

THE COAGULANT ACTIVE PHOSPHOLIPID (PL) CONTENT OF PROTHROMBIN COMPLEX CONCENTRATES (PCC) IS A CRITICAL DETERMINANT OF THEIR *IN VIVO* THROMBOGENICITY. A.R. Giles, M.E. Nesheim, P.B. Tracy, H. Hoogendoorn & K.G. Mann. Depts. Pathology & Medicine, Queen's University, Kingston, Ontario & The Mayo Clinic, Rochester, Minn.

It has generally been assumed that the thrombogenicity of PCC relates to their invariable contamination by activated products of their component clotting factors. In earlier studies we demonstrated a lack of correlation between the activated clotting factor content & *in vivo* thrombogenicity of some PCC. In contrast, *in vitro* measurement of coagulant active PL content appeared to be a more reliable determinant of subsequent *in vivo* activity. In recent studies we have confirmed the presence of PL of known coagulant activity in PCC & studied its activity both *in vitro* & *in vivo* after its extraction from the parent PCC.

PCCs were obtained from various agencies or prepared in our laboratory to a standard protocol either from fresh frozen plasma or plasma obtained from outdated platelet concentrates. Each was assayed for Xa (S-2222) & PL (thrombin generation assay with DAPA) & an index of thrombogenicity *in vivo* determined in a stasis model in rabbits. There was a significant correlation between the latter & PL ($r=0.8727$ $p<0.01$) but not Xa. Thin layer chromatography of an ethanol ether extract of the most thrombogenic PCC showed the presence of PL known to be coagulant active including Phosphatidylserine & -choline. This extract retained full PL replacing activity in the DAPA assay *in vitro* but was non-thrombogenic *in vivo*. When, however, a highly purified preparation of Xa was added, in the same relative proportions as in the parent PCC, thrombogenicity *in vivo* was restored.

It is concluded that the content of coagulant active PL is a critical determinant of the potential thrombogenicity of PCC *in vivo*. It appears to act in concert with Xa &, conceivably, other serine proteases perhaps by protecting them from inactivation by AT III. This may explain why relatively "non-activated" PCCs may still be associated with thromboembolic complications.

THREE DIMENSIONAL COMPUTER GRAPHICS MODELS OF BOVINE FACTOR Xa, FACTOR IXa AND THROMBIN. B. Furie, D.H. Bing, B.C. Furie, D.J. Robison, J.P. Burnier, and R.J. Feldmann. Tufts-New England Medical Center, Boston, MA, Center for Blood Research, Boston, MA and NIH, Bethesda, MD.

Three dimensional structural models of the heavy chain of Factor Xa, the heavy chain of Factor IXa, and the B chain of thrombin have been developed using a computer molecular graphics display system. These models are based upon the sequence homology of these proteins with chymotrypsin and trypsin and the assumption that the three dimensional structures of the peptide backbones of these proteins are nearly identical to the known backbone structure of trypsin and chymotrypsin. Sequence alignments of each protein with the digestive proteases were based upon regions of sequence homology, location of disulfide bonds, and preservation of β -barrels. Regions of insertion and deletion relative to the chymotrypsin backbone were identified and incorporated into the backbone structure. Factor Xa (heavy chain) and Factor IXa (heavy chain) contain 5 insertions and 4 deletions. Thrombin (B chain) contains nine insertions and two deletions. Factor Xa, Factor IXa, and thrombin models were generated by substituting their respective amino acid sequences into the model of the peptide backbone. The molecular surfaces of these models each suggest unique topographical and physicochemical properties. In contrast, the core of these proteins are nearly identical. One area of the molecular surface, the active site, is also highly conserved despite marked differences in substrate specificity among these enzymes. The extended substrate binding regions, surrounding the active site, are highly substituted and also contain areas of both insertions and deletions. These models predict that the functional differences that distinguish these serine proteases are manifestations of the molecular surface differences. It would appear that substrate recognition and specificity determinants of Factor Xa, Factor IXa, and thrombin reside in an extensive surface surrounding the active site, but not exclusively within the active site.

THE EXTRAVASCULAR COAGULATION SYSTEM: THE PRODUCTION OF PROTHROMBIN, FACTORS V, X, IX, VII AND TISSUE FACTOR IN MACROPHAGES. B. Østerud, U. Lindahl, J. Børgwald and R. Seljelid. Inst. of Med. Biol. Univ. of Tromsø, Tromsø, Norway and Dept. of Med. and Physiol. Chem., Swedish Univ. of Agricultural Sciences, Uppsala, Sweden.

The synthesis of coagulation factors and tissue factor (T.F.) has been investigated in macrophage cultures. The macrophages were obtained from mice by peritoneal washing. Serum free medium collected after 24 h macrophage culturing contained 4-12% of prothrombin, Factors VII, IX and X. These factors were not produced when either cycloheximide or warfarin were added to the growth medium. Factor V, secreted from the macrophages, was detected by the use of a clotting assay and a coupled amidolytic assay. Warfarin had no effect whereas cycloheximide blocked the synthesis of Factor V.

T.F. activity in lysed macrophages was determined by incubation of the test sample with purified F.VII, F.X and CaCl₂. The production of the T.F. was dependent on lymphocytes. Lymphocytes themselves did not synthesize T.F., but they were required for the production of T.F. in macrophages as the use of anti-theta antibody + complement blocked the generation of T.F.. Endotoxin added to the cultures enhanced the synthesis of T.F. 10-40 fold (i.e. expressed in F.Xa generated in the T.F. assay: macrophages alone 0.5%, macrophages + endotoxin 18.0%, lymphocytes alone + endotoxin < 0.1%).

Macrophages secreted variable amounts of activated F.X due to the tissue factor production. However, endotoxin stimulated macrophages with high concentration of T.F. secreted less F.Xa probably caused by the synthesis of an inhibitor to F.Xa.

This study demonstrates that there exists a complete extravascular coagulation system that can be triggered by the synthesis of T.F..

STUDIES OF FACTOR VIII INHIBITOR BYPASSING ACTIVITY (FEIBA). T.W. Barrowcliffe, E. Gray and G. Kemball-Cook. Division of Blood Products, National Institute for Biological Standards and Control, London NW3 6RB, U.K.

The activities of an activated Factor IX concentrate (FEIBA, Immuno AG) were studied by two *in vitro* assays: a one-stage method using VIII-deficient plasma as substrate, and a two-stage assay based on the thrombin generation test. The nature of the active principle was explored by measuring the reduction in activity when FEIBA was incubated with specific antibodies.

Incubation of FEIBA with human antibodies to Factor VIII reduced its activity by about 30% in the one-stage assay, and about 50% in the two-stage assay, suggesting that FEIBA contains Factor VIII procoagulant activity. Inactivation of the Factor VIII in FEIBA was somewhat slower than that of normal Factor VIII, indicating partial protection from inhibition. Human antibodies to Factor IX inhibited the one stage activity by about 30%, and incubation with both antibodies also produced a 30% reduction in activity. The remaining procoagulant activity decayed only slowly when incubated with non-inhibitor plasma. In contrast, purified human Factor Xa lost activity rapidly on incubation in normal plasma, as did a purified fraction from a Factor IX concentrate, which had high activity in the one-stage assay.

These results suggest that the *in vitro* activity of FEIBA is due to at least two components. One component appears to be dependent on both Factors VIII and IX and may be a complex of VIII and IXa. The other component acts later than Factors VIII and IX in the coagulation cascade but, unlike purified Factor Xa, is relatively resistant to inactivation by plasma inhibitors such as antithrombin III. FEIBA was also found to contain phospholipid, and it may be that the phospholipid protects both the Factor VIII and activated enzymes from their inhibitors.