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THE SPECIFICITY OF ANTIPLATELET ANTIBODIES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)*

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Shorter platelet survival in childhood idiopathic thrombocytopenic purpura (ITP) in remission and the presence of platelet-opsonizing activity in serum have been shown by us previously[1,2]. Since then the relation of this activity to the thrombocytopenic phase as well as to remission has been evaluated[3,4], and it has been shown that destruction of IgG caused the platelet-opsonizing effect to disappear. In addition, adsorbance of the opsonizing effect was shown to be specific to platelets, as the erythrocytes and lymphocytes did not on any occasion adsorb this effect. Therefore, we strongly suggest that use of the term ITP should be restricted to immune thrombocytopenic purpura cases in which only antiplatelet antibodies are detected.

Material and Methods

Appropriate sera were obtained from 13 children with ITP in the thrombocytopenic phase and 5 who had been in remission from 6 months to 6 years. The children ranged from 3 to 15 years of age. All sera were stored at −20°C until used.

The diagnosis of ITP was based on platelet counts of less than 50,000/μl, with an excessive or normal number of megakaryocytes in the bone marrow. Splenomegaly was not present, and there were no underlying diseases. None of the patients had a history of drug-induced etiology, and none of them had received platelet or blood transfusions prior to this study. Platelets were enumerated by phase contrast microscopy[5], and the control counts ranged from 150,000 to 425,000/μl.

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The platelet-opsonizing effect of the IgG, IgM, IgA fractions and whole serum was studied according to the method of Handin and Stossel[6], summarized as follows: One half milliliter of washed donor platelets (3-5x10^8) were incubated with 1 ml of patient serum (in the thrombocytopenic period or in remission) or control serum for half an hour at room temperature (opsonization of platelets) and washed in 2 ml of 0.1 mM EDTA saline. The platelets were then centrifuged at 1000 g for 10 minutes. The sedimented platelets were rewashed twice with Hank's solution and resuspended in 0.2 ml of this solution, mixed with 0.4 ml of autologous leukocyte suspension (4-7x10^7/ml), 0.4 ml nitroblue-tetrazolium (NBT) solution (0.2%), and 0.1 ml of 1 mM calcium chloride. Following the incubation of this mixture for 5 minutes at 37°C, the phagocytosis of sensitized platelets by autologous leukocytes was stopped by adding 3 ml of 0.1 mM EDTA saline; the mixture was immediately centrifuged at 500 g for 10 minutes and the supernatant discarded. Two ml of dioxane (Merck) was added over the sediment, and this mixture was incubated for an hour at 85°C, centrifuged for 10 minutes at 500 g and optical density (O.D.) recorded at 580 nm, which indicates the reduction of NBT.

For the separation of the IgG, IgM, and IgA fractions of the patients' sera, sephadex column chromatography[7,8], and for the separation of IgG alone, diethyl amino ethyl (DEAE) cellulose[9] was used. For the adsorption of antiplatelet opsonizing activity, the sera or IgG fractions were incubated with 0.1 ml of sedimented erythrocytes, lymphocytes or platelets for 5 minutes at 37°C. At the end of this period, supernatant of serum or IgG was separated from the cells by centrifugation at 1000 g for 10 minutes, dialyzed against phosphate-buffered saline, and the platelet-opsonizing activity was determined as mentioned above[6].

The IgG fraction of the sera of 4 patients with ITP was used for the opsonophagocytic test before and after breaking its disulfide bonds by dithiothreitol and alkylating them with iodoacetamide using the modification technique of Miller and Metzger[10] as described by Blezikian et al[8].

Results

The optical density changes at 580 nm for the 13 ITP cases in the thrombocytopenic phase ranged between 0.03 and 0.135 with a mean of 0.055 ± 0.018 (SD). During remission (platelet counts $\geq$ 150,000) in 5 patients, the range was between 0.03 and 0.07 with a mean of 0.039 ± 0.009. In 25 hematologically normal controls, the optical readings ranged between 0.018 and 0.03 with a mean of 0.023 ± 0.003 (SD). The thrombocytopenic phase and remission values of the patients were significantly different from the control values ($p < 0.001$ and $p < 0.01$, respectively). The difference between thrombocytopenic phase and remission values was also significant ($p < 0.01$), as reported previously[4].

IgG separated through DEAE cellulose in 7 sera (2 cases in remission) and by sephadex column in 11 sera (3 in remission) gave optical densities (0.038 ± 0.008) very close to the respective whole serum values in 15 cases, 4 of whom were in remission (0.0388 ± 0.07; $p > 0.05$). No platelet opsonizing effect related either to IgM or IgA was shown in the sera of 2 cases in remission and 8 in the thrombocytopenic phase (Figure 1). When the whole serum of 6 patients and the IgG fraction of 3 (all in the thrombocytopenic period) were used for the opsonophagocytic test before and after adsorption with erythrocytes, lymphocytes and platelets, the disappearance of their platelet-sensitizing effect was observed only after incubation with platelets. Optical densities of 9 cases before adsorption ranged between 0.03 and 0.055 with a mean of 0.037 ± 0.012. After incubation with lymphocytes and erythrocytes using three whole sera and three IgG fractions, the mean optical densities were found to be, respectively, 0.034 ± 0.008 (ranging between 0.025 and 0.049) and 0.32 ± 0.005 (with a range of 0.025 to 0.041). These were not significantly different from the pre-treatment values ($p > 0.05$ for both). However, following incubation with platelets, optical densities of three IgG fractions and 5 whole sera showed optical densities in a range of 0.01 to 0.018, with a mean of 0.013 ± 0.003, which was markedly decreased compared to their pre-incubation values ($p < 0.001$; Figure 2).

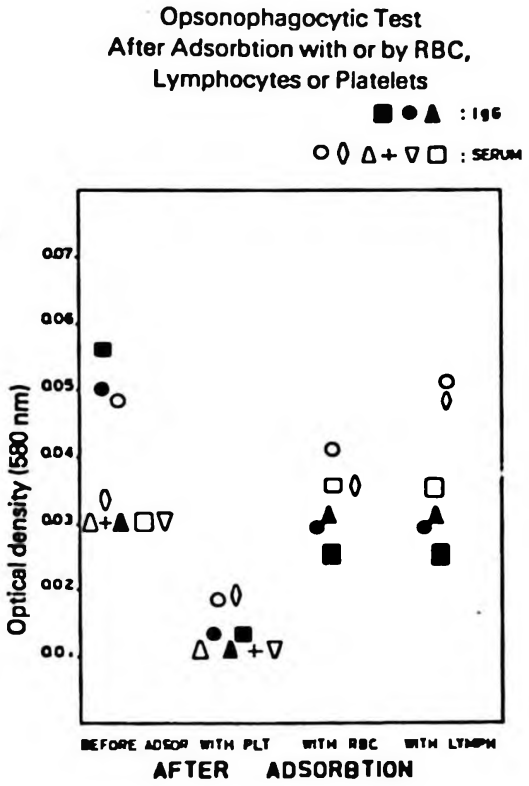

Figure 1.

The antiplatelet antibody effect of whole sera is related to the IgG fraction only. (Each mark indicates one patient.)

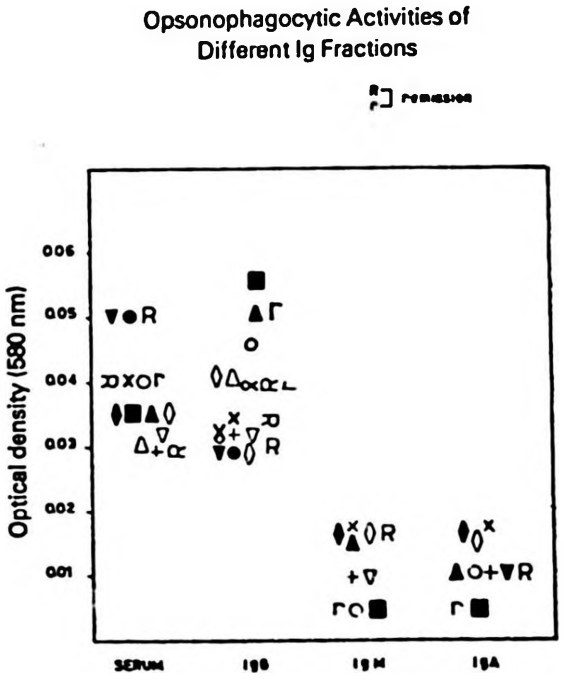

Figure 2.

The specificity of antiplatelet antibodies in whole sera or IgG fraction. They are adsorbed only by platelets but not by red blood cells or lymphocytes.

The platelet-opsonizing effect of IgG fraction of 4 patients was studied before and after their treatment with dithiothreitol and iodoacetamide. The post-treatment optical density values were quite low (0.012 to 0.014), comparable to IgM or IgA values (Figure 3).

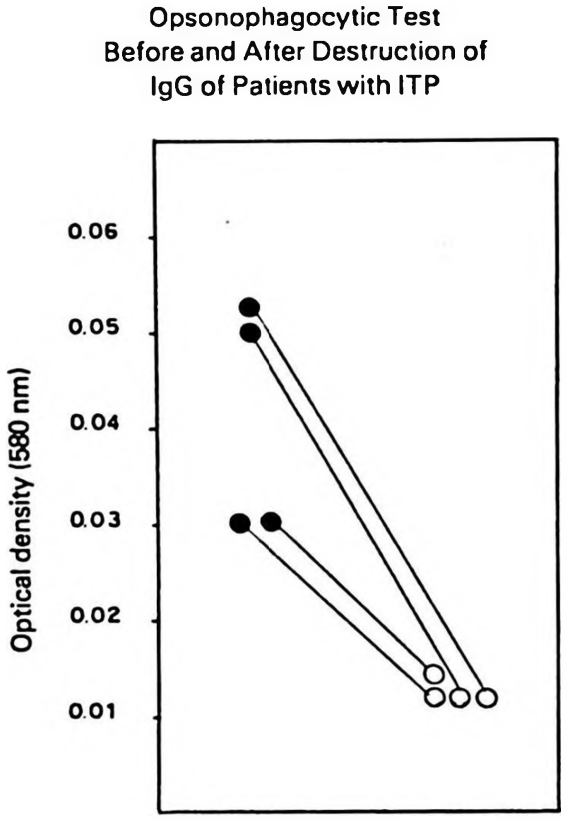

Figure 3.

The IgG fraction obtained from sera of 4 patients with ITP loses its platelet-sensitizing effect following treatment with dithiothreitol and iodoacetamide.

Discussion

Since both humoral and cell-mediated immunity alterations are involved in the pathogenesis of adult ITP, the term autoimmune thrombocytopenia has been used for this condition[11-18]. From the results of the present studies, it can be concluded that platelet-opsonizing effect to ITP serum in the acute phase and/or in remission is related to antiplatelet antibodies in IgG fractions. In this respect the first direct evidence of antiplatelet antibodies in remission in childhood ITP was shown by us[1,2]. A shortened platelet survival in ITP cases in remission was reported by us in three chronic and 5 acute ITP cases[1,2]. Since then, our studies have been extended to include 14 children with ITP (6 chronic and 8 acute) in remission[3]. These again showed a marked reduction in mean platelet survival (4.0 days), somewhat shorter than previously reported[1,2], and antiplatelet antibodies were also shown in all cases[3]. Differentiation of secondary thrombocytopenia can easily be accomplished

in the absence of these antibodies in acute or chronic childhood thrombocytopenia cases[19]. Three other children without antiplatelet antibodies, who were originally admitted with the probable diagnosis of ITP, were later found to have secondary thrombocytopenias due to preleukemia, hypersplenism and aplastic anemia. Thus Tate et al's[19] thesis that the presence of antiplatelet antibodies in the serum of adult patients is consistent with the diagnosis of ITP, also appears to hold true for children. Although some control values were very close to the patients' lowest optical density readings, it should be mentioned that these were not obtained on the same experimental day. Our findings of significantly lower values in remission compared to acute phase readings ($p < 0.01$) are compatible with those of Lightsey et al[20] who, using platelet-associated IgG, indicated that these changes are related to the prognosis.

Because patients with ITP show the platelet opsonizing effect only in the IgG fraction (Figure 1), and this disappears following destruction of IgG by dithiothreitol and iodoactamide (Figure 3), it should also be related to antiplatelet antibodies. Since these IgG fractions were adsorbed by platelets but not by red blood cells or lymphocytes (Figure 2), they must contain antiplatelet antibodies. Therefore, they should be looked for in questionable ITP cases, especially to distinguish the secondary thrombocytopenias without antiplatelet antibody production. Although antiplatelet antibodies decrease in remission, like circulating immune complexes[21] they can still be detected years later[2].

All the above mentioned studies, as well as one of vincristine treatment in chronic cases[22], would strongly support our proposal that the pathogenesis of childhood ITP is similar to that of adult ITP[2]. Therefore the clinical differences between childhood and adulthood ITP must be dependent upon age-related hormonal and/or environmental factors which influence the immunologic responses in general.

We proposed in 1978 that the abbreviation "ITP" might be used for infectious thrombocytopenic purpura because of the high incidence of antecedent infections[23]. Since the presence of antiplatelet antibodies has been shown by us so far in every case in which they were sought, it seems preferable to use ITP as an abbreviation for immune thrombocytopenic purpura. Although the term autoimmune thrombocytopenic purpura has been used by several authors[11-18], it is equivalent to idiopathic thrombocytopenic purpura. In view of our findings, therefore, it should be considered that all thrombocytopenic purpuras due to autoimmune diseases may not be accepted as ITP.

Summary

The platelet-opsonizing effect of serum in ITP is shown to be in the IgG fraction only and specific for platelets, and it is therefore concluded to be related to antiplatelet antibodies. These antiplatelet antibodies were shown in the sera of all children with

ITP in the thrombocytopenic period and in remission and can therefore be used for differentiation from secondary thrombocytopenias. Although the antibodies persist in remission, they decrease significantly. Since in each case the presence of antibodies specific to platelets has been shown, which is not the case in thrombocytopenias of autoimmune diseases, we suggest that the term ITP should be used as an abbreviation for immune thrombocytopenic purpura in childhood.

REFERENCES

1. Özsoylu Ş, Allahverdi H, Laleli Y. Platelet survival in cases of idiopathic thrombocytopenic purpura (ITP) in remission. Excerpta Medica International Congress Series No. 357, 1974, p. 496.
2. Özsoylu Ş, Allahverdi H, Laleli Y, Pırnar A. Platelet survival in childhood idiopathic thromboyctopenic purpura in remission. *J Pediatr* 89:388, 1976.
3. Özsoylu Ş. Studies on the etiopathogenesis of idiopathic thrombocytopenic purpura. First International Symposium. International College of Pediatrics, Helsinki, 1978.
4. Özsoylu Ş. Öztürk M. Antiplatelet antibodies in thrombocytopenic phase and remission in childhood idiopathic thrombocytopenic purpura (ITP) (Submitted to *Scand J Haematol*).
5. Brecher G, Cronkite EP. Morphology and enumeration of human blood platelets. *J Appl Physiol* 3:365, 1951.
6. Handin RI, Stossel TP. Phagocytosis of antibody-coated platelets by human granulocytes. *N Eng J Med* 290:989, 1974.
7. Hudson L, Hayf C. *Practical Immunology*. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Pub, 1976, p. 156.
8. Blezikian SB, Tsan M, Laleli YR, Bias WB. Immunological reactions involving leukocytes. II: Studies of a patient with multiple transfusion-induced allogeneic antibodies to leukocytes. *Johns Hopkins Med J* 138:73, 1976.
9. Reif AE. Batch preparation of rabbit gamma G globulin with DEAE cellulose. *Immunochemistry* 6:723, 1969.
10. Miller F, Metzger H. Characterization of a human macroglobulin II. Distribution of the disulfide bonds. *J Biol Chem* 240:4740, 1965.
11. Handin RI, Piessens WF, Moloney WC. Stimulation of nonimmuzined lymphocytes by platelet antibody complexes in idiopathic thrombocytopenic purpura. *N Engl J Med* 289:714, 1973.
12. Harrirgton WJ, Minnich V, Hollingsworth JW, Moore CV. Demonstration of thrombocytopenic factor in blood of patients with thrombocytopenic purpura. *J Lab Clin Med* 38:1, 1951.
13. Wybran J, Fudenberg HH. Cellular immunity to platelets in idiopathic thrombocytopenic purpura. *Blood* 40:856, 1972.
14. McMillan R, Longmire RL. Tavassoli M, et al. In vitro platelet phagocytosis by splenic leukocytes in idiopathic thrombocytopenic purpura. *N Engl J Med* 290:249, 1974.
15. Dixon R, Rosse W, Ebbert L. Quantitative determination of antibody in idiopathic thrombocytopenic purpura. Correlation of serum and platelet bound antibody with clinical response. *N Engl J Med* 292:230, 1975.
16. Dixon RH, Rosse WF. Platelet antibody in autoimmune thrombocytopenia. *Br J Haematol* 31:129, 1975.
17. Karpatkin S Autoimmune thrombocytopenic purpura. *Blood* 56:329, 1980.

18. Von Dem Borne AEG Kr, Helmerhorst FM, van Leeitwen EF, et al. Autoimmune thrombocytopenia; detection of platelet autoantibodies with the suspension immunofluorescence test. *Br J Haematol* 45:319, 1980.

19. Tate DY, Sorenson RL, Gerrard JM, et al. An immunoenzyme histochemical technique for the detection of platelet antibodies from the serum of patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP). *Br J Haematol* 37:265, 1977.

20. Lightsey AL Jr, Koenig HM, McMillan R, Stone JR Jr. Platelet associated immunoglobulin G in childhood idiopathic thrombocytopenic purpura. *J Pediatr* 94:201, 1979.

21. Öztürk M, Özsoylu Ş, Bakkaloğlu A. Circulating immune complexes in childhood (ITP) (Submitted to *Acta Paediatr Scand*).

22. Hiçsönmez G, Özsoylu Ş. Vincristine for treatment of chronic thrombocytopenia in children (letter). *N Engl J Med* 296:454, 1977.

23. Özsoylu Ş, Kanra G, Savaş G. Thrombocytopenic purpura related to rubella infection. *Pediatrics* 62:567, 1978.