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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 endotoxic syndromes. In order to establish the role of antithrombin III (AT III) in the modulation of DIC in the rabbit, characterization of rabbit AT III was undertaken. Rabbit antithrombin III, isolated according to modifications of the method of Thaler and Schmer, has a molecular weight comparable to that of human AT III (62,000 daltons) as measured by mobility on SDS-PAGE gels. Mixtures of rabbit and human AT III co-migrate as a single band on 7.5% SDS-PAGE gels. Rabbit AT III possesses both progressive and heparin activated (immediate) antithrombin activity in assays using human thrombin. Antisera raised against rabbit AT III demonstrates no cross reactivity with human AT III suggesting that despite physiologic and molecular weight similarities, antigenic differences are present. Incubation of 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 assays indicating that the antigenic determinants are not found in either the heparin binding or active thrombin binding sites. Crossed immunoelectrophoresis (IEP) demonstrates that antisera to rabbit AT III reacts with both free rabbit antithrombin III and AT III-thrombin complexes and can therefore be used in immunologic assays to quantitate total rabbit AT III (bound and free) and in crossed IEP to demonstrate the mobility of both free and complexed AT III.

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CHANGES IN THE ANTITHROMBIN III ACTIVITY AT THE INTERFACE PLASMA-PHOSPHOLIPIDS. F. Josso and S. Béguin. Department of Haematology, University Hospital Necker-Enfants Malades, Paris, France.

The role of antithrombin III (AT III) on kinetics of thrombin activity was studied in an inhibitor free coagulation system in which purified AT III 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 : (i) kinetics of thrombin formation as well as the peak level of thrombin activity were strongly dependent on AT III concentration ; (ii) Xa formation was unaffected by AT III level ; moreover the decrease of Xa 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 AT III level. This assumption was confirmed by AT III infusion to normal subjects. On the other hand our findings cast doubt on the physiological importance of the inhibitory activity of AT III 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 AT III seems to result mainly from the inhibition of the cooperative action of thrombin on its own formation.

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CONGENITAL ABNORMAL ANTITHROMBIN III. N. Sakuragawa, T. Takahashi and I. Horikoshi. Central Clinical Laboratory, Toyama Medical and Pharmaceutical University, Toyama, Japan

We found a family which had congenitally abnormal antithrombin III (AT-III) showing remarkable difference between biological and immunological activity. We studied genetic trait and effects of heparin and thrombin on AT-III.

Case: A 23 years old female developed thrombophrebitis on her left leg in July, 1979. After a while she seemed to have developed thrombi on bilateral iliac vein. She had also abdominal tumor, and a cystic kidney was found to be by an exploratory laparatomy. Five days after the operation, she was hemiparalyzed on her left. Cranial angiography disclosed obstruction of right middle cerebral artery by thrombi. Laboratory findings were as follows: Fibrinogen 145 mg/dl, FDP 40 ug/ml, platelets 8.6×10^4/cmm, a-PTT 30.6 sec and PT 100%. The biological activity of AT-III was 26%, which dissociated from immunological activity of 54 mg/dl. Two dimension immunoelectrophoretical analysis showed two or three peaks at the albumin area after the addition of heparin to normal AT-III. However her AT-III did not move from α_2-macroglobulin area. Her parents were cousins each other, and her parents and her sister also had abnormal AT-III. This indicates congenitally abnormal AT-III is transmitted autosomally dominantly. The administration of concentrated AT-III preparation raised its concentrate to 70%, and coagulation disorders disappeared. Immunoelectrophoresis revealed that administered AT-III disappeared in five or seven days after infusion. Oral anticoagulant therapy could prevent thrombi formation.

Conclusion: We found a patient who had abnormal AT-III which brought on intravascular coagulation. Biologically active AT-III was much less than immunologically active one. Immunoelectrophoresis disclosed this AT-III did not bind with heparin. We treated the patient with oral anticoagulant therapy. Abnormal AT-III is transmitted autosomally dominantly.

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FAMILIAL ANTITHROMBIN III DEFICIENCY ASSOCIATED WITH RECURRENT ARTERIAL THROMBOEMBOLISM. 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 thromboembolic disease sustained three arterial thromboembolic occlusions during a 7 mo. period. The patient had antithrombin III (AT III) levels of 19.2 mg/dl (N=17-30 mg/dl) by RID and an immediate (heparin activated) antithrombin level of 77% (N=88-120%). Crossed IEP showed normal electrophoretic mobility of the patient's AT III. 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 AT III deficient family members. A 19-year-old brother of the propositus, with no history of thrombotic events but with 13 mg/dl AT III and 83% 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 AT III 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.