Autoantibodies Against Platelet Membrane Glycoproteins in Children With Acute and Chronic Immune Thrombocytopenic Purpura

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The autoimmune nature of chronic immune thrombocytopenic purpura (ITP) in adults is widely accepted. In contrast, the pathogenetic mechanism in acute and chronic ITP in children is not known. In this report, we studied 39 children with destructive thrombocytopenia. 15 patients with acute ITP and 24 patients with chronic ITP. Platelet autoantibodies to platelet glycoprotein IIb/IIIa were detected in 14 of 24 patients (58.3%) in the chronic ITP group and in four of 15 (26.7%) with acute ITP. Binding ratios (±SD) of positive patients were significantly greater (P = .01) in chronic ITP (8.0 ± 9.1) when compared with those of acute ITP where the binding ratios were only slightly above the normal range (1.9 ± 0.4). The results show that autoantibodies against platelet glycoproteins are present in the majority of children with chronic ITP confirming the autoimmune nature of this disorder. The minimal elevation seen in the positive children with acute ITP suggests a different pathogenetic mechanism. These data suggest that this approach may be useful in differentiating acute from chronic ITP patients.

CHRONIC IMMUNE thrombocytopenic purpura (ITP) in adults is usually of insidious onset and is characterized by increased platelet destruction due to antiplatelet autoantibodies. Patients rarely undergo a complete remission and almost always require therapy. Platelet-associated autoantibodies to the platelet membrane glycoproteins (GP) IIb/IIIa or Ib/IX can be detected in approximately 75% of patients and plasma autoantibodies in about one half. In children, two forms of ITP occur. A syndrome similar to adult chronic ITP and an acute form of ITP. Acute ITP occurs abruptly, often after a viral illness and platelet counts recover spontaneously within weeks to months, although therapy with high-dose corticosteroids or intravenous (IV) gammaglobulin may result in earlier recovery. Several investigators have noted increased platelet-associated IgG in acute and chronic childhood ITP but there are few data on the presence of autoantibodies. Beardsley et al reported autoantibody against a 100,000 kilodalton (Kd) protein in nine patients with childhood chronic ITP; in three, the protein was shown to be GPIIIa by the lack of autoantibody reactivity with thrombasthenic platelets. Negative results were noted in eight patients with childhood acute ITP. In the present study, we have evaluated plasma for the presence of antiglycoprotein autoantibodies in children with either acute or chronic ITP.

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ITP PATIENTS

Sera were collected from 39 children with destructive thrombocytopenia. All had normal or increased numbers of megakaryocytes in the bone marrow. Fifteen patients (ten girls, five boys) with a median age of 4.5 years (1½ to 11½) were classified as acute ITP having thrombocytopenia without predisposing disease (eg, sepsis, DIC, drug-induced thrombocytopenia) but with recovery of the platelet count to normal (>150,000/μL) within 12 months following initial diagnosis. Twenty-four patients (eight girls, 16 boys) with a median age of 10½ years (3½ to 19½) were classified as chronic ITP with thrombocytopenia (platelet count <150,000/μL) for more than 12 months. The median duration of thrombocytopenia (range) for the two groups was: acute ITP —0.4 months (0.1 to 3) and chronic ITP —33.6 months (12 to 84). The duration of thrombocytopenia in the acute ITP group may be shorter than usually seen since these patients received either high-dose IV IgG or corticosteroids. The median platelet count (range) of the acute and chronic ITP patients was 10,000/μL (4 to 41,000) and 31,000/μL (4 to 139,000), respectively. Patients with splenomegaly (two cases) and possible lupus erythematosus (three cases) were not excluded from the study. Sera from ten healthy children were used as controls.

IMMUNOBEAD ASSAY

The immunobead assay was performed with minor modifications as described elsewhere. All ITP patient samples were assayed at the same time to exclude day to day variation. Immunobeads were prepared by incubating ½-inch polystyrene beads with monoclonal antibody (10 μg per bead) in 0.1 mol/L NaHCO₃, pH 8.5) against either GPIIb/IIIa (provided by Dr Virgil Woods, University of California, San Diego) or GPIb/IX (provided by Drs Theodore Zimmerman and Zaverio Ruggeri, Scripps Clinic and Research Foundation). Platelets obtained from normal donors were washed six times with isotonic citrate buffer, pH 6.2. Washed platelets (10⁶) were incubated with either patient or normal control serum (900 μL) for 60 minutes at room temperature and overnight at 4°C. After washing, the sensitized platelets were solubilized in 1% Triton X-100. The lysates were then centrifuged at 12,000 × g for five minutes and then each was sequentially incubated for 60 minutes with a ½-inch polystyrene bead coated with either mono-
clonal anti-GPIIb/IIIa (first incubation) or anti-GPIb (second incubation) to bind any immune complexes consisting of autoantibodies and either GPIIb/IIIa or GPIb/IX. The autoantibody was then detected by radiolabeled monoclonal antibody against human IgG (HB43; American Type Culture Collection, Rockville, MD). Data are expressed as a binding ratio of cpm of the patient sample divided by the mean cpm of four control samples. Patient samples with a binding ratio of >1.56 (>3 SD over control) were considered positive.

RESULTS

In the 24 children with chronic ITP, serum autoantibodies against GPIIb/IIIa were noted in 14 (58.3%) (Fig 1). The binding ratios (±SD) of the group averaged 5.1 ± 7.7 while mean values for positive patients were 8.0 ± 9.1 with a range of 2.1 to 32.4. There was no correlation between the platelet count and the anti-GPIIb/IIIa binding ratio. Of the 15 children with acute ITP, 4 (26.7%) had elevated anti-GPIIb/IIIa binding ratios (Fig 1). The positive values ranged from 1.6 to 2.4 with a mean (±SD) of 1.9 ± 0.4. The positive patients with chronic ITP had significantly greater values than the positive patients with acute ITP (P = .01, Student’s t test).

None of the patients with either chronic or acute ITP had detectable plasma autoantibodies to GPIb/IX. All samples obtained from healthy children gave negative binding ratios.

DISCUSSION

In this report, 39 children with immune thrombocytopenia were studied retrospectively for autoantibodies against GPIIb/IIIa or GPIb/IX. In the chronic ITP patients, 58% had anti-GPIIb/IIIa antibodies while only 27% of the acute ITP patients were positive. In addition, the binding ratios in the positive chronic ITP group were significantly higher than those of the patients with acute ITP.

The results in childhood chronic ITP are almost identical to those of adults. Previously reported results in adult chronic ITP patients showed 56% (32 of 54) had plasma autoantibodies to platelet glycoproteins compared with the present results of 58.3% positive in children with chronic ITP. However, there were some differences. Approximately 25% of adult patients had anti-GPIb/IX autoantibodies while no positive results against these glycoproteins were noted in this childhood group. Whether this is due to chance or reflects a difference between the groups will require a larger number of studies. In any case, our results suggest that chronic childhood ITP is an autoimmune syndrome similar to that seen in adults.

The results noted in the acute ITP patients suggest a different pathogenetic mechanism. Only four of 15 gave positive results and in each case the binding ratios were only slightly above the normal range. These weakly positive results could be explained in a variety of ways. They could reflect binding of viral proteins and attached antibody to the platelet glycoproteins resulting in a nonspecific positive result. It is known that some drugs bind to platelet glycoproteins (eg, quinidine to GPIb/IX) and that viral proteins can bind to platelets. Alternatively, the presence of viruses may result in a conformational change in the platelet glycoproteins resulting in the development of an immune response to neo-antigens, which will be no longer exposed once the viral infection abates. Further studies will be required to differentiate these possibilities. In addition, a large prospective study is indicated to demonstrate whether measurement of autoantibodies at diagnosis in these patients will help differentiate children with acute ITP from those with the chronic form.

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