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BINDING OF 37-DKa PLATELET AGGLUTINATING PROTEIN TO HUMAN PLATELETS. E. C.-Y. Lian and F.A. Siddiqui. The Hemophilia and Thrombosis Center, Center for Blood Diseases, University of Miami and Veterans Administration Medical Center, Miami, FL, USA

We have previously reported the purification of a 37-KDa platelet agglutinating protein (PAP p37) from the plasma of a patient with thrombotic thrombocytopenic purpura. Using ^{125}I-labeled p37, the properties of its binding to platelets were studied. The binding of p37 to washed human platelets from 4 normal subjects and two TTP patients after recovery was specific, concentration dependent and saturable. The Scatchard analysis revealed that the binding sites for p37 was about 100,000 per platelet with a dissociation constant of 48 X 10^{-9} M. The binding of p37 to erythrocytes was very little and non-specific. Stimulation of platelets by thrombin or ADP did not have any effect on the binding of p37 to platelets. The monoclonal antibodies to platelet GP Ib (6D1) and GP IIb-IIIa (10E5)(A gift of Dr. Barry Coller) did not inhibit the binding of p37 to platelets. Fibrinogen (1 mg/ml) and FVIII/vWF (250 ug/ml) reduced the binding slightly. The polyclonal antibodies to p37 as well as Concanavalin-A inhibited the binding of p37 to platelets through their direct interaction with p37. Other lectins such as phytohemagglutinin, potato lectin and helix pomatia lectin did not have any effect. At 40 mM, sialic acid, α-D-(+)-glucose, D-(+)-mannose and D-fructose caused 91%, 44%, 79%, and 63% inhibition of p37 binding respectively. D-(+)-galactose did not interfere with the binding. It is concluded that p37 binds to platelets on the sites other than GP Ib and Gp IIb-IIIa and its binding to platelets is inhibited by certain sugars.

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FETAL PLATELET TRANSFUSIONS IN THE MANAGEMENT OF ALLOIMMUNE THROMBOCYTOPENIA. A.H. Waters (1), R. Ireland (2), R.S. Mibashan (2), M.F. Murphy (1), D. S. Millar (2), J.F. Chapman (1), P. Metcalfe (1), L.S. de Vries (3), C.H. Rodeck (4) and K.H.Nicolaides (2). St. Bartholomew's (1), King's College (2), Hammersmith (3) and Queen Charlotte's Maternity (4) Hospitals, London, U.K.

Intracranial haemorrhage is the most serious complication of alloimmune neonatal thrombocytopenia (ANT). It has generally been assumed that this occurs during delivery, but evidence is accumulating that intracranial haemorrhage may have already occurred in utero. Management of the pregnancy at risk is therefore more exacting, and it has been suggested that intrauterine platelet transfusions may be of benefit (Daffos et al, Lancet, ii, 632, 1984). We have used this approach in two pregnancies in $P1^{A1}$ negative mothers with $P1^{A1}$ positive fetuses affected by ANT. Both were second pregnancies, the first in each case having produced a brain damaged infant due to CNS haemorrhage. First patient (CW): Ultrasound scans of the fetal head at 10,22,28 and 32 weeks were all normal. She was admitted at 35 weeks for fetal sampling and platelet transfusion. Ultrasonography showed dilated ventricles and a left anterior cerebral haematoma. The fetal platelet count was 12 x 10^9/1,rising after transfusion of $P1^{A1}$negative platelets to 139 x 10^9/1. The baby was delivered by Caesarean section and the cord blood platelet count was 126 x 10^9/1.Subsequent clinical assessment by CT scanning and NMR indicated both recent (1-2 weeks) and older (>4weeks) cerebral haemorrhages (de Vries et al, in press). Second patient (CR): Platelet transfusions were started earlier in this pregnancy. At 26 weeks the fetal platelet count was 32 x 10^9/1, rising to 160 x 10^9/1 after platelet transfusion. This was repeated at 27 wk (25 to 280 x 10^9/1), 29 weeks (5 to 320 x 10^9/1) and regularly until birth. Before the third platelet transfusion, the mother received intravenous IgG 0.4 g/Kg/d for 5 days, which had no effect on the fetal platelet count. These cases illustrate the potential value of ultrasound-guided intravascular, umbilical cord transfusions of compatible platelets in raising the fetal platelet count in ANT, but emphasise the short duration of this effect (<1 week). As the procedure is so labour intensive, further studies are needed to identify the high risk pregnancies, to determine the optimal time for intervention and to assess the success of this approach.

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CHARACTERIZATION OF SERUM PROSTACYCLIN BINDING DEFECTS IN THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP). K. Wu, C. Manner, and A. Tsai. University of Texas Health Science Center at Houston, TX, U.S.A.

To understand further the pathophysiologic significance of PGI_2 binding defects in TTP, we measured serum PGI_2 binding activity in 12 TTP patients and matched controls. Serum binding of PGI_2 was measured by Sephadex G-25 gel filtration. The mean binding activity in 33 healthy subjects ages 20-40 years was 39.9 ± S.D 4.4%. The mean value of TTP (n=12) was significantly lower (26.2 ± 4.1, P <0.01). Serum from 5 severe ITP, 5 DIC and 5 thrombocythemia exhibited normal binding activity. To determine the binding kinetics we utilized ^{3}H-iloprost in a gel filtration method described by Hirose and Kano (Biochim. Biophys. Acta. 751:376, 1971). To 50 mg of Sephadex G-50, 0.435 ml of 50mM Tris buffer (pH 7.4) was added. After swelling of the gel was completed, 0.195 ml of the buffer solution containing serum and ^{3}H-iloprost was added. The sample was mixed and the protein and ligand concentration was determined. The computer fitting of the binding isotherm according to the originally proposed equation yielded a binding curve consistent with a single class of binding sites. The Kd value of normal serum was 70 μM and the B_{max} 48 nmol/ml. Acute TTP serum exhibited a reduced binding affinity (Kd 236 μM) and a slightly elevated capacity (B_{max} 85 nmols/ml). The binding parameters improved following successful treatment but the Kd remained subnormal (120 μM). These data indicate that reduced PGI_2 binding activity is due to lower affinity of the PGI_2 binding proteins. The relationship between defective PGI_2 binding activity and PGI_2 production was then evaluated. Serial serum and 24 hour urine were collected. Urinary samples were extracted and their 6-Keto-$PGF_{1\alpha}$ (6KP) and thromboxane TXB_2 levels were measured by RIA. TTP patients in remission had normal levels of urinary 6KP and TXB_2 while urinary 6KP and TXB_2 were elevated in relapsing TTP. Defective binding was noted when relapse began to occur while elevated 6KP and TXB_2 were noted 48 hrs later. Both 6KP and TXB_2 were normalized when the disease was controlled. Our findings indicate that defective PGI_2 binding plays an important role in causing excessive platelet activation and platelet-vessel wall interaction in TTP.

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PLATELET FACTOR H REGULATES THE ACTIVITY OF THE ALTERNATIVE PATHWAY OF COMPLEMENT ON THE SURFACE OF NORMAL AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PLATELETS. D.V. Devine and W.F. Rosse. Duke University Medical Center, Durham, NC, U.S.A.

Paroxysmal nocturnal hemoglobinuria (PNH) is frequently complicated by thrombosis. It has been suggested that the abnormal interactions of PNH platelets with complement contribute to thrombosis. Using purified complement proteins, we have previously demonstrated that the platelets from some patients with PNH do not demonstrate elevated activity of C3bBb, the alternative pathway C3 amplification enzyme complex, even though they lack the C3bBb regulatory protein, decay accelerating factor (DAF). As measured by fluorescence flow cytometry, washed platelets from both normal donors and PNH patients released the fluid phase C3bBb regulatory protein, factor H, in response to the deposition of purified complement proteins. Platelet factor H was localized to the alpha granules by immunocytochemical techniques. A quantitative radioimmunoassay demonstrated that normal platelets released 54 ± 6 ng factor H/10^8 platelets in response to thrombin stimulation. PNH platelets contained less factor H (22 ± 7 ng/10^8 platelets) than normal platelets. Thrombin stimulated platelets from patients with elevated C3bBb activity released less than half of the factor H measured in detergent extracts. However, thrombin stimulated platelets from PNH patients exhibiting normal C3bBb activity released nearly all their factor H. The release of factor H from normal platelets was blocked by treating the platelets with metabolic inhibitors. In the absence of factor H release, the activity of the C3bBb complex increased three-fold. In addition, the number of molecules of 125I-factor B bound per C3b increased from 0.40 to 0.92 when factor H release was blocked. The inhibition of DAF by anti-DAF had no effect on the activity of C3bBb if factor H could be released from the platelets. However, when factor H release was blocked by treatment with metabolic inhibitors, the inhibition of DAF by anti-DAF increased the activity of C3bBb by 40%. Therefore, in the absence of DAF, platelets can regulate complement activation by the alternative pathway via the release of platelet factor H. Since factor H is an alpha granule protein, platelet release in the presence of activated complement may contribute to the occurrence of thrombosis.