The Pathophysiology of Immune Thrombocytopenic Purpura
An Evolving Perspective

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder characterized by thrombocytopenia, a decreased number of circulating platelets. It can lead to symptoms such as nose bleeding (epistaxis), localized hemorrhaging in skin or mucous membranes that appear as red or purple spots (petechiae), and bruising (purpura). More rarely, it can be associated with severe bleeding events. ITP results from platelet destruction, and evidence is increasing that platelet production is also impaired in the disorder. In children, the onset of ITP is often acute, and the condition usually resolves without treatment. In adults, ITP is frequently insidious in onset and chronic, rarely resolving spontaneously. Among middle-aged adults, ITP affects women more than men, although the female predominance is not evident in patients older than 60 years.

Although purpura has been known since antiquity, it was not until the late 19th and early 20th centuries that significant breakthroughs were made in recognizing its underlying mechanisms and 2 seemingly opposing hypotheses were put forth, both of which were subsequently accepted as correct. One hypothesis was that ITP results from excessive destruction of platelets by the spleen, and the other centered on the role of impaired platelet production by megakaryocytes, the bone marrow cells that produce platelets. An advanced understanding of molecular and cellular physiology has since provided important insights into the pathophysiology of ITP, most recently the discovery of the role of thrombopoietin (TPO), the hormone that regulates the production of platelets. This article maps the key events in this history, describing the path of our growing understanding of the pathophysiology of ITP and its implications for novel treatment strategies.

Historical Perspectives on the Pathogenesis of ITP

Purpura, termed after a gastropod mollusk that yields a purple dye, has been recognized as...
a clinical symptom as early as the Greco-Roman period by physicians such as Hippocrates and Galen, who described the condition as red “eminences” or spots associated with pestilential fevers. It was not until the Middle Ages that differentiation of types of bleeding was first recorded. In the 10th century, the Arab physician Avicenna briefly described chronic purpura, and in 1658, the French physician Lazarus Riverius suggested that purpura was due to a “thinness of the blood” and could occur in the absence of fever. In 1735, the German physician Paul Gottlieb Werlhof provided the classic clinical description of ITP, calling purpura “morbus muscosus haemorrhagicus” and describing the condition in a young woman with cutaneous and mucosal bleeding. In the early 1800s, the English dermatologist Robert Willan wrote that purpura hemorrhagica, a description consistent with ITP, was characterized by purpura and bleeding from the mucous membranes.

In the late 19th century, the central role of the low platelet count in ITP was identified. Krauss (1883) and Denys (1887) observed that platelets are diminished when purpura is most severe and increased when hemorrhages cease. Hayem (1895) confirmed these isolated observations with more accurate platelet counts. The issue of whether ITP results from platelet destruction or from suppression of platelet production was framed by the German physician Frank in 1915 and 1 year later by the Czech Kaznelson, a medical student at the time. Frank proposed that “essential thrombopenia” is the result of a reduction in platelet production by megakaryocytes. Kaznelson’s view was that thrombocytopenia, analogous to hemolytic anemia, is a result of increased platelet destruction in the spleen. He convinced a professor to perform a splenectomy on a woman with chronic ITP. The result was an increase in her platelet count (from 0.2 to 500 × 10^9/L of blood) and remission of the purpura.

**Evidence for Reduced Platelet Production and Increased Platelet Destruction**

In 1946, Dameshek and Miller took an important step forward in assessing reduced platelet production in ITP patients. They observed that the numbers of megakaryocytes and platelets were correlated across a variety of disorders, including leukemia and pernicious anemia; when megakaryocytes were reduced in number, so, consequently, were platelets. However, while platelet numbers were decreased in ITP, megakaryocyte numbers were normal or increased, but only a third or fewer of megakaryocytes showed evidence of platelet production. The researchers also found that the numbers of a larger intermediate megakaryocyte, the promegakaryocyte, were decreased in patients with ITP. They attributed the decrease in blood platelets to a severe reduction in platelet production by megakaryocytes. These were key findings at a time when even the normal mechanisms for megakaryocyte growth and platelet shedding were poorly understood.

Five years later, in 1951, Harrington et al showed that a factor capable of causing ITP could be transmitted via the blood plasma fraction of patients with ITP. Harrington et al reported that a woman with chronic ITP had given birth to a child with purpura, which resolved 3 weeks later, although the mother still had ITP. Harrington et al believed that a humoral antplatelet factor had been passed from mother to child. This insight inspired them to inject the blood plasma fraction from 10 patients with ITP into Harrington and 9 healthy volunteers. The blood plasma fraction from 8 of the 10 patients with ITP caused severe thrombocytopenia in the healthy volunteers, 2 of whom also developed purpura. Within several days, however, the platelet counts of all the volunteers, including Harrington, had returned to normal (Figure 1). Harrington et al concluded that an endogenous antplatelet factor is capable of destroying platelets and causative of ITP.

Harrington et al had shown that platelet destruction is at least one mechanism involved in ITP. Of additional interest, the blood plasma fraction, collected from 2 of the 10 patients with ITP after their platelet levels had returned to normal following splenectomy, elicited the same degree of thrombocytopenia in the volunteers as had their blood plasma fraction before they underwent splenectomy. The effect of splenectomy on the patients was mixed, ranging from remission to no rise in the platelet count. From this observation, Harrington et al surmised that the thrombocytopenic factor does not necessarily disappear from the plasma when the platelet count increases after splenectomy.

**The Autoimmune Nature of ITP**

The nature of the ITP plasma factor became clear with the research of Shulman et al, who showed that a protein fraction isolated from the plasma of patients with ITP induced transient thrombocytopenia in a dose-dependent manner in hematologically healthy subjects. The effects were less pronounced if the subjects were asplenic. The effects were also markedly blunted or indistinguishable from control with the administration of prednisone before the injection of ITP plasma (Figure 2). Moreover, it was observed that the thrombocytopenic factor was associated with immunoglobulin G (IgG), and the most abundant antibody type. Henceforth, ITP would be widely regarded as an autoimmune disease, and the “I” of ITP, which had once stood for “idiopathic,” was increasingly recognized to stand for “immune.”

Another advance in clarifying the autoimmune pathophysiology of ITP came with van Leeuwen et al in 1982. Sera of 42 patients with ITP and eluates prepared from the platelets of these patients were tested against the platelets of 8 healthy subjects, each of whom had a different platelet-
specific alloantigen phenotype, and the platelets of 2 patients with Glanzmann’s thrombasthenia, a condition in which a deficiency or dysfunction of platelet membrane glycoprotein (GP) complex IIb/IIIa (GP IIb/IIIa) precludes platelet aggregation. Van Leeuwen et al demonstrated that sera (12 of 14) or eluates of platelets (32 of 42) from patients with ITP were equally reactive with the platelets of healthy subjects, irrespective of the platelet phenotype of those subjects, but they would not bind to the platelets of patients with Glanzmann’s thrombasthenia. It was thus surmised that anti-GP IIb/IIIa autoantibodies are present in patients with ITP.11 However, 2 years later, Woods et al found that a much lower proportion of patients with ITP had platelet-reactive antibodies than van Leeuwen et al had reported.17,18 The different results may have been due to variability in the sensitivity, methodology, and specificity of the assays used, as well as differing patient populations and different methods of measuring anti-GP IIb/IIIa antibodies;17 these results signaled the need for a better assay.

By 1987, 2 assays for the measurement of platelet-associated autoantibodies (as well as of autoantibodies free in plasma) had been developed by McMillan et al and Kiefel et al.18,20 However, the diagnostic and prognostic utility of these assays remains debatable because fewer than 70% of patients with ITP have detectable autoantibodies and because antibodies directed against the relevant glycoproteins are also present in a number of other disorders.2,19 It has been shown that approximately three-quarters of patients with ITP have antiplatelet antibodies directed against GP IIb/IIIa and another glycoprotein complex, GP Ib/IX, whereas the rest of the patients with ITP have antibodies that bind other platelet membrane proteins.21 It is for this reason that antiplatelet antibody tests are of limited clinical use in the diagnosis of ITP.

Platelets

Suppression of Platelet Production in ITP

Using heterologous platelets, Harker and Finch observed normal platelet recovery, shortened platelet survival, and high rates of platelet production (see “Platelet Kinetic Studies,” page 4 for definitions of terms), ranging from 4 to 9 times normal, in 4 patients with ITP.24 They also found an increase in megakaryocyte mass. The authors proposed that the bone marrow was able to substantially increase its platelet production rate in an attempt to compensate for the ongoing platelet destruction.24 However, subsequent platelet survival studies using autologous platelets have suggested that platelet production in ITP is
usually not increased. Inconsistent with the working hypothesis that low platelet counts in ITP result solely from accelerated platelet destruction, Heyns et al found that platelet production was notably reduced in 75% of patients with ITP. Four years later, Heyns et al further assessed platelet production rates and their relationship to the site of platelet destruction and clinical severity of disease. The authors grouped patients with ITP on the basis of where the radioactively labeled platelets were being destroyed (as identified by scintigraphy): splenic destruction versus diffuse destruction in the reticuloendothelial system (RES) (primarily the bone marrow and liver). They discovered that platelet production rates were related to where the platelets were being destroyed; platelet production was increased in patients with a diffuse RES destruction pattern but decreased in patients with a diffuse RES destruction pattern. Heyns et al concluded that compared with splenic destruction, diffuse RES destruction (either bone marrow or hepatic) is associated with more severe disease, reflected by decreased platelet production and lower platelet counts.

Another autologous platelet study was performed in 1985 by Stoll et al, who reported that platelet production in patients with ITP remained surprisingly within normal limits even though the mean cell life of platelets was significantly reduced compared with that in healthy subjects. The authors also found that most of the patients with ITP had significantly increased levels of platelet-associated IgG and had larger platelets than healthy subjects. If platelet destruction were the only mechanism to cause thrombocytopenia, then platelet production would be expected to increase to offset low platelet counts. Their findings suggest that thrombocytopenia may result not only from platelet destruction, but also from antibody-mediated damage to megakaryocytes. Alluding to a study in 1982 by Vainchenker et al reporting that GP Ib/IIIa and GP Ib had been found in both megakaryocytes and platelets, Stoll et al concluded that ITP autoantibodies influence the maturation of megakaryocytes, perhaps leading to increased platelet size.

**Platelet Kinetics and Treatment**

In the late 1980s, 2 studies further examined the response to 2 historically successful treatments, corticosteroids and splenectomy (see “Comment on Splenectomy,” page 732-35). In 1987, Ballem et al analyzed the platelet production rates among untreated, prednisone-treated, and splenectomized ITP patient cohorts. Most untreated patients with ITP had either significantly decreased or normal platelet production rates, approximately half of prednisone-treated patients had increased platelet production rates, and splenectomized patients were equally distributed as having increased, decreased, or normal platelet production rates. To understand the mechanism by which the platelet production rate was altered in ITP, the investigators analyzed in vitro the megakaryocyte progenitors (megakaryocyte colony forming cells [Meg-CFCs]) from these patients. They found that the percentage of Meg-CFCs in the DNA synthesis phase of the cell cycle was increased in all groups of patients with ITP (by as much as 3-fold in untreated and prednisone-treated patients) in comparison with healthy subjects and was, moreover, inversely correlated with the platelet count, indicating that the cell cycle activity of megakaryocyte precursors normally increases as platelet demand increases in thrombocytopenia. Because the cell cycle activity of megakaryocyte precursors is increased in patients with ITP, but platelet production rates do not necessarily increase, the authors concluded that antibody-mediated damage to megakaryocytes may result not only from platelet destruction, but also from increased platelet production. To quantify platelet production, defined as the number of platelets released into the circulation, 2 parameters have commonly been used: megakaryocyte mass and platelet turnover. The megakaryocyte mass, which provides an indirect calculation of the platelet-producing capacity of the bone marrow, is based on photomicrographic measurements of megakaryocytes and is the product of the total number of megakaryocytes and the mean megakaryocyte volume. Platelet turnover is calculated by dividing the peripheral platelet count by the platelet survival time (measured in days) after correcting for platelet recovery (to account for platelets sequestered in the spleen). The rate of platelet turnover reflects the number of labeled platelets removed (cleared) from the circulation by processes other than sequestration. In healthy subjects, this is mostly the result of platelet apoptosis, and the remainder are removed by a stochastic (random) process probably involved in maintaining normal hemostasis. In pathologic situations such as ITP, in addition to normal platelet clearance, antibody-mediated destruction by the reticuloendothelial system (RES) occurs. At stable platelet counts, platelet clearance equals platelet production and hence the platelet turnover number indicates both the rate of platelet production and the rate of platelet clearance.
mediated suppression of platelet production must occur after the maturation of Meg-CFCs into megakaryocytes and that antibodies ultimately prevent mature megakaryocytes from shedding platelets into the circulation.36

Prednisone and splenectomy variably influence the rates of platelet production or destruction to improve the platelet count in patients with ITP. Prednisone was shown to increase platelet production in patients with ITP, with no presumed effect on platelet destruction by the spleen and liver.36 Splenectomy, on the other hand, decreased rates of platelet destruction and increased the rate of platelet production; Ballem et al interpreted these results to suggest that, in addition to inhibiting platelet destruction, splenectomy may enhance platelet production, possibly because the removal of the spleen eliminates a significant source of platelet-specific antibodies.36

In 1989, Gernsheimer et al also examined patients with ITP receiving prednisone or undergoing splenectomy.37 They found that most prednisone-treated patients with ITP exhibited a tripling of their platelet count, an improvement that was significantly correlated with an increase in platelet production. Most patients who underwent a splenectomy exhibited a clinically meaningful and stable 4-fold improvement in their platelet count. In contrast to the findings of Ballem et al, however,37 platelet production was not significantly changed. This increase was mainly the result of an improvement in platelet survival and recovery after splenectomy, suggesting a decreased rate of platelet destruction. As in the prednisone-treated patients, no meaningful pattern of changes in antiplatelet antibody levels was detected in the patients who had undergone splenectomy.37

The finding that prednisone treatment increased platelet production demonstrates its efficacy in raising platelet counts: more platelets were released from the bone marrow.36 In the patients who underwent splenectomies, successful treatment occurred as a result of increased platelet survival and recovery without a change in platelet production. Ultimately, Gernsheimer et al suggested that thrombocytopenia in ITP results primarily both from ineffective platelet production because of the intramedullary removal of platelets by RES cells in the bone marrow as the platelets are produced, and from a reduction in the survival of circulating platelets because of destruction in RES organs.37

Megakaryocytes

In the studies that followed Dameshek and Miller’s 1946 report on megakaryocytes in ITP,52 the suppressive effect of autoantibodies on megakaryocytopoiesis (the differentiation and maturation of megakaryocytes from stem cells) was made clearer. Taken together, the evidence indicates that ITP is caused by both increased platelet destruction and decreased platelet production.

Maturation and Morphology

In 1953, Pisciotta et al described an increase in the number of megakaryocytes in patients with ITP and stated that the cells most affected in ITP were immature promegakaryocytes and megakaryoblasts.35 Of the adult megakaryocytes observed, none exhibited platelet shedding. At a time when ITP antibodies were not yet confirmed, the authors concluded that a powerful platelet agglutinin present in ITP was equally capable of attacking megakaryocytes and the platelets surrounding and budding from megakaryocytes.35 Immunofluorescent antitryptase studies of ITP in both humans and animal models supported the notion that antiplatelet antibodies would bind to megakaryocytes. A further connection was made in 1978 when McMillan et al demonstrated the particular importance of IgG.29 They observed that IgG produced by cells (grown in vitro) from the spleens of patients with ITP would bind to megakaryocytes, whereas IgG produced by cells from the spleens of healthy controls did not bind to megakaryocytes.29 Kiefel et al later reported that autoantibodies of the IgG class were present in about 50% of patients with ITP, whereas IgM and IgA were rarely detected.39

The presence of IgG autoantibodies has implications for the maturation of megakaryocytes. Because GP Ib/IIa and GP Ib/IX are expressed on the surface of committed megakaryocyte progenitors, the binding of antibodies to megakaryocytes might have deleterious effects on megakaryocytopoiesis. Either by destroying megakaryocytes or platelets while they are still in the bone marrow, or by interfering with megakaryocyte maturation and platelet shedding, antibody binding might interfere with the production of platelets from megakaryocytes and the release of platelets from the bone marrow.41

In 2004, Houwerzijl et al published the results of a study that investigated the morphology of megakaryocytes in ITP. Using electron microscopy, the authors conducted an ultrastructural study of bone marrow megakaryocytes from patients with ITP.42 Most of the megakaryocytes in ITP were abnormal and showed features characteristic of nonclassic apoptosis, with an intact, mostly thickened peripheral zone that did not contain functional cellular material such as organelles (Figure 3). Of these, nearly all were surrounded by neutrophils and macrophages, some of them in a state of phagocytosis, suggesting an anti-mega-karyocyte inflammatory response. Approximately one-third of

Figure 3. Ultrastructure of healthy and ITP megakaryocytes.42
A, Mature megakaryocyte from a healthy donor. The right panel shows a higher magnification of intact mitochondria (arrowheads) and a normal demarcation pattern. Original magnification ×3,000 (left panel), and ×18,000 (right panel). B, Mature megakaryocyte of an ITP patient showing apoptotic characteristics. The right panel shows a detail of the nuclear fragmentation (arrow) and chromatin condensation to the margins of the nucleus (arrowheads). The shrinkage and rounding up of the cell have reduced cellular volume considerably. Original magnification ×7,000 (left panel), and ×18,000 (right panel). ITP, immune thrombocytopenic purpura; N, nucleus

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patients with ITP had abnormalities with apoptosis-like features in mature megakaryocytes.42

Although abnormal morphology was observed in the majority of ITP megakaryocytes in all stages of development, Houwerzijl et al found that most of the morphologic damage occurred in mature megakaryocytes, which are primarily responsible for platelet production. The percentage of abnormal megakaryocytes was found to increase as the megakaryocytes matured.42

When patients with ITP were compared with healthy controls, no significant difference was found in the total number of megakaryocyte progenitors (Meg-CFCs), yet the patients showed a significant decrease in the percentage of mature megakaryocytes, a similar decrease in the percentage of promegakaryocytes, and a similar percentage of earlier megakaryocytes, the megakaryoblasts.42

Houwerzijl et al found that most of the megakaryocytes derived from CD34+ cells grown in ITP plasma showed the same apoptotic abnormalities seen in ITP megakaryocytes in vivo. The implication of this finding, according to the authors, is that patients with ITP have defects not only at the platelet level but also at the megakaryocyte level. Furthermore, this study provided additional evidence of reduced platelet production by morphologically abnormal megakaryocytes.42

**Effects on Megakaryocyte Growth: In Vitro Studies**

Additional in vitro studies have further defined the role of autoantibodies in suppressing megakaryocyte production and maturation and platelet release. Two studies in particular, by Chang et al43 and McMillan et al,44 support the view that suppression of megakaryocytopoiesis and reduction of the platelet count are caused by autoantibodies in ITP. In the study by Chang et al, CD41+ cells were grown in the presence of plasma from pediatric patients with ITP and the number of megakaryocytes that were produced in vitro was measured.43 The number of megakaryocytes produced in ITP plasma that lacked antibodies to GP Ib/IIa and GP Ib was the same as the number produced in control plasma from healthy donors, whereas about half as many megakaryocytes were produced in the presence of ITP plasma that contained both anti-GP Ib/IIa and anti-GP Ib autoantibodies (Figure 4). ITP plasma that had only anti-GP Ib autoantibodies also produced approximately half the number of megakaryocytes, whereas the yield of megakaryocytes in ITP plasma with only anti-GP Ib/IIa autoantibodies did not significantly differ from that of control plasma. Chang et al concluded that the anti-GP Ib rather than the anti-GP Ib/IIa autoantibodies may be responsible for the suppression of megakaryocyte growth (at least in pediatric patients with ITP).43

Chang et al also found that when ITP plasma was depleted of anti-GP Ib autoantibodies, the yield of megakaryocytes was approximately double that of the unaltered ITP plasma. This suggested that anti-GP Ib autoantibodies contribute, at least in part, to the reduced yield of megakaryocytes that grew in the presence of ITP plasma.43 Despite other research finding apoptotic-like features in megakaryocytes in ITP,45 Chang et al found no evidence that the deleterious effect of ITP plasma autoantibodies on megakaryocytopoiesis is the result of an increase in apoptosis. Based on a study of an inhibitor of apoptosis, a fluoromethyl ketone (FMK)-based general caspase inhibitor, Chang et al found that the presence of FMK did not reverse the relative reduction in megakaryocyte production in ITP plasma versus that in control plasma.43 However, because FMK inhibits

![Figure 4](image-url)

*Figure 4. Yields of megakaryocytes in culture containing ITP or healthy control plasma, classified by the presence or absence of ITP autoantibodies.*43

Results presented are a percentage of controls (mean ± SD). GP, glycoprotein; ITP, immune thrombocytopenic purpura; SD, standard deviation

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**Comment on Splenectomy**

The remarkable symptomatic improvement seen in some patients following splenectomy has long suggested a central role of the spleen in the pathophysiology of ITP. Although splenectomy is effective for many patients, approximately 40% do not respond and/or relapse within 5 years. Patients with ITP in complete remission after splenectomy are estimated to have a 20% chance of relapsing based on long-term follow-up (5-10 years; Figure 5). Splenectomy is linked to a small, increased life-long risk for bacterial infections. Other limitations and disadvantages include the natural disinclination to remove a healthy organ and concerns over possible adverse events resulting from any major operation, such as infection, bleeding, or reactions to anesthesia. To predict the outcome of splenectomy and reduce the risk for complications and ineffective treatment, studies have sought to use preoperative 111In-labelled platelet scintigraphy in the spleen and liver of patients with ITP. The prognostic value of platelet scintigraphy, however, is low and not widely accepted because individual differences may be major sources of variance and because platelet destruction may occur in parts of the RES other than the spleen and liver.

Data from McMillan et al resemble those of Chang et al, with several notable differences. The pediatric patients studied by Chang et al showed reduced production of megakaryocytes in vitro, but only in the presence of anti-GP Ib antibodies, without involvement of anti-GP Ib/Illa antibodies. In contrast, both types of antibodies suppressed megakaryopoiesis in the adult cohort of McMillan et al. Furthermore, most of the pediatric patients in the study by Chang et al had acute ITP, in contrast to the patients with chronic ITP in the study of McMillan et al. Unlike the transient effects of autoantibodies in children with ITP, whose platelet counts recover quickly, the effects of autoantibody-mediated suppression of megakaryopoiesis may play a more critical role in adult patients with chronic ITP. Also, the sample size in the study of Chang et al may not have been large enough to detect a reasonable experimental effect. Regardless of these differences, the 2 studies taken together provide strong support for the contention that autoantibodies suppress megakaryopoiesis and thereby may reduce platelet production in patients with ITP.

**Thrombopoietin**

Many recent studies have improved our understanding of ITP and the physiology of platelet production, with particular implications for the development of novel therapeutics. Of specific interest was the isolation of TPO. First isolated and cloned in 1994, TPO is produced constitutively in healthy persons, primarily in the liver. It enters the circulation, where it binds to the surface membrane cytokine receptor c-Mpl, a member of the hematopoietic growth factor receptor superfamily. The primary hormone controlling megakaryocyte development, TPO promotes the proliferation of hematopoietic stem cells and of committed progenitors of all lineages, and especially promotes the development and maturation of megakaryocyte progenitors. Upon its binding to megakaryocyte progenitor cells, TPO activates several distinct signal transduction pathways: phosphorylation of tyrosine Janus kinase (JAK2) and signal transduction and transcription (STAT5) proteins induce cell proliferation, activation.

![Figure 5. Kaplan-Meier curve of remission rate (CR + PR) in adults with ITP (N=56) following splenectomy](image-url)
of mitogen-activated protein kinases (MAPK) induces cell differentiation, and activation of antiapoptotic pathways supports extended cell life (Figure 6).6,52 Although this process promotes the viability of pluripotent stem cells and a diverse array of multilineage progenitor cells, it primarily promotes the viability and growth of Meg-CFCs and early megakaryocyte progenitors. Thus, TPO enhances the maturation of megakaryocytes and platelet production.6

The number of platelets available to bind TPO regulates the level of free TPO in the circulation.51 Platelets bind TPO, internalizing it and leading to its eventual degradation.53 When platelet production is low, TPO increases, as in diseases such as aplastic anemia, in which patients are deficient in all blood cell types.54,56 But serum TPO levels are normal or only mildly elevated in patients with ITP in comparison with patients with aplastic anemia.56 This is most likely because patients with ITP have normal or increased numbers of megakaryocytes (immature and maturing),42 to which TPO also binds,53 as well as an increased clearance of platelets from the circulation. The relative deficiency of TPO in ITP may thereby limit platelet production.

The Regulation of TPO Levels

Two studies published in 1996, by Emmons et al and Kosugi et al, elucidated the mechanisms by which TPO levels are regulated in humans.56,57 Animal studies had shown an inverse relationship between TPO levels and circulating platelet mass when platelet production was reduced by chemotherapy.51,58,59 Emmons et al sought to determine whether this was also the case in humans.57

In the study by Emmons et al, patients with thrombocytopenia due to increased platelet destruction (ITP, post-transfusion purpura, drug-induced purpura) were compared with patients with decreased platelet production (aplastic anemia).67 All patients had equally low platelet counts, although patients with aplastic anemia had megakaryocytic hypoplasia and

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**Figure 6. Mechanism of activation of the thrombopoietin receptor (Mpi) by thrombopoietin.**

- The existence of preformed inactive dimers of the thrombopoietin receptor, as shown in this diagram, has not been conclusively proven.
- Other signal transduction molecules (not shown) are also activated by TPO binding.

**GRB2,** growth factor receptor–bound protein 2; **JAK2,** Janus kinase 2; **MAPK,** mitogen-activated protein kinases; **SHC,** Src homologous and collagen-like protein; **SOS,** son of sevenless protein; **STAT5,** signal transduction and transcription 5; **p42/44,** phosphorylated 42- and 44-kDa mitogen-activated protein kinases; **TPO,** thrombopoietin

*Modified with permission from reference 6.*
Figure 7. A representation of the pathogenesis of ITP.2,62,63

Reduced numbers of mature functional megakaryocytes and abnormally low platelet counts characterize ITP and result from the activity of anti-GP IIb/IIIa and anti-GP Ib/IX antibodies, which are produced by B cells. These autoantibodies bind to platelets, resulting in platelet destruction in the RES, and also bind to megakaryocytes, causing deleterious effects that interfere with platelet production. Assays for antiplatelet antibodies lack specificity and sensitivity. TPO, which promotes maturation of megakaryocytes and increases platelet production, presents a promising avenue for treatment. Since TPO levels are normal in ITP, raising TPO levels increases megakaryopoiesis, which in turn increases megakaryocyte numbers and platelet production.

GP, glycoprotein; ITP, immune thrombocytic purpura; RES, reticuloendothelial system; TPO, thrombopoietin

Adapted from references 2, 62, 63.
patients with ITP or other disorders had normal or increased megakaryocyte mass. All patients with ITP had less than the minimal detectable amount of TPO (<200 pg/mL), whereas most of those with aplastic anemia had highly elevated TPO levels (mean, 1,487 pg/mL). Because TPO levels differed between patient groups even though all patients had low platelet counts, Emmons et al concluded that the circulating platelet number is not the major determinant of TPO levels. Rather, the platelet production rate was proposed to be the critical determinant of TPO levels.57

Also in 1996, Kosugi et al compared TPO levels of patients with ITP with those of healthy controls and of patients who had aplastic anemia.56 In contrast with controls, patients with ITP had either mildly elevated (70%) or normal (30%) levels of TPO, whereas patients with aplastic anemia had highly elevated levels of TPO. In all patients, the TPO levels were not correlated with platelet counts, corticosteroid use, or the presence of anti-GP Ib/IIa autoantibodies.56

The results of Kosugi et al suggest that TPO levels are probably regulated by clearance by the platelet mass and by the bone marrow megakaryocyte mass. In disorders such as aplastic anemia, in which the megakaryocyte mass is low and the passage of platelets through the circulation is reduced, TPO is markedly elevated. In ITP, the megakaryocyte mass is expanded and the passage of platelets through the circulation is increased, resulting in a nearly normal level of TPO. Kosugi et al and Emmons et al both proposed that platelet production in ITP may be limited by the amount of TPO available and that supplemental infusion of TPO may be a viable therapeutic option.56,57

**TPO in the Context of Treatment**

In 2001, Kappers-Klunne et al focused on TPO levels in patients with ITP before and after treatment with corticosteroids, splenectomy, or high-dose I.V. immunoglobulin.55 Regardless of treatment, on average, there was a significant post-treatment decrease in mean levels of TPO. However, in some patients TPO levels increased after treatment, and in others there was no change. Both before and after treatment, there was no correlation of TPO levels with platelet counts, except in a subset of patients in whom the pretreatment platelet counts were markedly reduced (≤20 x 10^9/L); pre-treatment TPO levels in patients with very low platelet counts were significantly higher than those of patients with higher platelet counts. Furthermore, in 15 patients in whom platelet kinetics studies were performed, the TPO levels pretreatment correlated significantly with the rate of platelet production. The Kappers-Klunne et al finding is consistent with the view that TPO binding to circulating platelets is the main regulatory mechanism of platelet production.55

Kappers-Klunne et al noted that one-third of the patients who reached complete remission showed an aberrant pattern of low pretreatment TPO levels that remained unchanged or increased after treatment. The authors linked their research to the Ballem et al finding that patients with ITP could have normal or decreased platelet production (based on platelet turnover measurements) that correlated directly with platelet survival.55 There might be cases, Kappers-Klunne et al indicated, in which relative marrow failure and endogenous TPO deficiency might contribute to the pathophysiology of thrombocytopenia.55 Consistent with the results of Emmons et al and of Kosugi et al, the Kappers-Klunne et al results suggest that TPO levels in ITP are not increased and that the administration of exogenous TPO might be an effective treatment for thrombocytopenia.55

**TPO Growth Factors as Treatment for ITP**

These and related advances in the understanding of the biological basis of thrombopoiesis provide a biochemical rationale for the development of TPO-mimetic pharmacologic agents for the treatment of thrombocytopenia. Several novel approaches are currently in various stages of clinical development. A first generation of thrombopoietin growth factors, consisting of recombinant proteins, appeared in the late 1990, but these were ultimately abandoned. A second generation of thrombopoietic growth factors has since emerged, including TPO peptide mimetics, TPO nonpeptide mimetics, and TPO agonist antibodies, each of which uniquely binds to and activates c-Mpl.6,60,61 Recent studies with TPO growth factors have indicated that they can effectively increase platelet counts in patients with ITP.

**Conclusion**

Hypotheses proposed nearly a century ago suggest that increased platelet destruction and decreased platelet production both play key roles in the pathogenesis of ITP (Figure 7, page 9). Until quite recently, the platelet destruction hypothesis has prevailed at the expense of the suppression theory, whereas both mechanisms are now viewed as important. Since the first splenectomy aimed at treating ITP was performed in 1916, the complex and often confounding picture of the pathophysiology of ITP has gradually been clarified. Although much remains to be learned about the disease, the understanding of ITP has grown markedly.

In summary, it now appears to be established that in addition to platelet destruction in the circulation, antiplatelet antibodies damage megakaryocytes and reduce platelet shedding in patients with ITP. These antiplatelet autoantibodies are primarily directed against GP Ib/IX and GP Ib/IIa, although in pediatric patients with acute ITP the role of the latter is questionable. Platelet kinetic studies have shown that ITP is characterized by an impaired patient bone marrow response to diminished platelet counts. Analysis of the partial efficacy of the 2 current standard treatments for ITP, corticosteroids and splenectomy, provides insights into the complexity of ITP pathophysiology. Whereas the efficacy of prednisone is derived from its capacity to induce an increase in platelet production, the benefits of splenectomy appear to derive from its ability to increase platelet survival and platelet recovery, with some effect on platelet production. The view that the efficacy of splenectomy is at least in part due to the excision of a site for antibody synthesis remains to be definitively demonstrated.

The relatively recent isolation and cloning of TPO has added substantially to the understanding of ITP. TPO acts through c-Mpl to promote megakaryocyte and megakaryocyte precursor proliferation, differentiation, and maturation. Patients with ITP are observed to have lower TPO levels relative to patients with other thrombocytopenic disorders. This understanding has led to one of several new therapeutic approaches to thrombocytopenia in ITP, aimed in this instance at inducing platelet production through the c-Mpl signaling pathway.
References


Normalized spleen/liver ratios on 111In-labelled platelet scintigraphy 
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