0294

08:45 h

REDUCED INVAGINATED PLATELET MEMBRANE IN IDIOPATHIC THROMBOCYTOPENIC PURPURA. C. Nouvel, M. El-Khoury, C. Liverato, P. Sié, A. Robert, B. BONEU. Groupe d'Etude sur les Maladies du Sang, Hopital Purpan, 31059 Toulouse cedex, France.

An indirect approach of the amount of invaginated platelet membrane has been provided by size measurements of platelets submitted to an hypotonic shock. We investigated this new morphological parameter in 15 normal subjects and in 11 ITP.

Platelet volume was measured by the electronic method (Coulter counter, channelyser C 1000) on EDTA-PRP and on EDTA-PRP diluted with distilled water. One min. after dilution, paraformaldehyde (final concentration 0.08%) was added to prevent any return to the initial volume by an active process. Platelet lysis was quantified by LDH assay. Isoton was diluted in the same proportion. Arithmetic mean platelet volume (MPV) was calculated ; graphic analysis of the data allowed to determine the mode and the standard deviation (SD) of the log volume distribution.

An inverse linear relationship between MPV and the log of osmolarity was observed. The maximum MPV increase without any detectable platelet lysis was obtained at 120 m Osm. For lower osmolarities a progressive lysis occured without further volume increase.

Macrothrombocytosis and increased SD of the log volume distribution were found in undiluted ITP-PRP (12.2 fl and 0.31 respectively versus 9.2 fl and 0.27 in controls). After hypotonic treatment, the MPV increased in ITP and controls (respectively 17.2 fl and 15.9 fl) but the relative increase was lower in ITP than in controls (42% and 63% respectively) ; the SD of the log volume distribution was unmodified.

These results suggest that large platelets occuring in ITP possess a reduced amount of invaginated plasma membrane.

0295

09:00 h

VARIABLE RESULTS WITH PLASMA EXCHANGE IN THROMBOTIC THROMBOCYTOPENIC PURPURA. Toby L. Simon, University of New Mexico, United Blood Services, Albuquerque, USA

The clinical efficacy of plasma exchange with fresh frozen single donor plasma in thrombotic thrombocytopenic purpura (TTP) was evaluated. Six patients with the classical findings of TTP, had plasmapheresis using the Haemonetics Model 30. Treatments were initiated daily. Two to three liters total exchanges were done; 1-1½ liters fresh frozen plasma were infused with each exchange. In five of the six cases, therapy was begun immediately after diagnosis. Steroids and antiplatelet agents were also used. Five of the patients were comatose when treatment was begun; the sixth had neurological symptoms of memory loss and aphasia with severe hemolytic anemia and thrombocytopenia. Circulating immune complex measurements were performed in all patients prior to treatment using the Clq and Raji cell assays.

Three patients (2 in coma) responded to therapy. The two comatose patients required more than four daily treatments before beginning to respond (followed by four and six alternate day treatments, respectively). The third patient went into remission after four daily treatments. Three patients in coma died, two deteriorating after the first plasma exchange. The third, treated after a delay due to severe cardiac complications, subsequently died.

The three responding patients were younger than the three who died. The two in coma who responded to therapy had a longer course of symptoms (suggesting disease onset prior to onset of coma) than the three who died. Circulating immune complexes by the Clq assay were negative in all six patients. The Raji cell assay was positive in one patient.

Even severely ill comatose patients with TTP respond to plasma exchange, but aggressive, persistent therapy may be required before response is seen. More fulminant disease with rapid onset may fail to respond. Circulating immune complexes do not appear to mediate the disease process.

0296

09:15 h

INTERACTION OF TRITIATED-QUININE WITH HUMAN PLATELETS IN THE PRESENCE OF A QUININE-DEPENDENT ANTIBODY. D.J. Christie and R.H. Aster. The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin, U.S.A.

In drug-induced immune thrombocytopenia, it has not been established with certainty whether drug binds first to the platelet membrane or to antibody to promote platelet-antibody binding. The association of the drug, quinine, and a quinine dependent antibody (obtained from a patient with drug-induced thrombocytopenia) with human platelets was studied using a tritiated derivative of quinine prepared by catalytic hydrogen exchange. Tritiated quinine binding to platelets was measured in the presence of the antibody or normal human serum (NHS). Platelets were stirred with the appropriate reagents at room temperature for 20 minutes and washed. Bound tritiated quinine was measured by scintillation techniques.

Two types of drug binding sites, antibody-dependent and antibody-independent, were demonstrated on platelets. In the presence of NHS, fewer than 20,000 molecules of drug became tightly bound to the membrane. In the presence of excess antibody, approximately 200,000 molecules of quinine became membrane-bound. Antibody did not bind to platelets in the absence of drug. These findings appear to provide the first demonstration of antibody-dependent binding of quinine to platelets and suggest that interaction of drug and antibody in the soluble phase precedes the binding of either constituent to the platelets ("innocent bystander mechanism"). The use of radioactive drugs appears to provide a useful tool for the investigation of the molecular basis of drug-induced immune disorders.

0297

09:45 h

EFFECTS OF A MONOCLONAL ANTIBODY TO HUMAN PLATELET GLYCOPROTEIN I ON PLATELET - VON WILLEBRAND FACTOR SUBENDOTHELIUM INTERACTIONS. C.Ruan , G.Tobelem , A.McMichael , L.Drouet Y.Legrand and J.P.Caen . Department of Haemostasis and Experimental Thrombosis, Hôpital Saint Louis, Paris, France and The Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, England.

A monoclonal antibody (AN51) to human platelet glycoprotein I (GPI) secreted by a hybrid myeloma has been tested on platelet functions. AN51 bound to normal and thrombasthenic platelets while it failed to bind to platelets from 6 patients with Bernard-Soulier syndrome (BSS). The nature of the antigen recognized by AN51 was determined by demonstration that the antigen, which was chymotrypsin sensitive, gave a peak at 150,000 daltons on SDS-PAGE after immunoprecipitation. AN51 strongly inhibited Ristocetin, bovine factor VIII or porcine factor VIII induced aggregation but did not modify ADP, collagen type I or type III, thrombin or arachidonic acid induced aggregations. Furthermore the adhesion-aggregation of platelet induced by microfibrils was also inhibited by the antibody AN51. Platelet adhesion to untreated or collagenase treated rabbit aorta subendothelium, using the Baumgartner technique, was impaired by AN51, and the inhibition was more pronouced at high shear rate conditions. AN51 decreased the binding of ^{125}I-factor VIII/Willebrand factor (FVIII/WF) to human platelets in presence of Ristocetin. The use of this monoclonal antibody directed against platelet GPI permits a better understanding of the platelet-platelet and platelet-subendothelium interactions mediated by FVIII/WF and will facilitate the purification of the platelet membrane glycoprotein lacking in BSS which may be the receptor for FVIII/WF.