEANINGFUL?

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Purpose of review. Aspirin resistance is a term coined to indicate aspirin-treated patients having *ex vivo* tests of platelet activation insensitive to aspirin treatment and recurrence of cardiovascular disease. In this review we focused on new data that are pro or contra the existence of aspirin resistance

Recent findings. Aspirin resistance defined by ex vivo tests of platelet activation yielded values ranging from 18% to 78% so indicating that such tests are not a good tool to measure with. In long-term aspirin-treated patients studies demonstrated small but functionally relevant platelet thromboxane A2 formation, that was responsible for an enhanced platelet activation to platelet agonist. These studies, however, did not fully exclude that aspirin compliance may be implicated in such phenomenon. Two trials performed in patients with coronary artery disease demonstrated that laboratory evidence of aspirin resistance was no more detectable when aspirin compliance was accurately monitored.

Conclusions. Given the multifactorial nature of atherothrombosis, recurrence of cardiovascular events in aspirin-treated patients is not necessarily suggestive of ' drug failure'. A cause-effect relationship between platelet insensitivity to aspirin and cardiovascular recurrence has not been defined overall because aspirin compliance has been scarcely considered. Until such crucial information is not taken into account, the existence of 'clinical resistance' to aspirin should be wisely considered.