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FIBRINOGEN BINDS TO HUMAN NEUTROPHILS AT A SITE DISTINCT FROM GPIIb/IIIa. E.J. Gustafson, H. Lukasiewicz, A.H. Schmaier, S. Niewiarowski and R.W. Colman. Thrombosis Research Center and Department of Medicine, Temple University, Phila., PA, U.S.A.

Many observations suggest a potential role for neutrophils in the modulation of hemostasis and thrombosis. Arterial thrombi are characterized by the presence of large numbers of neutrophils lining the perimeter of platelet aggregates. While investigating binding of high molecular weight kininogen (HMWK) to neutrophils, we found that fibrinogen (Fb) could inhibit binding of ^{125}I-HMWK as well as displace HMWK already bound to neutrophils. We therefore initiated studies to determine whether Fb could bind to human neutrophils. Both Zn^{++} and Ca^{++} were required for maximal binding of ^{125}I-Fb to neutrophils. Binding did not occur with Ca^{++} (2mM) alone and was only 1/3 the maximal amount with Zn^{++} (50 μM) alone. At 4° the amount of ^{125}I-Fb bound to neutrophils reached a plateau by 15 minutes and remained at this level over the next 30 minutes. At 23° and 37° the amount of ^{125}I-Fb bound peaked by 4 minutes and then decreased over the next 30 minutes indicating receptor-mediated internalization. Excess Fb inhibited binding of ^{125}I-Fb to neutrophils while prekallikrein, factor XII, and fibronectin did not. Binding of ^{125}I-Fb was 99% reversible at 4° within 10 minutes with a 50-fold molar excess of Fb and 90% displaceable by excess HMWK. The apparent Kd was approximately 0.45 μM. Arg-Gly-Asp-Ser (RGDS) is a tetrapeptide common to Fb, fibronectin, vitronectin and other cell-attachment proteins. Fb has been demonstrated to bind to the glycoprotein IIb/IIIa (GPIIb/IIIa) complex which is the platelet membrane receptor for RGDS. Although this RGDS-GPIIb/IIIa interaction occurs with Fb binding to platelets, it is apparently not involved with Fb binding to monocytes. To investigate if Fb binding to neutrophils involved this interaction of GPIIb/IIIa - RGDS we performed further studies. Binding of ^{125}I-Fb to neutrophils was not inhibited by RGDS nor was it inhibited by a monoclonal antibody (10E5) to the platelet GPIIb/IIIa complex. In addition, the amount of ^{125}I-Fb that bound to neutrophils from a patient with Glanzman's thrombosthenia was the same as that bound to normal neutrophils. These studies indicate that human neutrophils specifically bind Fb at a site similar to HMWK and distinct from GPIIb/IIIa.

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STRUCTURAL DIVERSITY AMONG CELLULAR ADHESION RECEPTORS: FIBRINOGEN BINDING IS A NOVEL BIOLOGICAL PROPERTY OF THE MONOCYTE DIFFERENTIATION ANTIGEN OKM1.
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A family of related glycoproteins (GP) mediate the interaction between the circulating adhesive proteins and a variety of cells (cytoadhesins). In this study we have compared two cell-surface antigens which share the property to bind fibrinogen: the platelet GP IIb/IIIa, prototype of the cytoadhesins, and the receptor for fibrinogen costitutively synthesized by monocytes. Two anti-GP IIb/IIIa monoclonal antibodies (Mabs) (LJP9, LJP5), recognizing functionally distinct epitopes of the GP IIb/IIIa did not react with monocytes nor inhibited ^{125}I-fibrinogen binding to monocytes. Similarly, an Arg-Gly-Asp containing peptide which completely abolished platelet-fibrinogen interaction, had no effect on monocytes. Structurally, the monocyte fibrinogen receptor was dimeric and composed of two subunits with molecular weight (M_r) of 155,000 and 95,000. This structural organization was different from that of the GP IIb/IIIa (M_r= 116,000), but in close analogy with the family of leukocyte differentiation antigens OKM1, LFA-1. Therefore, this possible relationship was investigated. A Mab to OKM1 antigen (10 μg/ml) completely suppressed fibrinogen binding to monocytes while it was ineffective on plateles. Iodinated monocyte lysate subjected to immunoprecipitation with OKM1 Mab (60 μg/ml) showed a dimeric antigen with the same molecular size of the monocyte fibrinogen receptor. Moreover, preclearing of the monocyte lysate with OKM1 Mab removed the immunoprecipitate corresponding to the monocyte fibrinogen receptor. These data indicate that the immunologic differentiation antigen OKM1, in addition to function as a complement receptor, displays also the novel biological adhesion property to mediate the binding of fibrinogen to monocytes.

PLATELET INHIBITION (2) Thursday

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ADMINISTRATION OF TICLOPIDINE IMPEDES ADP-INDUCED ACTIVATION BUT DOES NOT INDUCE A THROMBASTHENIC STATE. F. Potevin, T. Lecompte, C. Lecrubier, M. Samama. Lab. Hématologie, Hôtel-Dieu, Paris.

The aim of this study was to reassess the pattern of aggregation and to investigate the platelet-fibrinogen interaction, in response to various agonists, following T. treatment: 250 mg b.i.d. for at least 1 week. Platelets were obtained from healthy volunteers (n=8) as well as from patients with cerebro-vascular disease (n=5). The regimen was able to induce the well-known and expected alterations of platelet behaviour in citrated PRP. Washed platelets were prepared according to MUSTARD et al. (1972). (1) In a first set of experiments it was shown that these alterations persisted after resuspension of twice-washed platelets, in the presence of fibrinogen (3.7 μM); with ADP there was a marked impairment of aggregation, quantified either as maximal change in light transmission (DTLmax), or as maximal velocity; after stimulation with 100 μM ADP there was still a 40% decrease in DTL max and an about 50-fold faster de-aggregation as compared to values obtained without T. treatment. PAF-acether (0.1 to 3 μM): decreased DTLmax mainly with the higher doses, persistent de-aggregation but unimpaired velocity. Collagen (from Stago, $\mu g.ml^{-1}$): DTLmax was decreased in response to low concentrations only (0.3 and 0.6). Arachidonic acid (20, 50, 100 μM) -induced aggregation remained unchanged, but the concentrations of U 46,619 able to induce an at least 50% DTLmax was shifted from 0.05 to 0.1 or 1.0 μM. (2) The association of human fibrinogen with stimulated platelets was quantified by plotting velocity against concentration of exogenous fibrinogen (0.06, 0.23, 0.92, 3.7 μM). The dose-response curves were modified in the presence of ADP (5 μM) but not PAF (0.5 μM). With selected low concentrations of collagen or arachidonic acid addition of fibrinogen is a prerequisite for aggregation; under these experimental conditions T. treatment only induced slight modifications of the responses. Fibrinogen-dependent aggregation (DTLmax) of platelets from T. treated patients in response to ADP (10 μM) or after pretreatment with alpha-chymotrypsin was 1/3 and 2/3 of control values respectively. Taken together our results do not support the induction of a functionally thrombasthenic-like state, but rather suggest a selective impairment of ADP-mediated platelet activation. Accordingly, the pattern of platelet behaviour observed under T. treatment can be mimicked by adding ATP (as ADP-antagonist) to normal platelets.

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HOW CAN WE INHIBIT 5HT-INDUCED PLATELET AGGREGATION AND WHY SHOULD WE BOTHER? Jane Bevan and S. Heptinstall, Department of Medicine, University Hospital, Nottingham, NG7 2UH, U.K.

Platelets are induced to aggregate when 5-hydroxytryptamine (5HT) is added to citrated whole blood and the extent of aggregation can be measured using a Whole Blood Platelet Counter. We have used this method to study a) 5HT-induced platelet aggregation in normal human blood and the effects of 14 5HT receptor antagonists and 2 Ca^{++}-channel blockers, and b) aggregation in blood from patients with peripheral vascular disease (PVD). Previous studies of platelet aggregation in platelet-rich plasma have indicated an increased platelet sensitivity to 5HT in PVD, and a multicentre study of ketanserin (a S_2 antagonist) is in progress.

5HT induces a transient reversible aggregation in human whole blood which can be prevented by 5HT receptor antagonists. The inhibitory effects of 7 relatively potent antagonists (IC_{50} 7 - 41nM, e.g. ketanserin and pizotifen) could not be surmounted by increasing the concentration of 5HT, but the inhibitory effects of 7 less potent antagonists (IC_{50} 0.28 - 53uM, e.g. mepyramine and amitriptyline) could be surmounted by 5-HT. One Ca^{++}-channel blocker (verapamil) inhibited platelet aggregation but another (amlodipine) had very little effect. Verapamil inhibited 5HT-induced aggregation at much lower concentrations (IC_{50} 1.6uM) than those required for aggregation induced by PAF, adrenaline or ADP (IC_{50} values 32, 33 and >100uM respectively) and the inhibition was insurmountable.

5HT-induced platelet aggregation in blood from patients with PVD does not differ qualitatively or quantitatively from aggregation in blood from healthy, age- and sex-matched controls: patients (n = 13), aggregation 30 seconds after adding 5uM 5HT = 55.1% ± 13.3(s.d.); controls (n = 13) aggregation = 48.3% ± 18.9(s.d.). Neither was the platelet aggregation induced by ADP, U46619 or PAF different in patients and controls.

We conclude that different 5HT receptor antagonists inhibit 5HT-induced platelet aggregation with different potencies and, apparently, different mechanisms of action, and that verapamil has a selective effect on 5HT-induced aggregation at relatively low concentrations. Results obtained in PVD do not encourage the use of a 5HT antagonist in this condition.

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