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PROTEIN C/PROTEIN S IN THE FOETAL BLOOD. ABSENCE OF BOUND PROTEINS AND C4 BINDING PROTEIN. E. Melissari, M.F. Scully, C. Parker, K.H. Nicolaides* and V.V. Kakkar. Thrombosis Research Unit, Dept. of Obstetrics and Gynaecology*, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, UK.

Protein C, free and bound protein S and C4 binding protein levels (C4bp), were measured by electroimmunoassay in 7 pregnant women aged 22-29 years at 16-18 weeks of gestation, immediately prior to termination of pregnancy for social reasons. Protein C and protein S levels were also measured in their foetuses from blood taken through the umbilical cord. In this group of pregnant women the mean levels for protein C were 104% of normal adult mean (range 80-128%), for C4bp 100% (52-150%), for free protein S 66% (43-89%). In the foetuses the mean value for protein C was 15.3% (10.5-21%) and for free protein S 36.85% (27-47%) of the normal adult mean. Bound protein S and C4bp levels were zero. Conclusions: (1) free protein S is significantly decreased (<2SD below the normal adult mean) in women after the first trimester of gestation whereas no change is seen in protein C concentration; (2) C4bp levels are at zero in the foetus as also are the levels of bound protein S; (3) foetal blood protein S level is approximately 2.5 times higher than protein C. Since all other vitamin K-dependent factors have been observed to be in the range of 10-20% of normal at this stage of gestation, our findings may be further proof of a non hepatic (endothelial) source of plasma protein S.

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MONOCLONAL ANTIBODIES TO HUMAN PROTEIN S AND C4b-BINDING PROTEIN. Martin Hessing, Joost C.M. Meijers, Jan A. van Mourik, Bonno N. Bouma. Dept. of Haematology, University Hospital Utrecht, CLB, Amsterdam, The Netherlands.

Protein S (PS) circulates in plasma both free and in reversible association with the complement component C4b-binding protein (C4bp). Only free PS is functional as a cofactor for activated protein C (APC). Cleavage of PS by thrombin at a site near the γ-carboxyglutamic acid domain is associated with a loss of cofactor activity. This may be a control mechanism for the anticoagulant activity of APC. These observations led us to investigate the role of C4bp and thrombin in the regulation of PS. Complex formation between purified PS and C4bp was studied in plasma and in a system with purified components. ^{125}I-labeled PS was first incubated with either C4bp or citrated plasma and then subjected to polyacrylamide gelelectrophoresis in the absence of SDS. The formation of the C4bp-PS complex in plasma and in the purified system was demonstrated by autoradiography. Crossed immuno-electrophoresis using an antiserum against PS was performed in the presence of 8 mM EDTA. Human citrated plasma showed two precipitin peaks. Free PS migrated rapidly in the first dimension, whereas the C4bp-PS complex was just anodal to the application slot. The addition of C4bp to either plasma or purified PS resulted in the disappearance of the free PS peak and an increase of the slower migrating peak. The effect of purified C4bp on the PS-cofactor function of APC was studied in citrated plasma. The prolongation of the APTT induced by the addition of APC could be inhibited by the addition of increasing amounts of C4bp. Monoclonal antibodies to PS and C4bp were prepared and characterized. The monoclonal antibodies to either PS or C4bp did not block the complex formation between C4bp and PS, as was demonstrated by dot blotting of C4bp with ^{125}I-PS and agarose gelelectrophoresis followed by Western blotting. Three out of 7 monoclonal antibodies to PS did not detect PS after thrombin cleavage on an immunoblot after non-reduced SDS polyacrylamide gelelectrophoresis. These 3 antibodies gave a significant shortening of the prolonged APTT induced by the addition of APC to normal plasma, indicating that these monoclonals inhibited the cofactor function of PS. The other 4 monoclonals to PS that did detect PS after thrombin cleavage on an immunoblot, gave only a minor inhibition of the PS cofactor function.

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PLASMA LEVELS OF PROTEIN S AND C4b-BINDING PROTEIN DURING TREATMENT WITH L-ASPARAGINASE. S. Viganò D'Angelo (1), L. Gugliotta (2) F. Gilardoni (1), A. Macagni (1), L. Chetti (2) and A. D'Angelo (1). Coagulation Service, Istituto Scientifico San Raffaele, Milano, Italy (1) & Institute of Hematology "L. e A. Seragnoli", University of Bologna, Italy (2).

Protein S (PS), the cofactor of activated protein C (PC), circulates in plasma as free PS (active) and in complex with C4b-binding protein (inactive). We have followed the changes of total PS, free PS, PS activity and C4b-binding protein in 5 adult patients with acute lymphoblastic leukemia treated with 20,000 IU/sqm L-asparaginase (L-ASE) administered three times weekly for two weeks (6 doses) and compared them to the changes of PC antigen and activity, antithrombin III and fibrinogen. The table shows the mean values of these parameters (% of pretreatment values) observed after three doses of L-ASE (A), after completion of treatment (B) and one week after the end of treatment (C).

	A	B	C
PS total antigen	75	55	60
PS free antigen	65	52	55
PS anticoagulant activity	62	39	50
PC antigen	51	59	85
PC activity	49	61	86
C4b-binding protein	78	68	76
Antithrombin III activity	70	65	67
Fibrinogen	59	37	51

These data suggest that at variance with PC and similar to fibrinogen and antithrombin III, total PS antigen is still reduced after one week from the end of L-ASE treatment. The reduction of total PS reflects that of free PS, as predicted by mass action. On the contrary, PS anticoagulant activity is decreased to a higher extent than total PS antigen. Acquired PS deficiency might play a contributory role to the development of thrombotic complications in patients receiving L-ASE.

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REDUCED PROTEIN S ANTICOAGULANT ACTIVITY IN ESSENTIAL MIXED CRYOGLOBULINEMIA. A. D'Angelo (1), F. Gilardoni (1), V. Toschi (2), C. Ciminiello (2), E.A. Sinico (2) and S. Viganò D'Angelo (1). Coagulation Service, Istituto Scientifico S. Raffaele, Milano, Italy, (1) & Dept. of Hematology, Ospedale S. Carlo, Milano, Italy (2).

Protein S (PS) is found in two forms in plasma, as free PS, which functions as a cofactor for activated protein C, and in equimolar complex with C4b-binding protein (C4b-bp), an inhibitor of the complement system. The Kd of the PS-C4b-bp interaction is one order of magnitude lower than the plasma concentration of the two proteins; thus 55-60% of total PS circulates in the bound form. Evidence has been provided that in vitro complement activation does not affect the equilibrium between PS and C4b-bp; however in patients with systemic lupus erythematosus and low C4 levels, a shift from free to bound PS has been observed. To further evaluate the relationship between complement activation and PS distribution we have measured PS and C4b-bp levels in 21 patients with essential mixed cryoglobulinemia (EMC), an autoimmune disorder characterized by cryoprecitable circulating immunocomplexes and associated with vasculitis and thrombotic episodes. EMC patients had cryocrit rangin from 1 to 66% and greatly reduced complement components (C1q: 45%, C3: 71%, C4: 15% of normal). Mean PS activity was significantly reduced in patients as compared to the control population consisting of 20 age-and sex-matched blood donors (69%, $p < 0.001$). Free PS was similar in patients and controls, but total PS was lower in EMC patients (82%, $p < 0.05$). Seven EMC patients had C4b-bp levels below 60%. Thus, reduction of PS activity in patients with EMC is not due to reduced free PS. Cultured endothelial cells synthesize and release PS with reduced specific activity. In EMC patients very high levels of von Willebrand factor (313%, $p < 0.001$) a protein released from endothelial cells, but not of ceruplasmin, another acute phase reactant protein, were observed. In vivo release of PS from endothelial cells might contribute to reduced PS specific activity in EMC.