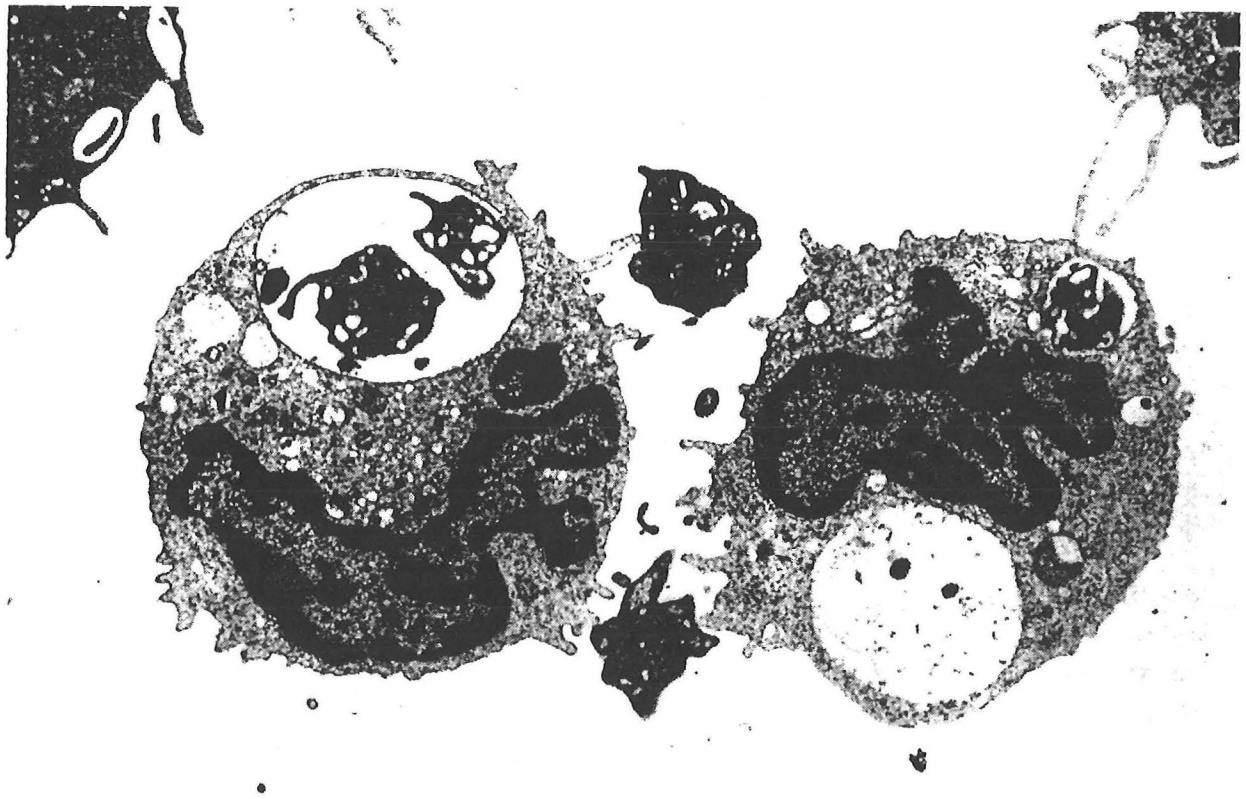
AUTO-IMMUNE THROMBOCYTOPENIA



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Cinical interests

"Benign" hematologic disorders
Disorders of hemostasis

Research interests

New immunological approaches to treating ITP

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A. NORMAL PLATELET PRODUCTION AND LIFE SPAN

Platelets are formed from megakaryocytes in the bone marrow, where the megakaryocyte precursor is one of the progeny of the common stem cell. Initially this diploid precursor is a small nondescript cell. Under the sequential influence of several thrombopoietic cytokines, it undergoes a series of unique endomitotic divisions, resulting in the gigantic bone marrow cell, the megakaryocyte, a cell with 16N or even 32N chromosomes, all contained within one huge, complex nucleus (1, 2). This development takes 7-10 days. While the nucleus is progressively enlarging there is a commensurate increase in the amount of cytoplasm. In this cytoplasm the organelles and granules develop which contain the compounds or their precursors vital for the platelet's role in primary and secondary hemostasis.

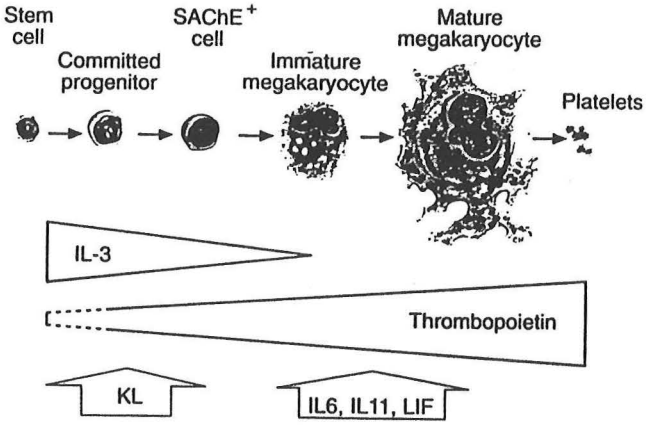


Figure 1: Megakaryocyte development
From Kaushansky 1995 (1)

When the megakaryocyte cytoplasm reaches maturity, the megakaryocyte membrane proliferates and invaginates into the cytoplasm. This forms a network called the demarcation membrane system, which divides the cytoplasm into areas called pro-platelets. Each megakaryocyte is able to produce two to three thousand platelets.

Individual platelets are believed to be released from long pseudopodia which extend from the megakaryocytes into the lumen of the marrow sinusoid. These platelets then enter the peripheral blood, where they circulate approximately seven to ten days.

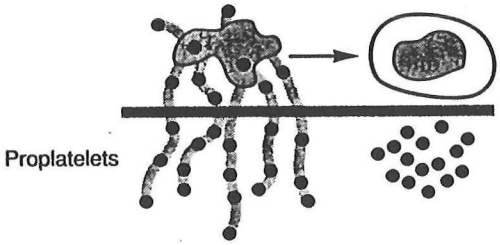


Figure 2: Platelet release from megakaryocytes

Under normal circumstances approximately one-third of the released platelets are sequestered within the spleen. There is also a random loss of platelets as they repair breaches in the endothelium, which consumes the equivalent of 25-40,000 platelets/mm^3 per day.

Individual platelets are enucleate structures, approximately 2μ in diameter, 10 femtoliters in volume. Their complex membranes, formed from the demarcation membrane system contain a number of functionally important glycoproteins. including adhesive molecules such as GPIb-IX (Von Willebrand's factor), GPIa/IIa (collagen), GPIc/IIa (fibronectin), and others such as GPIIb-IIIa, involved in platelet-platelet aggregation via fibrinogen "bridging." The membrane also contains receptors for the Fc (constant) portion of immunoglobulin molecules and complement receptors (3).

B. HISTORY OF ITP (4, 5)

IN 1881 Bizzorzero first correctly identified platelets as the essential component for primary hemostasis . It was a decade later before megakaryocytes were described, and it was not until the turn of the century that their role in platelet production was recognized. In 1915, Frank observed that patients with immune thrombocytopenic purpura (ITP), or as it was known then, *purpura haemorrhagica* (non-febrile purpura) had normal or even increased numbers of megakaryocytes in the bone marrow, but a markedly diminished number of platelets in the peripheral blood. Frank took this as evidence for defective platelet production, especially as the megakaryocytes looked different from normal, as their number included many immature forms. Many others, including the influential Minot in Boston, were convinced that platelet production in the marrow was normal, and the low platelet count was due to excessive platelet destruction in the periphery , a situation analogous to hemolytic anemia. They believed this *idiopathic* thrombocytopenic purpura (ITP) was the platelet equivalent of hemolysis, with platelets rather than red cells being destroyed prematurely in the spleen. The cause of this premature destruction was not known.

This line of reasoning led to a trial of splenectomy for ITP, which became the mainstay, essentially the only effective treatment for ITP for several decades (6). The surgeon's maxim "in wet, out dry" recognized that hemostasis was usually dramatically improved as soon as the splenic artery was cross clamped. The platelet count was observed to rise immediately, often reaching supranormal levels in the first 24-48 hours. The reason for the platelets' premature removal by the spleen was still not understood, although many believed some circulating factor was responsible, especially when it was observed that a pregnant woman with ITP could give birth to a baby with thrombocytopenia.

In 1950 William Harrington, a 27 year old Boston-trained physician (7), began a hematology fellowship with the eminent Dr. Carl V. Moore at Washington University Medical School in St. Louis. There was such intense competition for this position, that the two finalists, Harrington and James Hollingsworth agreed to share the salary. Harrington had become very interested in ITP while a medical student at Tufts in Boston, and was convinced that some substance circulating in the blood was responsible. He was determined to test this theory. One weekend, while Dr. Moore was away, a patient with severe ITP was admitted. This patient had

previously had her spleen removed, but continued to hemorrhage. Harrington realized this was the case he needed to prove his theory, and persuaded Hollingsworth to join him in the experiment, which is graphically described by Lawrence Altman in *Who Goes First. (8)*

Harrington determined that they would take a pint of this patient's blood and administer it to the one who matched her blood type. This individual would donate a pint of blood to be infused into the patient. Scientific rigor demanded a bone marrow examination before and after the infusion, to confirm the numbers of megakaryocytes had not been affected, and serial platelet counts on both patient and subject would be measured. Harrington, who as luck would have it, had the same blood type as the patient, donated his pint of blood to be given to the patient; he was so excited he did not feel the sternal bone marrow Hollingsworth performed on him. He was then infused with the patient's blood and both young physicians set out to perform the serial platelet counts. Harrington began to feel increasingly unwell, and was alarmed to find his platelet count had fallen to almost unrecordable levels. Hollingsworth was even more aghast when he observed Harrington have a *grand mal* seizure. It finally dawned on them that the experiment they had undertaken was potentially very dangerous. Fortunately Harrington recovered and went back to counting platelets, without further untoward events. When Dr. Carl Moore returned the next day they confessed, and the alarmed Moore insisted Harrington be hospitalized. His platelet count returned to normal with the next four to five days, fortunately without further incident.

Once the worry about Harrington was over the three became very excited about the success of this "experiment." Over the next few months nearly everyone working in the department, from Dr. Carl Moore himself to the department secretaries, became subjects for further experiments, although they used much smaller volumes of patient plasma. Harrington gave himself 35 more infusions of ITP plasma, proving without doubt that it contained an anti-platelet factor. By the time his paper describing this was published, the thrombocytopenic factor was known to be part of the globulin fraction of the plasma (9).

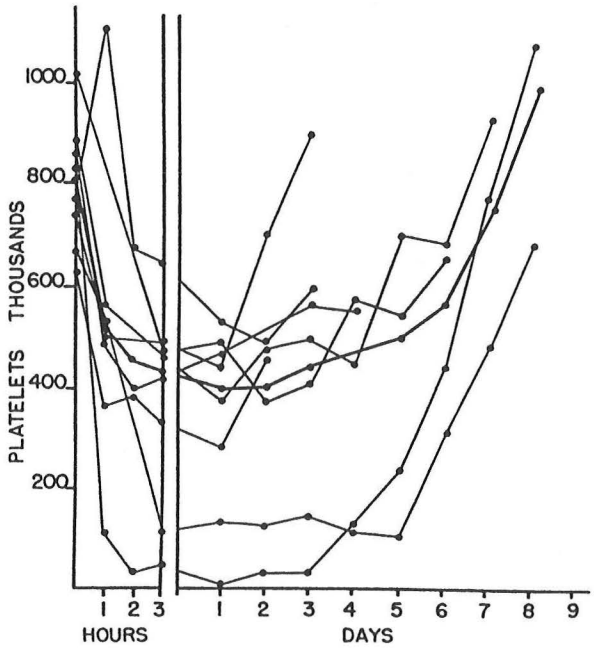


Figure 3: Infusion of normal subjects with blood or plasma from a patient with ITP (9)

The method pioneered by Harrington was subsequently used many times to further elucidate the mechanisms of ITP. The response of splenic and asplenic subjects, the response with and without corticosteroids, and the effect of stored autologous serum on patients in remission were studied. The thrombocytopenic factor was localized to the IgG immunoglobulin fraction, and found to have the characteristics of an antibody: it could be adsorbed from ITP serum by normal platelets, it had species-specificity and reacted *in vivo* like well-characterized anti-platelet allo-antibodies and drug antibodies. The I of ITP, which previously had stood for *idiopathic*, came to stand for *immune* thrombocytopenic purpura.

C. PATHOPHYSIOLOGY OF ITP

1. Antiplatelet antibodies

Patients with ITP have been shown to have increased number of platelet-associated IgG and IgM molecules, both adherent to platelet membrane and within the platelet cytoplasm (10, 11). However, even in normal subjects, immunoglobulins, particularly IgG, adhere non-specifically to the platelet membrane, through its many immunoglobulin Fc receptors (12). Thrombocytopenia from platelet consumption of any cause is usually associated with an increased number of molecules of IgG per platelet, some of which may reflect their larger size. Patients with an increased serum IgG for any cause (myeloma, liver disease, AIDS) similarly show increased amounts of IgG bound to their platelets through membrane Fc receptors (13). Thus measurements of platelet-associated IgG, a test akin to the direct antiglobulin (Coombs') test for red cells has been too non-specific to be clinically useful (14). Serum measurements of circulating anti-platelet antibodies have been even less helpful.

More recently auto-antibodies directed at important functional glycoproteins have been identified in ITP, particularly antibodies to GPIIb/IIIa and GPIa/IX (15-19). Antigen capture techniques, using monoclonal antibodies to specific glycoproteins, enable measurement of antibodies bound through their Fab component (20). These techniques involve exposing purified glycoproteins immobilized on beads or in microtiter wells to ITP serum or solubilized platelets, and demonstrating their presence by adding a labeled anti-human antibody. It is possible, especially in the acute ITP seen in children that the viral infection or other alterations of thesehese glycoproteins, renders them antigenic.

2. Mechanisms of Platelet destruction

Phagocytosis of antibody-coated platelets is well recognized, with the membrane-associated antibody binding to the Fc receptor of the macrophage (21, 22). These events have been dramatically captured by electron microscopy (23).

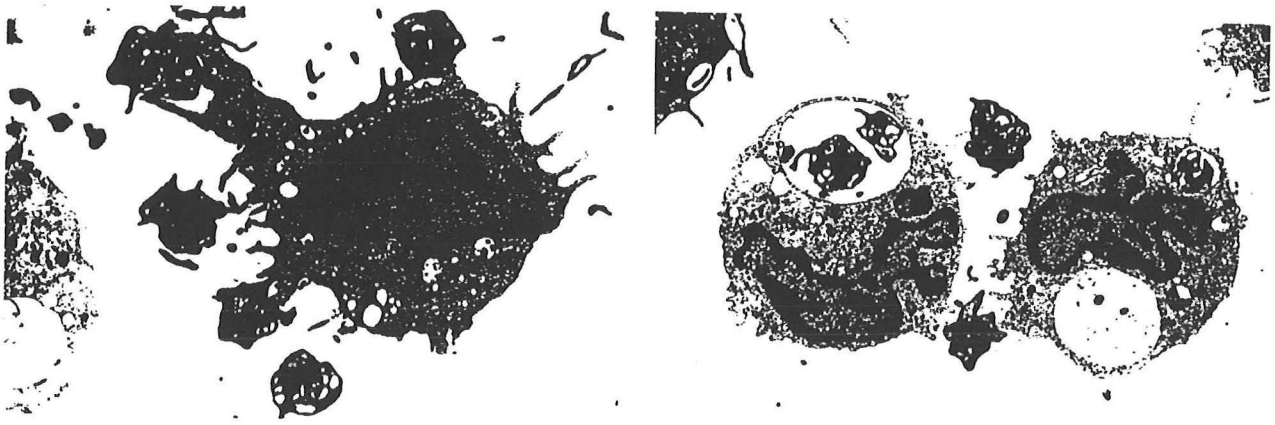


Figure 4: Transmission electron microscopy of phagocytes binding and ingesting platelets (23)

The early recognition of splenectomy as the most effective treatment modality in ITP suggested that much of this phagocytosis occurred in the spleen. However, other parts of the reticulo-endothelial system (RES) are clearly involved, suggested by the 20-30% of patients who do not respond to splenectomy and by studies using labeled platelets which demonstrate uptake in liver also (24). There may also be some intravascular destruction of platelets via the complement pathway (25). Removing the spleen has two potential benefits: it removes an important source of antibody production, and removes a stringent micro-environment, wherein the antibody-coated platelets come in close opposition to avid phagocytes.

Experiments using infusion of ITP plasma into splenectomized and non-splenectomized subjects, show that a significantly higher concentration of antibody is needed to produce thrombocytopenia in splenectomized subjects; the presence of the spleen increases the efficiency of destroying antibody-coated platelets.

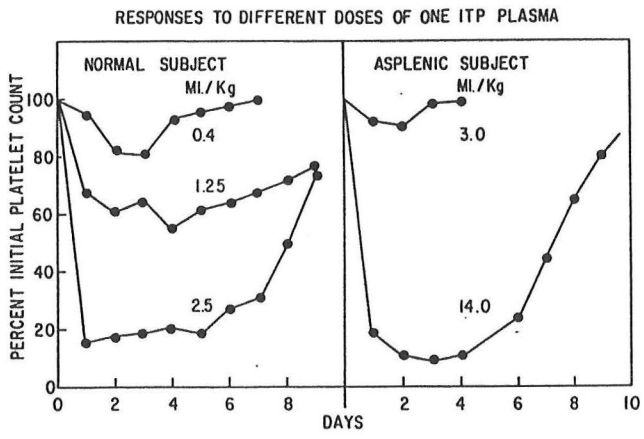


Figure 5: Infusion of ITP plasma to individuals with and without a spleen(26)

Chromium-labeling of platelets has shown they have a very short survival in ITP, with a half life of only a few minutes in severely affected individuals. As expected, half lives are proportional to the platelet count (27).

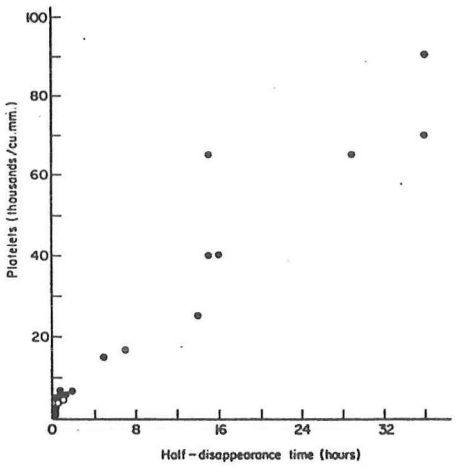


Figure 6: Platelet survival in ITP (27)

It was always assumed that production of platelets was increased in ITP, because of the frequently observed increase in number of megakaryocytes in the marrow and the larger-than-normal platelets observed in the peripheral blood. In 1977 Harker demonstrated that in ITP platelet production from megakaryocytes could increase up to five fold (28). However, recent evidence suggests impaired platelet production or release in up to half the cases of ITP. Antibodies from ITP spleens have been found to bind to megakaryocytes. This may interfere with platelet production, possibly by binding to the membrane demarcation system, or by labeling the released platelets so that they are removed by marrow macrophages, before they get a chance to enter the peripheral circulation (29-31)

The stimulus to megakaryocyte proliferation in ITP is poorly understood. Although thrombopoietin levels are a little higher than normal, they are significantly less than in hypoproliferative thrombocytopenic states (32).

3. Function of platelets in ITP

Because of their short half life, most of the platelets circulating are newly produced. This can be confirmed by determining the reticulated *platelet count,* whereby the percentage of young platelets, still containing RNA are measured by flow cytometry, analogous to the red cell reticulocyte. This technique is useful in distinguishing thrombocytopenia from defective production from that of consumptive disorders, but is still a research tool. In ITP, reticulated platelet counts are significantly higher than those in patients thrombocytopenic from AML or in normal subjects (33). These young platelets are larger than normal size when seen with electron microscopy or in the peripheral blood smear. The young platelets in ITP function very well(34). It is well recognized that bleeding times in ITP are much shorter than in thrombocytopenia from hypoplastic causes.

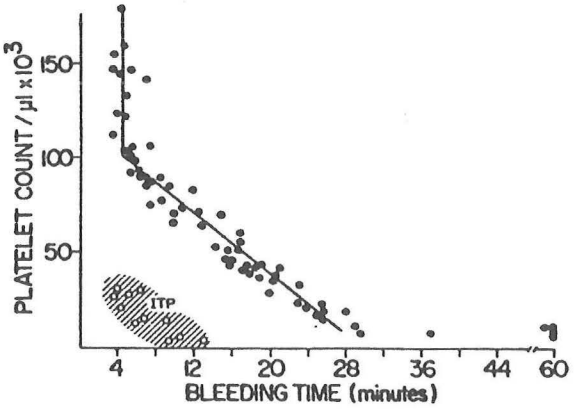


Figure 7: Relationship between bleeding time and platelet count (35).

D. THE CLINICAL PRESENTATION OF ITP

ITP is a relatively common hematological disorder, and occurs in both children and adults. Its estimated incidence is 1:10,000, but it may occur even more frequently. It is appreciated more often, now that mild asymptomatic cases are picked up by automated complete blood count (CBC) counting.

ITP in children is significantly different from that seen in adults, and is usually an acute and short-lived illness following a viral infection. This review will focus on the disorder as it occurs in adults, so-called chronic ITP (36).

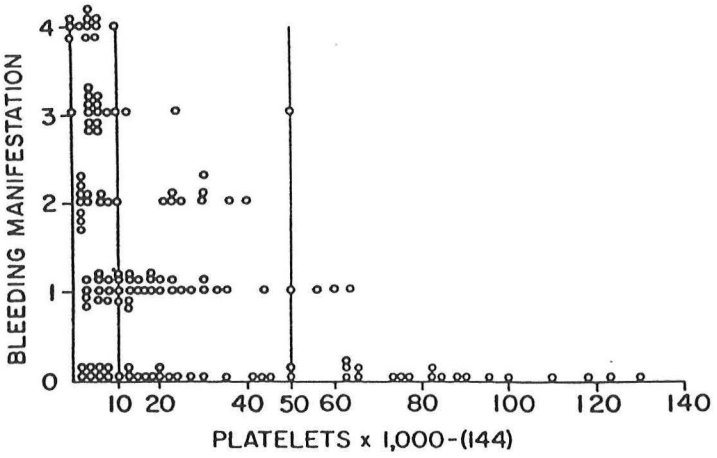
Table 1: COMPARISON OF ACUTE & CHRONIC ITP

	Acute	Chronic
Peak incidence	2-9 years	20-40 years
F:M ratio	1:1	3:1
Prior infection	Common	Uncommon
Platelet count	<20K	10-100K
Onset	Acute	Gradual
Duration	2-6 weeks	Years
Response to steroids	Uncommon	Common
Response to splenectomy	Variable	Common

The clinical presentation of chronic ITP is essentially that of a defect in primary hemostasis. Bleeding manifestations correlate with the degree of thrombocytopenia and include, in order of severity: skin bruising, muco-cutaneous bleeding such as epistaxis, petechiae in skin and mucous membranes. The most feared bleeding complications, fortunately rare, are internal, particularly intracerebral bleeding and bleeding obstructing the airway, seen in 1-2% of cases. With the highly functional platelets in this condition, few serious clinical manifestations are seen, unless the platelet count is very low, even then many patients have modest symptoms (37).

Figure 8: Bleeding manifestations in relation to platelet count (37)

4=massive bleeding;
3=serious spontaneous bleeding;
2=limited spontaneous bleeding;
1=minor bleeding
0=no bleeding



ITP is essentially a diagnosis of exclusion, both in its clinical presentation and in the laboratory. Clinical manifestations are limited to signs of bleeding. Clinical features such as splenomegaly should raise other diagnostic possibilities. The conditions in Table 2 should be considered. Most can be excluded by evaluation of the history, basic clinical examination and CBC with smear review. History taking should include questions about symptoms of viral infection, about medications, alcohol intake, HIV risk factors and symptoms suggestive of systemic lupus erythematosus. Physical examination should search carefully for splenomegaly, which is not expected in primary ITP, and for manifestations of the other disorders associated with thrombocytopenia.

Table 2: Differential diagnosis of chronic ITP

Spurious thrombocytopenia - EDTA or other agglutinins

Common causes of thrombocytopenia that mimic ITP
- Pregnancy - gestational thrombocytopenia
- Drugs - quinine, quinidine, heparin . . .
- Viral infections, especially AIDS
- Hypersplenism due to chronic liver disease

Disorders with 2^{o} immune thrombocytopenia
- Systemic lupus erythematosus
- Lymphoproliferative disorders: CLL, Non-Hodgkin's lymphoma
- HIV infection

Less common causes
- TTP/HUS
- Myelodysplasia
- Wiskott Aldrich syndrome
- Type 2B von Willebrand's disease
- Chronic disseminated intravascular coagulation (DIC)

Laboratory assessment with CBC should show an isolated thrombocytopenia, without abnormalities in other cell counts, unless there is anemia from significant bleeding. The blood smear should always be examined and should be normal apart from thrombocytopenia, usually with large platelets. It is particularly important to examine the smear for platelet clumping of "spurious" thrombocytopenia, and for schistocytes, to exclude thrombotic thrombocytopenic purpura (TTP).
See Appendix 2 for a scheme for laboratory evaluation of thrombocytopenia (38).

In 1996 our thinking about both diagnosis and management of ITP was challenged by the publication of practice guidelines by the American Society of Hematology (39). As the editorial accompanying its publication in *Blood* states (40)

> . . . our eyes were opened to the fact that most of what we do with ITP patients is based on case series of selected patients, whose course cannot be evaluated in the absence of a control group, and whose outcomes were only evaluated by platelet counts. There is a lack of knowledge about the natural history of untreated ITP and of the effect of treatment on clinical outcomes of major bleeding and death.

The guidelines were developed by a panel of 15 hematologists experienced in the management of ITP, who evaluated the available literature, using specific criteria to assess validity. They then voted on nearly 2000 questions regarding diagnosis and treatment, and presented the majority vote as their recommendations.

If history, examination initial CBC and peripheral smear are compatible with a diagnosis of ITP, minimal further testing is indicated. The panel's consensus proposed the only further tests needed were:

1. HIV antibody, if there are risk factors for HIV
2. Bone marrow examination in patients over 60 years, and in patients being considered for splenectomy.
3. Thyroid function tests before elective splenectomy

They corroborated our recommendation that routine bone marrow aspiration was not required(38). Figure 9 shows the wide range of opinions amongst the panel hematologists in answer to the question *Is it necessary/appropriate to order bone marrow aspiration/biopsy to establish the diagnosis of ITP in all adult patients at presentation?* This assumes a history, exam and basic CBC consistent with the diagnosis of ITP. "1" reflects total disagreement with the premise, "9" total agreement.

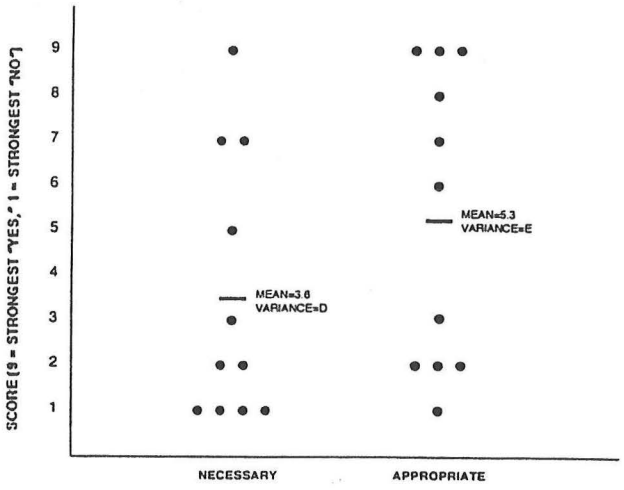


Figure 9

The guidelines also recommended against bleeding time, abdominal imaging studies to detect splenomegaly or routine platelet antibody tests, because of the latter's poor sensitivity and specificity.

E. TREATMENT OF ITP (31, 41-45)

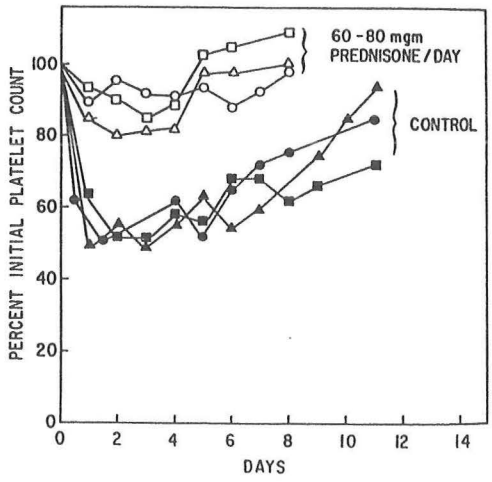
Many patients diagnosed with ITP do not require treatment. The decision to treat should be based on both the degree of thrombocytopenia and associated symptoms. (See Appendix 1 for details of the specific ASH guidelines). Treatment is not recommended in patients with platelet counts of 30,000/mm^3 or more, unless there are significant bleeding symptoms. Once the platelet count gets below 20,000/mm^3, at which level patients are usually symptomatic, therapy is recommended.

Treatment options for ITP are clearly divided into first line therapy and "the rest." First line therapy includes corticosteroids and splenectomy, the combination of which is effective in 60-70% of patients. For the significant numbers of symptomatic patients who do not respond to first line treatment, there is a long list of alternative options, none subjected to randomized analysis, and most reported in fairly small series.

1. Initial treatment of ITP

a. Corticosteroids: These have been the sheet anchor for initial treatment of ITP since the 1950s. They appear to work in two ways: in the early phase of treatment they reduce the number of Fc receptors on macrophages, particularly in the spleen; long term they appear to suppress platelet antibody production. After nearly 50 years of use there is no major randomized study of this treatment modality. There is consistent evidence, from uncontrolled studies, that gluco-corticoids improve platelet counts in most patients; unfortunately most responses are not durable. The ASH panel concluded that glucocorticoid therapy with prednisone at a dose of 1-2 milligrams per kilogram was the appropriate initial treatment for patients with platelet counts of less than 30,000/mm^3, or at higher levels if significant bleeding attributable to thrombocytopenia was present. Steroids should be continued until there is a response and than tapered over a month. If no response in platelet count is seen after 4-6 weeks, or if there is a drop in platelet count as prednisone is tapered, a prompt splenectomy is usually indicated.

Figure 10 shows the protective effect corticosteroids steroids in reducing the severity of thrombocytopenia from passive infusion of ITP plasma. This likely reflects the reduction of phagocyte Fc receptors (26).



b. Splenectomy: most adult patients with symptomatic ITP eventually require a splenectomy. For this reason, vaccination against *pneumococcus, meningococcus* and *hemophilus influenzae B* should be given, as soon as the platelet count will safely allow. For patients who eventually need splenectomy, approximately 60% will have sufficient control of their platelet count long-term that they do not need corticosteroids or other maintenance therapy. Patients who need splenectomy, but who are thrombocytopenic may need special measures to get their platelet count to safe levels for the surgery. Such treatments include RES blockade with intravenous gammaglobulin (IVIG) or Rh immune globulin, or a repeat course of corticosteroid therapy for patients who have initially responded to this medication. Most patients with ITP can safely have laparoscopic splenectomy.

2. Emergency Treatment of ITP

Sometimes patients with chronic ITP present with acute life-threatening bleeding. The ASH panel endorsed our practice in this situation: high-dose parenteral gluco-corticoid therapy in the form of methyl prednisolone, one gram daily for three days. In dire situations simultaneous IVIG may be used. Platelet transfusion usually gives very little increment, as expected from the tagged platelet survival studies, but will often be included in emergency management, particularly if there is intracerebral bleeding. IVIG given simultaneously with platelet transfusion may enable longer circulation of transfused platelets.

IVIG is an important medication in the acute management of ITP. Its mechanism of action is through RES blockade: Fc receptors, occupied by the infused gammaglobulins, cannot bind to antiplatelet antibodies on the platelet surface, so platelets escape phagocytosis. Unfortunately, IVIG has a short duration of action, usually one or two weeks at the most. This expensive (and sometimes scarce) medication for should be saved for bleeding emergencies or for preparing a patient for splenectomy or other surgery. Studies have shown that a more rapid rise of platelet count occurs with IVIG than with Prednisone alone. However, no difference in outcome was seen in a randomized study comparing initial treatment with Prednisone, IVIG, or both in combination (46). The dose of IVIG varies in different studies; we usually give a total dose of 2 grams per kilo, spread over 4-5 days. Apart from the expense of IVIG there are some untoward side-effects, including fevers, chills, headaches, vomiting. Some IVIG preparations contain immune complexes which can produce renal and other problems; for this reason we do not usually give doses above 0.5 gram per kilo per day.

Rh immune globulin is an alternative method for inducing temporary RES blockade (47-49). This preparation contains antibody directed at the Rh(D) antibody, and is only effective in patients who are Rh-positive. In such patients the administration of intravenous Rh immune globulin, usually in a single dose, binds to the red cells causing a mild hemolysis (hemoglobin drop of 1-2g/dL); these antibody coated red cells block macrophage Fc receptors. This effect lasts only as long as the blockade persists, which is about 7-14 days. Like IVIG it is only appropriate for

patients with acute bleeding problems or patients being prepared for surgery. Because of recent shortages of IVIG, we have been using Rh immune globulin therapy. However, it has not had any specific controlled trial in chronic ITP; moreover it is only licensed for use in patients who still have a spleen.

3. Treatment of refractory ITP

Approximately 30% of patients with symptomatic ITP do not respond to steroids and splenectomy, and constitute a major treatment challenge. Such patients appear to have high levels of antiplatelet antibody, in conjunction with very active extra-splenic RES activity. There are numerous alternative treatments for patients still at symptomatic platelet counts after splenectomy, but none are very effective. We usually start with the least morbid of these treatments, and will often try several different treatments in sequence. They include:

a. Danazol. Since this anabolic steroid treatment was first described fifteen years ago (50) there have been numerous reports of efficacy in uncontrolled studies. Danazol is usually given in doses of 200-800 mg. a day, with response rates varying from 10 to 80%. It often takes a couple of months to demonstrate efficacy. Platelet counts seldom normalize, but may increase to asymptomatic levels. Danazol's major risk is liver dysfunction. It is more regarded for its "steroid sparing" effect.

b. Immunosuppression with cyclophosphamide, cyclosporine and azathiaprine. None are very effective and all have a fairly high side effect protocol. None of these agents have had any controlled trials.

c. Vinca alkaloids, such as vincristine and vinblastine, may produce a transient improvement in platelet count, but few patients have a sustained response. These medications have troublesome side-effects, particularly peripheral neuropathy in the case of vincristine and neutropenia in the case of vinblastine.

d. Plasma exchange. While theoretically attractive, has not been found to have any long-term effect on ITP in the several series reported. Modification of plasma pheresis using staphylococcal protein A immunoabsorption columns has also been reported to have modest benefit in this group of patients with severe refractory thrombocytopenia (51).

e. Pulse decadron therapy. Great enthusiasm greeted a paper suggesting that monthly 4 day pulses of high dose Decadron benefited most patients with refractory ITP (37, 52, 53). Unfortunately several other groups could not corroborate these results (54).

Table 3 represents outcome data from 12 different large series of patient with ITP from 12 different countries. These suffer from the limitations of collated data, , mostly from small retrospective series. It is surprising that in relatively common disorder there is not more helpful information. These available data show ITP has a low rate of spontaneous remission, but a fairly effective treatment strategy. Even for those patients who remain thrombocytopenic there is a low mortality from bleeding complications.

Table 3: OUTCOME OF ITP IN ADULTS
1761 patients from 12 studies

Complete Remission without therapy	27	(1.5%)
Patients in complete remission	1027	(64%)
Hemorrhagic complications (first 6 months)		
Acute deaths		
Intracerebral hemorrhage	36	(2%)
Other Hemorrhage	7	(<1%)
Other deaths	35	(2%)
Patients with persistent thrombocytopenia	465	(26%)
Later spontaneous recovery	22	(5%)
Later hemorrhagic death	25	(5%)

from George et al 1996(39)

F. OTHER DISORDERS WITH AUTO-IMMUNE THROMBOCYTOPENIA

1. ITP in HIV infection

Thrombocytopenia has been known to be a manifestation of HIV infection since the earliest days of the epidemic (55). ITP is usually an early manifestation of HIV infection, often occurring at quite robust CD4 counts (390/mm^3 in one study), before clinically overt AIDS develops. HIV associated thrombocytopenia is not predictive for progression of disease (56). In HIV infection there are two components to the thrombocytopenia: an immune-mediated platelet destruction and failure of platelet production, associated with infection of the megakaryocyte with HIV. Viral particles have been seen within megakaryocyte cytoplasm.

HIV-associated ITP is clinically almost identical to primary ITP, other than the sex association. However, the antibodies coating the platelet are different. Eluates of these platelet antibodies seldom react against the antigens of normal platelets. The immunoglobulins bound to platelets are usually part of immune complexes, which include antibodies directed against different components of HIV, including GP120 and HIV p-24 (57). At times the platelets are very heavily coated, and a marked increase in platelet-associated IgG, IgM and complement components can be

measured. Platelet kinetics have shown that HIV-related thrombocytopenia is associated with increased splenic platelet sequestration and destruction.

As in primary ITP, asymptomatic mild or moderate thrombocytopenia may not require treatment and patients seldom have bleeding problems until the platelet count is below 30,000/mm^3. Anti-retroviral treatment is the initial treatment of choice, particularly with zidovudine. Only a minority of patients completely normalize their platelet count, but up to 60% have sufficient improvement to spare them other treatments or troublesome symptoms. This treatment particularly helps patients where production failure, associated with megakaryocyte infection, is a significant component of the thrombocytopenia.

For those whose thrombocytopenia is due more to immune destruction, the same treatment modalities used in primary ITP are effective. A majority of patients have improvement of their platelet count from corticosteroids. Responses to corticosteroids are often not sustained; moreover the side effects of steroids are particularly unwelcome in these patients. Very short pulses of dexamethazone can been effective (58). For patients with symptomatic thrombocytopenia, not responsive to medical therapy splenectomy is indicated (55). Despite the different immune mechanism for HIV-associated thrombocytopenia, splenectomy has proven a very effective treatment (59), with significant improvement in platelet count in 80% of patients. Interestingly, the CD4 count often increases after splenectomy, presumably because of different distribution. There were fears initially that splenectomy might accelerate the onset of AIDS and might be associated with significant worsening of infective problems. This has not been confirmed.

A number of the other second line treatments discussed in the section on primary thrombocytopenia also effective in HIV-associated thrombocytopenia and have had limited trial, including IVIG, Rh immune globulin, Dapsone and Danazol.

2. ITP and Systemic Lupus erythematosus (SLE)

Less than 2% of patients initially diagnosed with ITP subsequently develop SLE, although many patients with SLE have auto-immune thrombocytopenia during the course of their illness. The antinuclear antibody test (ANA) has limited utility in terms of predicting whether SLE will develop, as 10 -25% of patients with ITP have a positive ANA. The few patients with ITP who go on to develop SLE usually have multiple serum antibodies to nDNA, or other soluble cytoplasmic or nuclear antigens, so it may be appropriate to perform these more specific tests when the ANA is positive. A recent study from Mexico showed a much higher rate of progression to SLE (12%) in long-term follow-up of patients who had required splenectomy for ITP (60) ASH guidelines did not recommend an ANA in the initial evaluation of ITP.

The auto-immune thrombocytopenia of SLE is very similar in clinical and laboratory manifestations to that of primary ITP, and the same treatment modalities

are effective and appropriate, including corticosteroids, intravenous immunoglobulin for acute hemorrhagic problems and splenectomy (61). Concerns for long term infective complications post-splenectomy are higher in this patient group, and benefit is not as clear cut as in primary ITP. With this proviso, it seems reasonable to use the ASH ITP guidelines in selecting treatment (39). Patients who do not respond to steroids or splenectomy in SLE may be treated with the same second-line treatments; Danazol is particularly helpful because of its "steroid sparing" effect in this group of patients for whom corticosteroid complications are a significant problem (62). Dapsone, which has a limited role in ITP management (63), has been helpful in resistant SLE-ITP, and can benefit discoid skin lesions simultaneously (64).

Thrombocytopenia in SLE is a poor prognostic sign; one recent cohort study found the risk of SLE-associated mortality was twice as high among patients with thrombocytopenia as in those without (65).

Antiphospholipid antibodies were first described in SLE. They comprise a large family of circulating immunoglobulins, which include anti-cardiolipin antibodies and lupus anticoagulants, both of which may be associated with venous and arterial thrombosis, fetal loss. Thrombocytopenia occurs in 40% of these patients A primary antiphospholipid antibody syndrome is now well recognized, with a similar incidence of thrombocytopenia. In patients with either primary or SLE-associated antiphopholipid antibody syndrome and thrombocytopenia, a majority have been shown to have antibodies against platelet surface glycoproteins such as GPIIb-IIIa or GPIb-IX (66) . In contrast, in patients with the antiphospholipid antibody syndrome, but no thrombocytopenia, this type of anti-glycoprotein antibody is very uncommon.

G. NEW DIRECTIONS FOR TREATMENT OF ITP

ITP is one of a group of immune disorders in which a completely new approach to immune suppression is being tried, utilizing an antibody which interferes with lymphocyte signaling and activation. Antibody production by B lymphocytes and activation of other immune cells require a specific interaction with T lymphocytes(67). This B cell/T cell interaction is mediated through several receptor-ligand binding events, including CD40 with its ligand, CD40L (68-70). Human CD40 is expressed primarily on B cells, monocytes, endothelial cells, fibroblasts, dendritic cells, T cells, and renal epithelial cells. CD40 belongs to a class of receptors involved in cellular activation/proliferation and apoptosis. The interaction of CD40 and CD40L occurs independently of major histocompatibility complex (MHC) and T cell receptor interactions and typically provides anti-apoptotic signals and lymphokine stimulatory signals (71).

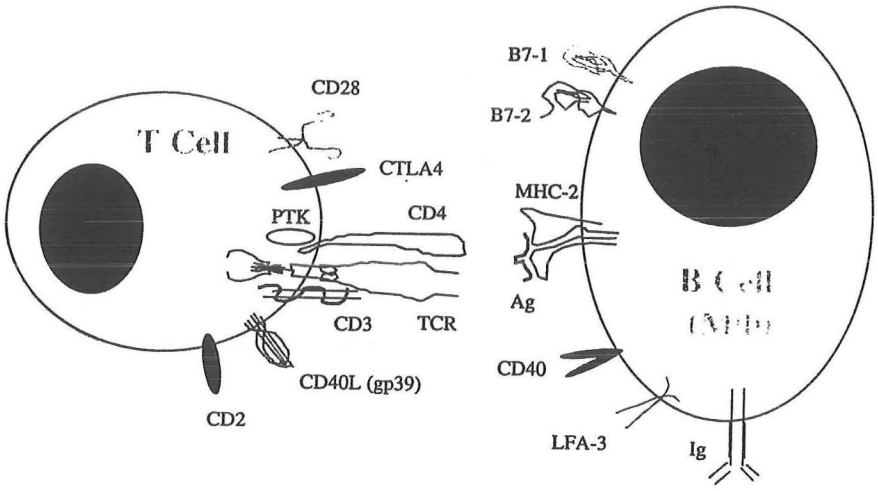


Figure 11: T and B lymphocyte interactions

Many animal models have demonstrated therapeutic effects to an antibody developed to the CD40L. Administration of anti-CD40L antibody to lupus-prone mice substantially reduced the anti-double stranded DNA, delayed the development of nephritis, and reduced mortality. Anti-CD40L antibodies given with murine models of rheumatoid arthritis, inflammatory bowel disease, fibrosis in the lung, multiple sclerosis, graft-vs-host disease, and arteriosclerosis showed prevention and delay of the disease.

A murine monoclonal antibody directed towards the CD40 ligand has been "humanized" for studies in ITP, lupus nephritis and post-transplant immune suppression. A phase I trial of this agent showed an excellent safety profile (72). A phase II trial is currently nearly completed, with preliminary results sufficiently encouraging that a Phase III trial is in the active planning stages.

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Appendix 1 Initial treatment options for adults with idiopathic thrombocytopenic purpura, according to an expert panel (42)

PLATELET COUNT AND BLEEDING STATUS*	PANEL AGREEMENT	APPROPRIATENESS UNCERTAIN
Platelet count 50-100 x 10^9/L		
Asymptomatic	Observation	NR
Minor purpura	Observation	NR
Mucous membrane bleeding	NR	Observation, glucocorticoids, hospitalization
Platelet court 30-50 x 10^9/L		
Asymptomatic	NR	Glucocorticoids
Minor purpura	NR	Glucocorticoids
Mucous membrane bleeding	Glucocorticoids±	Hospitalization, IV IgG§
Severe bleeding	Glucocorticoids, IV IgG, hospitalization	
Platelet court 20-30 x 10^9/L		
Asymptomatic	NR	Glucocorticoids, IV IgG
Minor purpura	Glucocorticoids	IV IgG
Mucous membrane bleeding	Glucocorticoids	Hospitalization, IV IgG
Severe bleeding	IV IgG, glucocorticoids, hospitalization	Splenectomy
Platelet court 10-20 x 10^9/L		
Asymptomatic	Glucocorticoids	Hospitalization, IV IgG
Minor purpura	Glucocorticoids	Hospitalization, IV IgG
Mucous membrane bleeding	Glucocorticoids, hospitalization	IV IgG
Severe bleeding	IV IgG, glucocorticoids hospitalization	Splenectomy
Platelet court < 10 x 10^9/L		
Asymptomatic	Glucocorticoids	IV IgG, hospitalization
Minor purpura	Glucocorticoids	IV IgG, hospitalization
Mucous membrane bleeding	Glucocorticoids	IV IgG
Severe bleeding	IV IgG, glucocorticoids hospitalization	Splenectomy

* Mucous membrane bleeding includes vaginal bleeding and other blood loss requiring clinical intervention; severe bleeding includes life-threatening bleeding; "Severe" category not included for platelet counts of 50 to 100 x 10^3 per mm^3 (50 to 1000 x 10^9 per L) because severe bleeding in such patients is unlikely to be caused by ITP

= No recommendation

± Prednisone 1 to 2 mg/kg/day or an equivalent drug

§ Intravenous immune globulin 1 to 2 g/kg, for 1 to 5 days

Appendix 2: A scheme for evaluating thrombocytopenia in the laboratory (from reference 38)