Adenovector-Mediated Expression of Human Thrombopoietin cDNA in Immune-Compromised Mice: Insights into the Pathophysiology of Osteomyelofibrosis

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Adenovector-Mediated Expression of Human Thrombopoietin cDNA in Immune-Compromised Mice: Insights into the Pathophysiology of Osteomyelofibrosis

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Thrombopoietin (TPO), the c-mpl ligand, is a hemopoietic growth factor that regulates megakaryocytopoiesis and platelet production in vivo and in vitro (1–3). TPO is highly conserved throughout mammalian evolution and shares structural homology with the amino-terminal region of erythropoietin, while the glycosylated carboxyl terminus of the molecule is unique (4). The human TPO gene has been mapped to chromosome 3q26–27 (5, 6), a locus known to be implicated in myeloproliferative diseases associated with thrombocytosis (7). The gene is organized into five coding exons, which span approximately 6 kb, and is interrupted by introns at the same sites as the erythropoietin gene (4). Liver and kidneys are the major sites for TPO production (8–10), although a variety of organs and tissues produce small amounts of TPO mRNA transcripts (4, 11). Administration of recombinant TPO to rodents, nonhuman primates, and humans results in the elevation of circulating platelet levels several times above normal value (3, 12) and ameliorates chemotherapy/radiation induced thrombocytopenia (13, 14).

Gene transfer offers an alternative strategy to the administration of the recombinant TPO protein. This has been achieved in experimental animals using retrovirus vectors to transfer the murine TPO cDNA into bone marrow cells of mice (15) as well as using an adenovirus vector to transfer the human TPO cDNA to mice via various routes of administration (16). Although both strategies are effective and attractive because they circumvent the necessity of ex vivo production and purification of TPO, gene transfer is associated with the risk of uncontrolled stimulation of the megakaryocytic lineage as has been observed with murine megakaryocyte growth and development factor (MGDF) cDNA delivered to the bone marrow (15, 17). We attempted to study the biologic consequences of chronic TPO overexpression by an in vivo transfection strategy. Using i.p. administration of AdTPO to mice, our experimental design differs from the retrovirus vector-based model (17) in several important aspects: First, the transfection of TPO cDNA happens in vivo and without perturbing the bone marrow, which will become one of the read-out tissues for the experimental outcome. Second, the artificial autocrine and paracrine mechanisms of
TPO release in the bone marrow microenvironment that were induced by the retroviral transduction of bone marrow cells can be excluded in our approach. Third, the CMV promoter used in our AdTPO construct assures high levels of TPO overexpression at extramedullary sites, which guarantees an optimal experimental challenge for the envisioned outcome parameter. Therefore, the present study may provide a clinically more relevant model for studying the consequences of chronically elevated plasma TPO level.

Materials and Methods

Experimental animals

Mice matched for age (8 to 10 wk), weight (>20 g), and sex were obtained from Charles River Laboratories (Wilmington, MA) and The Jackson Laboratory (Bar Harbor, ME) and maintained in germfree conditions. Four strains of mice were evaluated, all on BALB/c background, including 1) immunocompetent BALB/c (18, 19); 2) nu/nu cell defect, with diminished CTL response and impaired Ab production (20–23); 3) SCID (T and B cell defect, diminished CTL, low to absent Ab production) (24, 25); and 4) NOD-SCID (also referred to as NOD/LtSz-SCID; T and B cell defect, mononuclear phagocytes diminished in number and function, low to absent Ab production, decreased NK activity) (26, 27). All strains received the AdTPO and the AdNull vector (10⁵ PFU in a volume of 100 μl, single i.p. administration on day 0). Each study group contained three animals.

Adenovirus vectors

AdTPO is an Ad5-derived Ela1-, partially Elb1-, partially E3-deficient vector with an expression cassette that contains the human TPO cDNA driven by the CMV major immediate/early promoter/enhancer (28, 29). The control vector AdNull is similar in design, except that it contains no transgene in the expression cassette (29). All vectors were amplified, purified, and titered as previously described (28, 30).

Peripheral blood counts

Initially every 3 to 4 days and later on a weekly basis, retroorbital blood was collected with capillary pipettes (Unopette, Fisher Scientific, Springfield, MA). Platelets, total white blood cells and granulocytes (polymorphonuclear leukocytes) were counted using a Neubauer hemocytometer. The hematocrit was measured in heparinized microhematocrit capillary tubes (Fisher Scientific). The plasma was collected, stored at −80°C, and assessed later by ELISA for human TPO levels and anti-TPO Abs.

ELISA for human TPO and anti-TPO Abs

Human TPO present in mice serum was assessed according to Emmons et al. (31). Briefly, microwell plates were incubated overnight with 2 mg/ml rabbit F(ab')2 anti-human Fc (Jackson ImmunoResearch, West Grove, PA) and then for 2 h with 100 ng/ml of chimeric mAb-IgG. Serially diluted plasma samples and standard (recombinant full-length human TPO produced in mammalian cells) were added to wells and incubated for 1 h. Bound TPO was quantitated colorimetrically by the addition of biotinylated affinity-purified polyclonal rabbit anti-TPO Abs (Genentech, South San Francisco, CA) followed by streptavidin-conjugated peroxidase. The detection threshold of the ELISA was 0.057 ng/ml.

Abs to murine (m) TPO present in the plasma were tested using a TPO binding ELISA. Briefly, biotinylated mTPO was bound onto a streptavidin-coated microtiter plate, and the plates were incubated with mouse serum from the treated mice. Bound anti-mTPO Abs were then detected with a secondary HRP-conjugated Ab (Genentech). The mTPO-coated microtiter plate (Costar, Cambridge, MA) was developed by incubating wells with 100 μl of streptavidin (250 μg/ml) in PBS containing 0.1% hemoferal for 12 to 72 h at 2 to 8°C followed by three washes with PBS containing 0.5% BSA and 0.01% polysorbate 20 (assay diluent). After washing, 100 μl of biotinylated mTPO (100 μg/ml) in assay diluent were added and incubated with assay diluent for 1 h at room temperature (RT) and subsequently washed 3 times with wash buffer. Samples (mouse plasma) and positive and negative controls (goat anti-mTPO serum and goat nonimmune serum, respectively) in assay diluent were added to appropriate wells and incubated for 2 h at RT with agitation. Bound murine anti-TPO was detected by adding 100 μl of goat anti-murine IgG-HRP in assay diluent to the wells and incubating for 1 h at RT. The reaction was stopped by adding 400 μl of 4.5 M sulfuric acid. The plate was read at 490 to 492 nm for detection absorbance and 405 nm for reference absorbance. The presence of anti-mTPO Abs was determined by evaluating the optical density ratio (ODR) for each control and sample. ODR = OD of sample well/OD of blank well. An ODR > 1.8 was reported as positive.

Progenitor assay

All animals were killed 80 days after vector administration. One pernur per animal was flushed with cold (4°C) IMDM/20% FCS, and the total yield of bone marrow mononuclear cells (MNC) was determined by counting an aliquot at the 0.36% acetic acid using a Neubauer hemocytometer. MNCs (10⁶) were plated in duplicate in 1 ml of 0.8% methylcellulose containing 30% FCS (50% 1-t-glutamine, 2.5% hemin, 0.05 mM 5-ME, rmIL-3 (50 ng/ml; R&D Systems, Minneapolis, MN), rm c-kit ligand (20 ng/ml; Immunoex, Seattle, WA) and recombinant human erythropoietin (2 U/ml; Sandoz, Basel, Switzerland) in 35-mm suspension culture dishes (Nunc, Inc, Naperville, IL). Cultures were incubated at 37°C in 100% humidity and 5% CO₂ for 7 days. Scoring was performed with an inverted microscope with 40× magnification on day 7. Colonies containing more than 50 cells were counted as burst-forming unit-erythroid (BFU-E), CFU-granulocytic-monocytic (CFU-GM), CFU-granulocytic/erythroid/megakaryocytic-monocytic (CFU-GEMM), and CFU-megakaryocytic (CFU-Mk). CFU-Mk were defined as colonies containing cells with exclusively megakaryocytic features. The total number of colonies per femur was calculated.

Histology

Twelve weeks after vector administration, spleen, liver, and bone marrow (femur, sternum, and thoracic vertebrae) were harvested for histologic evaluation. The tissues were fixed in 4% formalin, paraffin embedded, cut and stained with hematoxylin and eosin, and silver stain according to standard protocols. Quantification of megakaryocytes was performed on bone marrow sections of thoracic vertebrae by counting mature megakaryocytes in 10 high power fields of comparable cellularity.

Statistical evaluation

Data are expressed as mean ± SEM of at least three animals for each per group. Statistical significance was determined using the two-tailed Student’s t test.

Results

Effect of TPO overexpression on platelets

As previously observed for the AdTPO vector (16), a single i.p. administration of the vector to BALB/c mice resulted in a 3- to 4-fold increase of platelet levels, peaking at day +7 and returning to baseline at day +14 (Fig. 1A). In contrast, all mice with immune defects demonstrated persistent elevation of platelet levels for the 80-day duration of the study. The nu mice had peak platelet levels 4-fold above baseline at day +10, falling to 2-fold above baseline by 3 wk, and sustaining that level for the remainder of the study. The SCID and NOD-SCID mice responded with a platelet peak of 12- to 14-fold above baseline 4 to 5 wk after vector administration. The pattern of thrombocytosis was similar in the two strains, with a parallel, gradual decrease over the 2 mo following peak levels. By 77 days, the platelet levels of the SCID and NOD-SCID mice were similar (p > 0.5), but both were higher than the platelet levels of the nu mice (p < 0.05, both comparisons) as well as the BALB/c mice (p < 0.01). The platelet levels of all control animals were unaffacted throughout the study (Fig. 1B). Despite the fact that platelets reached high levels in all mice (peak levels BALB/c 6.4 ± 0.5 × 10⁹/μl, nu 5.9 ± 0.6 × 10⁹/μl, SCID 14.5 ± 0.5 × 10⁹/μl, NOD-SCID 16 ± 2.1 × 10⁹/μl) and were maintained >8 × 10⁹/μl for 1 mo in the SCID and NOD-SCID mice; no lethality nor adverse effects related to thrombocytosis were observed.

All BALB/c mice developed thrombocytopenia by day +35 after vector administration, which persisted for the remainder of the study (Fig. 1). Anti-TPO Abs were found only in BALB/c mice (Table I) and first detected on day +7. In these animals, human TPO (45 ± 15 ng/ml) was detectable only on day +3. In contrast, nu, SCID, and NOD-SCID mice did not develop anti-TPO Abs,
and human TPO could be detected up to 80 days after vector administration (Table I). In the SCID animals, sequential evaluation of human TPO levels throughout the study revealed good correlation between TPO and platelet levels (Fig. 2). The increasing TPO concentration was followed by a rapid increase of circulating platelets. On day 21, the peak of TPO concentration (635 ± 106 ng/ml) was reached. The peak level of platelets (14.5 ± 0.5 x 10^6/μl) followed 7 days later. TPO and platelets dropped gradually in a parallel fashion with platelet counts decreasing roughly 1 wk following the drop in TPO concentration. None of the control animals had detectable levels of human TPO or anti-TPO Abs (not shown).

**Table I. Correlation of human TPO levels and anti-murine TPO Abs in plasma of AdTPO-treated mice 80 days after vector administration**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Human TPO (ng/ml)</th>
<th>Anti-Murine TPO Abs (ratio test plasma/background)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c</td>
<td>&lt; 0.06</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Nude</td>
<td>0.16 ± 0.08</td>
<td>&lt; 1.8</td>
</tr>
<tr>
<td>SCID</td>
<td>6.57 ± 4.64</td>
<td>&lt; 1.8</td>
</tr>
<tr>
<td>NOD-SCID</td>
<td>0.61 ± 0.47</td>
<td>&lt; 1.8</td>
</tr>
</tbody>
</table>

*Detection threshold of ELISA is 0.057 ng/ml.

Ratios above 1.8 are indicative of presence of relevant Ab titer.

**FIGURE 1.** Four strains of mice (□ BALB/c; ◊ Nude; ○ SCID; △ NOD-SCID mice) were treated with 10^9 PFU AdTPO (A) or AdNull (B) i.p. on day 0. Mice with SCID and NOD-SCID showed a dramatic increase of circulating platelet numbers 4 to 5 wk after AdTPO administration. Peak levels of platelets were followed by a biphasic decrease during the second half of the study. On day 80, the platelet levels were still twofold higher than the pretreatment value. Nude and BALB/c mice showed maximal platelet levels 7 to 10 days following administration of AdTPO. Subsequently, the platelets of nude mice dropped to high normal levels and remained slightly elevated throughout the study. BALB/c mice showed a complete normalization of the thrombocytosis by day 14. Later on these animals developed a chronic thrombocytopenia. None of the animals treated with the control vector AdNull showed changes in platelet numbers.

**FIGURE 2.** In SCID mice, the concentration of human TPO was measured throughout the study and compared with levels of circulating platelets. The rapid increase of TPO concentration was followed by dramatic platelet increase. Following TPO peak on day 21, the platelets continued to rise for 7 days. On day 80, there was still human TPO measurable in the circulation of SCID mice (6.57 ± 4.64 ng/ml, Table I).

**FIGURE 3.** Four strains of mice (□ BALB/c; ◊ Nude; ○ SCID; △ NOD-SCID) were treated with 10^9 PFU AdTPO (A) or AdNull (B) i.p. on day 0. SCID mice showed increased circulating granulocytes for 7 wk after AdTPO administration, with a peak level 12-fold higher than the pretreatment value on day 28. By the end of the study, the granulocyte numbers were normal. NOD-SCID mice showed an increase in circulating granulocytes 10-fold above the pretreatment value on day 21. The granulocytosis lasted for 4 wk. BALB/c and nude mice as well as all control mice did not show any increase of circulating granulocytes following treatment with adenovector.

**Effect of TPO overexpression on granulocytes and erythrocytes**

Although TPO is considered to be relatively megakaryocyte specific, the SCID and NOD-SCID mice exhibited a marked granulocytosis over several weeks (Fig. 3A). Granulocytes increased 10- to 12-fold above baseline and peaked parallel to the platelet counts 3 to 5 wk after vector administration. The granulocytes completely
returned to baseline by 6 to 11 wk after vector administration. In contrast, no significant changes of granulocyte number was observed in BALB/c and nu mice after AdTPO treatment or in control mice (Fig. 3B).

The AdTPO-treated SCID and NOD-SCID mice developed a significant anemia at the time of thrombocytosis and granulocytosis (Fig. 4A). In both strains, this was associated with low levels of circulating reticulocytes (not shown). The hematocrit nadir (33 ± 4%) was reached 5 wk after vector administration, coinciding with the peak of platelets and granulocytes. No change in hematocrit was observed in BALB/c and nude mice treated with AdTPO and in all control mice.

**Histologic effects of TPO overexpression**

Histologic examination revealed significantly more mature megakaryocytes in the bone marrow of AdTPO-treated SCID and NOD-SCID animals as compared with the control mice (SCID, $p < 0.001$, Fig. 6). In contrast, BALB/c mice had significantly decreased numbers of mature megakaryocytes ($p < 0.001$), consistent with the presence of circulating anti-murine TPO Abs (Table I). The nu mice had only slightly, not significantly ($p = 0.15$) elevated numbers of megakaryocytes as compared with the control. Histologically, BALB/c, nu, and NOD-SCID mice showed normal cellularity and architecture of the bone marrow (Fig. 7, A, B, and D). In contrast, AdTPO-treated SCID mice developed severe osteomyelofibrosis and osteomyelosclerosis throughout the hemopoietic tissue (Figs. 7C and 9A). Reticulin staining confirmed the finding of osteomyelofibrosis exclusively in SCID mice (Fig. 8, A–D). As a consequence of osteomyelofibrosis, only AdTPO-treated SCID mice had extramedullary/extrasplenal hemopoiesis, with the formation of blood islands and foci of megakaryocytes in liver and lung (Fig. 9, B and D). In the spleen, fibrotic tissue surrounding islands of hemopoiesis and megakaryocytes was observed (Fig. 9C). Splenomegaly was present also in NOD-SCID mice treated with AdTPO (not shown), but neither myelofibrosis nor extrasplenal hemopoiesis were found. No pathologic conditions of the liver and lung were seen in the respective control mice (data not shown).

**Cellularity and clonogenic characteristics of bone marrow in mice with TPO overexpression**

Bone marrow cellularity 80 days after vector administration, estimated by the yield of MNCs from one femur cavity, was in the normal range for BALB/c, nu, and NOD-SCID mice independent of treatment (Table II). However, AdTPO-treated SCID mice had very poor yields compared with control SCID mice ($p < 0.001$, Table II). In terms of progenitors, AdTPO-treated BALB/c and SCID mice had diminished amounts of CFUs per femur compared with the respective control mouse (BALB/c, $p < 0.05$; SCID, $p < 0.01$; Fig. 5). In contrast, AdTPO-treated nu mice had increased numbers of CFUs compared with control nu mice ($p = 0.06$). As shown in the clonogenic assay, these differences were mainly due to changes in the compartments of CFU-GM, GEMM, and -Mk (Fig. 5).

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findings in the bone marrow and other organs (lung, liver, spleen) were observed in AdNull-treated mice.

**Discussion**

We treated immune normal BALB/c mice and three strains of syngeneic mice with various immune deficiencies with AdTPO and AdNull to study the in vivo effects of chronic TPO overexpression. Adenovectors provide high levels of transgene expression and thus are good vehicles for somatic gene therapy (32, 33). However, cellular and humoral immune responses of the host limit sustained transgene expression (34–36). We have previously shown that adenovirus-mediated transfer of human TPO cDNA driven by a CMV promoter rescues thrombocytopenia in mice rendered thrombocytopenic by radiochemotherapy (16). We hypothesized that immune-deficient mice may have prolonged expression of adenovirally mediated TPO cDNA and may provide a model to address questions regarding the biologic effects of chronic TPO exposure in vivo. At the same time, the relative contribution of host immunity on adenoviral mediated transgene expression could be assessed.

One major finding of this study is the observation of fibrotic changes in the bone marrow and at extramedullary sites in response to chronic overexpression of TPO exclusively present in SCID mice. Recently, Yan et al. (17) described a mouse model for osteomyelofibrosis using a retrovirus-based transfer of TPO cDNA to bone marrow mononuclear cells. Our experimental design differs in several respects from the highly artificial retrovirus-based model and may reflect more closely the clinical picture of osteomyelofibrosis. 1) In contrast to the retroviral model, which was characterized by a low range of TPO production as measured by moderately elevated platelet counts and no effect on circulating granulocytes, we observed excessive thrombocytosis and granulocytosis as consequence of high level of TPO expression. The hyperproliferative phase followed by marrow fibrosis/failure mimics realistically the development of osteomyelofibrosis. 2) In our experimental design, the transfer of TPO cDNA does not require manipulations of the bone marrow or bone marrow reinfusion and may therefore facilitate the interpretation of the histologic changes in the bone marrow. 3) The CMV promoter of AdTPO assures high level of expression of TPO at extramedullary sites as shown by sequential determination of TPO plasma levels, which may provide an optimal, strong, and lasting challenge by TPO. 4) Since adenovectors deposit the transgene episomically, there is no risk of immunological autografting as has been debated in retroviral approaches (37). 5) The proposed model is simple and easy to perform.

Surprisingly, we observed myelofibrotic and myelosclerotic changes only in AdTPO-treated SCID mice. NOD-SCID mice with an identical pattern of TPO overexpression and hypermegakaryocytosis in the bone marrow did not develop osteomyelofibrosis. In vivo and in vitro studies have shown that bone marrow megakaryocytes produce TGF-β1, platelet-derived growth factor, and fibroblast growth factor, which stimulate fibroblasts to proliferate, produce, and secret type IV collagen (17, 38–40). Normally, these cytokines are packaged into platelets, transported through the blood stream, and released at the site of vascular injury (41). It has been proposed that secondary osteomyelofibrosis is related to ineffective megakaryocytopenia, leading to increased apoptosis of megakaryocytes and promegakaryocytes with local release of TGF-β1, platelet-derived growth factor, and fibroblast growth factor, which then stimulates bone marrow fibroblasts (41, 42). Our observation of the striking difference in the development of osteomyelofibrosis between SCID and NOD-SCID mice suggests an additional factor in the pathogenesis of secondary osteomyelofibrosis. It has been shown that bone marrow monocytes and macrophages of NOD-SCID mice are functionally deficient in terms of IL-1β secretion, cytokine receptor regulation, and protein kinase C expression (26, 27). Based on our data, we postulate that normal bone marrow monocytes and macrophages, as present in SCID but not in NOD-SCID mice, may be required for generation of secondary osteomyelofibrosis. Further studies are required to support this hypothesis.

From an interventional point of view, the aspects of hematologic responses to high level TPO expression are of greater relevance. The data show marked differences in the extent and magnitude of platelet, granulocyte, and hematocrit responses to AdTPO treatment depending on the immune status of the mice. Immune-competent (BALB/c) and nu mice (T cell deficient) had only transient thrombocytosis of 7 to 10 days with platelet peaks of 4 to 5 times normal value. In contrast, SCID and NOD-SCID mice (both T and B cell deficient) had thrombocytosis for several months, and their platelet peaks were 10 to 12 times above normal values. BALB/c mice had measurable human TPO levels only on day +3 post injection. Compared with BALB/c mice, the peak concentration of human TPO in SCID mice was 14-fold higher and was reached later (day +21). In BALB/c mice, the production of human TPO ceased within 1 wk, whereas nu, SCID, and NOD-SCID mice maintained low levels of human TPO production throughout the study. It has been shown in several animal studies that the stability and level of adenovirally mediated transgene expression is dependent on mouse strain (43), immune status of the host (34, 44), coexpression of adenoviral structure proteins (45), and administered vector dose (46). We show that the expression of TPO, as measured by elevated platelet numbers, can be substantially prolonged (7- to 10-fold) by eliminating the immune functions of the host. On the other hand, in an immunocompetent host, the adenovirally mediated expression of a xenogeneic transgene may induce a humoral immune response against the transgene product, which shortens the transgene expression and may cross-react with the homologous self protein, causing autoimmune phenomenon. This was observed in immune-normal BALB/c mice that developed anti-
FIGURE 7. Eighty days after adenovector administration, all mice were killed and the bone marrow was evaluated histologically (see Materials and Methods). Representative cross-sections of the vertebrae of mice treated with AdTPO are shown. BALB/c (A), nude (B), and NOD-SCID (D) mice showed normal architecture and cellular content of the bone marrow. There was no increase in fibrotic or osteosclerotic tissue. In contrast, SCID mice (C) showed severe osteomyelofibrosis and osteosclerosis, which replaced most of the normal hemopoietic tissue (H&E stain, ×400).

FIGURE 8. Eighty days after adenovector administration, all mice were killed, and the bone marrow was evaluated by reticulin staining (see Materials and Methods). Representative cross-sections of vertebrae of mice treated with AdTPO are shown. BALB/c (A) and nude (B) mice show normal cellularity without evidence of increased reticulin fibers. C, SCID mice showed massive increase of reticulin fibers replacing most of the hemopoietic tissue. Some pyknotic megakaryocytes (M) are still present. D, NOD-SCID mice did not show any increase of fibrotic tissue despite increased numbers of bone marrow megakaryocytes (M). The hemopoietic tissue was normal in terms of architecture and cellularity (silver stain, ×1000).
murine TPO Abs, resulting in chronic thrombocytopenia with deple-
tion of bone marrow megakaryocytes and hemopoietic progen-
itors 5 wk after administration of AdTPO. In another series of
experiments, we showed that BALB/c mice develop immune
thrombocytopenia only after treatment with adenovectors express-
ing human TPO but not after treatment with adenovectors express-
ing murine TPO (our manuscript in preparation). A similar break
of immune tolerance was observed by Tripathy et al. (47) upon
infecting BALB/c mice with an adenovector delivering human
erthropoietin cDNA. Therefore, the autoantibody production in
immunocompetent animals is due to species-specific epitopes in
the native protein rather than unspecific adenovirally mediated
epitopes. Such transgene-specific immune responses may become
therapeutically important in cancer treatment protocols that utilize
tumor vaccines. In immunocompetent cancer patients, adenovi-
rally mediated overexpression of a heterologous or mutated tumor
Ag may evoke a significant antitumor immune response.

TPO is not the only factor that regulates the circulating platelet
level. Clinical studies showed that plasma TPO concentration in
patients with thrombocytopenia depends on the pathomechanism
of thrombocytopenia: patients with thrombocytopenia, because of
peripheral platelet destruction and increased megakaryocytopoi-
esis in the bone marrow, had low levels of TPO, and patients with
bone marrow failure showed high levels of TPO (31). Furthermore,
NF-E2 knock-out mice (48), which lack the hemopoietic subunit (p45)
of the heterodimeric erythroid transcription factor
NF-E2, have severe thrombocytopenia despite normal plasma TPO
levels and normal megakaryocyte mass in the bone marrow. In our
model, the increase of circulating platelets occurred simulta-
neously with the increase of TPO plasma concentration. However,
platelets continued to increase at least 1 wk past the peak of plasma
TPO concentration. Later, platelets dropped markedly delayed to
the drop of TPO concentration. By the end of the study, SCID and
NOD-SCID mice still had substantially increased numbers of
megakaryocytes in the bone marrow along with residual human
TPO in plasma. These findings together with clinical and experi-
mental evidence in the literature (31, 48–53) support the hypoth-
esis that TPO primarily regulates the megakaryocyte mass in the
bone marrow and only indirectly influences the level of circulating
platelets.

In addition to thrombocytosis, granulocytosis was also observed
in mice with prolonged TPO overexpression. In the bone marrow,
this was associated with expansion of various hemopoietic pro-
genitor pools, favoring CFU-GM, -GEMM, and -Mk. This pleio-
tropic effect of TPO was reversed in mice with autoantibodies
against TPO. Besides peripheral thrombocytopenia, these mice had
significantly lower number of hemopoietic CFUs in the bone mar-
row as compared with control mice. Although TPO was discovered
as a growth factor of the megakaryocytic lineage (1, 2, 4, 8), in
vitro data have demonstrated a synergistic stimulatory effect of
TPO with c-kit ligand (54, 55), IL-3 (56), and erythropoietin (57)
on hemopoietic progenitors. In animal models, pegylated MGDF
has led to enhanced recovery of platelets and erythrocytes after
myelosuppressive treatment (13, 16), and studies in humans sug-
stest that TPO is effective for mobilizing progenitor cells into the
peripheral circulation (49). Taken together, these results identify
TPO as a hemopoietic growth factor that exerts multilineage stim-
ulation of progenitors in concert with other factors present in the
bone marrow microenvironment. Moreover, recently Young et al.

FIGURE 9. Only AdTPO-treated SCID mice showed extrasplenal hemopoiesis. A, Cross-section of proximal fèmur revealed severe osteomy-
elofibrosis and osteosclerosis similar to the findings in the vertebra of these animals. Extrasplenal hemopoiesis (H) and megakaryocytopoiesis (M)
were found in liver (B) and lung (D). The spleen (C) showed predominantly large megakaryocytes (M) (H&E stain, ×400).
References


