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STUDIES ON PLATELET ANTIGENS AGAINST SERA FROM PATIENTS WITH ITP. K Hirai (1), K. Yasunaga (1) and R. Ryo (2). The First Department of Internal Medicine, Kansai Medical University, Osaka, Japan (1) and Blood Transfusion Service, Kobe University Hospital. Hyogo, Japan (2).

Chronic idiopathic thrombocytopenic purpura (ITP) is a clinical syndrome characterized by destruction of platelets by antiplatelet antiboodies. The precise pathogenic mechanism of platelet destruction in ITP is not known, although many investigators have reported that platelet-associated IgG (PAIgG) is increased in this desease. We have evaluated PAIgG in 66 patients with ITP by a competitive solidphase microenzyme immunoassay and investigated its specificity aganist antiplatelet antibody in 24 patients with ITP by Western blotting. PAIgG values were elevated in most ITP patients with platelet counts of under 50,000/μl, but within normal range in most patients with platelet count of over 50,000/μl. PAIgG values were also elevated in ITP patients with megakaryocyte counts of over 200/μl, and within normal range in most patients with normal megakaryocyte counts. Western blotting was carried out by SDS-PAGE of whole platelet lysate or platelet membrane lysate and trasfer of the platelet fraction onto nitrocellulose strips. Bound immunoglobulins were detected with an avidin-biotin-peroxidase system. Several bands of bound immunoglobulins were detected in the whole platelet lysates of ITP patients, but most of these could not be detected in platelet membrane lysates. This finding suggests that some immunoglobulins from ITP patients may bind to cytoplasmic proteins in whole platelet lysate. These observations are consistent with the hypothesis that the pathogenesis of ITP involves an immunological mechanism.

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STIMULATED MEGAKARYOCYTES ARE FOUND IN CHILDHOOD ITP BUT NOT IN ADULT ITP. R.F. Levine and P.K. Shoff, VA Med Center and GW Univ, Wash. D.C., USA

ITP is thought to be caused primarily by peripheral platelet destruction, but recent work has suggested that platelet production may also be impaired. Although the clinical course in children usually differs from that in adults, no distinctions have been established with regard to marrow characteristics. To evaluate megakaryocyte (mega) responses in this disease we examined mega size, ploidy, maturation and morphology in 8 children and in 8 adults with ITP and in 8 "normal" marrows (4 children, 4 adults). Marrows were prepared by a buffy coat wedge smear or by a cover slip squash method. Control values differed according to the type of marrow smear used. From 100-300 Feulgen-stained megas were examined in each specimen, as previously described. Wright-stained material was also examined. With the squash method megas from normal children and adults had similar characteristics. The megas of each child with acute ITP showed marked increases in size (volumes were 4X normal), ploidy (as high as 1024 N; medians were 64N, or 2 doublings higher than normals), and maturation stage (86% mature forms vs 43%). In contrast, none of the marrows of adults with acute or chronic ITP (1 with mild, 4 moderate, and 3 with severe thrombocytopenias) showed any stimulation of megas. Overall, their megas were normal in size, ploidy and maturation. Occasional dissociation of mega ploidy and maturation was seen, but not enough to alter the profiles of any one parameter. There were no obvious or suggestive signs of "damage" to the megas of children or adults with ITP. In conclusion, the megas of childhood ITP showed a pattern of marked stimulation of size, ploidy and maturation, as seen in animals injected with antiplatelet serum. The failure of adult marrows to response in these parameters to thrombocytopenia may be pathogenetically related to the chronicity of adult ITP.

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THE EFFECT OF INTRAVENOUS IMMUNOGLOBULIN ON PLATELET KINETICS IN CHRONIC IMMUNE THROMBOCYTOPAENIC PURPURA (ITP). P.N.Badenhorst, H.F.Kotze, A.duP.Heyns, M.G.Lotter, P.Wessels and J.P.Roodt. MRC and University of the Orange Free State Blood Platelet Research Unit, Bloemfontein, South Africa.

Most patients with ITP respond to high doses of intravenous immunoglobulin (IVIg) with a transient increase in platelet count. The effect of IVIg on platelet kinetics was studied in 5 patients with chronic ITP. Autologous platelets were labelled with In-111 and mean platelet lifespan (MPLS) calculated; in vivo distribution and sites of platelet sequestration were determined with a scintillation camera and computer assisted image analysis. The studies were performed before and after treatment with 2 g/kg Sandoglobulin. Two groups of patients were identified: those with a splenic platelet sequestration pattern (spleen-liver In-111-activity ratio >1.4) and those with diffuse sequestration of platelets in the reticuloendothelial system (RES).

Sequestration pattern		Plt count (x 10^9/l) Pre	Post	MPLS (hours) Pre	Post	Spleen-liver ratio Pre	Post
Splenic	1.	36	188	42	171	4.8	2.2
	2.	35	90	2	19	2.8	1.4
	3.	18	76	26	54	2.3	0.5
Diffuse	1.	10	20	8	11	0.3	0.6
	2.	9	60	22	23	1.0	1.0
Reference		150-400		224±21		1.4±0.6	

There was a significant difference in mean platelet counts before and after treatment ($p<0.05$). Patients with a splenic sequestration pattern responded better to IVIg: the MPLS lengthened and the high spleen-liver ratio decreased. In the diffuse RES sequestration pattern group, IVIg had almost no effect on platelet kinetics. We conclude that platelet kinetic studies identify a subgroup of patients with ITP who will respond to IVIg therapy.

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COAGULATION STUDIES IN THROMBOTIC THROMBOCYTOPENIC PURPURA, WITH SPECIAL REFERENCE TO VON WILLEBRAND FACTOR ABNORMALITIES. Hoyu Takahashi (1), Wataru Tatewaki (1), Tadao Nakamura (2), Masaharu Hanano (1), Ken Wada (1) and Akira Shibata (1). The First Department of Internal Medicine, Niigata University School of Medicine, Niigata, Japan (1) and Division of Internal Medicine, Ojiya General Hospital, Ojiya, Niigata, Japan (2).

The profile of blood coagulation and fibrinolysis was studied in detail in 8 patients with thrombotic thrombocytopenic purpura (TTP), who had most of the characteristic findings such as fluctuating neurologic signs, schistocytic hemolytic anemia, marked thrombocytopenia, renal abnormalities and fever. Fibrinogen (2.2-3.6 g/L) and factor XIII levels were normal in all patients, while FDP values were slightly elevated (from below 5 to 40 mg/L). Antithrombin III, alpha 2-plasmin inhibitor and plasminogen were normal in all patients except one with elevated FDP. Alpha 2-macroglobulin was normal as well. Plasmin-alpha 2-plasmin inhibitor complex measured by an enzyme-immunoassay was either normal or marginally elevated. Tissue-type plasminogen activator antigen was elevated to 5.2-14.5 μg/L. Protein C activity and antigen were either normal or elevated, while protein S antigen was decreased in 3 patients. Factor VIII activity and von Willebrand factor antigen (vWf:Ag) and ristocetin cofactor (RCof) were either normal or elevated, but RCof/vWf:Ag ratio was decreased (mean 0.546 ± SD 0.1876). Crossed immunoelectrophoresis and SDS-agarose gel electrophoresis revealed that the hemostatically most active, high-molecular-weight vWf multimers were absent from or relatively decreased in TTP plasma. In addition, a vWf fragment with a faster mobility than the major vWf was demonstrated in some patients. Histidine-rich glycoprotein and fibronectin were decreased in 3 and 4 patients, respectively. Most of these abnormal findings were nearly normalized in remission. Although the pathogenesis of TTP is still uncertain, these results indicate that in contrast to disseminated intravascular coagulation, the intravascular generation of thrombin and plasmin was minimal in TTP, and suggest that the high-molecular-weight multimer vWf and fibronectin are consumed probably due to their participation in platelet thrombus formation in addition to platelet aggregating factor.