Thrombopotein: its ups and downs

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Chapter 12

Summary and General Discussion
Chapter 12

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12.0 Introduction

Since the discovery of thrombopoietin (Tpo), it has been amply shown by both in vitro and in vivo studies that Tpo is the main regulator of thrombocytopoiesis. Tpo promotes proliferation, maturation and differentiation of cells from the megakaryocytic lineage, which ultimately results in the formation of platelets. Tpo exerts its function via binding to the Tpo-receptor, Mpl, which is expressed on all cells from the megakaryocytic lineage, including platelets. In vivo, administration of Tpo leads to enhanced thrombocytopoiesis and expansion of the number of circulating platelets (reviewed in Chapter 1).

To gain insight in the process of thrombocytopoiesis and platelet homeostasis, knowledge about the structure and function of Tpo and its receptor and about the control of circulating Tpo levels is essential. The data described in this thesis confirm and extend previous observations that measurement of plasma Tpo levels is of clinical importance. In addition, this thesis provides information about the mechanisms underlying different platelet disorders and it increases the knowledge with respect to the interplay between platelets and Tpo. In this chapter, the gathered data will be summarised and discussed with respect to other published studies.

12.1 Thrombopoietin ELISA: Test characteristics and reference values

As described in Chapter 2, we developed a sensitive and specific ELISA with which Tpo concentrations can be reproducibly measured. In contrast to other published Tpo ELISAs [1-3], our ELISA was the first that was solely based on monoclonal antibodies, two for capturing Tpo and one for its detection. An advantage of the use of monoclonal antibodies as compared to polyclonal antibodies is that they can be supplied continuously with a stable quality. With the assay, the full-length, biologically active Tpo molecule is detected. The truncated form, containing the biologically active N-terminal domain only, is not detected with our assay, but is in all other described assays. As a standard, we used a pool of plasma from patients with high Tpo levels. The Tpo concentration of the pool was arbitrarily set at 100 arbitrary units/ml (AU/ml). As an alternative, we tested several recombinant Tpo preparations (rhTpo) and found different results. For instance, 1 AU equalled 9 pg of rhTpo from Research Diagnostics Inc., but when calibrated against rhTpo from Genentech Inc., 1 AU equalled 1 pg. Since a universal standard is not yet available and, in our hands, all recombinant Tpo preparations were found to degenerate in time, we preferred to use a plasma pool as a standard. In the assays described so far, various standards were incorporated (recombinant Tpo preparations, either truncated or full-length) and different read-out units were used, referring either to the absolute mass (pg/ml) [3-6], and the commercially available Quantikine Tpo ELISA, R&D systems, Minneapolis, MN, USA) or, in one case, to the molar content (fmol/ml) [2]. When comparing data from different studies, the characteristics of the assay system should be taken into account, and the data should be normalised to the reference values as assessed in the same assay system.

We have shown that it is important to note whether Tpo level measurements were performed with plasma or with serum. We found Tpo levels in serum to be on average 3.4 times higher.
than Tpo levels in plasma from healthy individuals (n=137) (Chapter 2). In Chapter 9 it was demonstrated that platelets can release Tpo upon activation, suggesting that in serum, both circulating Tpo, and Tpo released by platelets during the clotting process, is measured. Indeed, in patients who have a low circulating number of platelets as a result of impaired platelet production, the ratio between plasma and serum concentration is decreased compared to individuals with normal platelet counts, i.e. 1.3 vs. 3.4, respectively. Platelet counts and the serum/plasma ratio were positively correlated ($R_p=0.7$, $p<0.01$) (see figure 1). In concordance with our findings, Ishiguro and co-workers [7] reported a four-fold difference in serum and plasma Tpo concentration when samples from 22 disease-free children with normal platelet counts were analysed [2]. With their ELISA system, both the N-terminal truncated form and the full-length form of Tpo can be measured. A difference in plasma and serum Tpo concentration, albeit to a smaller extent, was also detected with the Tpo ELISA developed by Amgen, which uses a polyclonal antibody recognising only the receptor-binding domain (N-terminal domain) for capturing and another polyclonal antibody raised against the full-length form of Tpo for detection [3]. Serum levels were reported to be on average 14 to 30% higher than plasma levels in healthy donors [4,8]. According to the product information of the commercially available Quantikine Tpo ELISA (R&D systems), which is based on two monoclonal antibodies, Tpo levels cannot be measured in (all) plasma samples (<31.2 pg/ml= detection limit). However, Tpo could be measured in 98% of the serum samples and was on average 67.1 pg/ml. Of the two other ELISA systems, one is not sensitive enough to detect normal Tpo levels [1]. With the other assay system, differences between plasma and serum levels were not assessed [6]. Although serum levels were generally higher than plasma levels, the extent of the difference varies. This might be due to differences in the characteristics of the ELISA system used, i.e. specificity's of the capture and detector antibodies and the standard applied. Alternatively, the procedure used to isolate plasma (anticoagulant used) or serum, might influence the results. In
our system, the use of EDTA, citrate or heparin as an anticoagulant did not influence the test results.

Reference values for the endogenous concentration of Tpo were established by the measurement of plasma Tpo levels of 193 healthy (adult) volunteers and ranged from 4-32 AU/ml [2.5th-97.5th percentile] (Chapter 2). Plasma Tpo levels were not different between males and females but tended to decrease slightly with age, which is in concordance with other reports [2,4]. Plasma Tpo levels in healthy newborns were slightly higher than the concentrations found in adults (see Chapter 6). Others also reported this relative increase in both serum and plasma samples [7,9-11]. Ishiguro et al.[7] reported elevated serum Tpo levels compared to Tpo levels in adults, in newborn infants and children up to the age of 15 yrs. With increasing age, Tpo levels declined, with the largest decline occurring in the first two months after birth and the highest Tpo levels found 2 days after birth. As in adults, Tpo levels were not different between males and females. As discussed in Chapter 6, the origin of the relatively elevated Tpo levels in children is unknown.

12.2 Measurement of Tpo levels: Diagnostic value

12.2.1 Thrombocytopenia

In case of thrombocytopenia of unknown aetiology, measurement of thrombopoietin levels provides a valuable diagnostic tool to differentiate between thrombocytopoietic failure and peripheral platelet destruction (Chapter 5&6). High Tpo levels, ranging from more than 5 to 30 times the normal upper limit, are associated with thrombocytopenia caused by a lack of megakaryocytes in the bone marrow. Normal to slightly elevated Tpo levels (less than twice the normal upper limit) are associated with thrombocytopenia resulting from peripheral platelet destruction, such as occurs in primary and secondary AITP. Other investigators have reported similar results [2,12-20]. Results were consistent when Tpo was measured in either serum or plasma. Thus, both sources can be used to discriminate between the two causes underlying thrombocytopenia. Apparently, in AITP, the reported Tpo production by bone-marrow stromal cells and increased megakaryocyte numbers in the bone marrow is not sufficient to overcome thrombocytopenia. Therefore, this patient group might benefit from treatment with Tpo or a Tpo-mimetic peptide.

In the aforementioned patient groups, plasma Tpo levels seemed to reflect the thrombocytopoietic capacity of the bone marrow rather than the number of circulating platelets, i.e. platelet production is normal or even slightly enhanced in AITP whereas in severely suppressed platelet production, Tpo levels are highly elevated. Patients with aplastic anaemia (AA) also have highly elevated Tpo levels [2,3,12,13,15,18,21-24]. In patients who show an increase in platelet count when recovering from immunosuppressive therapy or bone-marrow transplantation, Tpo levels decreased but remained above normal levels [25,26]. It has been suggested that in these patients, the elevated Tpo levels reflect the persistence of a haematopoietic defect.
in the bone marrow. Possibly, increased Tpo levels are required to maintain sufficient thrombocytopoiesis.

Patients with myeloid malignancies, such as myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML), form an exception to the rule that Tpo levels are increased when thrombocytopoiesis impaired. Normal, as well as elevated plasma Tpo levels were found in these patients (figure 2). In MDS, Tpo levels have been reported to vary with disease classification: Tpo levels are generally lower in refractory anaemia with excess blasts (RAEB) or RAEB in transformation (RAEB-t) than in refractory anaemia [27-29]. It is noteworthy that Tpo-R expression by blast cells has been reported in 30 to 40% of RAEB and RAEB(t) but not in RA [30-32]. Similarly, the Tpo-R is expressed by blast cells of 50% of the patients with AML [30,33-39]. Tpo-R expression correlates with an unfavourable prognosis. It is conceivable that the blast cells expressing the Tpo-R bind Tpo and thereby prevent a rise in Tpo levels. Further studies should clarify whether Tpo levels and expression of its receptor by blast cells are related, and whether Tpo level measurements can be of prognostic value, with low Tpo concentrations being unfavourable.

12.2.2 Thrombocytosis

For the differential diagnosis of disorders with thrombocytosis, Tpo level measurements seem to be of limited value. We found plasma Tpo levels to be within the normal range in reactive thrombocytosis as well as in the myeloproliferative disorders, polycythaemia vera and essential thrombocythaemia (Chapter 7). Thus, in these disorders, Tpo measurements do not have a discriminatory value. Similar results, i.e. plasma Tpo levels within the normal range or slightly elevated, have been reported by others for both PV and ET, but also for chronic myeloid leukaemia (CML) and myelofibrosis (MF) [40-43]. The reported serum Tpo levels in these patient groups are also within the normal range or elevated [2,22,44-49]. However, as
compared to plasma Tpo levels, maximal Tpo levels seem to be higher and relatively more patients have serum Tpo levels above the upper normal limit. This difference might be attributed to the measurement of both circulating Tpo and Tpo released by platelets in serum. Measurement of Tpo levels in thrombocytosis can be used to exclude the presence of overproduction of Tpo as the cause of the increased platelet count. In hereditary thrombocythaemia Tpo levels were found to be highly elevated. To date, four families have been reported in whom thrombocytosis occurred as a result of overexpression of Tpo [50-55]. The increased Tpo production, leading to thrombocytosis, was caused by a mutation in the 5' untranslated region of the Tpo gene (albeit different ones in each family), resulting in loss of translational repression. Serum Tpo levels in affected family members were five to twenty times normal, which is higher than the Tpo elevations seen in PV, ET and reactive thrombocytosis. Whether overexpression of the Tpo gene is involved in all cases of hereditary thrombocytosis remains to be investigated.

Overproduction of Tpo can also occur in patients with hepatoblastoma, as we observed in two patients with hepatoblastoma and thrombocytosis. Komura et al. have reported highly elevated Tpo levels (over five times normal) in children with thrombocytosis resulting from Tpo overproduction by hepatoblastoma cells [56].

In summary, in thrombocytopenia with unknown aetiology, measurement of Tpo levels can be of value in discriminating thrombocytopenia resulting from impaired platelet production (high Tpo levels) from thrombocytopenia resulting from peripheral platelet destruction (normal to mildly elevated levels). In disorders with thrombocytosis, measurement of Tpo levels can be used to exclude Tpo overproduction as the cause of increased platelet numbers. In case of Tpo overproduction such as occurs in patients with hereditary thrombocytosis or patients with a hepatoblastoma, highly elevated Tpo levels are found. The highly elevated Tpo levels in these patients contrast with the normal or mildly elevated Tpo levels seen in reactive thrombocytosis (i.e. secondary to surgery, infections or malignancies) or myeloproliferative disorders. Figure 3 shows a schematic representation of the Tpo levels of the different patient groups as reported in this thesis.

12.3 Measurement of Tpo levels: Implications for the regulation of Tpo

Measurement of Tpo levels in individual patients and patient groups with different thrombopoietic disorders contributes to the knowledge on the regulation of thrombopoiesis. The main mechanism that is thought to determine the amount of circulating Tpo is based on constitutive Tpo production by the liver and kidney, and clearance of Tpo via receptor-mediated uptake by platelets and megakaryocytes [57].

The results from Chapter 4 suggest that the kidney does not play a major role in Tpo production. In patients with severe renal failure, platelet counts were within the normal range and Tpo levels were normal or even slightly increased. Apparently, loss of kidney function does not lead to an overall decrease in Tpo production and thrombocytopenia.
Figure 3: Summary of the Tpo levels in the different patient groups as described in this thesis. The hatched bars represent control values for the different age groups. Dark grey: decreased platelet counts. Light grey: normal platelet counts. Black: increased platelet counts. * serum Tpo level, all other measurements were performed in plasma.

The capacity of platelets to bind and clear Tpo from the circulation is demonstrated in Chapter 3. Plasma Tpo levels decreased in thrombocytopenic patients, after they had received a platelet transfusion. Similar results had been previously reported from animal studies [57,58]. Moreover, we demonstrated that the infused platelets bound Tpo in vivo because the Tpo content of the infused platelets rose. Previously, Fielder and co-workers [58,59] and Li and co-workers [60] already showed in vitro that upon binding, platelets internalise and degrade Tpo. In patients that underwent major surgery (Chapter 5), the mean platelet Tpo content rose after surgery, preceding an increase in circulating Tpo. This suggested that the rise in Tpo was first reflected in an enhanced Tpo uptake by platelets and indicates that under steady-state conditions platelets are not saturated with Tpo but still have the capacity to bind Tpo.

According to the model of constitutive production and Tpo-R-mediated clearance, a decrease in platelet production will lead to a rise in the available amount of thrombopoietin and thereby stimulate thrombocytopoiesis. The inverse relationship between platelet counts and plasma Tpo levels as detected in patients undergoing chemotherapy (Chapter 2 [61]) [1,62-64] is consistent with this model. In these patients, Tpo levels increased when platelet counts decreased and vice versa. As shown in Chapter 5,6 and 9, thrombocytopenia does not always involve highly elevated plasma Tpo levels. In Chapter 5 [65] it is shown that plasma Tpo levels were indeed highly elevated in patients with thrombocytopenia resulting from a decreased platelet production, but in patients with thrombocytopenia resulting from peripheral platelet destruction, Tpo levels were normal or only slightly elevated. The latter finding was consistent in foetal and neonatal patients with alloimmune thrombocytopenia (Chapter 6). The explanation for the relatively normal Tpo levels in auto- and alloimmune thrombocytopenia, but also in drug-induced thrombocytopenia, post-transfusion purpura and X-linked thrombocytopenia...
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[13,66] is that platelet production is normal or even enhanced in these patients. In our study, this was reflected by the normal GC levels, i.e. a marker for total platelet mass, and the elevated megakaryocyte numbers in the bone marrow. The increase in the number of megakaryocytes is probably due to the local production of Tpo by bone-marrow stromal cells as previously reported [23,67-69]. Despite the shortened platelet life-span in these patients, the total megakaryocyte and platelet mass seems to be sufficient to remove Tpo from the circulation at a normal rate. In view of the low numbers of circulating platelets, the apparently normal Tpo clearance leads to a relative Tpo deficiency in these patients.

In patients with thrombocytopenia resulting from peripheral platelet consumption such as occurs in disseminated intravascular coagulation (DIC), platelet production is also not impaired. In Chapter 9 [70] it was shown that the plasma Tpo levels in 15 patients with DIC were normal in 3 patients but were increased in 12 patients. On average, Tpo levels in patients with DIC were higher than in ITP, but lower than in patients with thrombocytopenia resulting from decreased platelet production. We postulated that the elevated Tpo levels in DIC resulted from the release of Tpo by activated platelets. We showed in the same Chapter that when platelets are stimulated with different activators in vitro such as thrombin or collagen, they release Tpo in the supernatant. In DIC, massive platelet activation occurs, and Tpo levels were found to correlate with markers for thrombin generation. The rise in Tpo level might therefore result from Tpo release by platelets. Alternatively, acute-phase reactants or proteins involved in the DIC-associated inflammatory process might induce enhanced Tpo production. In Chapter 8 we showed that a peak in plasma IL-6 preceded the peak in plasma Tpo concentration in patients that had undergone major surgery. IL-6 was found to enhance Tpo production by the hepatoblastoma cell lines HepG2 and Hep3B (Chapter 8 and [71]). It is possible that also in vivo, IL-6 or other inflammatory cytokines enhance Tpo production. In healthy individuals who were infused with endotoxin (Chapter 10), circulating platelet counts decreased and Tpo levels rose. In these individuals, IL-6 levels and C-reactive protein were increased. Here as well, the elevation in Tpo concentration could be the result of platelet Tpo release, enhanced Tpo production under the influence of pro-inflammatory cytokines, diminished Tpo clearance, or a combination of these mechanisms. Further studies should be performed to dissect the exact mechanisms behind the increase in Tpo concentrations. For instance, it would be interesting to investigate the Tpo levels in patients treated with IL-6, to see whether IL-6 can directly affect Tpo levels.

Based on the previously mentioned model of Tpo-R-mediated clearance of Tpo, it was expected that Tpo levels would be decreased in patients suffering from thrombocytosis. In contrast, we found Tpo levels to be in the normal range in all patients with reactive thrombocytosis, polycythaemia vera and in all but one patient with essential thrombocythaemia (lowered Tpo concentration). It has previously been suggested that the Tpo-R expression is decreased on platelets of patients with ET [45] [42] and PV [41,72] and in PV a defect in the receptor glycosylation has been described [73]. A decreased Tpo uptake in these patients might explain the relatively high Tpo levels. Indeed, we showed that the platelet Tpo content
in these patients was decreased, although an overlap with the Tpo content in healthy individuals existed. Direct assessment of the amount of receptor-bound Tpo in the platelets from PV and ET patients versus controls might result in a larger difference. As described in Chapter 9 this can be done by the addition of truncated Tpo or a Tpo-mimetic peptide to the platelets before platelet disruption or stimulation.

In patients with reactive thrombocytosis, the platelet Tpo content was not decreased, and platelet counts and Tpo levels were positively correlated. We therefore postulate that in these patients enhanced Tpo production might underlie the relatively elevated Tpo levels. Here as well, inflammatory cytokines might play a role in enhancing Tpo production because in conditions in which secondary thrombocytosis occurs, such as thrombocytosis resulting from infection or after surgery, elevated levels of for instance IL-6, IL-8 and C-reactive protein have been reported [47,74-76].

In Chapter 11 it was shown that serum Tpo levels were elevated in patients with multiple myeloma (MM), despite the presence of normal platelet counts. IL-6 levels in these patients were not elevated. Since platelet counts were normal, serum Tpo levels in MM patients could be compared with serum levels in healthy controls. The elevated Tpo levels might be involved in the maintenance of a normal thrombopoiesis, even when malignant myeloma cells infiltrate the bone marrow, thereby suppressing haematopoiesis. An explanation for the elevated Tpo levels would be that MM cells themselves produce Tpo or, in analogy with IL-6 [77,78], induce Tpo production in bone-marrow stromal cells. We recently found that two out of seven tested myeloma cell lines produced Tpo in vitro (unpublished data; the Tpo measured in the ten-fold concentrated supernatant of 100,000 cells at day 6 was 7 and 3.5 units). Tpo production could not be enhanced or induced by coculturing the cell lines with bone-marrow stromal cells. With a quantitative PCR, Tpo mRNA was detected in all myeloma cell lines, with the highest Tpo mRNA content found in the two Tpo-producing cell lines (i.e. 6 and 60%, respectively, of the amount found in the HepG2 cell line, which was set at 100%. All other cell lines showed a Tpo mRNA expression of less than 2%). Studies are underway to assess whether this finding can be extrapolated to multiple myeloma. The presence of Tpo mRNA in blast cells from myeloma patients will be examined together with their capacity to produce the Tpo protein. This will be related to the Tpo levels measured in the patient. Also, it remains to be investigated whether Tpo is of value as a disease marker, and whether it is involved in the pathogenesis of the disease.

In summary, the reported Tpo levels in the different patient groups are, in general, consistent with a model in which Tpo levels are produced constitutively and Tpo is cleared by receptor-mediated Tpo uptake. However, based on the results described in this thesis, some refinements can be made (Figure 4).

1) The kidney does not seem to contribute in a major way to Tpo production
2) Under some conditions (ITP) bone-marrow stromal cells can produce Tpo
3) An increase in circulating Tpo levels is not always caused by diminished clearance but might also result from Tpo release by activated platelet or
4) by enhanced Tpo production under the influence of inflammatory cytokines.
5) Diminished Tpo clearance might occur as a result from decreased expression of the Tpo receptor on platelets
6) Malignant cells might participate in the clearance (AML/MDS) as well as in the production of Tpo (MM)

It should be noted that regulation at the level of gene translation, such as has been reported to occur in hereditary thrombocythaemia [51-53,55], and the possible modulation of Tpo function by post-translational processing has [79,80] not been addressed in the investigated patient groups described in this thesis. Whether post-translational processing actually plays a role in the modulation of Tpo activity in vivo remains to be determined.

12.4 The potential function of Tpo release by platelets

In Chapter 9 we showed that platelets release biologically active Tpo when stimulated with platelet activators such as thrombin, collagen and CD41. The exact location is still unclear but
from the fractionation experiments it seemed that the platelet granules are a likely candidate. However, immuno-electron microscopy studies with antibodies directed against Tpo showed that Tpo is mainly associated with the subcannaliclar system in the platelet (Dr. M. Klinger, University of Luebeck, Germany, personal communication). A study of Tpo release by platelets from patients with storage pool deficiencies (SPD), lacking either one of the granules, might shed light on this question. Also, the origin of the "stored" Tpo remains to be determined; i.e. is it stored in the megakaryocytic stage, in the platelet stage or both.

The amount of receptor-bound Tpo in platelets seems to be higher than the amount of unbound Tpo because addition of truncated Tpo or a Tpo-mimetic peptide before platelet stimulation resulted in a three- to fivefold increment of measurable Tpo. Since the release of other markers did not increase, we concluded that the release of the "extra" Tpo resulted from the dissociation of Tpo from its receptor after competition with the truncated Tpo or the Tpo mimetic peptide. Some comments with regard to these experiments should be made. First, the amount of released Tpo might be an underestimate of the actual amount present in platelets. The washing procedure during the platelet isolation might have resulted in a loss of receptor-bound Tpo from the cell surface (but is not likely to affect the receptor-bound Tpo in the cell). Second, the procedure of platelet isolation might have resulted in selection of the platelet population, resulting in either a selective removal or enrichment of platelets with a high Tpo content. In this respect, it would be also interesting to investigate the platelet Tpo content of young versus old platelets.

The mechanism of Tpo release by platelets upon activation might have more functions than stimulating thrombocytopoiesis per se. For instance, it might play a role in tissue repair and neovascularization of damaged tissues. It has been demonstrated in vitro that Tpo can have a priming effect on platelet responses to agonists such as ADP, thrombin and epinephrine [81-90]. At very high concentrations, Tpo was found to activate platelets in vitro. Platelets contain several factors in their granules that are involved in processes like wound healing and neovascularization. For instance, PDGF and VEGF, which are contained in platelet granules, are growth factors for smooth muscle cells and vascular endothelial cells, respectively. When tissue damage occurs, platelets will accumulate and be activated at the site of injury. During the release reaction, Tpo will be released and can prime other cells to become activated or, when the local Tpo concentration is very high, can activate other platelets directly. As was recently shown in an in vitro flow model [91], Tpo might also enhance platelet adhesion at the site of injury.

Patients with solid tumours who are treated with recombinant Tpo preparations might be at risk for an increase in their tumour burden. It is conceivable that platelets adhere to the vascular tumour endothelium upon rebound thrombocytosis. The high circulating Tpo levels might prime, or directly induce the platelet release reaction, resulting in the release of substances that enhance neovascularization, thereby supporting the tumour. Whether this phenomenon does actually occur remains to be investigated.
12.5 Future perspectives

From the measurement of Tpo levels in patients with different thrombocytopoietic disorders, much has been learned about the regulation of thrombocytopoiesis. In addition, it proved to be of diagnostic value, especially in the differential diagnosis of thrombocytopenia. From a therapeutical viewpoint, insight in the regulation of thrombocytopoiesis has led to the identification of several patient groups that might benefit from treatment with Tpo. Initially, several clinical trials have been initiated that included patients in whom Tpo was administered to accelerate platelet recovery after myelosuppressive or -ablative therapy. Further studies should show whether Tpo treatment in this patient group is beneficial and what the optimal dosing schedule is. For these patient groups, the ex vivo expansion of thrombocytopoietic progenitors might prove to be an effective treatment regimen. Other patient groups that are likely to benefit from treatment with Tpo are patients with a relative Tpo deficiency (relatively low Tpo levels despite a low number of circulating platelets), such as patients with allo- or autoimmune thrombocytopenia and other groups of patients who suffer from immune-mediated platelet destruction. A similar approach has already been proven successful in patients with autoimmune neutropenia. Upon treatment of these patients with granulocyte colony-stimulating factor, neutrophil counts rose and titres of neutrophil-specific autoantibodies decreased [92-95].

For some disorders, such as the myeloproliferative disorders, it still needs to be investigated whether there is a role for Tpo in the aberrant overproduction of platelets and potentially other lineages, and if so, what this exact role is. In addition, several issues remain to be investigated, such as the exact mediators involved in inducing Tpo production by bone-marrow stromal cells, the role and occurrence of post-translational processing of the Tpo molecule under normal and pathological conditions, and the biological functions of the Tpo and Tpo-R variants.

References


13. Emmons RVB, Reid DM, Cohen RL, Meng G et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 1996; 87: 4068-71.


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91. Personal communication. Dr G. van Willigen; Dept. of Haematology, University Medical Centre, Utrecht, The Netherlands