Chronic Thrombopoietin Overexpression Induces Mesangioproliferative Glomerulopathy in Mice

Haruko K. Shimoda,[1,*] Masahiro Yamamoto,[3] Kotaro Shide,[1,2] Kenjirou Kamezaki,[2] Tadashi Matsuda,[4] Katsuhiro Ogawa,[3] Mine Harada,[2] Kazuya Shimoda[1]

[1] Department of Internal Medicine, Gastroenterology and Hematology, Faculty of Medicine, Miyazaki University, Miyazaki, Japan
[2] Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan
[3] Department of Pathology, Asahikawa Medical College, Asahikawa, Hokkaido, Japan
[4] Department of Immunology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

Running title: Glomerulopathy in TPO Transgenic Mice

Keywords: thrombopoietin, mesangial cell, mesangial matrix, TGF-β1, PDGF-BB, glomerulopathy

Corresponding author; Haruko K. Shimoda, M.D., Ph.D.
Department of Internal Medicine, Gastroenterology and Hematology,
Faculty of Medicine, Miyazaki University
5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
Phone: +81 985 85 9121, Fax: +81 985 85 5194
E-mail: hshimoda@fc.miyazaki-u.ac.jp

Abstract: 150 words
Text: 2744 words
Figures: 1-5

Abstract

We previously reported that mice transgenic (Tg) for thrombopoietin (TPO) developed progressive fibrosis and osteosclerosis of the bone marrow. Here, we show that TPO-overexpressing mice also exhibited notable histological changes in the kidneys, including an increased number of mesangial cells, expansion of the mesangial matrix in the glomerulus, and atrophy of the renal tubuli. Plasma transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF)-BB, which could induce mesangioproliferative responses in glomeruli, were both elevated in TPO Tg mice, even though TPO itself has no effect on mesangial cells due to their lack of c-Mpl. The mesangial proliferative change in TPO Tg mice was thought to be induced by the elevation of these cytokines. In conclusion, our finding that TPO-overexpressing mice developed mesangioproliferative glomerulopathy might represent an undesirable effect of chronically elevated TPO *in vivo*, which should be taken into consideration before new TPO-like growth factors become available in clinical practice.

Introduction

Thrombopoietin (TPO) is a hematopoietic cytokine involved in the regulation of megakaryopoiesis and platelet production [1-3], and its receptor is c-Mpl. Mice deficient in *TPO* or *c-mpl* exhibited an ~80% reduction in platelet numbers [4, 5], indicating that both TPO and c-Mpl are essential for megakaryopoiesis. TPO is produced primarily in the liver and kidneys [6, 7] and affects cells expressing surface c-Mpl. The expression of c-Mpl is restricted to hematopoietic stem cells, progenitor cells, megakaryocytes, and platelets [8-10], and c-mpl mRNA can be detected only in hematopoietic or lymphoid tissues such as bone marrow, spleen, and fetal liver [11]. Therefore, the effects of TPO are thought to be restricted to these organs and cells.

In addition, several groups, including ours, have established TPO-overexpressing mice and reported that these mice develop fibrotic changes in their bone marrow and spleens [12-16]; little is known, however, about the effects of chronic TPO overexpression on other organs. Using TPO-transgenic (Tg) mice, we investigated whether systemic overexpression of TPO affected any organs besides the bone marrow and spleen. We show here that the continuous secretion of TPO in mice induces mesangial proliferative changes in the glomeruli. Since TPO itself did not seem capable of affecting non-hematopoietic or non-lymphoid tissue, due to the absence of c-Mpl, we expected that TPO overexpression would have some indirect effects. We show here that the plasma levels of transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF)–BB, both of which were previously proposed to be the primary cause of glomerulosclerosis, are elevated in TPO Tg mice.

Results

Changes in the bone marrow and spleen of TPO-overexpressing mice (Fig. 1)

We previously reported that TPO transgenic mice under the control of the mouse IgH enhancer and promoter developed myelofibrosis and osteosclerosis in the bone marrow

with extramedullary hematopoiesis in the spleen at about 9 months of age [16]. In this study, we examined the bone marrow and spleen from these TPO Tg mice at 18 months of age, and observed massively increased intracellular matrix in the bone marrow (Fig.1A) and islands of hematopoiesis in the spleen (Fig.1B). As we previously reported [16], TPO Tg mice at 9 months of age exhibited thrombocytosis and leukocytosis in peripheral blood, even though few mononuclear cells were left in the bone marrow due to the developed myelofibrosis. This was attributed to the belief that extramedullary hematopoiesis in the spleen of TPO Tg mice was enough to produce the increased numbers of platelets and white blood cells in the presence of a high amount of TPO. In this study, we did not examine the hematological values of TPO Tg mice at 18 months of age, but the fact that histological examination of the spleen showed as active hematopoiesis at 9 months of age and that these mice were expected to continuously secrete TPO from the bone marrow and spleen for the duration of their lifespans, suggested that the numbers of platelets and white blood cells were still increased in these TPO Tg mice at 18 months of age.

Histological findings in the kidneys of TPO-overexpressing mice (Fig. 2, 3 and 4)

We next explored whether TPO Tg mice had any abnormalities in other organs. We systematically examined TPO Tg mice at 18 months of age, as well as corresponding wild-type mice, and discovered histologically significant differences only in the kidneys of TPO Tg mice. Macroscopically, the kidneys from TPO Tg mice were slightly larger than those from wild-type mice (Fig. 2A). Hematoxylin and eosin examination of kidney sections from TPO Tg mice showed enlarged glomeruli compared to those of wild-type mice (Fig. 2B). The mesangium, which is one of the important components of the glomerulus structure, comprises mesangial cells (phagocytic support and secretory cells) and their amorphous secretory product, the mesangial matrix, and it occupies the central region of a glomerular lobule. Periodic acid-Schiff staining of kidney sections from TPO Tg mice revealed the significant change of the mesangium in the glomeruli, showing an increased number of mesangial cells along with expanded

mesangial matrices (Fig. 3A, upper panels). To evaluate this proliferative change of the mesangium in the glomeruli, we first defined a mesangial region as an area in the mesangium surrounded by glomerular capillary lumens (indicated as * in Figure 3A, lower panels). When the box (□) sections in the upper panels of Figure 3A were magnified digitally (Figure 3A, lower panels), one mesangial region showed four mesangial cell nuclei in a TPO Tg mouse, but only one in a wild-type mouse (illustrated within a solid oval), and the enlargement of the mesangial matrix was significant in a TPO Tg mouse (illustrated within a dot oval). These changes were seen throughout the glomeruli of TPO Tg mice. Then, we examined ten glomeruli from each mouse, counted the numbers of mesangial cell nuclei within one mesangial region (Fig. 3B, left), and measured the expansion of the mesangial matrix by defining the size of a mesangial cell nucleus as one unit and counting how many units the matrix within one mesangial region could contain (Fig. 3B, right). The number of mesangial cells per mesangial region was higher in TPO Tg mice (4.550 ± 0.998) than in wild-type mice (2.550 ± 0.751, $p<0.05$), and the mesangial matrix within one mesangial region was larger in TPO Tg mice (3.125 ± 0.222 units) than in wild-type mice (1.275 ± 0.668 units, $p<0.01$). In more than 90% of glomeruli of TPO Tg mice, the numbers of mesangial cells were increased and the mesangial matrix had expanded when compared to the mean values of wild-type mice. The glomerular tubuli leading to the glomeruli appeared atrophic (Fig. 4, indicated by arrows), indicating that the nephron had degenerated after the progressive damage of the glomeruli.

We also assessed renal function by measuring blood urine nitrogen (BUN), which is more sensitive than serum creatinine, to evaluate glomerular filtration capacity in chronic renal disease in mice. There was no significant difference in the level of BUN between TPO Tg mice and wild-type mice at 18 months of age (26.1 ± 3.2 mg/dl in TPO Tg mice (n = 5) vs. 30.7 ± 4.7 mg/dl in control mice (n =6), $p>0.05$).

Plasma cytokine levels (Fig. 5)

As we reported previously, TPO Tg mice produced approximately two to three times as

much plasma TPO as did wild-type mice [16]. However, the elevated plasma TPO did not seem directly responsible for the renal effects because kidney cells lack c-Mpl. Since the elevation of two specific cytokines is considered the main cause of glomerulosclerosis, we measured the plasma levels of TGF-β1 and PDGF-BB to clarify the cause of mesangial proliferation in TPO Tg mice. The plasma levels of total TGF-β1 were 2.7-fold higher in TPO Tg mice (87 ± 22 ng/ml) than those of wild-type mice (32 ± 9 ng/ml, $p<0.001$) (Fig. 5A). Plasma PDGF-BB levels in TPO-Tg mice were approximately double those of normal littermates (3.9 ± 1.4 ng/ml vs. 2.1 ± 0.7 ng/ml, respectively, $p<0.05$) (Fig. 5B). There was no significant correlation between the levels of TGF-β1 and PDGF-BB ($p>0.05$).

Discussion

We have shown here that *in vivo* TPO overexpression induces mesangial proliferative changes in glomeruli, including atrophic glomerular tubuli (Fig. 2, 3 and 4). The number of mesangial cells per mesangial region was increased 1.8-fold, and the mesangial matrices were increased by 2.5-fold in TPO Tg mice relative to wild-type mice (Fig. 3B).

The histological changes in the kidneys of TPO Tg mice were similar in part to those observed in TGF-β1 Tg mice, which produce TGF-β1 protein in the liver and show high levels of plasma TGF-β1 [17-19]. The TGF-β1 Tg mice showed significant expansion of the mesangial matrices and thickening of the glomerular basement membranes. We previously showed that TPO Tg mice exhibited higher levels of plasma TGF-β1 [16], but this elevation could not fully explain the histological abnormalities of TPO Tg mice. TPO Tg mice exhibited mesangial cell proliferation in addition to matrix accumulation, whereas TGF-β1 Tg mice did not exhibit such obvious cell proliferation. TPO itself did not seem capable of acting directly on mesangial cells, since its receptor (c-Mpl) is absent from murine mesangial cells [11]. We therefore examined plasma cytokine levels, which might induce the proliferation of

mesangial cells. PDGF-BB is another critical factor in the pathogenesis of mesangioproliferative glomerulopathy; PDGF-BB overexpression by intravenous administration of PDGF-B cDNA in an adenoviral vector induced significant mesangial cell proliferation accompanied by mesangial matrix expansion and is considered a model for glomerulosclerosis [20]. Strikingly, plasma PDGF-BB levels were about double in TPO Tg mice relative to those in normal littermates (Fig. 5B), and total TGF-β1 levels were similarly elevated (by 2.7-fold) in TPO Tg mice (Fig. 5A).

Both TGF-β1 and PDGF-BB were previously proposed to be primary causes of glomerulosclerosis in several animal models and human renal diseases [21-25]. Isaka *et al*. (1993) reported that the *in vivo* transfection of the *PDGF-B* gene into rat kidneys induced mesangial cell proliferation and a slight increase in the mesangial matrix. They also introduced the *TGF-β1* gene into rat kidneys and showed that overexpression of TGF-β1 induced mesangial matrix expansion rather than cell proliferation [26]. As mentioned above, TGF-β1 Tg mice developed progressive glomerulosclerosis without obvious mesangial cell proliferation [17-19]. These observations indicate that TGF-β1 plays a predominant role in increasing the mesangial matrix, whereas PDGF-BB is instead responsible for increasing cellularity; either TGF-β1 or PDGF-BB alone can induce glomerulosclerosis. The mesangial proliferative response observed in TPO Tg mice, in terms of both matrix and cellularity, was considered the result of the cooperative effects of TGF-β1 and PDGF-BB, which were elevated simultaneously in TPO Tg mice.

It remains unclear as to which cells are involved in the abnormal secretion of TGF-β1 or PDGF-BB in TPO Tg mice. Megakaryocytes, the numbers of which were elevated in TPO Tg mice, have been considered the most probable source of these elevated cytokines [27, 28]. Given that TPO Tg mice showed numerous megakaryocytes in the bone marrow and spleen, it is reasonable that the elevated cytokines could have been produced by the increased megakaryocytes in the bone marrow and spleen. The increased numbers of circulating platelets in TPO Tg mice might be also responsible for the elevation of plasma TGF-β1 and PDGF-BB, because platelets contain an abundance of both cytokines [27, 29, 30]. Here, we were aware of

and had considered the problem of *ex vivo* platelets activation and release of TGF-β1 and PDGF-BB during blood sampling. However, we optimized our sampling procedure so that we would exclude this kind of artifact (see Methods section).

After a systemic investigation, the kidney was the only organ (besides the bone marrow and spleen) in which TPO Tg mice showed significant abnormalities. Other organs, including the liver, were normal in TPO Tg mice, whereas the TGF-β1 Tg mice exhibited multiple tissue lesions, including significant fibrosis of the liver, arteritis, myocarditis and fibrosis in the heart, and atrophic changes in the pancreas and testis [17]. Mice overexpressing PDGF-BB also revealed changes to the liver (hepatic stellate cell proliferation and fibrosis), bone (endosteal proliferation), and lung (perivascular lymphoid cell infiltration) [20]. These discrepancies between the TPO Tg mice and the TGF-β1- or PDGF-BB-overexpressing mice might be due to expression level differences of these two cytokines; for instance, the TGF-β1 Tg mice exhibited 8-fold higher plasma TGF-β1 and the PDGF-BB-overexpressing mice had 4- to 10-fold higher plasma PDGF-BB than did the littermate controls. In TPO Tg mice, the plasma levels of these two cytokines were elevated by 2- to 3-fold at most. Such a low elevation of TGF-β1 or PDGF-BB might be enough to cause mesangioproliferative glomerulopathy, despite the fact that such concentrations of TGF-β1 and PDGF-BB have no effect on other organs, including the liver and lungs. The autocrine mechanism of these cytokines, however, might be involved in the changes to the kidneys. In TGF-β1 transgenic mice driven by the albumin promoter, TGF-β1 protein and mRNA were detected in the glomeruli and liver, whereas they were both absent from wild-type glomeruli [19]. On the other hand, PDGF in cultured mesangial cells has been shown to induce its transcription in response to its peptide [31]. These results indicate that elevated concentrations of circulating TGF-β1 and PDGF-BB induce the local production of TGF-β1 and PDGF-BB by an autocrine mechanism in the glomeruli, leading to greatly increased local amounts of these cytokines in the kidneys. In addition, low concentrations of TGF-β1 have been shown to stimulate mesangial cell proliferation in culture by up-regulating PDGF-B-β-receptor expression [32]. In this regard, it is possible that elevated levels of circulating plasma TGF-β1 or PDGF-BB

primarily stimulate mesangial cells and interstitial fibroblasts in glomeruli, followed by autoinduction of the same cytokines, resulting in the observed mesangial proliferative change.

Our whole hypothesis of the indirect role of a high amount of TPO in the mesangial proliferation is based on the phenomena observed in an *in vivo* overexpression model of TPO, which are the increased numbers of megakaryocytes and the elevated plasma levels of two specific cytokines, TGF-β1 and PDGF-BB. To elucidate the responsibility of TPO for the mesangial proliferative change in TPO Tg mice, further *in vitro* investigation might be required such as the experiments of co-cultures of megakaryocytes and mesangial cells with or without the stimulation of TPO.

It seems that new TPO-like growth factors might soon be clinically available for treating thrombocytopenic disorders [33]; however, these thrombopoietic growth factors have not yet been used *in vivo* for a long period. Our finding that TPO-overexpressing mice showed mesangioproliferative glomerulopathy might suggest one of the problems of long-term elevation of TPO *in vivo* and should be taken into account before practicing novel therapy for thrombocytopenic disorders with new TPO-like growth factors.

In conclusion, we have shown that mesangial cells increased in number and the mesangial matrices expanded in TPO Tg mice. We hypothesize that mesangial cell proliferation and mesangial matrix accumulation result primarily from increased plasma concentrations of TGF-β1 and PDGF-BB. This phenomenon may represent an undesirable effect of chronically elevated TPO *in vivo* and should be noted before TPO-like growth factors become available in clinical practice.

Methods

Mice

Transgenic mice expressing full-length murine TPO under the control of the mouse IgH enhancer and promoter were generated as previously described [16]. These TPO Tg mice constitutively produced 2- to 3-fold higher levels of plasma TPO than did their normal littermates from as early as 2-4 months of age, and they developed myelofibrosis and osteosclerosis of the bone marrow at 9 months of age. Splenomegaly with extramedullary hematopoiesis was observed at 3 months of age in the Tg mice.

Mice were housed and bred in the Kyushu University Animal Center.

Histologic analysis and glomerular findings

Kidney tissues were fixed in formalin, embedded in paraffin, and cut for hematoxylin and eosin staining or periodic acid-Schiff staining according to established protocols. Histologically, the mesangium consists of mesangial cells and the mesangial matrices, and it occupies the central region of a glomerular lobule. A mesangial region is defined as an area surrounded by glomerular capillary lumens (indicated as * in Figure 3A, lower panels). To evaluate the proliferation of the mesangium in glomeruli, the number of mesangial cell nuclei within one mesangial region was counted, and the expansion of the mesangial matrix was measured by defining the size of a mesangial cell nucleus as one unit and counting how many units the matrix within one mesangial region could contain in terms of area. Ten glomeruli from each mouse were examined, and the average values were compared.

Blood sampling

Blood was collected from the retro-orbital plexus into heparin-coated microcapillary tubes (Terumo, Tokyo). To minimize platelet activation, platelet-free plasma was immediately obtained by the following protocol: cells were sedimented by centrifugation at 800 x g for 10 min, after which the supernatant was collected and the separated plasma centrifuged again at 10,000 x g at 4°C for 10 min to completely

remove the platelets. The platelet-free plasma was aliquoted and stored at –20°C until analysis.

Cytokine quantification

The levels of plasma TGF-β1 and PDGF-BB were determined using enzyme-linked immunosorbent assays (ELISAs), using Quantikine Kits for each murine cytokine (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. TGF-β1 was measured after acidification to activate the latent forms into immunoreactive forms. Acidification was performed according to the manufacturer's instructions without modification. The limits of detection of the assays were 2.89 pg/ml for total TGF-β1 and 7.7 pg/ml for PDGF-BB.

Statistical analysis

The results are presented as means ± SD. Statistical significance was determined using the two-tailed Student's *t*-test, and p values <0.05 were considered statistically significant.

References

1. Kaushansky K, Lok S, Holly RD, et al.: Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. Nature 1994; 369(6481): 568-71.

2. Lok S, Kaushansky K, Holly RD, et al.: Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. Nature 1994; 369(6481): 565-8.

3. Wendling F, Maraskovsky E, Debili N, et al.: cMpl ligand is a humoral regulator of megakaryocytopoiesis. Nature 1994; 369(6481): 571-4.

4. de Sauvage FJ, Carver-Moore K, Luoh SM, et al.: Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. J Exp Med 1996; 183(2): 651-6.

5. Gurney AL, Carver-Moore K, de Sauvage FJ, Moore MW: Thrombocytopenia in c-mpl-deficient mice. Science 1994; 265(5177): 1445-7.

6. Bartley TD, Bogenberger J, Hunt P, et al.: Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. Cell 1994; 77(7): 1117-24.

7. Chang MS, McNinch J, Basu R, et al.: Cloning and characterization of the human megakaryocyte growth and development factor (MGDF) gene. J Biol Chem 1995; 270(2): 511-4.

8. Vigon I, Mornon JP, Cocault L, et al.: Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: identification of a member of the hematopoietic growth factor receptor superfamily. Proc Natl Acad Sci U S A 1992; 89(12): 5640-4.

9. Methia N, Louache F, Vainchenker W, Wendling F: Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryocytopoiesis. Blood 1993; 82(5): 1395-401.

10. Solar GP, Kerr WG, Zeigler FC, et al.: Role of c-mpl in early hematopoiesis. Blood 1998; 92(1): 4-10.

11. Souyri M, Vigon I, Penciolelli JF, Heard JM, Tambourin P, Wendling F: A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell 1990; 63(6): 1137-47.
12. Yan XQ, Lacey D, Fletcher F, et al.: Chronic exposure to retroviral vector encoded MGDF (mpl-ligand) induces lineage-specific growth and differentiation of megakaryocytes in mice. Blood 1995; 86(11): 4025-33.
13. Yan XQ, Lacey D, Hill D, et al.: A model of myelofibrosis and osteosclerosis in mice induced by overexpressing thrombopoietin (mpl ligand): reversal of disease by bone marrow transplantation. Blood 1996; 88(2): 402-9.
14. Villeval JL, Cohen-Solal K, Tulliez M, et al.: High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. Blood 1997; 90(11): 4369-83.
15. Frey BM, Rafii S, Teterson M, Eaton D, Crystal RG, Moore MA: Adenovector-mediated expression of human thrombopoietin cDNA in immune-compromised mice: insights into the pathophysiology of osteomyelofibrosis. J Immunol 1998; 160(2): 691-9.
16. Kakumitsu H, Kamezaki K, Shimoda K, et al.: Transgenic mice overexpressing murine thrombopoietin develop myelofibrosis and osteosclerosis. Leuk Res 2005; 29(7): 761-9.
17. Sanderson N, Factor V, Nagy P, et al.: Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci U S A 1995; 92(7): 2572-6.
18. Kopp JB, Factor VM, Mozes M, et al.: Transgenic mice with increased plasma levels of TGF-beta 1 develop progressive renal disease. Lab Invest 1996; 74(6): 991-1003.
19. Mozes MM, Bottinger EP, Jacot TA, Kopp JB: Renal expression of fibrotic matrix proteins and of transforming growth factor-beta (TGF-beta) isoforms in TGF-beta transgenic mice. J Am Soc Nephrol 1999; 10(2): 271-80.
20. Hudkins KL, Gilbertson DG, Carling M, et al.: Exogenous PDGF-D is a potent mesangial cell mitogen and causes a severe mesangial proliferative glomerulopathy. J

Am Soc Nephrol 2004; 15(2): 286-98.
21. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. Proc Natl Acad Sci U S A 1993; 90(5): 1814-8.
22. Chen S, Hong SW, Iglesias-de la Cruz MC, Isono M, Casaretto A, Ziyadeh FN: The key role of the transforming growth factor-beta system in the pathogenesis of diabetic nephropathy. Ren Fail 2001; 23(3-4): 471-81.
23. Niemir ZI, Stein H, Noronha IL, et al.: PDGF and TGF-beta contribute to the natural course of human IgA glomerulonephritis. Kidney Int 1995; 48(5): 1530-41.
24. Lai KN, Tang SC, Guh JY, et al.: Polymeric IgA1 from patients with IgA nephropathy upregulates transforming growth factor-beta synthesis and signal transduction in human mesangial cells via the renin-angiotensin system. J Am Soc Nephrol 2003; 14(12): 3127-37.
25. Chen S, Iglesias-de la Cruz MC, Jim B, Hong SW, Isono M, Ziyadeh FN: Reversibility of established diabetic glomerulopathy by anti-TGF-beta antibodies in db/db mice. Biochem Biophys Res Commun 2003; 300(1): 16-22.
26. Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Imai E: Glomerulosclerosis induced by in vivo transfection of transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. J Clin Invest 1993; 92(6): 2597-601.
27. Fava RA, Casey TT, Wilcox J, Pelton RW, Moses HL, Nanney LB: Synthesis of transforming growth factor-beta 1 by megakaryocytes and its localization to megakaryocyte and platelet alpha-granules. Blood 1990; 76(10): 1946-55.
28. Wickenhauser C, Hillienhof A, Jungheim K, et al.: Detection and quantification of transforming growth factor beta (TGF-beta) and platelet-derived growth factor (PDGF) release by normal human megakaryocytes. Leukemia 1995; 9(2): 310-5.
29. Roberts AB, Sporn MB: Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). Growth Factors 1993; 8(1): 1-9.
30. Heldin CH, Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999; 79(4): 1283-316.
31. Silver BJ, Jaffer FE, Abboud HE: Platelet-derived growth factor synthesis in

mesangial cells: induction by multiple peptide mitogens. Proc Natl Acad Sci U S A 1989; 86(3): 1056-60.
32. Haberstroh U, Zahner G, Disser M, Thaiss F, Wolf G, Stahl RA: TGF-beta stimulates rat mesangial cell proliferation in culture: role of PDGF beta-receptor expression. Am J Physiol 1993; 264(2 Pt 2): F199-205.
33. Kuter DJ: New thrombopoietic growth factors. Blood (in press)

Figure Legends

Fig. 1. Developed myelofibrosis and osteosclerosis in the bone marrow with extramedullary hematopoiesis in the spleen of TPO Tg mice at about 18 months of age. Histologic (A) bone marrow and (B) spleen sections from TPO Tg mice at 18 months of age. Hematoxylin-eosin stains (x400).

Fig. 2. Kidney alterations in TPO-overexpressing mice. (A) Macroscopic appearance of kidneys from 18-month-old TPO Tg mice (right) and corresponding wild-type mice (left). (B) Histologic kidney sections from wild-type (left) and TPO Tg mice (right). Hematoxylin-eosin stains (x400).

Fig. 3. Mesangioproliferative change in the kidney of TPO-overexpressing mice. The mesangium is the one of the components of the glomerulus structure and consists of mesangial cells (phagocytic support and secretory cells) and their amorphous secretory product, the mesangial matrix. A mesangial region is defined as an area in the mesangium surrounded by glomerular capillary lumens (indicated as * in Figure 3A, lower panels). (A) Upper panels: periodic acid-Schiff stains of histologic kidney sections from wild-type (left) and TPO Tg mice (right) (x400). Lower panels: the digitally magnified view of the box (□) section in the upper panels to look in at one mesangial region (original magnification is the same as upper panels, x400). Four mesangial cell nuclei are illustrated in a TPO Tg mouse, but only one in a wild-type mouse (within a solid oval), and the mesangial matrix in the TPO Tg mouse is significantly expanded (within a dot oval). (B) The proliferation of the mesangium is stated in figures. The number of mesangial cell nuclei within one mesangial region was counted (indicated as ■), and the expansion of the mesangial matrix was measured by defining the size of a mesangial cell nucleus as one unit and counting how many units the matrix within one mesangial region could contain (indicated as ◆). From each mouse, ten glomeruli were examined and the average values were compared. Four mice were examined for both TPO Tg and wild-type mice. −, mean values.

Fig. 4. Atrophic glomerular tubuli in the kidney of TPO-overexpressing mice. Periodic acid-Schiff stains of representative histologic kidney sections from wild-type (left) and TPO Tg mice (right) (x400). Arrows in TPO Tg mice indicate the atrophic glomerular tubuli leading to the glomeruli.

Fig. 5. Plasma TGF-β1 and PDGF-BB levels. (A) Total plasma TGF-β1 and (B) PDGF-BB levels from TPO transgenic (Tg) and wild-type (WT) mice (n=5 for both). Levels were determined by ELISA, and TGF-β1 was measured after acidification of the samples, as described in the Materials and Methods. Values for the TPO Tg mice that are statistically different from those of wild-type littermates are indicated by $p<0.01$ and $p<0.05$. –, mean values; n, number of mice.