FIBRINOLYTIC ACTIVITY (FA) OF NORMAL HUMAN PERIPHERAL BLOOD MONONUCLEATED CELLS (MC). E. Drau and L.A. Monroe. 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. Plasma 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 32 ± 4 ng/h. When MC (0.5 x 10^5/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 PA activity in MC mixted 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 ng/mg) was 67 ± 9 ng/3 h, while FA with mPLG (40 μg/ml), an approximate 3-fold polar excess was 156 ± 6 ng/h, indicating a possible role for the N-terminal portion of the PLG molecule (containing kringle domains 1-4) in interaction of MC, and PLG, or of MC-derived PA and PLG. FA of MC (0.5 x 10^5/ml) in autologous plasma (85 ± 3 ng/3 h) was markedly reduced by 40% by an α2-antiplasmin (α2-AP) of 0.2 μg/ml and further by an addition of tranexamic acid (10 mmol/L) (5 ± 1 ng/3 h). Thus, normal peripheral blood monocytes, like PMN, may contribute, albeit at a low level, to 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.

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 in a characteristic finding of tissue mediated tissue 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 (IPA) 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 UK-plasminogen cleavage, and formation of complexes between 125I-proteinase and UK-I. PA-I secretion is dose dependent, and antigen specific, i.e. a nonsensitizing antigen does not induce a PA-I response.

PLATELET DISAGGREGATION IN PLASMA--A NOVEL EFFECT OF TISSUE PLASMINOGEN ACTIVATOR. J. Lasca lzo and D. E. Vaughan. 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 conditions. 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, it has been shown that tPA promotes 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. Precisuation of platelet-rich plasma with alpha-2-antiplasmin inhibited disaggregation by tPA. While platelet surface fibrinogen receptors did not appear to be proteolized by plasmin in this plasma system, platelet-bound cohesin fibrinogen was selectively proteolized with compromise of activity of fibrinogen. The rate of disaggregation correlated best with the rate of loss of platelet-bound fibrinogen and not with the rate of fibrinolysis. These data demonstrate that tPA facilitates platelet disaggregation through the plasmin-mediated proteolysis of cohesin fibrinogen. This phenomenon may be important in the dispersal of circulating platelet aggregates and may be operative in the thrombolysis of platelet-rich clots.

THE BINDING OF TISSUE PLASMINOGEN ACTIVATOR TO PLATELETS. J. Lascalzo 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, radiiodinated, recombinant tPA specifically and saturably with an apparent, estimated dissociation constant (Kd) of 458 mM, binding approximately 570 molecules per platelet at saturation. Washed platelets activated with adenosine 5'-diphosphate in the presence of 0.1 mM fibrinogen and 1 mM CaCl2 bound tPA with greater affinity, having an estimated apparent Kd of 30.6 mM and binding approximately 25,000 molecules per platelet at saturation. Binding 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 in an estimated Kd of 2.5 μM 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 likely that for these domains within the platelet aggregate that likely supports tPA binding and facilitates plasminogen activation.