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MONOCLONAL ANTIBODIES AGAINST THROMBIN-ANTITHROMBIN III COMPLEX: EPITOPE SPECIFICITY AND EFFECT ON THROMBIN-ANTITHROMBIN III INTERACTION. S. Asakura, N. Yoshida and M. Matsuda. Institute of Hematology, Jichi Medical School, Tochigi, Japan.

Among monoclonal antibodies (MCA's) raised against human thrombin (T)-antithrombin III (AT) complex (TAT), two MCA's designated as JITAT-16 and 17 with high affinity, Kd = 4.6 nM and 4.1 nM, respectively, were selected and characterized for specificity and functions. Their respective immunoglobulin subclasses are IgG_1 and IgG_2a, and epitopes were found to be different from each other as shown by crisscross inhibition experiments. Immunoblotting of normal plasma and serum electrophoresed on non-SDS polyacrylamide gel showed that these antibodies reacted with normal serum but not with plasma. This was verified by an enzyme-linked differential antibody immunosorbent assay using either one of the MCA's as the first antibody and the other MCA labeled with peroxidase as the second one. By immunoblotting after SDS-PAGE, we found that both antibodies reacted with TAT, but not with its respective nascent constituent, AT or T. However, they reacted with reactive site-cleaved AT (or thrombin-modified AT, ATM) and also a complex of AT with activated factor X (Xa-AT). These results indicate that both of these antibodies recognize enzyme-treated forms of AT, including AT molecules complexed with enzymes reversibly or irreversibly as well as ATM. Upon incubation of T with AT in the presence of JITAT-16, T activity remained nearly unchanged and formation of irreversible TAT did not proceed as expected. Moreover, AT was preferentially converted to ATM. When JITAT-16 was added after completion of TAT formation, however, neither recovery of T activity nor generation of ATM was observed. These findings were not obtained when JITAT-17 had been substituted for JITAT-16. These data suggest that JITAT-16 may have converted AT from an inhibitor to a substrate for T after having recognized a possible intermediate reversible complex of AT with T. Undoubtedly, in the presence of a polyclonal antibody against AT, neither TAT formation nor ATM generation was observed at all. The mechanism of the unique function of JITAT-16 has not been fully clarified as yet, but this antibody seems to give us new information on the kinetic study of TAT formation and ATM generation when AT was allowed to react with enzymes.

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THROMBIN-ANTITHROMBIN III COMPLEX - A NEW PARAMETER FOR DETECTION OF THROMBOTIC STATES
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Recently, we have developed an enzyme-linked immunosorbent assay (ELISA) for determination of thrombin-antithrombin III complex (TAT) in human plasma (1). The test system follows the sandwich principle and uses two different antibodies directed against human thrombin and human antithrombin III, respectively. The antibodies bind selectively to the corresponding antigen moieties of TAT. The assay was calibrated with definite concentrations (2.0 to 60 µg/l) of preformed purified TAT added to TAT-poor plasma. Plots of absorbance 492 nm against TAT concentration revealed a linear correlation (r = 0.98). The lower limit of sensitivity of the assay was 0.5 µg/l. Mean coefficients of variation of 4 % (intraassay) and 7.5 % (interassay) were found for TAT concentrations between 2 and 60 µg/l. A reference range from 0.85 to 3.0 µg/l was calculated from TAT concentration in plasma samples from 88 healthy donors (mean value ± SD: 1.45 ± 0.4 µg/l). In plasma samples from patients with pulmonary embolism confirmed by lung-scan (n = 17), TAT concentrations between 10 and 20 µg/l were measured. In patients with deep vein thrombosis confirmed by phlebography (n = 15), TAT were found up to 7 - 13 µg/l. Patients with septicaemia associated with a consumption coagulopathy (n = 10), and patients with acute hepatic failure (n = 5) showed markedly increased TAT values. In plasma samples from patients with various metastatic malignant diseases, TAT concentrations of 30 to 60 µg/l could be measured. From these data we conclude that measurement of TAT can be a sensitive parameter for specific detection of a latent activation of the clotting pathway.

(1) PELZER, H., Schwarz, A., Heimburger, N. Thromb. Res., Supplement VI (1986), 51

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THROMBIN-ANTITHROMBIN III (TAT) COMPLEXES: A STUDY IN CONSECUTIVE PATIENTS SUSPECTED OF DIC. J.A. Hoek, R. Lamping, A. Sturk, J.J.J. Borm, F. Berends, J.W. ten Cate. Div. Hemostasis and Thrombosis, Academic Medical Center, Amsterdam, The Netherlands.

DIC is a clinical syndrome which is initiated by excessive thrombin generation, fibrin formation and fibrin deposition in organs, thus resulting in multiple organ failure. It is a frequent, often fatal complication in intensive care patients. We evaluated a recently developed ELISA (Behringwerke) for TAT complexes in 50 healthy volunteers and in 43 consecutive intensive care patients referred by the attending physician on the clinical suspicion of DIC. The ELISA is based upon thrombin-antibody coated test tubes and measurement of bound TAT complexes with peroxidase-conjugated AT III antibody. The method has a detection limit of 0.7 µg TAT/L, a normal range of 0.97-5.4 µg/L. The intra- and inter assay v.c. at 3.3, 11.2 and 42.4 µg/L TAT levels were 5, 7, 8% and 9, 9 and 12% resp. Patients suspected of DIC were in a clinically septic condition (n=33), confirmed by positive bloodcultures in 18, malignancies (n=5), multiple trauma (n=3) or solutio placentae (n=2). Of the 43 patients 29 had increased TAT levels: mean 21.5, range 5.4-62.5. TAT levels correlated ($p < 0.05$) with concurrently assayed plasma fibrinogen (r=0,543), Factor V (r=0,539), platelet count (r=0,307), AT III (r=0,317), and with plasminogen (r=0,365) (Spearman rank correlation test). The accuracy of the TAT-test for the diagnosis of DIC was assessed in all patients. DIC was assumed if patients fulfilled 3 of the 5 following laboratory criteria, i.e. AT III <0.7 U/ml, factor V <0.6 U/ml, fibrinogen <1.5 g/L, platelet count $<100 \times 10^9$/L or a positive fdp/FDP test. The sensitivity and specificity of the TAT-test was 86 and 46% respectively, the positive and negative predictive values were 60 and 77%.

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Increase of Thrombin-Antithrombin III (TAT) Complex Plasma Levels in Thromboembolic Diseases during Thrombolysis. R. Seitz, G. Prätorius, H. Blanke, R. Egbring, B.E. Strauer. Div. of Internal Medicine, University of D-3550 Marburg, F.R.G.

Recently an enzyme immuno assay of thrombin-antithrombinIII complex (TAT) plasma levels was developed by PELZER et al. (Thromb. Haemost. 54:24,1985). This test appears to be useful in the detection of intravasal thrombin generation, since all of 17 patients (pts.) with pulmonary embolism and 15 of 16 pts. with deep vein thrombosis (DVT) showed elevated values above 3 ng/ml.

In 9 pts. with acute myocardial infarction (AMI) the TAT levels increased significantly ($p < 0.001$) 3 to 6 hours after thrombolytic therapy with 1.5 million units streptokinase (SK) over 30 minutes. A concomitant increase of fibrinopeptide A (FPA) levels ($p = 0.048$) was observed. In contrast, 8 AMI pts. treated with heparin showed an insignificant increase of TAT and FPA. In 7 DVT pts. the TAT levels rose significantly ($p < 0.001$) within 6 hours after start of urokinase (UK) infusion, while the FPA levels were enhanced prior to treatment and showed no further increase.

In order to assess the in vitro effects of SK and UK on TAT levels, clots obtained by recalcification of citrated plasma were incubated in heparin (2 units/ml) plasma. An increase of TAT occurred after addition of SK or UK, which was less pronounced when the clots were rinsed extensivly or squeezed before incubation. When SK or UK were added to plasma in the absence of a clot, still a small increase of TAT occurred which was absent in saline controls.

The data suggest that SK and UK action is associated with the generation of TAT complexes. In vivo, thrombin or thromboplastic material might be released by enhanced "wash out" from the recanalized coronary artery or from the reperfused infarcted myocardium. Thrombin might also be released from binding sites on fibrin clots or fibrinogen. It is conceivable that these findings contribute to the understanding of reocclusion of infarct vessels after thrombolytic therapy. This points to the importance of careful anticoagulation in patients receiving thrombolytic therapy.