Autoantibodies to Platelets in Children with Thrombocytopenia

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Antiplatelet antibodies were titered in children and adults with idiopathic thrombocytopenic purpura. The levels of platelet-associated IgG were elevated in 100% of the examined children and 86% of the adults, their mean titers being at least 10 times higher than in the control and differing little within the groups. Serum antibodies reacting with normal donor platelets were detected in 43% of adults and in only 18% of children. Improvement of the clinical picture and increase of the platelet count in these patients according to different protocols always correlated with a drop in the level of antiplatelet antibodies. The results point to the immune nature of idiopathic thrombocytopenic purpura in children.

Key Words: *idiopathic thrombocytopenic purpura; antiplatelet antibodies; reaferon*

Thrombocytopenias represent a group of diseases in which the platelet count in the blood drops below 150,000 per μl. Idiopathic thrombocytopenic purpura (ITP) and symptomatic thrombocytopenia resulting from another underlying disease (leukemia, systemic lupus erythematosus, etc.) are distinguished. Although ITP is largely a diagnosis ruling out other pathology capable of causing similar symptoms, it is considered at present as an autoimmune disease characterized by the presence of antiplatelet antibodies inducing massive destruction of platelets. Hence, the detection of antibodies to platelets in patients is evidence of the immune nature of the disease and a diagnostic criterion for ITP [1,5].

ITP affects both adults and children. An acute form of ITP, usually heteroimmune, that is, associated with changes of platelet antigens, is more often observed in childhood [1,8]. On the other hand, in many cases the disease may assume a chronic course in children as well. In contrast to ITP in adults, such cases have received less attention.

The present study was devoted to measurements of antiplatelet antibodies in children with chronic ITP. This group was compared with a group of adult patients with ITP, and the effects of various treatment modalities on the level of antiplatelet antibodies and disease duration were assessed.

MATERIALS AND METHODS

Twenty-two patients with ITP aged up to 16 (children) and 14 patients aged over 16 (adults) were examined. The control group consisted of 10 healthy donors with more than 200,000 platelets per μl of blood. A tentative diagnosis of ITP was made on the basis of a drop of the platelet level below 30,000 per μl in any patient, against the background of an adequate or high content of megakaryocytes in bone marrow puncture specimens. Hemorrhagic syndrome of various severity was observed in the majority of patients. Children were treated with corticosteroids, reaferon (α_{2b}-interferon), and human immunoglobulins. If therapy was ineffective, splenectomy was resorted to.

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Direct radioimmunoassay was used to detect antibodies associated with the platelet surface [3,6]. The number of platelet-associated immunoglobulins (PAIgG) was assessed from the binding of ^{125}I-labeled antibodies to human IgG (^{125}I-anti-IgG$_h$) with patient platelets washed free of plasma and expressed in ng/10^6 platelets. Antiplatelet antibodies in the serum were assessed from antibody binding to normal donor platelets immobilized on plastic using a previously described radioimmunoassay technique [2,6]. In studies by both methods responses surpassing the control level in healthy donors at least twofold were considered positive.

RESULTS

The content of PAIgG and serum antibodies was measured by radioimmunoassay and enzyme immunoassay, respectively, in both groups of patients (children and adults with ITP) (Table 1). Elevated levels of PAIgG were found in all 22 children examined and in all but 2 of the 14 adult patients. These results are in line with previous reports of elevated levels of PAIgG in more than 80% of patients with ITP [6,7]. The number of patients with antiplatelet antibodies in the serum was appreciably lower. In only 4 (18%) of the 22 children did we find serum antibodies. The PAIgG level was high in all these patients. In the group of adult patients this parameter was more than twice as high: serum antibodies were detected in 6 (43%) of the 14 patients. This is close to previously reported values [4]. In one patient serum antibodies were detected in the presence of a normal PAIgG level. It is possible that thrombocytopenia in this case was nonimmune. This patient was subjected to repeated transfusions in the course of treatment, which might have led to the production of alloantibodies directed against donor but not his own platelets, which were detected by enzyme immunoassay. In a female patient without antibodies in the serum or on the platelet surface the tentative diagnosis of ITP was not confirmed, and later aplastic anemia was diagnosed. The lower percentage of patients with elevated levels of serum antibodies in comparison with the PAIgG level seems to be due to the fact that sometimes they are produced as a result of impairment of the structure of the platelets themselves, rather than disorders in immune system function, and in such cases antibodies in the serum do not bind normal donor platelets. In the group of children the number of patients with serum antibodies reacting with donor platelets was only half as high as in the adult group. Hence, it is possible that in children ITP more often develops as a result of changes in the platelet antigenic structure than it does in adults.

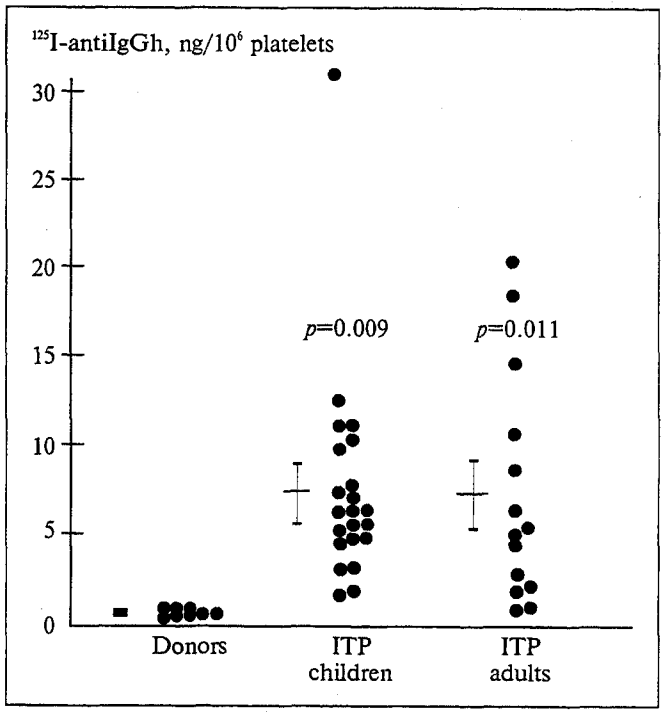

Fig. 1. PAIgG in donors and ITP patients aged up to 16 (children) and over 16 (adults). Individual values and mean±errors of the mean values are presented. Reliable differences (p) between the groups of patients and donors were calculated using Student's t test for mean values.

Figure 1 presents individual and mean levels of PAIgG in the groups of children and adult patients with ITP and of control donors. The mean PAIgG levels were at least ten times higher in the patients than in the donors. However, there were no appreciable differences between the two groups of patients.

In the group of children 21 of the 22 were treated with corticosteroids and one with reaferon without a preliminary course of corticosteroids. In 12

TABLE 1. Titration of Antiplatelet Antibodies in the Serum and on the Platelet Surface in Patients with ITP

ITP patients	Number of examinees	PAIgG+sAb$^+$	PAIgG+sAb$^-$	PAIgG-sAb$^+$	PAIgG-sAb$^-$
Children	22	4 (18)	18 (82)	0	0
Adults	14	5 (36)	7 (50)	1 (7)	1 (7)

Note. PAIgG were radioimmunoassayed and serum antibodies were detected by enzyme immunoassay. Responses which surpassed the control level in healthy donors twofold were considered positive. The number of patients with the corresponding PAIgG values and levels of serum antibodies (sAb) in percent to the total number of patients in the group is shown in parentheses.

TABLE 2. Effect of Immunosuppressive Therapy and Splenectomy on the Count of Platelets in the Peripheral Blood and the Level of Antiplatelet Antibodies on the Platelet Surface in Children with ITP

Patient	Treatment	Platelets per μl		Antiplatelet antibodies (PAIgG, % of control)	
		before	after	before	after
F.G.	Reaferon	<5 000	200 000	665	100
K.A.	" "	<5 000	10 000	776	1 460
S.A.	" "	<5 000	<5 000	860	780
K.Yu.	" "	<5 000	<5 000	824	600
P.P.	" "	<5 000	20 000	250	250
S.D.	" "	<5 000	<5 000	355	782
U.Ya.	" "	<5 000	<5 000	240	260
K.Z.	Immunoglobulins	<5 000	<5 000	1 830	1 110
N.O.	" "	<5 000	20 000	500	664
Kh.D.	" "	<5 000	<5 000	250	355
K.A.	Splenectomy	10 000	600 000	1 460	100
S.A.	" "	<5 000	550 000	780	100

Note. Reaferon (1 and 3×10^6 U every other day in children aged under 10 and 10 to 16, respectively, for 3 months) and human immunoglobulins (2 g/kg b.w. for 5 days). Splenectomy was performed in 2 patients after failure of reaferon therapy. The level in donors was taken as 100%.

patients corticosteroid therapy was effective; out of the 9 patients resistant to corticosteroids a course of reaferon therapy was administered to 6 and of human immunoglobulins to 3. Due to the inefficacy of the therapy, splenectomy had to be performed in 2 of the 9 children. The level of antiplatelet antibodies was measured before and after treatment in the children treated with reaferon, immunoglobulins, or splenectomy. Table 2 shows that the pretreatment platelet count did not surpass 30,000/μl in any of the patients, and the PAIgG level was appreciably higher than in the controls. Reaferon therapy was effective in only one case (patient F. G.), whose platelet count normalized in parallel with a drop of the PAIgG level. It should be noted that this was a patient to whom corticosteroids were not administered prior to reaferon. In the rest of the patients reaferon therapy did not lead to an increase in the platelet count, and the level of PAIgG remained elevated. In three cases (patients K. A., S. A., and K. Yu.) a short-term decrease of hemorrhages was observed after reaferon. Treatment with immunoglobulins (3 patients) did not lead to normalization of the platelet count or a drop of the PAIgG level, but it is noteworthy that the severity of the hemorrhagic syndrome eased for some time. Splenectomy performed in two patients led to a complete remission: cessation of hemorrhages and a rise of the platelet count to more than 200,000 μl. The efficacy of splenectomy is apparently not just due to the fact that the spleen is the principal site of platelet destruction. In both cases we observed a drop of PAIgG to the normal level, suggesting that the spleen in these patients was not only the place of platelet sequestration, but of antibody production as well.

Hence, in all children with ITP examined thrombocytopenia was associated with a high level of antiplatelet antibodies, and the rise of the platelet count in the course of treatment correlated with the fall of the PAIgG level. In cases where the treatment was of no avail, the level of PAIgG remained elevated. These results indicate that autoimmune mechanisms and the production of antiplatelet antibodies play a key role in the pathogenesis of chronic ITP not only in adults, but in children as well.

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