Autoimmune Thrombocytopenic Purpura

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SUMMARY

Adult autoimmune thrombocytopenic purpura (ATP) is a platelet disorder that develops in certain individuals with a genetic as well as sex (female) predisposition following an environmental event (?viral). This results in the production of an IgG antiplatelet antibody capable of reacting with the host’s platelets, as well as crossing the placenta. This leads to the rapid clearance and destruction of opsonized platelets by the reticuloendothelial system, particularly the spleen, by greater than tenfold the normal rate. Bound platelet IgG correlates with disease severity, whereas serum antiplatelet IgG does not. It has not been rigorously established whether bound platelet IgG is directed against a platelet antigen or represents an immune complex bound to the platelet Fc receptor. Nevertheless, several lines of evidence suggest that antiplatelet IgG binds directly to a platelet antigen(s).

Megakaryocyte number, volume, and mass are increased commensurate with increased platelet turnover. Platelets of increased size, megathrombocytes, are noted on peripheral smear or via platelet volume distribution analysis. Megathrombocyte number is proportionate to megakaryocyte number and to platelet turnover. Megathrombocyte diameter is inversely proportional to platelet survival.

Antiplatelet antibody is also associated with qualitative platelet functional defects, which are indistinguishable from those noted with thrombopathia (i.e., apparent platelet release defect). Antibody-induced functional defects are probably more common than quantitative thrombocytopenic defects and may represent a significant portion of those women with the “easy bruising” syndrome and normal platelet count.

Adults who develop ATP generally develop the chronic variety, which remains permanently with the patient. Treatment should be directed towards maintaining the patient free of purpura, not restoring the platelet count to normal. This can generally be accomplished with a platelet count of >40,000/cu mm with patients having this disorder. Approximately 50% of patients respond to steroids by a significant elevation of platelet count and improvement of purpura. However, cessation of therapy results in eventual relapse if the disease is of the chronic variety. Splenectomy is successful in approximately 65%-75% of patients, resulting in a restoration of the platelet count to normal or safe levels by removing a major source of platelet destruction as well as antibody production; platelet survival improves. At least 50% of patients “in remission” following steroids or splenectomy generally have a compensated thrombocytolytic state in which increased platelet production keeps up with increased platelet destruction. Antiplatelet IgG can often be found in the serum of these patients.

Patients refractory to steroids and/or splenectomy present with a serious therapeutic problem. Immunosuppressive therapy is effective in approximately one-third of refractory patients, but often relapses occur, requiring maintenance therapy with potentially mutagenic drugs. The use of vinca alkaloids has recently been proposed. Its efficacy has yet to be established and its use should be confined to research centers.

HISTORICAL BACKGROUND AND PATHOGENESIS

In the 1950’s, the term idiopathic thrombocytopenic purpura (ITP) referred to a clinical disorder of unknown etiology associated with thrombocytopenia.
and purpura. The disorder could be acute and often transient, as in the case of childhood ITP, recurrent with episodes of “remission,” or chronic, as in adult ITP. It was noted that mothers with ITP (with active disease or in remission) often gave birth to children who developed transient ITP, suggesting the transfer of a humoral factor.\textsuperscript{1-4} It was also noted that patients with Coomb's positive (autoimmune) hemolytic anemia often had episodes of ITP (Evan's syndrome) both transient and recurrent, suggesting an autoimmune etiology.\textsuperscript{5} Furthermore, the favorable response to steroids (antilymphocytic) and/or splenectomy (antireticuloendothelial) encouraged the thought that this disease might be mediated by the production of anti-platelet antibody.

In 1951, Harrington clearly established the humoral nature of such an antiplatelet factor and provided the strongest evidence that this disease was antibody-mediated.\textsuperscript{3} He infused the plasma equivalent of a unit of blood from a patient with ITP intravenously into himself, and promptly developed thrombocytopenic purpura (Fig. 1). A drop in platelet count occurred immediately, reaching its nadir in 1-3 hr and returning to normal in 4-6 days. Of interest was the observation that only 63\% of patients with the diagnosis of ITP contained a factor in their plasma that was capable of inducing thrombocytopenia when infused into volunteer recipients.

**SEERUM ANTIBODY**

Harrington then developed a simple platelet agglutinin test that consisted of the addition of heat-inactivated “test” sera to an aliquot of normal platelet-rich plasma followed by incubation overnight at 5°C.\textsuperscript{3} The sera of patients with ITP were positive in approximately 65\% of cases.\textsuperscript{6} This antiplatelet factor was shown to be present in the gamma globulin fraction of sera. In 1965, Shulman et al.,\textsuperscript{7} employing the same in vivo test as Harrington,\textsuperscript{3} demonstrated that the antiplatelet factor was in the 7S gamma globulin fraction of serum, was adsorbed by human platelets, was species specific, and affected autologous as well as homologous platelets.

The platelet agglutinin test\textsuperscript{3,7-14} as well as other tests\textsuperscript{10,15} for antiplatelet antibody, particularly the antiglobulin consumption test,\textsuperscript{7,8,16-18} although reproducible in some\textsuperscript{4,10,16} laboratories, was not reproducible in others\textsuperscript{7,8,10-13,19} Indeed, some workers claimed that the antiplatelet factor of serum was no more than thrombin,\textsuperscript{11} a potent platelet agglutinin. It was argued that the absence of platelets and their phospholipid platelet factor 3 (PF-3) in these patients prevented adequate utilization of prothrombin, leading to a high residual prothrombin in their sera. This could then be converted to thrombin by the addition of platelets to the test system.\textsuperscript{13}

In 1968, Karpatkin and Siskind\textsuperscript{20} employed an ammonium sulfate globulin fraction of sera to develop a PF-3 immunoinjury technique for measuring antiplatelet antibody that circumvented the problem of residual prothrombin or thrombin. This technique was capable of detecting an antiplatelet factor in approximately 60\%-65\%\textsuperscript{6,21} of patients with ITP, confirming Harrington's original work with platelet agglutinins, as well as the work of Steffen,\textsuperscript{16} Dausset,\textsuperscript{17} and Van deWiel et al.\textsuperscript{18} with the antiglobulin consumption test. The PF-3 immunoinjury technique takes advantage of the requirement of platelet phospholipid to act as a catalytic surface for the coagulation cascade and is measured by the acceleration of the clotting of platelet-rich plasma following addition of a factor capable of injuring platelets.\textsuperscript{4,20,21} The PF-3 immunoinjury technique has been recently modified so that it can be employed as a rapid, routine laboratory test employing frozen platelets and requiring 4 hr for its performance.\textsuperscript{21} Positive results have been found in 60\% of patients with ITP. Results are also positive in systemic lupus erythematosus (SLE), rheumatoid arthritis, lymphoma, autoimmune hemolytic anemia, chronic...
hepatitis, and following numerous blood transfusions. This antiplatelet factor has the characteristics of an IgG immunoglobulin in that it is stable at 56°C; it can be neutralized with a globulin fraction of rabbit anti-human IgG antisera; but not with normal rabbit globulin. The sensitized platelet can be adsorbed to protein A, which binds to the Fc domain of IgG. The "sandwiched" radioactive material can be eluted and the eluted activity neutralized with rabbit anti-human IgG but not with normal rabbit globulin. The eluted material has a molecular weight of 150,000 and S value of 7-7.5 similar to that of human IgG; and it can be transferred across the placenta. The heavy chain subclass of the IgG immunoglobulin appears to be γG3, and the IgG contains both kappa and lambda light chains. Some degree of platelet specificity does exist, since the anti-platelet antibody of 3 of 5 patients studied reacts more intensely with their own platelets than with platelets from unrelated donors. The serum antibody is useful for diagnostic purposes only, since its titer does not correlate with severity of diseases. Results confirming the reliability of the PF-3 immunoinjury technique and indicating the presence of antiplatelet antibody in ITP patients have been published by a number of workers employing a variety of different techniques. Because of the immunologic nature of this disorder, in which the host's immune system destroys the host's platelets, it was recommended that the term idiopathic be changed to autoimmune and the disorder designated ATP or autoimmune thrombocytopenic purpura. This designation has been accepted by other investigators.

BOUND ANTIPLATELET ANTIBODY

In 1975, Rosse and coworkers made a major contribution to this field by developing a complement-mediated red cell lysis-inhibition technique for the quantitative detection of bound antiplatelet antibody, wherein 17 of 17 patients tested were positive. Bound platelet IgG was found to correlate with severity of disease. Treatment of patients with steroids or splenectomy lowered platelet IgG; and patients with considerably elevated platelet IgG were refractory to corticosteroids or splenectomy. This work was quickly confirmed by several laboratories employing similar as well as different techniques, which include immunofluorescence. Fab-anti-Fab IgG assay, radioactive 125I Coomb's test, and a solid-phase radioimmunoassay for IgG, employing rabbit anti-human IgG "sandwiched" to radioactive 125I staphylococcal protein A, which binds to the Fc domain of IgG. The various techniques described measure 1-11 ng of nonspecific IgG on 10⁶ normal platelets and 5-13 times this amount on platelets from ATP patients (tests are positive in >92% of thrombocytopenic patients).

The immunofluorescent technique is relatively insensitive. The complement lysis-inhibition technique is complicated, detects IgG at the 10-25-ng level, and can be employed with as little as 10⁶ platelets. The Fab--anti-Fab technique is also relatively complicated, requires 3 days to perform, is sensitive at the 15-20-ng level, and can be performed on approximately 2 x 10⁷ platelets. The radioactive IgG Coomb's test can be applied to routine use but has a complicated method of standardization (IgG fixed to red blood cells), is sensitive at the 5-10-ng level, and requires approximately 10⁷ freshly collected platelets. The solid-phase radioimmunoassay can be performed with commercial reagents and is relatively simple, except for the iodination of protein A. It is 10-50-fold more sensitive than other techniques, can be performed with as little as 2 x 10⁷ platelets, and can be employed with frozen platelet extracts (samples can be stored at −20°C in the presence of protein inhibitors for future assay).

The inverse relationship between platelet count and platelet IgG is best expressed by a log-log equation with a correlation coefficient, r = −0.71, p < 0.001, rather than an arithmetic plot, r = 0.56, p < 0.001. This indicates considerably more antibody bound to platelets from patients with low platelet counts than would be expected from a simple inverse arithmetic relationship. This could represent more antiplatelet antibody bound to larger younger platelets, megathrombocytes, which are increased in number in this disorder. However, a recent study suggests otherwise. It is conceivable that younger platelets may be more resistant to destruction and clearance by the reticuloendothelial (RE) system.

Platelet-bound IgG has also been recently reported in 46% of patients following gram-positive or -negative septicemias, where positive test results are thought to represent nonspecific binding of bacterial antigen-antibody complexes to platelets. Accordingly, positive results associated with septicemias should be interpreted with caution, before making the diagnosis of ATP.

COMPLEMENT

Serum complement levels are generally normal, and attempts to measure serum complement-dependent antibody have been negative. Nevertheless, C3 has recently been reported to be bound to platelets in the presence of bound IgG (9 of 14 patients with elevated platelet IgG; 9 of 16 total patients tested). The C3 bound was proportional to the IgG bound. In another study employing a radioactive 125I Coomb's test, 12
of 21 patients had elevated C3 in the absence of elevated IgG levels. The pathophysiologic significance of fixation of C3 to platelets in 58% of the 36 patients (from both studies) remains to be determined.

ROLE OF THE SPLEEN IN ANTIBODY PRODUCTION

In 1971, Karpatkin et al. demonstrated decreased circulating antiplatelet antibody postsplenectomy in 7 of 8 patients studied. In 1972 both Karpatkin et al. and McMillan et al. independently demonstrated synthesis of specific IgG antiplatelet antibody by the spleen, which reflected approximately 0.6%-5% of total IgG produced. Both laboratories also noted a considerable increase in the synthesis of nonspecific IgG by splenic tissue from these patients that was 5-55 times that produced by control spleens, an observation that remains unexplained.

ON THE NATURE OF THE ANTIGEN

Present technology for the detection of antiplatelet antibody in the serum or on the platelet does not distinguish between IgG and IgG complexed to an antigen, i.e., an Ag-Ab complex bound to the platelet Fc receptor. It is conceivable that ATP is an immune complex disease wherein the IgG antibody reacts with a foreign (?viral) antigen or a (nonplatelet) circulating host antigen, to form an immune complex that then binds to the platelet; or a foreign antigen binds to the platelet and then reacts with an antiforeign antibody. Six lines of evidence suggest that this may not be the case: (1) Antiplatelet factor classically crosses the placenta in this disease, resulting in the passive transfer of neonatal thrombocytopenia. IgG is known to cross the placenta, whereas immune complexes are less likely to do so. (2) The in vivo infusion experiments had demonstrated the thrombocytopenic factor to be in the 7S gamma globulin fraction of sera. (3) The serum factor demonstrates greater specificity for the patient's platelets. (4) Lymphocytes from cultured spleens are capable of synthesizing IgG, which specifically binds to washed platelets. (5) The eluate from bound antiplatelet IgG migrates in the 7S (IgG) region of a sucrose gradient (although it is conceivable that the complex may have dissociated with the extraction procedure, or that the Ag may be too small to appreciably affect the IgG sedimentation value). (6) Antibody activity, as defined by IgG platelet adsorption, is eluted from Sephadex G200 and DEAE cellulose at the area where IgG is found.

Nevertheless, circulating immune complexes have been recently reported in this disorder by Lurhuma et al. These workers employed two methods for the detection of circulating immune complexes based on their inhibitory effect on the agglutination of IgG-coated latex particles by rheumatoid factor or Clq. The sera of 48 of 72 patients with ATP (58%) displayed a significant inhibitory effect toward both agglutinating agents. A negative correlation was obtained between the platelet count and the titer of the inhibitory factors. When the sera were subjected to Sephadex G-200 gel filtration, IgG and DNA could be detected in "heavy factions" corresponding to Ag-Ab complexes in 6 different patients. However, DNA was not necessarily eluted with "heavy IgG" or fractions with inhibitory activity for agglutination of IgG-coated latex particles. Dissociation and association of "immune complex" inhibitory activity could be obtained with sera from 2 patients. These authors also found hepatitis HbsAg in 20 sera, Epstein-Barr virus (EBV) antigen in 5 sera, and adenovirus antigens in 6. This very interesting report remains to be confirmed. However, confirmation of the presence of immune complexes would still not rule out the presence of specific IgG against platelet antigens. These complexes could represent an epi-phenomenon, wherein immune complexes are developed against platelet or other cellular antigens produced during cell destruction by "specific" platelet antibodies. In order to conclusively demonstrate the presence of specific antibody against platelet antigens, it will be necessary to demonstrate that Fab fragments of eluted platelet IgG or serum antiplatelet IgG specifically binds to platelets. Experiments designed to answer this question have as yet not been reported.

ROLE OF CELL-MEDIATED IMMUNITY

In 1970, Piessens et al. demonstrated increased lymphocyte transformation (incorporation of tritiated thymidine into DNA) with autologous platelets from a patient with ATP as well as homologous platelets plus the patient's lymphocytes, suggesting a role for cell-mediated toxicity. Wybran and Fudenberg performed similar studies on a group of patients with this disorder and noted positive lymphocyte stimulation with autologous platelets in 7 of 8 patients with severe disease and 3 of 6 patients with mild disease; 10 normal subjects and 6 patients with autoimmune thrombocytopenic disorders gave negative results. Similar observations were made by Clancy in 6 of 7 patients with the disorder. He also noted inhibition of leukocyte migration by autologous platelets in 9 of 10 patients tested. This technique is based on the fact that when antigen is incubated with sensitized lymphocytes, a number of soluble factors are released, including a migration inhibition factor that inhibits the migration of macrophages, polymorphs, and lymphocytes. However, it now appears likely that the results reported in their studies actually represented nonspe-
cific lymphocyte stimulation by platelet–antibody complexes, which have been shown to stimulate lymphocyte transformation.44 Platelets have recently been shown to be coated by antiplatelet antibody (see Bound Platelet Antibody). In 1979, Quagliata and Karpatkin56 reported impaired lymphocyte transformation in whole blood with mitogenic agents (phytohemagglutinin and concanavalin A) in patients with ATP (but not with washed lymphocytes). The number of T cells, B cells, and null cells were normal. An inverse relationship was found for the number of T cells and platelet count in 31 ATP patients, with \( r = -0.55, p < 0.001 \). However, “killer” cells could not be demonstrated employing lymphocytes from 7 ATP patients incubated with \( ^{51}\text{Cr}-\text{labeled allogetic or syngenic platelets. Lymphocyte capping with rabbit antihuman lymphocyte IgG was impaired in 7 of 7 patients with ATP.} \)

The apparent disappearance of impaired lymphocyte transformation with washed lymphocytes and the abnormal lymphocyte capping suggests the presence of a blocking factor(s) or cell or humoral antibody(s) that could be responsible for the observed qualitative abnormalities of cell-mediated immunity.

PLATELET KINETICS

It is generally accepted that thrombocytopenia is secondary to increased platelet destruction of antibody-coated platelets by phagocytosis,51-54 particularly in the spleen. Platelet survival is markedly shortened to less than 10% of the normal 10-day survival.55-59 Megakaryocytes are increased in number, volume, and immaturity.40-56,57 Platelets are decreased in number but generally increased in volume (see Platelet Size).

More specifically, Branehog et al.40 have demonstrated that platelet survival is proportional to the circulating platelet count (at a platelet survival of 0–3 days). Harker56 and Branehog et al.57 have both studied a total of 49 patients with comparable platelet counts, mean 30,000/cumm. The mean platelet survival was 0.346 and 0.6752 days, respectively. The mean platelet turnover was increased to 4.96 and 2.3 times normal,57 respectively. Megakaryocyte mass has been quantified by Harker et al. who studied 14 patients. Megakaryocyte number averaged 3 times the control value, and megakaryocyte volume averaged 1.6-fold. Thus, the increase in total megakaryocyte mass averaged 4.8-fold, indicating that under suitable stress, the bone marrow can increase platelet production by approximately fivefold. The ratio of platelet turnover to megakaryocyte mass in ATP patients was the same as that found in normal subjects. This implies that thrombopoiesis is effective. Thus, the increase in megakaryocyte mass in ATP patients (compared to normal subjects) parallels the increase in platelet turnover (compared to normal subjects). Although the older literature refers to “abnormal megakaryocytes,” reflecting decreased platelet production secondary to splenic inhibition of the bone marrow via a humoral antagonist,61-64 the kinetic evidence of Harker56 as well as Garg et al.40 suggests otherwise. Nevertheless, common antigens for the megakaryocyte and platelet have been demonstrated with heterologous antiplatelet sera,65-67 and more recently, with radioactive homologous splenic antibody synthesized in vitro.68 It is conceivable that impaired megakaryocyteopoiesis may obtain in some situations; however, this has not been demonstrated as yet.

PLATELET SIZE

Large platelets or megathrombocytes are routinely noted in this disorder on EDTA smear or by volume determination in a Coulter Counter with EDTA employed as anticoagulant40 (Fig. 2). Megathrombocytes generally correlate with megakaryocyte number in most acquired platelet disorders of increased destruction or decreased production, \( r = 0.7, p < 0.001 \).40 Mean platelet diameter is 1.6-fold greater than normal in ATP. The megathrombocytes in this disorder are probably “stress” platelets, which are released during conditions of increased megakaryocyte turnover. The increase in megathrombocytes parallels the increase in megakaryocytes noted and can be in the order of 3–4-fold.40 There is generally a shift in the platelet volume distribution curve to the right towards larger platelets. Approximately 50% of patients in

![Fig. 2. Coulter platelet volume distribution curves in 20 normal subjects + 2 SD (grey area). (A) A patient with a normal platelet count (160,000/cu mm) in “remission” from ATP (with elevated platelet IgG, 25 ng Ig/10⁶ platelets). (B) A patient with ATP and a platelet count of 55,000/cu mm. Calibration: 1 window = 0.25/cu μ.](image-url)
"apparent" clinical remission have increased megathrombocytes70 (as well as bound platelet IgG70), indicating increased platelet turnover despite a normal platelet count. This is known as a "compensated thrombocytolytic state." There is a significant inverse relationship between platelet diameter and platelet mean life-span, \( r = -0.8, p < 0.001 \).77 There also is a significant relationship between platelet diameter and platelet production rate, \( r = 0.7, p < 0.001 \) and between platelet size and percent young megakaryocytes, \( r = 0.6, p < 0.005 \).77

Patients with particularly severe disease also have evidence for intravascular thrombocytolysis, as noted by the presence of small fragments that can be detected with the Coulter Counter by a shift of the platelet volume distribution curve to the left or by a separate small particle peak. These have been shown to contain platelet fragments as well as red blood cell fragments by electron microscopy. The red blood cell fragments are associated with weak complement sensitization of red blood cells in 7 of 12 patients, suggesting that autoantibody might also be directed (subclinically) against red blood cells.54

QUALITATIVE PLATELET DEFECTS

In 1972, Clancy, Jenkins, and Firkin71 reported the presence of qualitative (functional) platelet defects in 11 patients in "remission" (normal platelet counts). Although no correlation was found between the presence of antplatelet antibody (PF-3 technique) and abnormal platelet function, an antplatelet function factor could be isolated from the globulin fraction of the patient's serum that could inhibit platelet function when mixed with normal platelet-rich plasma. The antplatelet function factor could be specifically adsorbed to washed human platelets and specifically neutralized with anti-human IgG, suggesting that it was an immunoglobulin. However, a similar antplatelet function factor was also found in 3 patients with mild intermittent disease in whom platelet function was normal.

In 1974, Regan, Lackner, and Karpatkin72 described abnormal platelet function in 12 of 21 consecutive patients with SLE that correlated with clinical severity of disease. No correlation was found between the presence of serum PF-3 antplatelet antibody and abnormal platelet function in the "nonresponder" group. However, an antplatelet function factor could be isolated from the serum globulin fraction of 3 of the 7 nonresponder patients, which could inhibit platelet function of normal platelet-rich plasma, whereas no such factor could be isolated from the serum of 4 of 4 "responder" patients with SLE who had normal platelet function.

In 1975, Lackner and Karpatkin73 studied 75 patients with the "easy bruising syndrome" and normal platelet count. Platelet function was normal in 31 patients (29 were female) and was indistinguishable from the "aspirin-like" platelet aggregation defect reported in patients with ATP,71 SLE,72 and thrombopathia.74-79 Of particular interest was the presence of antplatelet antibody in 38% of these patients. Increased number of megathrombocytes was also noted in 71% of these patients, suggesting increased platelet turnover despite a normal platelet count.

In 1967, five independent groups74-79 described an apparently new qualitative platelet disorder in which the prolonged bleeding time, impaired collagen-induced platelet aggregation, and impaired epinephrine-induced secondary wave of aggregation were attributed to defective release of platelet adenosine diphosphate (ADP). The disorder was termed thrombopathia and is indistinguishable from the acquired aspirin defect in normal subjects with respect to platelet aggregation. Most of the patients described have been females. Three of six patients studied had increased megathrombocytes. Four patients had histories that included ankylosing spondylitis, intermittent arthritis, arthralgia, and myositis. Upon further study, two subgroups were documented: one in which the storage pool of adenine nucleotide and serotonin associated with platelet dense granules was diminished, termed "storage pool" disease,79 and the other in which the storage pool was normal, but the release from this pool with initiation of irreversible platelet aggregation was impaired, termed "release defect."74-79

The patients with thrombopathia (both varieties) are indistinguishable from the 31 easy bruising patients described by Lackner and Karpatkin77 with respect to sex, clinical history (a mild bleeding disorder), and platelet function. The presence of antplatelet antibody in 38% of these patients, as well as the finding of similar platelet function abnormalities in such autoimmune disorders as compensated ATP in remission and SLE, strongly suggests an autoimmune etiology or association for many of the patients with thrombopathia and/or the easy bruising syndrome. This is supported by the disappearance of abnormal platelet function in at least 3 of the 31 patients studied, as well as the description of an acquired storage pool disorder associated with antplatelet antibody that responded to prednisone therapy in a patient with nephritis, polyarthritis, chondritis, thrombophlebitis, Raynaud's phenomenon, and a thrombotic tendency.80 It is intriguing that 2 of the nonresponder SLE patients, 3 of the easy bruising patients, 2 of the thrombopathia patients, and the patient with the acquired storage pool disorder had transient episodes
of thrombocytopenia. It is therefore likely that antiplatelet antibody can contribute to or be responsible for both quantitative as well as qualitative platelet disorders. This can explain those patients with autoimmune thrombocytopenic purpura with so-called “safe” platelet counts of >50,000/cumm who have purpura and bruise easily. It is further proposed that some patients with thrombopathia and/or the easy bruising syndrome may have a forme fruste of ATP, wherein the antiplatelet antibody damage is insufficient to result in thrombocytopenia, but sufficient to elicit functional platelet defects.73,81 Perhaps two varieties of antiplatelet antibody are present: one capable of destroying platelets (? high-affinity antibody), and the other capable of altering platelet function (? low-affinity antibody). It is suggested that thrombopathia and/or the easy bruising syndrome may be the bottom of the ATP iceberg. The incidence of this qualitative disorder(s) is considerably more common than classic ATP.81 84

THE ATP ICEBERG

It should be emphasized that thrombocytopenic purpura is merely the top of the iceberg, and that probably many thousands of individuals have platelet counts below 150,000/cum mm without purpura (95% probability of being abnormal182). These individuals are unaware of their illness, since symptoms of purpura or bleeding do not occur until the platelet count reaches dangerous levels, i.e., <30–50,000/cum mm. There are still other individuals with a compensated thrombocytolytic state, i.e., normal platelet count but decreased platelet survival and increased megathrombocytes.82 These individuals probably have subclinical compensated autoimmune thrombocytolysis, wherein increased bone marrow production of megakaryocytes keeps up with increased peripheral platelet destruction. These individuals are in precarious balance, and any stress on the productive capacity of the bone marrow, such as nutritional (folate or B12), drugs, toxic substances (alcohol), or viral or bacterial infections as well as stress on platelet survival such as alcohol, infection, etc., can precipitate thrombocytopenia. These individuals may possibly include the numerous women with normal platelet counts and easy bruising syndrome and/or thrombopathia, some of whom have antiplatelet antibody and defective platelet function.

HLA TYPING AND GENETIC PREDISPOSITION WITH DRw2

Recent studies on the genetic predisposition to ATP have revealed a high association with an alloantigen of the HLA-D locus, which is responsible for the mixed lymphocyte stimulation reaction. Specific antisera are now available that have specificities closely “related” to alleles of the D locus and are therefore called DR or DRw for D-related workshop designation. The DRw2 alloantigen has been found in 75% of 20 consecutive ATP patients in the New York City area, compared to 23% (<0.001) in an ethnically matched control population.85 This provides a genetic predisposition with a relative risk of 10. (A relative risk of 1 would indicate no association between the presence of the antigen and the disease.) Of particular interest was the apparent typing of a single allele, DRw2, in 10 of the 20 patients tested. Also noted were a high association of the apparent haplotypes A3-B7 and A26-Bw38 of the HLA-A and B loci, which appear to be in linkage disequilibrium with DRw2 in the population studied.

These data indicate a genetic predisposition to ATP that is inherited with a DRw2 gene of the major histocompatibility system. It is of interest that SLE has recently been shown to be associated with DRw2 and DRw3, with relative risks of 3.9 and 6.5, respectively.86,87

DIAGNOSIS

The term autoimmune thrombocytopenic purpura should be applied to those patients fulfilling the following criteria.

1. Increased platelet destruction as manifested by thrombocytopenia or shortened platelet survival i.e., compensated thrombocytolytic states.46,57,82 Both conditions are associated with an increased percent or number of megathrombocytes (young platelets).46,57,82

2. Increased number of megakaryocytes in the bone marrow. In the older literature,46 emphasis has been placed on abnormal megakaryocyte morphology: i.e., absence of platelet budding, lack of granularity, vacuolization, cytoplasmic and nuclear degenerative changes. These observations are probably a reflection of megakaryocyte turnover and maturation. Any disorder with increased megakaryocyte turnover is likely to contain an increased number of young megakaryocytes, which in turn would probably reflect most of the above findings.

3. Presence of bound antiplatelet antibody in the absence of septicemia42 or hypergammaglobulinemia157.

4. Exclusion of other primary clinical disorders that are capable of giving all of the above criteria or some of the above criteria: systemic lupus erythematosus, lymphoma, disseminated intravascular coagulation, hypersplenism, drug-induced thrombocytopenic purpura, sepsis, etc. The distinction between ATP and drug-induced immunologic thrombocytopenia is often crucial. All drugs should be withheld and the platelet count closely observed. Failure to return to a normal platelet count in 7–10 days (in the absence of serious
hepatic or renal disease) rules out the diagnosis of drug-induced immunologic thrombocytopenia.

(5) Absence of splenomegaly in 97% of patients. Its presence usually,88 but not always (as might be the case with children89 90), excludes the diagnosis of ATP.

The term ITP should be reserved for those 5%-10% of patients without demonstrable bound antiplatelet antibody but who fulfill the remaining criteria.

CLINICAL SIGNS AND SYMPTOMS

The signs and symptoms of this disorder are directly related to the platelet count and are not specific for ATP or ITP but will be present in any quantitative platelet disorder. When the platelet level is insufficient to support hemostasis, generally <30–50,000/cu mm, capillary bleeding or purpura ensue. The degree of thrombocytopenia required for hemorrhage can be quite variable, 10–50,000/cu mm, and is probably dependent on such factors as platelet age (greater functional capacity of young platelets,92 94 or megathrombocytes95) antiplatelet antibody binding leading to functional defects,88 and capillary vessel integrity. The type of bleeding is predominantly dermal and mucosal. Bleeding into the skin with pin-point hemorrhage (petechiae), particularly in dependent areas where there is increased capillary pressure (i.e., lower extremities), is often the rule. Petechiae are violaceous, flat, round, intradermal, and submucous hemorrhages that later turn blue and yellow. The petechial purpura may become confluent and present as ecchymoses. Common areas of hemorrhage are in the nasal, buccal, gastrointestinal, and vaginal mucosa. Examination of the mouth often reveals violaceous as ecchymoses. Common areas of hemorrhage are in the nasal, buccal, gastrointestinal, and vaginal mucosa. Examination of the mouth often reveals violaceous hemorrhagic blebs, 0.25–0.5 cm in diameter, on the buccal and glossal mucosa with bleeding gums. Conjunctival and retinal hemorrhages may be noted. In chronic ATP or ITP, there is often a history of easy bruising in the absence of trauma, frequent epistaxes, and prolonged menses. If the thrombocytopenia is chronic, gastrointestinal blood loss may ensue, and iron deficiency anemia with microcytic hypochromic red blood cells may be evident on peripheral smear.

The most serious complication and one which is rarely seen (particularly when steroids are employed) is hemorrhage into the central nervous system. This can be fatal98 and must be rapidly and vigorously prevented by suitable therapy when there is purpura and signs or symptoms of CNS bleeding, such as retinal hemorrhages, convulsions, meningismus, headache, change in personality, or more specific neurologic signs.

Laboratory findings reveal a prolongation of the bleeding time, a positive tourniquet test, and absent to normal tourniquet test, and absent to normal clot retraction, depending on the platelet count. There are diminished platelets and increased megathrombocytes on peripheral smear. Platelet and red blood cell fragmentation may be detectable via Coulter Counter if the thrombocytopenia is severe enough. Anemia secondary to blood loss may be present. The white blood cell count is generally normal or slightly increased. There is often a relative lymphocytosis with atypical lymphocytes (particularly in children) and eosinophilia. The bone marrow contains increased numbers of young megakaryocytes and often an increase in eosinophiles.89 96 The myeloid: erythrocyte (M:E) ratio may be decreased if blood loss is a prominent feature.

TEMPORAL CLASSIFICATION AND NATURAL HISTORY

Autoimmune thrombocytopenic purpura may be temporally classified into three categories: acute, intermittent, and chronic.

The acute variety most often occurs in children, particularly following a seasonal (winter–spring) viral illness96 in 50% of patients (or, rarely, following a vaccination against a viral illness.133) These include typical childhood infections and upper respiratory infections. The average interval between purpura and preceding infection is 2 wk.90 Of interest is the recent report of high levels of bound platelet IgG in this disease, which the authors postulate represents Ag–Ab complexes.98 The disease has an average duration of 1–2 mo (usually less than 6 mo). The male:female ratio is 1.38,90,96,97 Although the acute disease is usually considered a milder disorder with a better prognosis than the chronic disorder, 27 deaths have been reported out of a total of 709 cases not treated with steroids.99 90,96,99 Approximately 7%-28% of children go on to develop the chronic variety.89 90,96

The chronic form most often occurs in adults, is persistent, lasting years to indefinitely, and has a female:male ratio of 2–4:1.138,97,100,101 The so-called remissions of the chronic form are remissions of purpura only. The platelet count usually remains at one-third to one-half the normal value.97 Some patients have been followed with the chronic disease for 30 yr.97

The intermittent variety may occur in childhood or adulthood and may by spaced by intervals free from disease, wherein both the platelet count and platelet survival are normal.102 The usual pattern is for the platelet count to return to normal in less than 6 mo, and remain normal for at least 3 mo prior to the next episode.90 As many as 5 recurrences have been reported over a 4–18-yr interval.102
POSSIBLE INTERRELATIONSHIP BETWEEN ATP AND SLE

ATP may be an early manifestation of SLE in 3%-16% of patients. In a series of 34 patients, the median time for the development of SLE post-splenectomy was 2.5 yr, with a range of 3 mo to 10 yr. Data supporting the similarity of these two autoimmune disorders (chronic ATP and SLE) can be obtained from the young female preponderance, the presence of antiplatelet antibody, and the high incidence of DRw2 in both disorders. Indeed, the claim has been made that splenectomy for ATP accelerates the expression of SLE or activates a “dormant” form of the disease. This claim has been challenged, however, and is not accepted by most workers in the field.

TREATMENT AND DIAGNOSIS

The classical treatment for autoimmune thrombocytopenic purpura is palliative, not curative, and is directed toward blockage or removal of a major site of platelet destruction, the spleen.

Treatment With Steroids

Administration of steroids is usually successful in either raising the platelet count to safe levels or normal levels and is often dose-dependent. It is equivalent to a “medical splenectomy” in that it prevents sequestration of damaged or antibody-coated platelets by the spleen (Fig. 3). Steroid administration to normal individuals has no effect on platelet survival or platelet count. Steroids are known to reduce the ability of phagocytes to digest intracellular particles and destroy bacteria. Steroids inhibit erythropagocytosis by granulocytes, adherence of antibody-coated platelets to granulocytes, and phagocytosis of platelets by granulocytes. Steroids inhibit chemotaxis of monocytes as well as monocyte receptors for IgG and C3. There is indirect evidence that steroids inhibit binding of autoimmune antibody to red blood cells as well as platelets. There is controversy as to whether steroids in the dosages currently employed significantly inhibit antibody production to a previously recognized antigen. However, steroids have been shown to decrease the levels of free and cell-bound autoimmune anti-red blood cell antibody in man following 4-90 days of treatment with 60 mg/day of prednisone. And, with high-dose methylprednisolone, 96 mg for 3-5 days, a significant 22% decrease in serum IgG has been reported in 86% of volunteers 2-4 wk later. Steroids however, do increase the catabolic rate of circulating IgG.

Prednisone at 40 mg/day can inhibit the response to delayed hypersensitivity. Steroids can enhance capillary resistance. The effect of steroids is usually noted within the first 24-48 hr if sufficient steroids are employed, i.e., prednisone, 1 mg/kg. However, a delay of 3-4 days may sometimes be required to effectively inhibit splenic sequestration. In some situations, a “response to steroids” may not be noted until 1 or more weeks of therapy. An effective response to steroids (i.e., platelet count rising to >100,000/cu mm) occurs in 36%-44% of all patients treated. Raising the steroid dosage to 1.5-2 mg/kg will have some effect on the platelet count (although not necessarily to an effective level) in 70%-80% of patients. The sole purpose of steroid implementation is to maintain the platelet count at a safe level, i.e., >50,000/cu mm. There is little value in attempting to bring the platelet count to normal levels. This is often associated with prohibitive levels of steroids, which lead to the well known deleterious side effects of hypercorticism. There is no available evidence that long-term steroid therapy has any effect on the natural history of the disease (following cessation of therapy). On the contrary, there is evidence that long-term large doses of prednisone can occasionally cause thrombocytopenia.

Indications for Splenectomy

A favorable response to moderate steroid dosage, 1 mg/kg, is probably an indication that the spleen is the major site for platelet destruction and that the patient will benefit from splenectomy. Harrington et al. have reported a success rate of 100% in such steroid-responsive splenectomized patients. However, the
duration of follow-up has not been reported. Furthermore, Doan et al. have noted a 79% response to splenectomy in patients who failed to respond to steroids. Splenectomy removes the potential site of destruction of damaged platelets, as well as a significant source of antiplatelet antibody production. After splenectomy, platelet survival may return towards normal within 65 hr to 5–8 days. The antiplatelet antibody, however, is still present in the plasma of patients with ATP, despite an apparent clinical remission, as well as on the platelets of some individuals. The antiplatelet antibody can be demonstrated in vivo by passive transfer to normal recipients and by the classical occurrence of neonatal thrombocytopenia in infants of mothers with splenectomy-induced remission from ATP. It is of interest that splenectomy in a normal individual or animal has no effect on platelet survival. The response of the platelet count to splenectomy is usually immediate with a peak obtained between the first and second week. In one study, all 15 of 22 patients who responded to splenectomy had a peak platelet count >500,000/cu mm, while those who did not have a remission had a peak below this level. Approximately 10%–12% of patients will relapse following initially successful splenectomy, the majority within the first year, but some as long as 5 yr later.

Shulman et al. have shown that six times more antiplatelet factor (obtained from a patient with ITP and infused into a volunteer recipient) is required to significantly lower the platelet count in a splenectomized recipient compared to a nonsplenectomized recipient. The response of the platelet count to splenectomy is usually immediate with a peak obtained between the first and second week. In one study, all 15 of 22 patients who responded to splenectomy had a peak platelet count >500,000/cu mm, while those who did not have a remission had a peak below this level. Approximately 10%–12% of patients will relapse following initially successful splenectomy, the majority within the first year, but some as long as 5 yr later.

Shulman et al. have shown that six times more antiplatelet factor (obtained from a patient with ITP and infused into a volunteer recipient) is required to significantly lower the platelet count in a splenectomized recipient compared to a nonsplenectomized recipient (Fig. 3). This indicates that other reticulendothelial organs, such as the liver, although less sensitive as a "sieve," are still capable of destroying highly sensitized antibody-coated platelets. The response to splenectomy varies from 50%–85% in reported series. However, Aster has suggested that hepatic and splenic sequestration can vary in individual patients with the course and severity of the disease. Hepatic sequestration was found in patients who were more severely affected, e.g., lower platelet counts and shorter platelet survivals. There is some experimental evidence in animals that a compensatory hepatic sequestration follows splenectomy, reaching its maximum 6–8 wk postsplenectomy. This was associated with increased erythropagocytosis by Kupffer cells following treatment with damaged red blood cells. Indeed, patients who relapse following "successful" steroid response can be shown to have developed hepatic sequestration. Nevertheless, Najean et al. have reported a 30% response to splenectomy in patients with exclusively or predominantly hepatic sequestration. Shulman, Baldini, and Aster and Jandl have presented evidence suggesting that the quantity of antiplatelet factor determines the site of sequestration. Splenic sequestration was extremely sensitive to low concentrations of antiplatelet factor following in vivo infusion of "ITP" plasma into volunteer recipients as was platelet survival in nonsplenectomized, immunosuppressed dogs. Higher concentrations of antiplatelet antibody infused in vivo into volunteer recipients resulted in hepatic sequestration.

The above observations indicate that splenectomy is in order for all chronic cases of ATP, i.e., >6 mo duration, regardless of the site of sequestration or response to steroids (if they cannot be maintained free of purpura on 5–15 mg prednisone/day). This is further supported by the clinical observations that unsuccessfully splenectomized patients sometimes respond better to steroids and/or immunosuppressive therapy and that splenectomy makes available to the active platelet pool the 40% of platelets normally sequestered in the spleen. These platelets are enriched with megathrombocytes, which aggregate better in vitro than other platelets.

Risk of Infection and Death Postsplenectomy

The hazard of severe infection (particularly pneumococcal) and rapid death in splenectomized infants and children has been recognized for the past 20 yr. This has been associated with disseminated intravascular coagulation and Waterhouse-Friderichsen syndrome. It has been argued that only children with associated primary disease, such as thalassemia major, Wiskott-Aldrich syndrome, histiocytosis, portal hypertension, etc., are at greater risk to infection and death. What has not been fully appreciated is the accumulative evidence of similar hazards in adults. Whittaker retrospectively studied 77 adult patients with severe pneumococcal infection (meningitis or bacteremia). Eight of these patients (10%) had absent splenic function (seven had splenectomies and one had splenic atrophy). Six patients died. Reasons for splenectomy varied from trauma (2 patients), incidental to surgery (2 patients), acquired hemolytic anemia, spherocytic hemolytic anemia, autoimmune thrombocytopenic purpura, and thalassemia major. The onset of pneumococcal sepsis post-splenectomy varied from 1.5 to 14 yr and averaged 5 yr. Hemostatic defects were observed in 7 patients, and in 4, the diagnosis of disseminated intravascular coagulation could be confirmed at autopsy: hemorrhagic diathesis, fibrin thrombosis of renal glomerular capillaries and adrenal sinusoids, focal ischemic and hemorrhagic lesions of the adrenal cortex, kidneys, liver, gastrointestinal mucosa, ovary, choroid plexus, and myocardium, including 2 patients with the Waterhouse-Friderichsen syndrome. It is of interest that 14
of 19 cases of Waterhouse-Friderichsen syndrome reported in the literature and associated with pneumococcal sepsis had absent spleens or splenic atrophy.144

In contrast, of 523 patients with nonpneumococcal infection, only 1 patient had an absent spleen.144 The findings strongly suggest that the spleen is a main line of defense in the handling of pneumococcal sepsis as well as disseminated intravascular coagulation. The former may operate via the ability of the spleen to produce antipneumococcal opsonins150,153 as well as act as a filter; the latter presumably acts via the clearance of activated coagulation factor products, since reticuloendothelial blockade or splenectomy enhances the hypercoagulable state.152

The splenectomized patient should be carefully scrutinized when developing any fever or infection because of the rapidly fulminant nature of pneumococcal sepsis and death in these patients.

Treatment of Patients Refractory to Steroids and Splenectomy

The role of extended immunosuppressive therapy for the treatment of ATP (particularly azathioprine), although theoretically sound, has not proved overwhelmingly successful in the few patients studied.126,127,135,138 Its use should be reserved for those patients who are refractory to steroids and splenectomy. A controlled study in this regard is lacking. Twelve of 36 documented cases have had an excellent response to immunosuppressive therapy.126,135 Cytoxan has also been used for the treatment of refractory patients with possibly better success than azathioprine.136,135 The disadvantage of cytoxan as well as azathioprine is the approximately 2-mo duration required to obtain an effect. Cytoxan also has the undesirable side-effect of hemorrhagic cystitis. The alkaloid, vincristine, has recently been employed for the treatment of such refractory patients by Ahn and coworkers,154 with benefit in 6 of 7 patients refractory to steroids with intact spleens, 9 of 13 patients refractory to both steroids and splenectomy, and 10 of 10 patients with SLE and thrombocytopenia. Follow-up studies have revealed that 4 of the original 13 patients are in prolonged remission (>6 yr). Five patients require the continued use of vinca alkaloids, and four patients are treatment failures. The drug is unique in that it is both immunosuppressive and thrombocytotic, and when effective, acts promptly (i.e., 7–10 days). Our own limited experience with vincristine in patients refractory to steroids and/or steroids and splenectomy is less enthusiastic. We have experienced the rare patient with prompt and dramatic recovery; as well as the more common situation where there is a small rise in the platelet count of 10–40,000/cu mm, with usual relapse several days later. Side-effects of peripheral neuropathy and intense bone (particularly jaw) pain are not uncommon. Ahn and coworkers have recently introduced a new approach for the delivery of vinca alkaloids to those patients.155 This consists of the in vitro incubation of the patient's platelets (or donor platelets) with vinca alkaloids, prior to the infusion of 1 U of vinca alkaid (vinblastine)-loaded platelets into the patient. The patient's circulating platelet antibody presumably reacts with these platelets and thereby serves to deliver vinblastine-loaded platelets to the RE system. The RE cells that ingest the vinblastine-loaded platelets are presumably "killed" by the vinblastine. They have treated 11 patients who were refractory to intravenous vincristine, as well as splenectomy, steroids, and other immunosuppressive agents, with 1–4 such infusions over a 2–wk period (each containing 2–5 mg of vinblastine). Six patients have gone into remission; 3 of these relapsed and 3 patients have remained in remission for 5–10 mo. Of the remaining 5 patients, 3 were partial responders and 2 failed to respond. Side effects included alopecia, mild confusion, and intense jaw pain. Similar studies have recently been reported by another group156 who noted a complete remission in 2 of 10 patients (7-mo duration). There were 6 transient responses (2–3 wk duration) and 2 complete failures. Of interest was their observation that bound antiplatelet antibody disappeared from the two patients who went into remission, with a T1/2 of 15 days, whereas it did not disappear from the treatment failures. It should be stressed that this interesting approach is still experimental. The long-term side-effects of continued vinca alkaid treatment, as well as RE damage has as of yet not been evaluated.

It cannot be overemphasized that the goal of therapy should not necessarily be directed toward restoration of a normal platelet count, but toward the restoration of a sufficient number of functioning platelets to maintain hemostasis for normal daily activity. This can generally be achieved with a platelet count of 50–80,000/cumm. It is rare for patients to develop purpura in this range if the defect is purely quantitative. Indeed, the claim has been made that only young platelets are hemostatically effective.44

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