Thrombopoietin: The Novel Hepatic Hormone

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The glycoprotein thrombopoietin (TPO) is the major stimulator of megakaryopoiesis and platelet production. Hepatocytes express TPO mRNA at a constant rate. The plasma TPO level is inversely correlated to the mass of megakaryocytes and platelets, which degrade the hormone following its binding to specific membrane receptors.

The classic work by James H. Wright (18) has established that blood platelets are detached particles of megakaryocyte cytoplasm. Platelets are anuclear, have a life span of ~10 days, and are important in primary hemostasis (14). The tiny discs become activated when they pass a blood vessel with a disrupted endothelium. They quickly exhibit membrane receptors and pseudopodia, adhere to subendothelial elements such as collagen, and form a hemostatic plug. The releasable contents of the different platelet granules (alpha, dense, and lysosomal) stimulate coagulation, increase vascular tone and permeability, and support endothelial repair and wound healing. Persons with a significant deficiency in platelet number or functionality develop increased vascular fragility and purpura, i.e., bleeding into skin and mucous membranes and extensive hemorrhage after cuts. Furthermore, platelets play a role in antimicrobial host defense, and thrombocytopenia increases the susceptibility to certain pathogens (19).

Healthy humans have 150–400 platelets/nl blood. In the steady state, thrombopoiesis balances platelet destruction. Megakaryocytic progenitors are continuously generated from hemopoietic stem cells in bone marrow (Fig. 1). Megakaryopoiesis and thrombopoiesis are stimulated by a variety of cytokines, including stem cell factor, granulocyte/monocyte colony-stimulating factor (GM-CSF), erythropoietin (EPO), and the interleukins (IL)-1, -3, -4, -6, -7, and -11 (2). In addition, there is true regulation of platelet production in terms of a control circuit, because an acute loss of platelets results in a stimulation of thrombopoiesis.

Recently, the gene encoding the specific thrombopoiesis-stimulating factor thrombopoietin (TPO) has been cloned and in vitro expressed, and the hormone has been identified as the dominant regulator of platelet production. Major progress has since been made in understanding the biodynamics and molecular mechanisms of thrombopoiesis. The therapeutic use of recombinant human TPO (rhTPO) is under clinical investigation (5). This review describes the present concept of the physiological regulation of platelet mass, which involves the continuous synthesis of TPO in the liver and its degradation by megakaryocytic cells and platelets.

Molecular biology of TPO

The human TPO gene comprises seven exons and six introns located on chromosome 3q26.3-27. Because of the use of different transcription initiation regions and alternative splicing, TPO mRNA transcripts may contain either one or both of the noncoding exons 0 and 1. The five coding exons span a region of 6 kb. Splice isoforms also vary in the junction of the last two exons (exons 5 and 6), but their role and regulation of these isoforms is not yet known. The primary translation product of the human TPO gene is composed of 353 amino acids, including a secretory leader peptide of 21 amino acids. The major form of circulating TPO consists of 332 amino acids and has a molecular mass of ~70 kDa (Table 1). In addition to its six glycosylation sites, the molecule possesses multiple O-glycosylation sites. The carbohydrate content amounts to 40%. Apart from the complete glycoprotein, smaller forms of TPO exist in blood. These result from alternative splicing of TPO mRNA, heterogeneity in the synthesis of the carbohydrate side chains, and extracellular proteolytic cleavage.

Two parts of the TPO molecule can be distinguished when it is separated between the arginine residues in positions 153 and 154. The NH2 terminal part binds to the TPO receptor, its amino acid sequence exhibits 23% identity with that of human EPO or 50% similarity when conservative substitutions are taken into account. It is also called the cytokine domain because of its four conserved cysteine residues and the typical four-helix bundle structure of cytokines of the class I family. The efficiency of the therapeutic administration of a truncated and recombinant TPO product of the 163 NH2 terminal amino acids [recombinant human megakaryocyte growth and development factor (rhMGDF)] demonstrates that the cytokine domain suffices to stimulate platelet production (5). The COOH terminal glycan domain of TPO (amino acids 226-332) contains multiple N- and O-linked carbohydrate chains. These are required for efficient secretion of TPO and to ensure the relatively long in vivo survival of the hormone (half-life 20–30 h).

Targets and effects of TPO

Megakaryocytes are the progeny of pluripotent hemopoietic stem cells and megakaryocytic progenitors (Fig. 1). The less differentiated CD34+ stem cells are also termed CFU-GM/CFC (colony-forming units with descendants that may differentiate into granulocytes, erythrocytes, monocytes, and megakaryocytes). In addition, hemopoietic stem cells generate identical copies of their own. TPO stimulates the growth of CFU-GM/CFC and the proliferation and differentiation of megakaryocytic progenitors. Analogous to the effect of EPO, the primary mechanism of the action of TPO is inhibition of apoptosis of its target cells. The many steps of proliferation, differentiation, and
maturation from a CFU-GEMM to a horde of platelets takes ~10 days. The earliest differentiated megakaryocytic progenitor is the burst-forming unit-megakaryocytic, which is characterized by a high proliferative activity and a requirement for IL-3 or GM-CSF. Its follower in differentiation is the colony-forming unit-megakaryocytic, which is sensitive to various hemopoietic growth factors and cytokines. Eventually, morphologically identifiable megakaryocytes emerge. By nuclear endomitosis, megakaryocytes develop into large polyploid cells. Finally, during the process of transmural migration or in the vascular bed, megakaryocytes break into 6–8 elongated projections per cell, the proplatelets, each of which can spawn ~1,000 platelets. Whether the sequestration of the megakaryocytes occurs mainly in bone marrow or subsequently in pulmonary capillaries is still a matter of debate.

The TPO receptor is present mainly on hemopoietic stem cells, megakaryocytic progenitors, and megakaryocytes. Apart from bone marrow, spleen, and fetal liver, nonhemopoietic tissues such as placenta and brain may express TPO receptor transcripts. The functional role of TPO in these latter organs is not fully clear. Of major significance is the fact that TPO receptors are maintained on platelets. There are ~30–60 high-affinity binding sites for TPO per platelet. The TPO receptor gene (17 kb) is located on the short arm of chromosome 1 (1p34). It is composed of 12 exons and 11 introns. The TPO receptor was initially identified as the product of the protooncogene c-mpl (associated with myeloproliferative leukemia) and is therefore called MPL. It is a member of the cytokine receptor superfam-

![Image](http://physiologyonline.physiology.org/)

**FIGURE 1.** Simplified model of hematopoiesis, indicating growth factors (TPO, thrombopoietin; SCF, stem cell factor; IL, interleukin; CSF, colony stimulating factor; EPO, erythropoietin) in control of viability, proliferation, and differentiation of hemopoietic stem and progenitor cells (CFU, colony-forming unit; BFU, burst forming unit) of megakaryocytes (Meg), granulocytes (G), monocytes (M), and erythrocytes (E). Bas, basophils; Eo, eosinophils; GEMM, granulocytes, erythrocytes, monocytes and megakaryocytes.

### TABLE 1. Molecular biology and chemistry of thrombopoietin

<table>
<thead>
<tr>
<th>DNA</th>
<th>mRNA All mammals studied</th>
<th>Protein Human</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td>Single copy gene located on chromosome 3q26.3-27</td>
<td>Prehormone of 353 amino acids, including a 21-amino-acid signal peptide</td>
</tr>
<tr>
<td>7 exons (2 noncoding, 5 coding), 6 introns</td>
<td>Expressed predominantly in liver and to a minor degree in kidney, spleen, lung, intestine, brain, and bone marrow</td>
<td>Monomeric glycoprotein with 6 potential N-linked and multiple O-linked glycosylation sites</td>
</tr>
<tr>
<td></td>
<td>Transcription from different promoters and splice variants of exons 0 and 1</td>
<td>2 disulfide bridges</td>
</tr>
<tr>
<td></td>
<td>Splice variants of exons 5 and 6 with unknown function</td>
<td>Mol. mass of ~70 kDa (40% carbohydrate)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibly proteolytically cleaved in circulation to smaller functional units</td>
</tr>
</tbody>
</table>

...ily characterized by two extracellular immunoglobulin-like domains, WSXWS boxes close to the transmembrane domain, and a lack of intrinsic kinase activity of the cytoplasmic region (11). Two isoforms of the TPO receptor have been identified.
(MPL-P and MPL-K), which differ in their intracellular domains as a result of alternative splicing. The calculated molecular mass is 71 kDa for MPL-P and 65 kDa for MPL-K. Human MPL from platelets is glycosylated and has an apparent molecular mass of 85–92 kDa.

On TPO binding, two receptor molecules form a tightened homodimer. Several cytoplasmic kinases are then activated, including Janus kinase-2, tyrosine kinase-2, mitogen-activated protein kinase, and extracellular signal regulated kinases-1 and -2. Furthermore, distinct factors of the signal transducers and activators of transcription family (STAT-1, -3, and -5) and the cAMP-responsive element binding transcription factor are activated. The identification of the exact cascade of events in TPO signaling is an attractive object of ongoing research.

The importance of TPO in the physiological control of platelet homeostasis has been demonstrated in mutant mice. Platelet counts are reduced to ~10% of normal in mice deficient in either the TPO gene or the TPO receptor gene (9). The animals show no apparent physical abnormalities. They do not bleed spontaneously, but their bleeding times are prolonged when tested by the tail clip assay. Blood cell counts other than platelets are normal. In bone marrow of TPO or TPO receptor knockout mice, the numbers of megakaryocytes, colony-forming units-megakaryocytic, and progenitors of other hematopoietic lineages are reduced (9). These findings indicate that TPO activity is necessary to maintain a high number of platelets, although it is not absolutely required for megakaryopoiesis and thrombopoiesis. The growth factors responsible for the basal rate of platelet production in the absence of TPO action are still to be identified. IL-3, IL-6, IL-11, or stem cell factor as single components cannot substitute for TPO. Studies in humans and experimental animals have shown that the administration of rhTPO or rhMGDF results in a dose-dependent stimulation of thrombopoiesis and thus increases the concentration of circulating platelets.

Another issue of physiological and pharmacological relevance relates to the effects of TPO on platelet function. In vitro studies indicate that TPO augments agonist-induced platelet aggregation, ADP release, and thromboxane formation. However, clinical studies with rhTPO have provided no evidence that TPO acts directly as a prothrombotic agent (1).

Sites and control of TPO synthesis

TPO mRNA is expressed mainly in liver, and to a lesser extent in kidney, spleen, lung, bone marrow, and brain. Thus there are not only similarities in the molecular structures but also in the production sites when EPO and the novel hematopoietic hormone TPO are compared. However, although there is a postnatal switch in the main site of EPO synthesis from the liver to the kidneys, the liver remains the principal site of TPO synthesis throughout mammalian life. The liver accounts for 95% of the total body TPO mRNA in human fetuses (15). An in situ hybridization study on adult human tissues has shown that the TPO mRNA-expressing cells are mainly hepatocytes in the liver and proximal tubular epithelial cells in the kidneys (13). Human hepatoma cell lines are a useful model for in vitro study of TPO gene expression. Production of TPO by primary cultures of human proximal tubular cells has also been reported (17).

The thrombocytopenia that develops in patients suffering from liver cirrhosis goes along with reduced hepatic TPO mRNA levels and relatively low plasma TPO concentrations. Hepatic TPO mRNA levels, blood platelet numbers, and circulating hepatic proteins decrease with the severity of liver failure (16). Plasma TPO levels and platelet numbers normalize after orthotopic liver transplantation in the afflicted persons (8). Further evidence for the predominant role of the liver in TPO synthesis has come from animal studies. When normal livers in mice were replaced by livers from TPO knockout mice, platelet numbers in the recipients decreased to <50% of normal (10). Thus the loss of TPO synthesis in the liver cannot be compensated for by other organs.

FIGURE 2. Feedback control circuit of the plasma TPO concentration (left) compared with that of EPO (right). TPO is mainly produced in liver and to a minor degree in kidney, spleen, and other organs. This production is constitutive, i.e., independent from the actual platelet concentration in blood. Regulation of the plasma TPO concentration, and thus platelet production, is accomplished by the targets, because megakaryocytes and platelets metabolize the hormone. In contrast, erythropoiesis is regulated through PO2-dependent EPO gene expression. Whether TPO and EPO produced in bone marrow play a significant role as paracrine mediators is being investigated.
sated for by other organs such as the kidney or the bone marrow.

Hepatic and renal TPO gene expression is generally thought to proceed at a constant rate, i.e., it is unregulated (Fig. 2). Both human and animal studies have shown that the TPO mRNA levels in liver, kidney, and spleen are similar in thrombocytopenic, normal, and thrombocytotic subjects and not influenced by the concentration of circulating platelets (13). Whether there is control of hepatic TPO synthesis at the translational level still remains to be investigated.

Regulation of the level of circulating TPO

Published values of the concentration of immunoreactive TPO in blood of healthy humans vary greatly, with median or mean values ranging from 20 to 240 pg/ml plasma or serum (~1 pM). Major reasons for these disparities include the release of TPO from platelets during clotting (yielding inappropriately high values in sera), differences in antibody specificity (polyclonal antibodies are more sensitive to truncated forms of TPO than monoclonal antibodies), and the lack of an international standard preparation of TPO. Thus, it is in general not possible to compare data between different laboratories. Despite these difficulties, however, it seems to be fairly well established that the plasma TPO concentration is usually elevated in thrombocytopenic states, whereas it is lowered on the occurrence of thrombocytosis (4).

In considering the constitutive expression of the hepatic TPO gene, the question arises as to how the fine regulation of the TPO concentration and of the number of platelets in blood is accomplished. Fielder et al. (3) have shown that the plasma TPO concentration is increased in thrombocytopenic TPO receptor knockout mice. The injection of platelets into these mice results in a marked decrease in the plasma TPO concentration within 2 h. Indeed, the level of circulating TPO is regulated through clearance by the platelets that bind the hormone (Fig. 2). The plasma TPO concentration of ~1 pM is low compared with the TPO receptor binding affinity, with a dissociation constant (Kd) of ~200 nM in normal humans (7). On TPO binding, the ligand-receptor complex is internalized and TPO is degraded. Internalized TPO is mainly localized in the surface-connected open canalicular system and the cytoplasm of platelets (6). The fate of TPO in platelets needs to be further investigated with respect to the molecular sites and velocity of proteolytic cleavage. The possibility still remains that platelets release biologically active TPO fragments on stimulation with aggregatory agonists. Note that megakaryocytes and their progenitors also bear TPO receptors and remove the hormone. However, the mass of this pool of cells is usually small compared with the large pool of platelets. Only in diseases such as immune thrombocytopenia may the enlarged total mass of megakaryocytic cells significantly reduce the level of circulating TPO.

The thrombocytic feedback control circuit, namely that the end cells regulate the concentration of their growth promoter, is not unique. Similar mechanisms exist in the regulation of other cytokines and hemopoietic growth factors, including EPO, granulocyte colony-stimulating factor, and monocyte colony-stimulating factor. In conclusion, there are three consequences of the binding of TPO to its receptor. The first is the initiation of specific signaling pathways that affect the viability, proliferation, and differentiation of hemopoietic stem cells and megakaryocytic progenitors. Second, the binding of TPO to platelets may render these hemostatically more reactive. The third consequence is TPO uptake, which lowers the concentration of TPO in the extracellular fluid and results in an inverse relationship between the TPO concentration and the mass of platelets and megakaryocytes.

Some reports indicate that TPO mRNA expression is induced by thrombocytopenia in bone marrow stroma. TPO mRNA has been detected in human myeloid stroma cell cultures. Since TPO expression is lowered by the platelet-borne glycoproteins thrombospondin and platelet factor 4, a second feedback loop has been proposed. It is possible that TPO synthesis in bone marrow is blocked by proteins released from the alpha granules of megakaryocytes and platelets if these cells are abundant (12). Clearly, however, the role of myeloid TPO in the paracrine control of thrombopoiesis needs to be further elucidated.

TPO as an acute-phase protein

Reactive thrombocytosis is a common complication of inflammatory processes. In these cases, the inverse relationship between the concentrations of platelets and TPO in blood is lost. The concentration of circulating TPO is abnormally high in reactive thrombocytosis, as has been shown in autoimmune diseases, infections, or malignancies. In vitro evidence suggests that IL-6 mediates this reaction by enhancing hepatic TPO mRNA expression (17). The 5'-untranslated region of the TPO gene contains 13 segments that match the type II IL-6 response element consensus sequence (CTGGGA). IL-6 upregu...
ulates the transcription of several acute-phase protein genes in the liver, such as those for fibrinogen and serum amyloid A. The concentration of circulating IL-6 is elevated in patients with inflammatory diseases. IL-6 also directly stimulates the growth of megakaryocytic progenitors in bone marrow. These links point to a role of platelets in inflammation. Platelets transport and, on activation, release vascular permeability-enhancing factors and mitogens for endothelial cells, smooth muscle cells, and fibroblasts, such as vascular endothelial growth factor and platelet-derived growth factor. Platelets are also transporters of chemokines and microbicidal proteins (19). They inactivate microbial pathogens by internalization and formation of cytotoxic O₂ species. In case of an infection, the abundance of platelets is of adaptive value, because it will support the inflammatory reaction, the attraction of leukocytes, and the killing of microbia (Fig. 3).

**Perspectives**

The discovery of the glycoprotein TPO as the primary viability and growth factor for the megakaryocytic progenitors in the bone marrow is an example of the ongoing success in endocrine research. Circulating TPO derives mainly from hepatocytes, which express TPO mRNA at a constant level. The plasma TPO concentration is inversely correlated to the mass of platelets and megakaryocytes, which internalize and degrade the hormone via their specific TPO receptors. Whether platelets also function as TPO storage sites releasing biologically active TPO on aggregation still needs to be clarified. Another observation still in question relates to the significance of TPO mRNA expression in bone marrow stroma. Clinical trials are ongoing to evaluate the potential of rhTPO for prevention of the severe thrombocytopenia associated with chemotherapy, radiotherapy, and myelodysplastic syndromes. The future will show whether the novel hormone TPO will be of similar clinical value to the closely related EPO.

**References**