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MEGAKARYOCYTIC ORIGIN OF PLATELET HLA CLASS I ANTIGEN. N. Kieffer, M. Titeux, A. Henri, J. Breton-Gorius and W. Vainchenker. Unite INSERM U.91, Hôpital Henri Mondor, 94010 Creteil, France.

The existence of HLA class I antigens on human platelets is well established. However, several authors have suggested that platelet HLA antigens are not integral membrane components but are acquired from soluble plasma sources and adsorbed to the platelet surface.
In the present study, we used the monoclonal antibody W6/32, directed against a monomorphic epitope of the HLA class I antigen for the immunochemical characterization of platelet HLA. Immunoprecipitation experiments, performed after in vitro metabolic radiolabeling of human platelets revealed a band of molecular weight 44,000 identical to that precipitated from metabolic labeled U937 or HEL cells. When the same antibody was tested by indirected immunofluorescence in a double labeling technique on in vitro cultures of human megakaryocytes, performed in the absence of human serum in the culture medium, megakaryocytes identified by an anti-vWF MoAb revealed a membrane staining with W6/32 identical to that observed on other bone marrow cells, e.g. macrophages. Our results provide evidence that platelet HLA has a megakaryocytic origin and that residual biosynthesis of HLA antigen does still occur in circulating platelets. However, our results do not exclude the ability of human platelets to adsord circulating HLA class I antigen from plasma.

PLATELET ADHESION

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ETHANOL INHIBITS RABBIT PLATELET RESPONSES TO COLLAGEN IN VITRO BUT DOES NOT AFFECT RABBIT PLATELET ADHERENCE TO DE-ENDOTHELIALIZED AORTAE IN VIVO. M.L. Rand (1,2), H.M. Groves (2), R.L. Kinlough-Rathbone (2), M.A. Packham (1) and J.F. Mustard (2). Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada (1) and Department of Pathology, McMaster University, Hamilton, Ontario, Canada (2).

Epidemiological studies indicate that moderate consumption of alcohol is associated with a reduced risk of coronary heart disease, but it is not known whether inhibition of platelet functions by ethanol is involved. We studied the effects of ethanol on rabbit platelet responses to collagen in vitro and in vivo. Addition of ethanol (4 mg/ml) to suspensions of washed platelets prelabelled with [^{14}C]serotonin inhibited aggregation and secretion in response to low (0.4 µg/ml) concentrations of acid soluble collagen (14% secretion without ethanol, 3% secretion with ethanol). With a higher concentration of collagen (1.25 µg/ml), 4 mg/ml ethanol had no inhibitory effect. The inhibitory effect of ethanol on collagen-induced aggregation was also observed in citrated platelet-rich plasma (c-PRP) to which ethanol was added in vitro and in c-PRP from rabbits given ethanol acutely by gavage (3.5 g/kg) 30 min before blood sampling. The accumulation of [^{51}Cr]-labeled platelets on the subendothelium of rabbit aortae de-endothelialized with balloon catheters was measured in vivo in rabbits given ethanol (blood ethanol concentration at time of vessel wall injury: 4.1 ± 0.2 mg/ml, mean ± S.E., n=6). Ten min after de-endothelialization, there was no difference between the number of platelets adherent per square mm of injured aorta of control rabbits (39,400 ± 2,600, mean ± S.E., n=6) and intoxicated rabbits (36,800 ± 3,700, mean ± S.E., n=6). Thus, although ethanol inhibits platelet aggregation and secretion in response to collagen in vitro and ex vivo, it does not alter platelet adherence to the subendothelium, including its constituent collagen, in vivo. Therefore, it is unlikely that ethanol exerts its beneficial effects against coronary heart disease by altering the initial adherence of platelets to injured vessel walls.

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THE INTERACTION OF BLOOD ELEMENTS WITH ENDOCARDIAL ENDOTHELIUM DAMAGED BY LACTIC ACID. G. Carter and J.B. Gavin. Department of Pathology, University of Auckland School of Medicine, Auckland, New Zealand.

It has already been demonstrated that ischaemic metabolites, which could diffuse from a myocardial infarct in vivo, can cause substantial damage to the endocardial endothelium and this could predispose to mural thrombosis.
To investigate the role of ischaemic metabolites in the pathogenesis of mural thrombosis, lactic acid (pH6.4) was passed through a two-way concentric catheter ligated into the left ventricle of isolated beating rat hearts that were perfused with oxygenated Krebs-Henseleit buffer (KHB) through an aortic cannula. After periods of 1, 2, and 4 hours, the lactic acid was followed for 10 minutes by 10 mls of whole blood from heparinized donor rats. Ventricles were then flushed with KHB, fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide in cacodylate buffer.
Scanning and transmission electron microscopy showed that platelets adhered to exposed basal lamina, microfibrils and collagen but not to intact or damaged endothelial cells. However densely aggregated thrombi only formed on regions of exposed connective tissue and never on basal lamina. Fibrin, leukocytes and red blood cells were associated with these platelet thrombi. Thus lactic acid and other ischaemic metabolites which could possibly diffuse in vivo from an infarct can contribute to endocardial damage which predisposes to mural thrombosis.

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