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Thrombopoietin and Platelet Production in Chronic Immune Thrombocytopenia

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Introduction

In 1663 Lazarus de la Riviere (Riverius) recognized that purpura was a manifestation of a systemic bleeding disorder, something he called “thinness of the blood.”⁷¹ Although most of his subjects probably did not have immune thrombocytopenic purpura (ITP), he stated “But there is one Symptome proper and peculiar to a pestilential Feaver, which doth not happen in other Feavers; viz. Purple Specks, or Spots on the whol Body, but especially in the Loyns, the breast and back, like unto Flea-bitings for the most part; which the Italian Physitians name Peticule or Petechio; and these Feavers which have these Symptoms, are commonly named Purpuratae or Petechiales, Purple or Spotted Feavers... wherein the blood boiling, do send forth it's thinner Exhalations to the surface of the Skin.”

The first modern description of ITP was probably that of the German physician Paul Gottlieb Werlhof who in 1775 described a case of “morbus maculosus haemorrhagicus.”⁸² Subsequently in 1808 the English dermatologist Robert Willan reported a case of mucosal and cutaneous hemorrhage he called “purpura hemorrhagica.”⁸³ Willan also suggested that “moderate exercise in the open air, a generous diet, and the free use of wine” might be an appropriate treatment. Other reports over the ensuing century clarified the characteristic hemorrhagic features of ITP and ultimately James Homer Wright related these clinical findings to the lack of platelets in his reports of 1902, 1906 and 1910.^{84–86} By the beginning of the 20th century what we now know to be ITP was a known diagnostic entity.⁶²

Theories as to the causation of ITP were developed early in the 20th century and set the stage for our current understanding of ITP. In 1915 the German physician Ernest Frank suggested that ITP was due to impaired production of platelets from megakaryocytes.²⁴ In contrast, in

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1916 the Czech medical student Paul Kasnelson speculated that ITP was due to splenic destruction of platelets, as in hemolytic anemia.^{44,91} He also succeeded in persuading his supervisor to perform a splenectomy in one such patient, with therapeutic success.

The debate as to whether ITP was due to decreased platelet production or increased platelet destruction was seemingly settled by the experiments of Harrington and Schulman which suggested a primary role for immune mediated platelet destruction. In his classic experiment, Harrington infused plasma or whole blood from patients with ITP into himself and his fellows; thrombocytopenia soon ensued (Figure 1).³⁷ Sternal bone marrow biopsies performed on these “volunteers” showed an increase in megakaryocytes during the thrombocytopenia. Subsequent studies by Shulman showed that the thrombocytopenia induced by ITP plasma infusion was reduced in subjects who were splenectomized or pretreated with corticosteroids.⁷³⁻⁷⁵ When combined with the platelet survival and kinetic studies of Harker in 1968, a model emerged that suggested that ITP was due to immune-mediated platelet destruction accompanied by a maximal 6-fold compensatory increase in platelet production by the bone marrow.^{30,31,33}

Since 1968, a greater understanding of platelet biology and its regulation by thrombopoietin (TPO) have emerged. It is now recognized that ITP is a disorder of both reduced platelet production and increased platelet destruction. New therapies for ITP with TPO mimetics have emerged that have exploited this new pathophysiological understanding to the benefit of many patients. In this chapter we review the biology of TPO, the regulation of its circulating level in ITP, the platelet kinetic data supporting inappropriate platelet production in ITP, and the TPO molecules available to treat ITP. Elsewhere in this volume, the clinical studies in ITP with these TPO mimetics are described in detail (link to other chapters).

Thrombopoietin is the primary regulator of normal platelet production

TPO is a 94 kD protein primarily made in the liver and secreted into the circulation; there is no storage form. Although there is some suggestion of local TPO production in the bone marrow, there is little evidence to show that this is physiologically relevant.⁶⁰ Indeed in patients with liver failure, TPO levels are low and patients are thrombocytopenic; upon orthotopic liver transplantation, TPO levels rise and the platelet count is restored to normal.^{68,69}

There does not appear to be any direct “sensor” of the platelet count and levels are regulated by an efficient, albeit primitive, feedback system (Figure 2).⁵¹ Hepatic TPO production appears to be constant with no transcriptional or post-transcriptional regulation yet identified.⁹⁰ Other than liver disease or hepatic resection, no disease or medication is known to affect TPO production. Upon entering the circulation, TPO is not bound to any specific carrier molecule but does bind with high avidity to TPO receptors on circulating platelets and target bone marrow tissues (megakaryocytes and megakaryocyte precursors).⁵⁶ Most of the circulating TPO is cleared by platelets (and possibly megakaryocytes) by binding to the TPO receptor, followed by internalization and catabolism of the bound ligand. Indeed, when platelets were transfused into patients with thrombocytopenia due to aplastic anemia, the elevated TPO levels fell 30%.⁷² In summary, the circulating TPO level is inversely related to the rate of platelet production: when platelet production is low, less TPO is cleared and levels rise; when platelet production is elevated, more TPO is cleared and levels fall.²¹

When TPO binds to the TPO receptor on platelets there are no major physiological sequelae other than an increase (at high non-physiological concentrations of TPO) in intracellular calcium concentration and an increase in the sensitivity of platelets to weak agonists (half-maximal platelet activation by ADP occurs at half the concentration of ADP usually required for this effect).³⁵ However upon TPO binding to megakaryocytes and megakaryocyte precursors, the TPO receptor is activated and increases megakaryocyte growth, endomitosis, and maturation as well as reducing the apoptosis rate of these cells.⁴³ In so doing,

megakaryocyte number, ploidy and viability increase; thereby increasing platelet production (Figure 3).

The importance of TPO for normal platelet production is illustrated by animals in which either TPO or the TPO receptor were “knocked out.” In animals homozygous for the absence of TPO or its receptor, the platelet count was reduced by 90-95%.^{15,16} As expected, the number of megakaryocytes and megakaryocyte precursors were reduced by a similar extent. Interestingly, myeloid and erythroid precursors were reduced by 70%, but with no effect on the white blood cell (WBC) count or hemoglobin.^{2,11} At the time of birth, humans lacking TPO or its receptor have marked thrombocytopenia as a result of the reduced numbers of megakaryocytes and megakaryocyte precursors; however over time such individuals become pancytopenic.^{5,40,79} Together these data suggest that TPO is necessary for the viability of the pluripotent stem cell and precursors of all lineages, but specifically important for the maturation and development of late megakaryocytes.

TPO levels and platelet production are usually not increased in ITP

Seemingly at odds with the long-held understanding that ITP is a disease of antibody-mediated platelet destruction, ITP is also a disorder of inappropriately low platelet production, i.e. platelet production remains close to normal and is not elevated 6-fold as suggested by Harker.³³ Both of these processes (increased destruction and reduced production) occur simultaneously and probably to a different extent in each patient; hence the widely varying rates of response to many agents. The apparent common mechanism is that the anti-platelet antibody opsonizes platelets leading to their destruction by the reticuloendothelial (RE) system and inhibits the growth (by accelerated apoptosis) of megakaryocytes and their progenitors (link to chapter by McMillan).⁶¹ Two main avenues of investigation support this statement and are reviewed next.

TPO levels are not significantly elevated in patients with ITP—As shown in Figure 4, at comparable degrees of thrombocytopenia, TPO levels are 10-fold elevated in patients with aplastic anemia (median = 1002 pg/mL, range: 37-161,538 pg/mL) but are virtually normal (median = 64 pg/mL, range: 22-256 pg/mL) in patients with ITP (median = 98 pg/mL, range: 17-313 pg/mL).^{21,65} The model described above (see Figure 2) helps to explain why.⁴⁷ In both patient groups, TPO is being constitutively made by the liver at the same rate. In the aplastic patient, platelet production is reduced far below normal, fewer platelets and megakaryocytes are available to bind and clear TPO, and levels rise.⁴⁷ In the ITP patient, platelets are still being made at an approximately normal rate (see below) but are being cleared at an increased rate; despite their shortened survival, the platelets can still bind and clear TPO at a normal rate, and TPO levels do not significantly increase.^a

Platelet turnover rates are not appreciably elevated in ITP—Dameshek and Miller made a noble attempt to measure platelet production in 1946¹⁴ by scoring the morphologic appearance of megakaryocytes and platelet budding in the bone marrows of patients with chronic and acute ITP, liver disease and splenomegaly. They found megakaryocytes were present in increased numbers in ITP patients, but noted that these megakaryocytes frequently appeared immature with a “greatly diminished productivity of platelets” and considered the possibility that the spleen exerted “an unusual effect upon the production of platelets from the megakaryocytes”.

^aThis clearance function is slightly more complex if explored further. There is actually a log:linear relationship between the TPO level and the rate of platelet production⁴⁷; hence as the rate of platelet production rises in ITP, clearance increases but TPO levels rapidly approach the lower limit of quantitation of the TPO assay. This is the explanation as to why TPO levels become readily elevated in disorders of reduced production but usually remain in the broadly normal range when the platelet production rate increases, even if greatly increased.

Taking advantage of the uptake of ^{51}Cr by platelets upon in-vitro incubation, Aster and Jandl developed methodology for the measurement of in-vivo platelet survival³. Radioactivity in serial blood samples collected after transfusion of ^{51}Cr labeled platelets revealed platelet lifespan in normal subjects averaged about 8 days and approximately 30% of the transfused platelets did not circulate and were pooled in the spleen. Platelets were lost from circulation mainly due to senescence with predominantly RE removal in the liver. Najean, *et al*⁶³ utilized ^{51}Cr labeling to study platelet survival in 317 patients with ITP on 450 occasions using allogeneic platelets when the patients' platelet counts were $<80,000/\mu\text{l}$ and autologous platelets at higher counts. Platelet survivals were always shorter than normal in these patients and directly related to the patients' platelet count whether autologous or allogeneic platelets were used.

Using these same platelet radiolabeling methods Harker and Finch³³ correlated thrombokinetic measurements with studies of megakaryocyte mass (i.e., number and volume) to further define thrombocytopenic disorders. They calculated the daily rate of platelet loss from the circulation by the formula:

$$\text{turnover} = \frac{\text{platelet count} \times 10^9 \text{ per liter} \times 90\%}{\text{platelet survival (days)} \times \text{platelet recovery (\%)}}$$

At a steady state, when the peripheral blood platelet count is stable and the number of platelets going into or out of the system is constant, platelet production must equal platelet destruction ("turnover"). At any given platelet count, as platelet survival shortens, the denominator decreases and the resultant production (turnover) rate increases. This makes sense physiologically as the marrow would be expected to increase production in response to thrombocytopenia to reach homeostasis, and is consistent with increased numbers of megakaryocytes observed in disorders of increased platelet destruction.

In 15 normal subjects studied using autologous radiolabeled platelets, the average platelet count was $250,000 \pm 35,000/\mu\text{l}$, platelet recovery $65\% \pm 4$, platelet survival 9.9 ± 0.6 days, and platelet production $35,000 \pm 4,000$ platelets/ $\mu\text{l/day}$ ³³. Radiolabeled platelet survivals were markedly shortened in 4 patients with ITP, from a mean of 9.9 days in normal subjects to 48-230 minutes, and calculated production rates in the ITP patients that were 4 to 9 times normal. Platelet kinetic measurements in 7 patients with secondary autoimmune thrombocytopenia were similar to the ITP group. The finding of marked shortening of platelet survival and by implication markedly increased rates of platelet production in ITP was later confirmed by Branehog⁷.

$^{111}\text{Indium}$ was introduced as an alternative radiolabel to ^{51}Cr in 1976⁷⁷. Higher labeling efficiency (64% for ^{111}In and 12% for ^{51}Cr) allowed kinetic measurements with autologous platelets rather than allogeneic platelets in patients with severe thrombocytopenia, and lower levels of erythrocyte binding (1% for ^{111}In and 7.4% for ^{51}Cr) gave more accurate measurements. Higher gamma emissions with ^{111}In also permitted investigators to reliably localize platelet uptake using external scanning techniques. Platelet kinetic experiments utilizing ^{111}In labeled autologous platelets^{4,38} avoided potential confounding and adverse effects of homologous (donor) platelets. In dual labeling experiments with ^{111}In - and ^{51}Cr -labeled autologous and homologous platelets in 13 patients with chronic ITP, autologous platelets survived significantly longer in the circulation than homologous platelets⁴.

A comprehensive evaluation of thrombokinetics using either ^{51}Cr or ^{111}In radiolabeled autologous platelets in 38 patients with ITP (18 patients receiving no treatment, 13 receiving prednisone, and 7 post-splenectomy)⁴ found platelet survivals to be less than normal with the exception of two post-splenectomy patients in complete remission, and 82% (36/44) of platelet

survivals were disproportionately less than expected based on the corresponding platelet count²⁹. There was a significant relationship between platelet count and autologous platelet survivals only for the splenectomized patients ($r=0.84$, $p<0.001$) and in patients with platelet counts $<170,000/\mu\text{l}$ there was no relationship between platelet count and platelet survival ($r=0.36$, $p>0.10$). There was a significant direct correlation between log platelet count and platelet production in all groups ($r=0.68$, $p<0.001$) (Figure 5) suggesting that platelet production rate rather than platelet survival determines platelet counts in ITP patients. Among patients on no treatment, 41% (7/17) had decreased platelet production and 53% (9/17) were in the normal range. Thus despite normal to increased numbers of marrow megakaryocytes in all ITP patients studied, 94% (16/17) of the untreated patients had an inappropriate thrombopoietic response to their low platelet counts. Possible causes of the ineffective thrombopoiesis were postulated to be: 1) antibody binding to megakaryocytes interfering with platelet production or release; or 2) antibody-mediated platelet phagocytosis in the bone marrow RE system preventing their release into circulation.

Improvement in thrombocytopenia following treatment of ITP is mediated by an increase in platelet production—

In contrast to kinetic measurements in untreated ITP patients who characteristically had decreased or normal platelet production rates, 47% (7/15) of ITP patients on prednisone were found to have high platelet production⁴. These data suggest that corticosteroid therapy may improve thrombocytopenia in many ITP patients by increasing platelet production. Measurement of platelet kinetics in patients before and after successful treatment with prednisone revealed that an increase in platelet count occurred without a corresponding increase in platelet survival²⁷ (Figures 6, 7) supporting the concept that the rate of platelet production is important in determining the circulating platelet count and a rise in platelet count is effected by increasing platelet production and/or release from the bone marrow. By contrast, successful removal of the spleen in ITP, the primary site of ¹¹¹In labeled platelet destruction by gamma camera imaging⁷⁶ is associated with an increase in platelet survival (Figure 8)^{7,27,59}.

Li and coworkers observed lower percentages of polyploid and apoptotic megakaryocytes and decreased in vitro platelet production compared to controls when bone marrow mononuclear cells and autologous CD8+ T cells from patients with chronic ITP were cocultured⁵⁸, but the addition of dexamethasone to the culture could correct these abnormalities. These studies give further credence to the idea that an improvement in platelet count is mediated by a rise in platelet production and that megakaryocytes can be driven to increase platelet production in ITP.

TPO and TPO mimetics

Over the past 15 years a number of molecules have been developed that bind and activate the TPO receptor.⁴⁸ The first generation of TPO molecules were recombinant proteins similar to native TPO but development was halted when antibodies formed against one of them. A second generation of TPO molecules was then developed to avoid autoantibody formation and two of these are now FDA-approved for the treatment of ITP. The structure and function of these molecules are discussed next with the detailed results of their clinical studies provided elsewhere in this volume (link to Bussel chapter).

First Generation TPO Molecules—Soon after the discovery of TPO, two recombinant TPO molecules entered clinical development. One was a recombinant human TPO (rhTPO) produced in CHO cells that, except for small differences in its pattern of glycosylation, closely resembled native TPO. The other, pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), was composed of the amino-terminal 163 residues of

human TPO, was not glycosylated, and had a 30 kD polyethylene glycol (PEG) moiety attached to keep the molecule stable in the circulation.^{45,46,48-50}

Preclinical studies in mice and rhesus monkeys showed that both of these recombinant molecules were very potent stimulators of megakaryocyte growth and platelet production. These animal studies demonstrated the following important pharmacodynamic aspects of all TPO treatments. After a single dose of TPO, there was a five day lag time before the platelet count started to rise (presumably due to TPO stimulation of early, but not late, megakaryocyte progenitors). What followed next was a platelet count rise peaking on days 12-14 that showed a log-linear relationship between the dose of TPO and the peak platelet count.³⁵ After the platelet peak was reached, the platelet count returned to normal by day 28 with no rebound thrombocytopenia. TPO had no effect on RBC or WBC. Prolonged exposure of animals to high doses was well tolerated but did produce reversible marrow fibrosis at these doses.^{25,80,89}

Subsequent human studies in cancer patients not receiving chemotherapy showed the same platelet response kinetics: a five day lag, peak platelet count at day 12-14, and no rebound thrombocytopenia.^{6,23} Neither recombinant TPO directly activated human platelets but both reduced by about 50% the threshold for platelet aggregation to weak platelet agonists like ADP.³⁴

Between 1995 and 2002 many clinical studies were conducted with both recombinant TPO molecules (for comprehensive reviews see ^{45,46,52}). In general, both molecules were well-tolerated, produced a robust rise in platelets (sometimes to over several million), were not associated with thrombosis, reduced the extent of thrombocytopenia in non-myeloablative (but not myeloablative) chemotherapy regimens, and improved the platelet counts in patients with ITP or myelodysplastic syndrome (MDS). In platelet apheresis donors given rhTPO or PEG-rHuMGDF there was a great increase in platelet yields.^{28,54,78} However subsequent larger safety studies with PEG-rHuMGDF in healthy volunteers demonstrated the appearance of a neutralizing antibody to TPO and development of both first generation TPO molecules was stopped.⁵⁷

Second Generation TPO Molecules—The results of the clinical trials with the first generation TPO molecules were sufficiently encouraging to prompt a search for non-immunogenic TPO molecules. Two general classes of molecules were developed: TPO peptide mimetics and TPO non-peptide mimetics. One drug in each class, romiplostim and eltrombopag, has now been FDA-approved for the treatment of ITP.⁴⁸

Romiplostim (AMG 531, Nplate): In 1997 a 14-amino acid peptide with no sequence homology with TPO was identified that bound and activated the TPO receptor.¹³ Given the requirement of any TPO to bind simultaneously two TPO receptor molecules, it was found that dimerization of these peptides increased their specific activity about 10,000-fold. Unfortunately, the usually short circulatory half-life of peptides often makes them poor pharmacologic agents. Efforts were then directed toward stabilizing the peptide (and yet preserving a dimeric structure) by attaching the peptides to a modified Fc receptor.¹⁰

Initially named Amgen Megakaryopoiesis Protein-2 (AMP-2), and subsequently developed as AMG-531, romiplostim is a 60 kD structure composed of 4 of these 14-amino acid peptides attached to a novel IgG heavy chain Fc region (a “peptibody”) by glycine bridges (Figure 9).^{10,81} To each arm of the Fc region are attached 2 TPO mimetic peptides, again creating a dimeric molecule capable of activating the TPO receptor. The peptides have no sequence homology with endogenous TPO such that if antibodies were to form against romiplostim, they would not cross react with endogenous TPO. Romiplostim has a circulatory half-life of 120-160

hours and is initially removed by the endothelial FcRn receptors, recycled, and eventually cleared by the reticuloendothelial system.⁸¹

Romiplostim binds to the distal HRD of the TPO receptor and activates the receptor just like recombinant TPO (Figure 3). In cultures of bone marrow and TPO-dependent cell lines, romiplostim produces the same effect as recombinant TPO.⁸ It does not directly activate platelets, but does lower the threshold for activation by ADP by 50%. Prolonged administration of large doses of romiplostim to mice produced marrow fibrosis that was reversible upon stopping the drug.⁵⁵

In healthy human volunteers, single doses of romiplostim produced a dose-dependent rise in platelet count beginning on day 5 and peaking at days 12-14.⁸¹ Auto-antibody formation did not occur. Romiplostim is now FDA approved for the treatment of ITP and is available as a lyophilized powder that is reconstituted with sterile water and injected subcutaneously usually once a week at doses of 1 – 10 µg/kg.^{1,10,53,81}

Eltrombopag (SB497115, Promacta): Screening of libraries of molecules to identify structures that activate the TPO receptor has yielded a large number of “lead compounds”.^{18, 19} Further modification to improve their biological activity and pharmacological properties has resulted in a number (eltrombopag⁴², AKR-501¹⁷, LGD-4665,²⁰ NIP-004⁶⁴, NIP-022⁶⁶, and butyzamide^{87,88}) that have undergone clinical development. Only one, eltrombopag, has completed Phase III trials and is FDA-approved for the treatment of ITP.

Although previously known as SB497115, eltrombopag is a small (442 Da) molecule (C₂₅H₂₂N₄O₄) with an acidic (COOH) group at one end, lipophilic (CH₃) groups at the other end, and a metal chelate group in the center that is a potent, orally available TPO non-peptide agonist (Figure 10). Single doses have no effect on the platelet count but daily doses for 10 days produce a peak platelet count on Day 16.⁴¹

Eltrombopag has a number of important features that are representative of this class of molecules:

- It is a small (442 Da) chemical structure that is an orally administered tablet given once a day at a dose of 25-75 mg.⁹
- It has very specific binding to the TPO receptor and binds only to TPO receptors in humans and chimpanzees.^{22,66} This has markedly limited preclinical efficacy studies in small animal models.
- This species specificity is attributed to which amino acid is present at position 499 in the transmembrane region of the TPO receptor: histidine in humans and chimps, leucine in all other species.
- It activates the TPO receptor by binding not to the distal HRD like TPO, but to the transmembrane region (Figure 3).^{45,66,88} This makes its biological effect at least additive to that of TPO.²⁶
- It does not directly activate platelets or alter their threshold for activation.⁴²
- At maximally tested doses, it increases the platelet count in healthy volunteers 1.5-fold above baseline compared with a 6-fold increase for romiplostim. Whether this difference in potency is of clinical importance is unclear.

The rationale for using TPO in ITP

Given the new understanding that ITP is a disorder of both increased platelet destruction and inappropriately low platelet production, new therapeutic options open up. In addition to using

drugs (IVIG, anti-D) or splenectomy to reduce the rate of platelet destruction, TPO mimetics (the “platelet producers”) can now be employed to increase platelet production and thereby ameliorate thrombocytopenia.

Early studies with PEG-rHuMGDF demonstrated that 3 of 4 Japanese patients with ITP had their platelet count increase after a 7-day administration (Figure 11).⁶⁷ In addition, one patient with an unusual form of cyclic ITP was maintained on weekly injections of PEG-rHuMGDF for over 8 years with good control of the platelet count.⁷⁰ As is presented elsewhere in this monograph (link to bussel chapter), both romiplostim and eltrombopag have been found to be highly effective in treating patients with ITP.⁴⁸

What has not been thoroughly explored in any of these clinical studies is exactly how TPO mimetics improve the platelet count. Although not directly studied with platelet kinetic approaches, they appear to have little effect on platelet survival in ITP. Indeed, upon stopping a TPO mimetic, the platelet count may plummet to life-threatening levels within 5-7 days; suggesting (but not formally proving) that platelet survival remains reduced.⁵³ The simplest explanation for their effect is that TPO mimetics are increasing the number of Mk precursors, increasing their maturation rate and thereby “overwhelming” the ability of the anti-platelet/megakaryocyte antibody to destroy the increased amount of platelets.

However, studies by Harker suggest a more sophisticated mechanism, i.e. TPO and TPO mimetics reverse the apoptotic effect of the anti-platelet/megakaryocyte antibody (or local T cell inhibition) on late megakaryocyte progenitors (probably not on the late megakaryocytes themselves because it takes 5-7 days before the platelet count rises in response to a TPO agonist).^{12,32,36} Harker extensively studied 8 patients with HIV thrombocytopenia.³² 6 were given 8 doses of 5 µg/kg PEG-rHuMGDF over 16 weeks and all had their platelet count rise 10-fold within 14 days; 2 patients received placebo and had no increase in platelet count. The megakaryocyte mass before treatment was $69 \pm 37 \times 10^{10}$ fL/kg (normal = 31 ± 5 fL/kg) and remained constant at 76 ± 45 fL/kg during treatment. Since there was no change in platelet survival, anti-platelet antibody or viral load, these results suggested that TPO therapy reduced apoptosis of megakaryocyte progenitors and megakaryocytes and allowed them to produce platelets. Whether this mechanism is true in non-HIV infected ITP patients awaits further studies; clearly bone marrow megakaryocytes in non-HIV infected ITP patients are undergoing accelerated apoptosis.³⁹

Summary

Despite normal or increased numbers of megakaryocytes observed in their bone marrows, platelet production appears to be inappropriately low in patients with chronic ITP. Levels of thrombopoietin in the circulation are only moderately elevated, if at all, likely due to uptake by platelets targeted for destruction in the RE system. In vitro studies reveal abnormal apoptosis and maturation of megakaryocytes which share antigenic targets with platelets such as GP IIb/IIIa. Indirect measurements of platelet production utilizing radiolabeled platelet survival measurements are consistent with an abbreviated response in the marrow compartment to the thrombocytopenia. Therapeutic intervention in ITP may increase the platelet count by increasing the numbers of platelets released into the circulation (corticosteroids), or increasing their circulating life span (splenectomy). The findings of inappropriately low levels of thrombopoietin and decreased platelet production by the bone marrow have afforded new opportunities for the therapy of chronic ITP. New agents mimicking the action of thrombopoietin at the megakaryocyte are capable of increasing platelet production and raising the platelet count.

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Figure 1. Infusion of anti-platelet antibody into healthy humans produces thrombocytopenia
Platelet counts after blood or plasma from patients with ITP were infused into health volunteers.

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Figure 2. The physiological regulation of thrombopoietin levels

The constitutive hepatic production of thrombopoietin (center) is cleared by avid thrombopoietin receptors on platelets resulting in normal levels when the platelet production is normal (left) and elevated levels when the platelet production is reduced (right). The bone marrow megakaryocytes are stimulated to make more platelets as the circulating thrombopoietin concentration rises.⁵¹

Figure 3. Activation of the TPO receptor

The TPO receptor has been proposed to exist as an inactive preformed dimer (left receptor dimer) with a proximal (HRD-1) and distal (HRD-2) hematopoietic receptor domain (HRD). Upon binding of thrombopoietin/romiplostim to the distal HRD-2 or binding of eltrombopag to the transmembrane region, the receptor (right receptor dimer) conformation changes and a number of signal transduction pathways are activated that increase platelet production.

Figure 4. TPO levels are not elevated in patients with ITP

TPO levels (+/-SD) were measured in thrombocytopenic patients with ITP or aplastic anemia and compared with healthy volunteers.¹

Figure 5. Relationship between platelet count and platelet turnover

There was a direct relationship between log platelet count and autologous platelet turnover; $r=0.068$, $P < 0.001$. Patients were on no treatment ($\circ$), prednisone ($\bullet$), or postsplenectomy (Δ). Hatched area represents the normal range.

Figure 6. Platelet survival before and after prednisone treatment

The ^{111}In labeled autologous platelet survivals of 12 ITP patients were unchanged by prednisone therapy before (baseline) and after therapy with corticosteroids increased their platelet count. The regression line for the data was determined by the equation $y = 0.06 + 1.60x$; $r^2 = 0.90$. The correlation coefficient between the platelet survival measurements before and after steroid treatment in the same patient was 0.21 ($P < 0.005$).

Figure 7. Platelet turnover and platelet count before and after prednisone treatment

In the 11 patients whose platelet counts improved with treatment there was a direct correlation between the base-line platelet count and platelet turnover rate ($\circ$) and between the post-prednisone values ($\bullet$) (correlation coefficient, 0.86; $P < 0.0005$). The retreatment and post-treatment data for the one patient who had no improvement in the platelet count are denoted by $\square$ and $\blacksquare$, respectively. The regression line for the pretreatment and post-treatment data for the 11 patients who responded to treatment (solid line) was predicted by the equation $y = 6.50 + 0.41x$; $r^2 = 0.75$. Individual pretreatment and post-treatment values are connected by dashed lines. The hatched area shows the expected normal relation between platelet count and platelet turnover ± 1 SD.

Figure 8. Platelet survival and platelet count before and after successful splenectomy

The relation between presplenectomy platelet counts and survival data is denoted by ○ and that between postsplenectomy values by ●. The solid regression line for the data was determined by the equation $y = 2.12 + 0.01x$; $r^2 = 0.61$. The correlation coefficient was 0.78 ($P < 0.0005$). Individual presplenectomy and post-splenectomy values are connected by dashed lines. The hatched area shows the expected normal relation between platelet count and platelet survival ± 1 SD.

Figure 9. Structure of romiplostim

Romiplostim is composed of the Fc (fragment “crystallized”) portion of IgG to which two 14 amino acid TPO peptides (purple) are coupled via glycine bridges (green) at the carboxy-terminus of each γ heavy chain. Interchain (cysteines C7 and C10) and intrachain (cysteines C42-C102, C148-C206) disulfide bridges are indicated in red. Heavy chain constant domains 2 (CH2) and 3 (CH3) are also shown. Romiplostim has a molecular weight of ~60 kDa.

Figure 10. Structure of eltrombopag

Eltrombopag [3'-{N'-[1-(3,4-Dimethylphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-2'-hydroxybiphenyl-3-carboxylic acid] is a hydrazone small (442 Da) molecule with an acidic (COOH) group at one end, lipophilic (CH₃) groups at the other end, and a metal chelate group in the center.

Figure 11. PEG-rHuMGDF increased the platelet count in an ITP patient. PEG-rHuMGDF was administered daily for 7 days and the platelet count and reticulated platelets measured daily. The rise in platelet count was preceded by an increase in reticulated platelets.⁶⁷