

367

## GRANULOCYTE ELASTASE RELEASE DURING BLOOD COAGULATION

T.Miura (1), M.Inagaki (1), M.Taki (1), N. Saito (1), T.Meguro (2) and K.Yamada (2).

Dept. of Pediatrics, School of Medicine, Keio Univ., Tokyo (1), Dept. of Pediatrics, School of Medicine, St. Marianna Univ., Kawasaki (2), Japan.

Granulocyte elastase (ELP) has a high-potency fibrinolytic activity. Hence, there is a possibility that ELP acts as a thrombolytic enzyme like plasmin in thrombolysis. We investigated the release of ELP from granulocytes, especially during blood coagulation.

The biological activity of ELP was measured using a synthetic substrate, Suc-Ala-Tyr-Leu-Val-pNA. The immunological activity assayed as an alpha-1-antitrypsin-ELP complex was measured using an anti-ELP antibody (Merck), because more than 90% of ELP in blood forms alpha-1-antitrypsin-ELP complexes.

The ELP activity in granulocytes extracted by 2 mol/l KSCN was 10 mU/10<sup>6</sup> cells. This fibrinolytic activity corresponds to 1-2 U of plasmin in the fibrin plate method.

The ELP release from separated granulocytes was observed by adding Ca<sup>2+</sup>, and the release was increased by Ca ionophore A 23187. The release was dose-dependent as far as 10 mM Ca<sup>2+</sup> (final concentration) and the maximum release was obtained within 15 minutes. However, the ELP release was not produced by thrombin.

The level of alpha-1-antitrypsin-ELP complex in serum was twice higher and that in heparinized plasma was 1.5 times higher than that in sodium citrated plasma. ELP was not released from granulocytes incubated in both prekallikrein deficient plasma and Factor XII deficient plasma containing 10 mM Ca<sup>2+</sup>. But addition of normal plasma (about 10%) resulted in ELP release.

These results suggest that the ELP release from granulocytes is dependent on Ca<sup>2+</sup> and the release is relevant to the blood coagulation system, especially to contact factors.

369

## MECHANISMS OF PROCOAGULANT GENERATION BY ALVEOLAR MACROPHAGES DURING MATURATION. Maria McGee and Henry Rothberger. Bowman Gray School of Medicine, Winston-Salem, NC 27103 USA.

During maturation in vivo and in vitro alveolar macrophages generate procoagulant(s) capable of activating the extrinsic pathway. It is generally agreed that at least part of the activity is due to TF (tissue factor). However, whether or not macrophages also generate functional factor VII or X is controversial. To characterize procoagulant activity increases, we measured kinetic parameters defining interactions between components of the TF-VII complex on membranes of alveolar macrophages either freshly isolated or cultured in serum free medium. In incubation mixtures with fixed concentrations of macrophages and added factor VII, the rate of factor Xa formation (measured by S-2222 hydrolysis) approached a maximum as factor X concentration was increased. Estimated concentrations of factor X yielding 1/2 maximal activation rates, (apparent Km) were 127.1±26 nM and 99.7±34 nM for fresh and cultured cells, respectively. Vmax (maximal velocities) were 1.2±0.24 and 8.9±5 nM Xa/min/10<sup>6</sup> cells. When concentrations of added factor X were kept constant, the rate of factor X activation increased as the added factor VII concentration was increased. For fresh and cultured cells, the respective apparent Kd were 1.8±0.7 and 1.4±0.25 nM. Maximal rates observed with X concentration fixed at 108 nM were 0.46±0.06 and 5.7±1.6 nM Xa/min/10<sup>6</sup> cells. In the absence of either added factor X or added factor VII, no factor Xa generation was detected in fresh or cultured cells, during 10-20 min incubation periods used for kinetic studies. The observed increase in Vmax without changes in apparent Km and Kd indicate that gains in procoagulant activity during macrophage maturation are due to increases in the number of functional binding sites for factor VII, without significant generation of functional vitamin K dependent factors (VII and X) by the cells. The data also indicate that maturation does not alter the rate behaviour of the TF-VII enzymatic complex on macrophage membranes. Mechanisms of complex assembly that we observed on macrophage membranes are similar to those described for the TF-VII complex assembly on purified systems.

105

368

## PLASMINOGEN ACTIVATOR ACTIVITY OF NORMAL AND MALIGNANT

MONONUCLEAR HUMAN CELLS. J.F. Filippi, D. Arnoux, N. Tubiana, B. Boutière, F. Le Caër, J. Sampol, Lab. Hématol. (Pr. J. Sampol, Pr. Y. Carcassonne), Fac. Méd. et Pharm., Marseille, France.

Plasminogen activators (PA) are thought to play a role in the invasive and metastatic properties of many types of cancer cells. Though, discrepancies in correlations between fibrinolytic activity and metastatic potential of malignant cells have been described.

In this study, we evaluated both tissue type (tPA) and urokinase type (UK) cellular PA activities in different mononuclear cell types: normal T and B human peripheral lymphocytes, B cells from patients with chronic lymphocytic leukemia (CLL), human blood monocytes, alveolar macrophages, U 937, RAJI and JM cell lines.

Mononuclear cells were isolated by Ficoll-hypaque gradients and monocytes by plastic adhesion. T and B cells were separated by a rosetting technique using sheep red blood cells. Cellular extracts were prepared by 0.5 % Triton X 100 buffer treatment followed by sonication and centrifugation 10' at 2000 g. PA assays were performed on the supernatants.

UK-type PA was evaluated by a liquid-phase assay in presence of human plasminogen (Kabi) and chromogenic substrate S 2251 (Kabi).

tPA was determined using a solid-phase fibrin activity assay which involves an affinity separation step and thus allows selective detection of tPA.

In both cases, results were reported in international units by reference to standard curves of UK (Choay) or tPA (Kabi).

In all cell types tested, PA detected was essentially urokinase-type. Highest PA activity was found in U 937 cells (0.7 IU/5x10<sup>6</sup> cells). In normal blood lymphocytes, mean PA activity was 0.08 IU/5x10<sup>6</sup> cells. Examination of lymphocytes from patients with CLL revealed a marked decrease in UK activity as compared to normals (< 0.01 IU/5x10<sup>6</sup> cells in more than 50 % cases).

The function of PA in normal lymphocyte physiology and the potential pathogenic role of diminished PA in CLL lymphocytes remains to be investigated.

370

## BEHAVIOUR OF ADRENOCROME PATHWAY IN PATIENTS WITH CEREBROVASCULAR DISEASES. C. Alessandri (1), F. Violi (1), M. Rasura (2), C. Caliendo (1), P. Pelaia (2).

Institute of Clinical Medicine I (1) and Department of Neurology (2) University of Rome "La Sapienza", Rome Italy.

Histopathological studies in segments of cerebral ischaemia show local inflammation with leucocytes infiltration. This event has been confirmed in vivo by means of radiolabelled leucocytes. This inflammatory response could be of detriment to cerebral tissue since leucocytes release toxic substances such as oxygen free radicals. A free radical mechanism, in fact, has been supposed as an event worsening the evolution of ischemia. Evidence of neutrophil activation in stroke patients was shown by us in previous reports, where we have described that the plasma of these patients contained an excess of a neutrophil oxidase able to convert, in vitro, adrenaline to adrenochrome. Aim of present study was to evaluate if neutrophil activation can be observed in patients with brain hemorrhage (BH) also. Six patients (females 1, males 5; age 68-79 years) suffering from BH and 15 patients (females 5, males 10; age 58-86 years) affected by brain infarction (BI) were studied within 20-48 hours from acute episode. Diagnosis of stroke was made by computerized tomography. Neutrophil activation was studied in plasma evaluating the oxidation of adrenaline to adrenochrome according to Matthews and Campbell method. 20 matched for age and sex healthy subjects were studied as control. A significant rise of plasma adrenaline oxidase activity was observed in patients with BI. This preliminary investigation suggests that neutrophil activation could be restricted to patients with BI. In fact, patients with BH had plasma oxidase activity similar to controls. Clinical data should be necessary to evaluate if a relation between leucocyte activation and the natural course of cerebral ischemia does exist.