CHARACTERIZATION OF RABBIT ANTITHROMBIN III. D. Estry, J.C. Mattson, T.G. Bell and G.H. Tishkoff. Department of Pathology and Lansing Regional Red Cross, Michigan State University, East Lansing, MI.

The rabbit is a well established model for studying the disseminated intravascular coagulation (DIC) associated with endotoxin syndromes. In order to establish the role of antithrombin III (ATIII) in the modulation of DIC in the rabbit, characterization of rabbit ATIII was undertaken. Rabbit antithrombin III (ATIII) was isolated according to modifications of the method of Thaler and Schmer, which has a molecular weight comparable to that of human ATIII (62,000 daltons) as measured by mobility on SDS-PAGE gels. Mixtures of rabbit and human ATIII co-migrate as a single band on 7.5% SDS-PAGE gels. Rabbit ATIII possesses both progressive and heparin activated (immediate) antithrombin activity in assays using human thrombin. Antisera raised against rabbit antithrombin III with specific antisera, either prior to or after addition of heparin, did not alter the ability of antithrombin III to inhibit thrombin in either the immediate or progressive assay indicating that the antigenic determinants are not found in either the heparin binding or active thrombin binding site. Crossed immunoelectrophoresis (IEP) demonstrates that antisera to rabbit ATIII reacts with both free rabbit antithrombin III and ATIII-thrombin complexes and can therefore be used in immunological assays to quantitate total rabbit ATIII and heparin bound ATIII. IEP to demonstrate the mobility of both free and complexed ATIII.


The role of antithrombin III (ATIII) on kinetics of thrombin activity was studied in an inhibitor-free coagulation system in which ATIII was added at various concentrations. Coagulation was initiated either by kaolin or by Stypven in the presence of phospholipids. Kinetics of the respective activities of factor Xa and thrombin were followed on chromogenic substrates S-2222 and S-2238 and on fibrinogen for the latter.

These experiments gave the following results:
1. kinetics of thrombin formation as well as the peak level of thrombin activity were strongly dependent on ATIII concentration;
2. in normal subjects, thrombin formation was unaffected by ATIII; moreover the decrease of thrombin activity was much slower than that of thrombin and was negatively correlated with phospholipid concentration.

These results illustrate the impact on blood coagulation of minor deviations from normal of ATIII level. This assumption was confirmed by ATIII infusion to normal subjects. On the other hand our findings cast doubt on the physiological importance of the inhibitory activity of ATIII on serine-proteases other than thrombin. At the interface plasma/phospholipids, when complexed with phospholipids and protein cofactors (i.e. factors V and VIII) the affinity of the enzymes for the inhibitor becomes negligible compared to their affinity towards their respective zymogen substrates. In a complete coagulation system, inhibition of thrombin formation by ATIII seems to result mainly from the inhibition of the cooperative action of thrombin on its own formation.

FAMILIAL ANTITHROMBIN III DEFICIENCY ASSOCIATED WITH RECURRENT ARTERIAL THROMBOSIS. C.S. Hale, J.C. Mattson and J.A. Zuhlke. Departments of Medicine and Pathology, Michigan State University, East Lansing, MI.

A 28-year-old male with a strong family history of thrombembolic disease sustained three arterial thrombotic events during a 27 mo. period. The patient had antithrombin III (ATIII) levels of 19.2 mg/dl (N=17-30 mg/dl) by RID and an immediate (heparin activated) antithrombin (AT III) level of 77% (N=88-120%). Crossed IEP showed normal electrophoretic mobility of the patient's ATIII. Of 30 family members tested, 10 demonstrated decreased AT III by both immunologic and functional assays. Two children of the propositus and three children of the sister of the propositus were tested and none were found to be abnormal. Because of the unusual presence of arterial thrombotic events in this family, platelet function studies were performed on the propositus and on two ATIII deficient family members. A 19-year-old brother of the propositus, with no history of thrombembolic events but with 13 mg/dl AT III and 85% activity had normal platelet aggregation studies and demonstrated no evidence of hyperaggregability with suboptimal concentrations of aggregating agents. He did, however, demonstrate a slight increase in platelet adhesion in a collagen adhesion assay. The second family member, a 65-year-old aunt of the propositus with 13.7 mg/dl ATIII and 75% activity was on Coumadin at the time of evaluation of platelet function. Platelet aggregation and adhesion studies were normal in this individual. The propositus was also tested while on Coumadin and showed no abnormality of platelet aggregation, no hyperaggregability and no increase in platelet adhesion.

In view of our experience, we recommend screening for AT III deficiency in patients with unexplained recurrent arterial thromboembolism as well as those with venous thromboembolism, especially if the family history is suggestive.