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 x 10^6/ml) had striking activity (292 ± 25 SEM mg fibrin lysed/h), (n=10 normal subjects) while MC alone (0.5 x 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 x 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 a time-dependent appearance of FA in plasma 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 x 10^6/ml) with PLG (40 μg/ml) was 447 ± 70 ng/h, while FA with mPLG (40 μg/ml), an approximate 3-fold polar excess was 156 ± 5 ng/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 x 10^6/ml) in autologous plasma (83 ± 3 ng/h) was markedly reduced by additions of single chain, radioiodinated, recombinant tPA (1 ng/3 h). Recombinant tPA was ineffective at saturation.

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 platelet aggregates, 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 α2-antiplasmin inhibited disaggregation by tPA. While platelet surface fibrinogen recep­tors did not appear to be proteolyzed by plasmin in this plasma system, platelet-bound fibrinogen was selectively proteo­lyzed with proteolysis of acid fibrinogen. The rate of disaggregation correlated best with the rate of loss of platelet-bound fibrinogen, and not with the total fibrinogen. These data demonstrate that tPA facilitates platelet disaggregation through the plasmin-mediated proteolysis of fibrinogen. This phenomenon may be important in the dispersal of circulating platelet aggregates and may be operative in the thrombolytic activity of t-PA-rich clots.

PLATELET DISAGGREGATION IN PLASMA--A NOVEL EFFECT OF TISSUE PLASMINOGEN ACTIVATOR. D.E. Vaughan and J.L. Loscalzo. 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, radiiodinated, recombinant tPA specifically and saturably with an apparent, estimated dissociation constant (Kd) of 450 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 CaCl2 bound tPA with greater affinity, having an esti­mated apparent Kd 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 fibrinogen with an estimated IC50 of 37.5 μg/ml of fibrinogen. 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 tPA is involved in the activation of platelet surface fibrinogen, which in turn, expresses domains that are important to the tPA binding domains of fibrin. It is noteworthy that these domains within the plateletaggregate that likely supports tPA binding and facili­tates plasminogen activation.

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

The presence of fibrin is a characteristic finding of tissue mediated lesions. It is known that peripheral blood mononuclear cells (PBMC) express tissue factor in response to recognition of a specific protein antigen. We have found PBMC secrete a plasminogen activator (PA) inhibitor (1) 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 125I-fibrin plate assay, inhibition of 125I-plasminogen cleavage, and formation of complexes between 125I-proteinase UK-I. PA-I secretion is dose dependent, and antigen specific, i.e. a nonsensitizing antigen does not induce a PA-I response.