RECORD trials) were initiated in late 2005. The results of phase II trials of rivaroxaban for the treatment of proximal deep vein thrombosis are also expected soon. With so many compounds in development, alternatives to the established anticoagulants may be available soon.

AREAS OF CERTAINTY – AND UNCERTAINTY – ABOUT BEST DOSAGES OF NEW ANTICOAGULANTS

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Recommended dosages of new anticoagulants to be administered to patients arise from clinical trials and should represent best estimates, with awareness that subsequent patients may differ in response. Nonetheless, decisions must be taken in selecting the dosages to be used in early human studies. One decision relates to balancing the twin goals of maximizing knowledge and minimizing risk to human subjects. Another related decision particularly relevant for anticoagulants relates to whether risk tolerance (e.g., for bleeding and efficacy) may differ depending on the indication, e.g., primary prophylaxis vs treatment of established thrombosis. A third decision relates to how subject risk is balanced against desired speed of drug development. There are other related decisions, including sample size, which can help make estimates of risk (for efficacy failure or adverse events) more or less robust, affecting future risk to other study subjects. Sample size choices can be driven by hypotheses that allow faster study completion but with riskier dosages.

These decisions are best informed by maximizing, within reason, the quality and quantity of data preliminary to each step. Relatively inexpensive studies performed with past anticoagulants include those in living animal models, tissue explants, *in vitro* systems, and ex vivo samples from human volunteers. Examples of these will be presented. Past published examples of choices in anticoagulant development will also be presented. Final suggested principles will be presented for consideration by the audience. They include: (1) The tolerance for treatment failure in primary prophylaxis, assessed by venography, can be higher than the tolerance for treatment failure of established thrombosis, (2) Finding the *minimum effective dose* is not an appropriate goal for treatment of a life-threatening condition if available therapy is highly effective, and (3) Monitoring for possible surrogate markers of efficacy and safety is desirable in clinical trials, whether or not their monitoring is planned to be a part of eventual usual treatment.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS - II

THE INTERACTIONS BETWEEN INFLAMMATION AND COAGULATION

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Recent studies have emphasized the important contribution played by inflammation in stimulation of the coagulant responses. Inflammatory mediators initiate coagulation, decrease the activity of natural anticoagulant mechanisms and impair the fibrinolytic system. The natural anticoagulants function to dampen elevation of cytokine levels. Components of the natural anticoagulant cascades, like thrombomodulin, can minimize endothelial cell dysfunction by rendering the cells less responsive to inflammatory mediators, facilitate the neutralization of some inflammatory mediators and decrease loss of endothelial barrier function. Thus, decreased anticoagulant pathway function not only promotes thrombosis but also amplifies the inflammatory process. The magnitude of the down regulation of the specific anticoagulant mechanisms varies markedly from vascular beds, possibly contributing to the reasons that some organs are more prone to injury in inflammatory diseases than others. Once the inflammation-coagulation interactions overwhelm the natural defense systems, catastrophic events occur such as manifested in severe sepsis or inflammatory bowel disease.

EXPRESSION AND REGULATION OF ENDOTHELIAL PROTEIN C RECEPTOR IN MONOCYTE-DERIVED DENDRITIC CELLS.

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Endothelial protein C receptor (EPCR) is a transmembrane protein, homologous to MHC class-1 molecules, that enhances the rate of protein C activation on the endothelial cells of the vessel wall. It has been reported to be present also in polymorphonuclear leucocytes and in monocytes. We showed by immunohistochemistry that dendritic-like cells in the normal gut mucosa express EPCR. We now confirm that these *dendritic-like cells* have a phenotype characteristic of dendritic cells, namely they express CD80, CD83 and HLA-DR. We could not identify EPCR+ dendritic cells in other tissues, such as lymph node, spleen, tonsil, lung, and skin. To further characterize dendritic cell EPCR, we set up cultures of monocyte-derived dendritic cells (MoDCs) that are commonly used as a model of dendritic cell physiology *in vitro*. $CD14^+$ monocytes were separated from buffy coats of healthy donors and cultured for 7 days with IL-4 and GM-CSF to obtain immature DCs. EPCR surface expression was monitored by flow cytometry together with expression of the DC markers HLA-DR, CD1a, CD80 and CD83. After 7 days of culture, approximately 25% of immature DCs expressed EPCR on their surface. De novo expression of EPCR was not correlated with modulation of apoptosis or cell cycle. Lipopolysaccharide-induced terminal maturation of DC down regulated the surface expression of EPCR by 40 % while up regulating the expression of CD83. Incubation

of cultured DCs with prostaglandins up regulated EPCR mRNA and protein expression by about 3 fold. Cytofluorimetric studies were compounded by confocal microscopy, which showed that dendritic cell EPCR has the same membrane distribution pattern as in endothelial cells. In conclusion, contact with bacterial antigens modulates EPCR expression on MoDCs, suggesting EPCR might be involved in antigen recognition or processing.

HYPERHOMOCYSTEINEMIA: STILL A TARGET FOR INTERVENTION?

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Homocysteine is a sulfur amino acid whose metabolism stands at the intersection of two pathways: remethylation which requires folic acid and B12 coenzymes and transsulfuration which requires pyridoxal-5-phosphate, the B6 coenzyme. Data from several studies suggest that mild elevations of homocysteine in plasma are a risk factor for occlusive vascular disease. In the Framingham studies we have shown that elevated homocysteine concentrations in plasma are a risk factor for prevalence of extracranial carotid-artery stenosis > 25% in both men and women. Prospectively elevated plasma homocysteine is associated with increased total and CVD mortality, increased incidence of stroke, increased incidence of dementia, cobnitive dysfunction and Alzheimer's disease, increased incidence of bone fracture and higher prevalence of chronic heart failure. However, recent homocysteine lowering trials produced mixed results with some showing benefits while others showing negative results or even adverse effects. Neverthelss, the relationships between homocysteine and disease are too strong to be ignored, and our task to understand what these relationships between homocysteine and disease mean. Our data on the pleiotropic relationship between homocysteine and various diseases, and the fact that in the majority of cases differences in homocysteine concentrations between healthy and disease populations are small, are supportive of the idea that homocysteine is a marker, rather than the cause for the respective disease. This would imply that not all cases of elevated homocysteine in plasma contribute to a disease risk. For example high niacin intake to lower blood lipids results in hyperhomocysteinemia because of *detoxification* of niacin through methylation by SAM. It is doubtful that this increase in homocysteine will pose any risk. It is therefore important to understand what elevated homocysteine in plasma represents. Deficiency of folate and other related vitamins provide more reasonable bases for relating pleiotropic relationships with diseases. Observed discrepancies between the outcome of the intervention trials should be used to understand what works and what does not.

AUTOIMMUNE THROMBOSIS

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The protein C system plays a central role in the pathophysiology of autoimmune thrombosis. Autoantibodies directed against protein C and protein S, with different characteristics, have been described in association with disseminated intravascular coagulation, skin necrosis and purpura fulminans. More recently, autoantibodies against the endothelial protein C receptor (EPCR) have been described in women with the antiphospholipid syndrome, in association with an increased risk of fetal death. Acquired activated protein C (APC) resistance has been reported in patients with anti-phospholipid antibodies. Beta2-GPI is a major antigen for antiphospholipid antibodies present in patients with the anti-phospholipid syndrome. High titers of anti-beta2-GPI IgG are considered a major risk factor for thrombosis and may interfere with the protein C system. In purified systems, anti-beta2-GPI monoclonal antibodies inhibit APC function by disrupting APC-protein S interactions induced by oxidation of membranes containing phosphatidylethanolamine (PE). Lipid oxidation enhances the anticoagulant function of activated protein C (APC) and facilitates detection of its inhibition by anti-phospholipid antibodies. The anticoagulant activity of APC was explored in Xa-one stage clotting assays with non-oxidized or oxidized phospholipid (PL) containing 40% PE, after the addition of total IgG fraction obtained from 25 patients with lupus anticoagulant (LA) and no history of thrombosis (LA+/Thr-)and 25 LA patients with history of thrombosis on oral anticoagulant treatment. LA was secondary to autoimmune diseases in 32% of LA+/Thr+ patients and in 84% of LA+/Thr- patients ($p = 0.0002$), but LA potency (PTT-LA, Staclot and Staclot + hexagonal PL, Stago) and aCL IgG titers at diagnosis were similar in the two groups of LA patients ($p = 0.29$). With non-oxidized PL, clotting times were prolonged by APC to a similar extent in both groups ($p = 0.26$). The APC response with oxidized PL was lower with IgG from LA patients than from controls(median ratio 2.46 in LA+/Thr+ patients and 2.63 in LA-/Thr+ patients vs 3.20 in controls, $p< 0.0001$). With respect to controls,impaired PL oxidation-dependent response to APC, mainly affected by presence of beta2-GPI in the test plasma, was observed for 5 patients in the LA+/Thr- group (OR = 6.75, $p = 0.07$)) and 13 patients in the LA+/Thr+ group (OR = 29.3, $p = 0.0002$). LA patients with impaired APC response had higher PTT-LA ratios ($p =0.001$) and aCL IgG titers ($p = 0.006$) than LA patients with normal APC response. In a logistic regression model, independent predictors of impaired oxidation-dependent APC response in LA patients were aCL IgG titers (OR = 1.05 for each unitary increment, $p = 0.015$) and association with autoimmune diseases (OR = 26.3, $p = 0.04$). These data strongly suggest that APC resistance with oxidized phospholipids is a major risk factor for thrombosis in patients with lupus anticoagulants. Because patients with LA and thrombosis are currently maintained on oral anticoagulant treatment for an indefinite time, this marker may be helpful in future studies for the identification of patients with a reduced risk of recurrence.