Circulating Immune Complexes In Childhood Idiopathic Thrombocytopenic Purpura (ITP)

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SUMMARY
Circulating immune complexes were determined in sera of 5 children with acute ITP and in 5 when they were in remission. Circulating immune complexes were shown in all cases in acute phase (x: 68.8—9.37%) and in remission (x: 44.4—18.6%) but only in 2 cases of appropriate controls (4,4% and 12% (x:0.82). Circulating immune complexes were found significantly higher in acute phase compared to remission values (p<0.05). These results are compatible with our previous findings that antiplatelet antibodies are present in thrombocytopenic phase and in remission; being less in remission.

Key Words: Circulating immune complex, ITP

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INTRODUCTION
The presence of platelet opsonizing activity in remission sera in addition to shorter platelet survival of all ITP cases in childhood were shown by us previously (1,2). Later we have presented evidence that plateled opsonizing activity was related to IgG fraction as in adults and its concentration decreases in remission.

Circulating immune complexes were also looked for in the sera of these patient, in acute phase as well as in remission.

MATERIAL AND METHODS
Appropriate sera were obtained from children with acute ITP (less than 6 months duration), 5 in acute phase and 5 in remission of 2 to 6 months duration. The ages of the patients ranged from 3 to 15 years. Sera of the 20 children in the same age range, without any hematological problems, served as controls.

The diagnosis of ITP was based on platelet of less than 50,000/ul, with an excessive or normal number of megakaryocytes in the bone marrow. Splenomegaly was not present and no underlying disease was shown. L.E. preparation (3 times), throat culture, Coombs test or fluorescent antinuclear determination. In no patients there was a history of drug induced etiology and to our obtaining sera for the study. Platelets were enumerated by phase contrast microscopy (4) and our controls’ counts ranged from 150,000 to 425,000/ul.

Suspension of normal human peripheral blood lymphocytes (PBL) were obtained by centrifugation over Ficoll-metrizoate as described by Byomu (5). Monocytes were removed by adherence to plastic, leaving virtually pure (97%) PEL suspension of which 98% were viable as determined exclusion of trypan blue. Two million PBL were suspended in 250 MI undiluted serum and incubated at 37°C for one hour followed two washes in buffered saline PBL, re-suspended in a final volume of 250 ul. An equal volume of 1% optimally sensitized chicken erythrocytes (EA) was added and Rosetteforming cells were determined as described by Bakkaloglu et al (6). In this assay, immvin complexes were detected in the serum
by measuring its ability to inhibit the binding of erythrocyte antibody complexes (EA) to the Fc receptors of B lymphocytes. The results were expressed as the percentage inhibition of EA rosette formation induced by serum when compared with control tests in which serum was replaced by the buffered saline. Later the patients’ sera were separated by ultracentrifugation at 50,000 g and EA tests were repeated by using supernatant and sedimented fractions.

RESULTS AND DISCUSSION

Only 2 of the sera from control subjects showed EA (Fc) rosette inhibition (4.4and 12%) while the remaining 18 sera produced no rosette. The mean inhibition of controls was 0.82 %. Sera from 10 children with ITP (in acute phase or remission) showed inhibited rosette formation (56.63 %) exceeding very significantly that of the control sera (P<0.001). The inhibition was also more marked with acute phase sera of 5 cases (mean of inhibition 68.8 %) than remission sera (44.4 %; P<0.05). The EA rosette inhibition was present predominantly in sedimented fraction of all sera in which ultracentrifugation could be carried out (Table I).

Although the EA rosette inhibition assay has not been widely applied to the conditions with immunopathology, it has been shown to be helpful in diagnosis of systemic lupus erythematosis (7), retinal vasculitis (8), glomerulonephritis (9,11), nephrotic syndrome (12) and in primary biliary cirrhosis (13).

Immune complexes have been detected by different assay methods (10-14). Since each assay detects only a particular type of immunocomplexes, it is better to use as many of them as possible in parallel in order to minimize the risk of missing certain types of complexes. Circulating immune complexes could be detected in acute phase as well as in remission sera of all cases of childhood ITP in this study, by using only the EA rosette inhibition test.

This finding fits well of our detection of antiplatelet antibodies in relapse and remission in all childhood ITP cases (1,2). The EA rosette inhibition seems less prominent in remission which is also parallel to our finding that antiplatelet antibodies are decreased in this phase of the disease. The more prominent EA rosette inhibition in the sedimented fraction of the patients’ sera is an indication that the immune complexes are responsible for the positivity of the test. This finding may be an additional evidence that ITP is an immunologic disorder.

### REFERENCES

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