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FIBRINOLYTIC ACTIVITY (FA) OF NORMAL HUMAN PERIPHERAL BLOOD MONOCYTES (MC). E. Grau and L.A. Moroz. Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada.

FA of blood encompasses a large cellular phase in addition to a fluid (plasma) phase. Polymorphonuclear neutrophils (PMN) have been implicated in this cellular activity, and MC have demonstrated fibrinolytic potential. Using a solid phase radiofibrin assay, we have examined FA of normal blood and plasma, and of purified PMN and MC alone, and with purified plasminogen (PLG), mini-plasminogen (mPLG) produced by PMN elastase digestion, or autologous plasma. PMN alone (0.5×10^6/mL) had striking activity (292 ± 25 SEM ng fibrin lysed/h), (n=10 normal subjects) while MC alone (0.5×10^6/ml) had mean FA of 32 ± 4 ng/h, all of which could be accounted for by contaminating PMN in the MC preparations (36 ± 8 ng/h). In comparison, mean whole blood FA was 72 ± 4 ng/h, and plasma FA was 22 ± 4 ng/h. When MC (0.5×10^6/ml) were assayed with PLG (2-40 µg/ml) or autologous plasma for 1 h, no significant FA was generated, indicating that neither intrinsic nor PLG-dependent (plasminogen activator, PA) activity of MC contribute significantly to the FA of whole blood as measured by routine 1 h assay, where 70% of measured FA involves the cellular phase. However, with longer assay times (2-6 h), there was time-dependent appearance of FA when MC were mixed with PLG or with autologous plasma. This FA was dependent upon interaction between MC and PLG, since no FA was generated by supernatants of MC preincubated alone, while FA was readily detected in the medium when MC and PLG were mixed. Comparing effects of PLG and mPLG, FA of MC (0.5×10^6/ml) with PLG (40 µg/ml) was 447 ± 9 ng/3 h, while FA with mPLG (40 µg/ml, an approximate 3-fold molar excess) was 156 ± 5 ng/3 h, indicating a possible role for the N-terminal portion of the PLG molecule (containing kringle domains 1-4 absent in mPLG) in interaction of MC and PLG, or of MC-derived PA and PLG. FA of MC (0.5×10^6/ml) in autologous plasma (83 ± 3 ng/3 h) was markedly reduced after lysine-Sepharose PLG depletion (14 ± 1 ng/3 h), and by addition of tranexamic acid (10 mmol/L) (5 ± 1 ng/3 h). Thus, normal peripheral blood monocytes, like PMN, may contribute, albeit in a minor manner, to normal blood fibrinolytic activity, via PLG activation rather than direct proteolysis, and constitute an additional mechanism for interaction between cellular and fluid (plasma) phases in blood fibrinolytic activity.

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INCREASED SECRETION OF A FIBRINOLYTIC INHIBITOR BY HUMAN MONONUCLEAR LEUKOCYTES PARALLELS THE PROCOAGULANT RESPONSE TO SPECIFIC ANTIGEN. B.S. Schwartz and M.C. Monroe. University of Wisconsin, Madison, WI, U.S.A.

The presence of fibrin is a characteristic finding of immune mediated tissue lesions. It is known that peripheral blood mononuclear cells (PBM) express tissue factor in response to recognition of a specific protein antigen. We have found PBM secrete a plasminogen activator (PA) inhibitor (I) in parallel to expression of tissue factor upon exposure to a sensitizing antigen. Increased PA-I can be detected by inhibition of urokinase (UK) in an ^{125}I-fibrin plate assay, inhibition of ^{125}I-plasminogen cleavage, and formation of complexes between ^{125}I-urokinase and UK-I. PA-I secretion is dose dependent, and antigen specific, i.e. a nonsensitizing antigen does not induce a PA-I response.

Dose of PPD (µg)	PPD Immune mU PCA	PPD Immune mU UK-I	PPD Nonimmune mU PCA	PPD Nonimmune mU UK-I
0	<3	75	<3	40
0.8	5	100	<3	60
4.0	21	160	<3	45
20.0	140	825	<3	70

The PA-I is secreted by monocytes, however the recognition of antigen is a T-cell function. Inhibition of PBM protein or RNA synthesis abrogates the PA-I response. The PA-I appears to be the type 2 inhibitor, in that 1) it is a much more efficient inhibitor of UK than of tissue type PA, 2) it is labile in acid and detergent, and 3) it is neutralized by IgG to human placental PA-I, but not by antiserum to PA-I of endothelial cells. It is concluded that PBM respond to a foreign stimulus by elaborating molecules that lead to both the deposition and persistence of fibrin.

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PLATELET DISAGGREGATION IN PLASMA--A NOVEL EFFECT OF TISSUE PLASMINOGEN ACTIVATOR. D.E. Vaughan and J. Loscalzo. Brigham and Women's Hospital and Harvard Medical School, Boston, MA, U.S.A.

Platelet aggregates are thought to play a significant role in many clinically important ischemic vascular events. Recently it has been shown that the platelet surface binds plasminogen and, in so doing, enhances its conversion to plasmin by tissue plasminogen activator (tPA). Since fibrinogen, an alternative substrate for plasmin, serves as the cohesive link among platelets induced to aggregate by a variety of agonists, we hypothesized that the local production of plasmin at the platelet surface may be important in promoting the disaggregation of aggregated platelets. When added to a suspension of human platelets induced to aggregate in plasma with adenosine 5'-diphosphate, tPA promoted disaggregation over several minutes. The rate of disaggregation and its extent were dependent on the concentration of tPA as well as on its time of addition. Preincubation of platelet-rich plasma with excess alpha-2-antiplasmin inhibited disaggregation by tPA. While platelet surface fibrinogen receptors did not appear to be proteolyzed by plasmin in this plasma system, platelet-bound cohesive fibrinogen was selectively proteolyzed compared with proteolysis of ambient fibrinogen. The rate of disaggregation correlated best with the rate of loss of platelet-bound fibrinogen and not with the rate of ambient fibrinogenolysis. These data demonstrate that tPA facilitates platelet disaggregation through the plasmin-mediated proteolysis of cohesive fibrinogen. This phenomenon may be important in the dispersal of circulating platelet aggregates and may be operative in the thrombolysis of platelet-rich clots.

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THE BINDING OF TISSUE PLASMINOGEN ACTIVATOR TO PLATELETS. J. Loscalzo and D.E. Vaughan. Brigham and Women's Hospital and Harvard Medical School, Boston, MA, U.S.A.

Since the platelet surface has been shown to be a site for plasminogen conversion by tissue-type and other plasminogen activators, we examined the binding of tissue plasminogen activator (tPA) to human platelets. Resting, washed platelets were found to bind single chain, radioiodinated, recombinant tPA specifically and saturably with an apparent, estimated dissociation constant (K_D) of 458 nM, binding approximately 570 molecules per platelet at saturation. Washed platelets activated with adenosine 5'-diphosphate in the presence of 0.1 mg/ml fibrinogen and 1 mM $CaCl_2$ bound tPA with greater affinity, having an estimated apparent K_D of 30.6 nM and binding approximately 29,000 molecules per platelet at saturation. Bound tPA could be completely displaced by an excess of unlabeled tPA. Interestingly, bound tPA could also be displaced from activated platelets with increasing concentrations of soluble fibrin with an estimated IC_{50} of 37.5 µg/ml of fibrin. In contrast, increasing concentrations of fibrinogen failed to reduce binding. These data show that tPA binds to the activated platelet surface by a mechanism that involves platelet-bound fibrinogen. In addition, these data suggest that on binding to the platelet surface, fibrinogen expresses domains that are similar to the tPA binding domains of fibrin. It is the presence of these domains within the platelet aggregate that likely supports tPA binding and facilitates plasminogen activation.

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