TGF-β1 in liver fibrosis: an inducible transgenic mouse model to study liver fibrogenesis

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Kanzler, Stephan, Ansgar W. Lohse, Andrea Keil, Jürgen Henninger, Hans P. Dienes, Peter Schirmacher, Stefan Rose-John, Karl H. Meyer zum Büschenfelde, and Manfred Blessing. TGF-β1 in liver fibrosis: an inducible transgenic mouse model to study liver fibrogenesis. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1059–G1068, 1999.—Transforming growth factor-β1 (TGF-β1) is a powerful stimulus for collagen formation in vitro. To determine the in vivo effects of TGF-β1 on liver fibrogenesis, we generated transgenic mice overexpressing a fusion gene [C-reactive protein (CRP)/TGF-β1] consisting of the cDNA coding for an activated form of TGF-β1 under the control of the regulatory elements of the inducible human CRP gene promoter. Two transgenic lines were generated with liver-specific overexpression of mature TGF-β1. After induction of the acute phase response (15 h) with lipopolysaccharide (100 µg ip), plasma TGF-β1 levels reached >600 ng/ml in transgenic animals, which is >100 times above normal plasma levels. Basal plasma levels of uninduced transgenic animals were about two to five times above normal. As a consequence of hepatic TGF-β1 expression, we could demonstrate masked transient upregulation of procollagen I and procollagen III mRNA in the liver 15 h after the peak of TGF-β1 expression. Liver histology after repeated induction of transgene expression showed an activation of hepatic stellate cells in both transgenic lines. The fibrotic process was characterized by perisinusoidal deposition of collagen in a linear pattern. This transgenic mouse model gives in vivo evidence for the important role of TGF-β1 in stellate cell activation and liver fibrogenesis. Due to the ability to control the level of TGF-β1 expression, this model allows the study of the regulation and kinetics of collagen synthesis and fibrolysis as well as the degree of reversibility of liver fibrosis. The CRP/TGF-β1 transgenic mouse model may finally serve as a model for the testing of antifibrogenic agents.

transforming growth factor-β1; stellate cell activation; collagen I; C-reactive protein promoter

HEPATIC FIBROSIS is a dynamic process caused by chronic liver injury due to various etiologies (viral, toxic, metabolic, autoimmune), eventually leading to cirrhosis. It is predominantly characterized by excessive accumulation of extracellular matrix caused by both an increased synthesis and decreased or unbalanced degradation of extracellular matrix. Transforming growth factor (TGF)-β1 seems to play a central role in the cytokine network involved in fibrogenesis (for review, see Refs. 5, 7, 21, 32, 48). High levels of TGF-β1 have been described in different acute and chronic liver diseases (4, 11, 40, 49). TGF-β1 is the prototype and best characterized of three TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3) encountered in mammalian species. It is a highly conserved molecule with a variety of signalling functions regulating cell growth, differentiation, migration, death, and expression of extracellular matrix (for review, see Refs. 31, 47, 62). TGF-β1 is secreted in a biologically inactive form as a complex consisting of two units of the large precursor segment of the TGF-β1 propeptide linked to the mature TGF-β1 dimer. In its biologically active form, TGF-β1 consists of a 25-kDa homodimer linked by disulfide bonds. Conversion of the latent complex to the mature, biologically active form is achieved in vitro by acid, alkali, heat, or proteases (24, 35). In vivo mechanisms of TGF-β1 activation are not fully understood but seem to involve proteases (1, 54).

In hepatic fibrogenesis, TGF-β1 is believed to be involved in the synthesis and deposition of extracellular matrix components like fibronectin, collagen type I, III, and IV, tenasin, elastin, osteonectin, biglycan, and decorin by fibroblasts, in the liver predominantly by activated stellate cells (myofibroblasts) (32, 33, 36, 37, 46). It has been shown that the level of mRNA for TGF-β1 and procollagen I, the main extracellular matrix component of fibrotic liver, is tightly correlated in liver biopsies of patients with chronic liver disease (11). Besides the fibrogenic effect, TGF-β1 decreases the degradation of extracellular matrix by inhibition of metalloproteinases via activation of their inhibitors (tissue inhibitors of metalloproteinases; see Refs. 28, 38, 42). In vitro data give support for a paracrine and autocrine secretion of TGF-β1 by activated stellate cells in chronic liver injury (2).

Importantly, animal models of experimental liver fibrosis, such as carbon tetrachloride-induced fibrosis (8), bile duct ligation (39), or schistosomiasis infection (17), support the in vivo relevance of TGF-β1 for liver fibrosis since increased concentrations of TGF-β1 were found very early in the course of fibrogenesis in these animal models. In a first transgenic mouse model of hepatic overexpression of TGF-β1 under control of the albumin promoter, Sanderson et al. (53) found evidence for a causative role of TGF-β1 for liver fibrogenesis in vivo, but this model is afflicted with a variety of extraintestinal complications ascribed to the constant expression of TGF-β1. These extrahepatic manifestations frequently lead to early death of the animals due
to severe glomerulonephritis, thus limiting the usefulness of this model. Recently, Clouthier et al. (16) characterized another transgenic mouse model of TGF-β1 overexpression using the regulatory sequences of the phospho-enolpyruvate carboxykinase (PEPCK) gene. This promoter is postnatally constitutive and leads to transgene expression in multiple organs, including liver, kidney, gut, and adipose tissue. Transcription of PEPCK and PEPCK-driven transgenes can be modulated, both negatively and positively, by altering the carbohydrate composition of the diet (57). This animal model has confirmed the results of Sanderson et al. (53) but has the same limitations.

To develop a transgenic mouse model with highly liver-specific, inducible expression of TGF-β1, we generated transgenic mice with a fusion gene consisting of a cDNA coding for the mature form of TGF-β1 under the control of the C-reactive protein (CRP) gene promoter. This promoter combines the advantage of very low basal expression with the potential of a massive induction of transgene expression by induction of an acute phase response, exceeding basal levels >100 times. These transgenic mice allow the study of the regulation of genes involved in fibrogenesis and fibrosis in the adult animal and may serve as a model for testing of antifibrogenic agents.

MATERIALS AND METHODS

Construction of the TGF-β1 expression vector and generation of transgenic mice. For transgene expression, the inducible human CRP gene was chosen. Of several truncated versions of this gene, construct 79 (41) was kindly provided by U. Ruether (Hannover, Germany). This version consisted of 1.7 kb of 5′ sequences and 3.8 kb of 3′ sequences and contained the BamHI-HindIII fragment at −1.7 kb to +3.14 kb as well as the KpnI-BamHI fragment from +7.5 kb to +10.5 kb as described in detail elsewhere (41). This version of the CRP gene is characterized by a low basal level of expression but a very high degree of inducibility. Out of construct 79 we excised an expression cassette (Fig. 1) or by PCR. The 5′-primer was derived from the human CRP intron from position 330 to 351 (5′-TAAGGGCACCCCCAGGCTATG-3′; see Ref. 59), and the 3′-primer was derived from the simian TGF-β1 from position 416 to 437 (5′-AGCAGCAGTTGA-CAGGATC-3′; see Ref. 56).

Induction of TGF-β1 expression in transgenic mice. Hepatic expression of the transgene-derived TGF-β1 was achieved by induction of the acute phase reaction by intraperitoneal injection of 100 µg lipopolysaccharide (LPS; L-8274; Sigma Chemicals, Heidelberg, Germany). Long-term induction of transgenic and wild-type animals was achieved by repeated LPS injections (3 times/wk). For these experiments, 8-wk-old transgenic and wild-type animals were chosen.

Measurement of TGF-β1 plasma levels. TGF-β1 plasma levels were measured using an ELISA kit (Genzyme) according to the manufacturer’s guidelines. This assay measures both the mature and the latent forms of TGF-β1. Blood samples were obtained either by cardiac puncture or by serial testing by incision of the tail vein. Plasma samples were stored at −20°C until analyzed.

Northern blot analysis. Total cellular RNA was isolated from various mouse organs by extraction in guanidinium thiocyanate and centrifugation in cesium chloride as described previously (13). Aliquots (10 µg) of total RNA were separated on 1% agarose gels containing 2.2 M formaldehyde and were blotted on nylon membranes (Hybond N; Amersham, Braunschweig, Germany). Filters were processed at high stringency as described (14) and were hybridized with the 32P-labeled simian TGF-β1 cDNA (1.5 kb). After electrophoresis, ethidium bromide was added to the RNA samples to enhance staining to assess the loading and the transfer efficiency of each RNA sample.

Poly(A)+ mRNA was prepared by using the oligotex mRNA kit (Qiagen, Hilden, Germany). Two micrograms of poly(A)+ mRNA per slot were taken for Northern blot detection of

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**Fig. 1.** Schematic representation of the 9.7-kb SfiI vector-free DNA fragment that was microinjected into pronuclei of one-cell mouse embryos (FVB/NHSD). EcoRI fragment (1.3 kb) derived from the human (h) C-reactive protein gene (CRP) promoter was used for Southern blot hybridization. ψ, Pseudogen of the hCRP gene; open area, intron of the hCRP gene; dotted area, 5′ cap site and polyadenylation site of the hCRP gene; hatched area, cDNA for the active form of simian TGF-β1; cross-hatched area, 5′-upstream and 3′-downstream sequences of the hCRP gene. Restriction enzymes in parentheses indicate destruction of the recognition sequence during vector construction. TGF-β1, transforming growth factor-β1.
mRNA for procollagen I and procollagen III using a human

Detection of TGF-β1 protein expression in the liver by Western blot hybridization. For Western blot analysis, liver proteins of transgenic and nontransgenic mice were separated by SDS-PAGE and were electroblotted on a polyvinylidene difluoride membrane (Millipore, Eschborn, Germany). For detection, 1 µg/ml of polyclonal antibody specific for the mature form of TGF-β1 (AF-101-NA; R&D Systems, Wiesbaden, Germany) or the proreg of TGF-β1 (anti-LAP, AB-246-NA; R&D Systems) was used. Immune complexes were visualized using a horseradish peroxidase-conjugated secondary antibody and chemiluminescence detection (Dianova, Hamburg, Germany and DAKO, Hamburg, Germany, respectively).

In situ hybridization experiments. In situ hybridization was performed on frozen liver sections (5 µm). Cryosections were fixed for 15 min in 4% paraformaldehyde in PBS and were washed in PBS. Next, slides were preincubated with 20 µg/ml proteinase K (MERCK, Darmstadt, Germany) in proteinase K buffer for 5 min and were rinsed in PBS, and 0.1 M triethanolamine with 0.25% acetic anhydride were added and incubated for 10 min. Thereafter, slides were washed in PBS, dehydrated in graded ethanol, and air-dried. Sections were then hybridized at 42°C in hybridization buffer [2 × saline sodium citrate (SSC), 5% dextran sulfate, 0.2% milk powder, and 50% formamide] containing 50 µl 35S-labeled TGF-β1 antisense or sense probes (106 counts per minute). Later (12 h), sections were rinsed in 2 × SSC at 42°C for 30 min and were treated with 50% formamide and 10 mM dithiothreitol in 2 × SSC for 2 × 15 min. Next, sections were washed for 15 min in 2 × SSC and 10 min in RNase buffer. RNase (20 µg/ml; Behring Diagnostics) was added and incubated for 30 min. Slides were then washed with RNase buffer, treated with 50% formamide at 65°C for 15 min, and rinsed in decreasing concentrations of SSC at 42°C. Thereafter, sections were dehydrated in graded ethanol, air-dried, and coated with photographic emulsion (Eastman Kodak, Rochester NY). After exposure, slides were developed, washed and fixed, and finally counterstained with hemalum.

Tissues for in situ hybridization were prepared 9 h after induction with LPS or interleukin (IL)-6, since peak mRNA expression under the CRP promoter was shown to occur at this time point (see Ref. 15, our own observation, and Fig. 2A).

Bioassay for TGF-β1. The amount of biologically active TGF-β1 in the plasma was quantified with an assay measuring the inhibition of growth of Mv 1 Lu mink lung epithelial cells (27). In brief, Mv 1 Lu cells (CCL-64; 30,000 cells/200 µl) were subjected to trypsination, washed in PBS, and suspended in assay medium [RPMI culture medium (GIBCO, Paisley, Scotland) supplemented with 1% 1-glutamin, 1% penicillin, 0.4% mercaptoethanol, and 5% fetal bovine serum (GIBCO)]. Next, cells were plated at a concentration of 1.5 × 105 cells/ml in 96-well microtiter plates. After incubation at 37°C for 1 h in a humidified CO2 incubator, TGF-β1 test samples and standards of known TGF-β1 concentration (Boehringer Mannheim) were added to the wells, and cells were cultured for 24 h. After this preincubation, cells were cultured in the presence of [3H]thymidine at 37°C for another 24 h. The amount of acid-precipitable radioactivity in the cells exposed to the test samples and TGF-β1 standards was determined with a liquid scintillation counter. All standards and samples were tested in triplicates. The concentration of biologically active TGF-β1 was determined by comparison with the appropriate standard curve.

Histology and immunohistochemistry. Tissues were fixed in 10% formaldehyde in PBS, embedded in paraffin, sectioned at 2 µm, and stained with hematoxylin/eosin and Sirius red by standard methods. Immunohistochemical staining for desmin (Dako M-0760, Hamburg, Germany) and α-actin (Sigma A-2547) was performed using 2-µm deparaffinized sections. The degree of fibrosis after repeated inductions of transgene-derived TGF-β1 expression was evaluated semiquantitatively by the Chevallier score, the scoring system for liver fibrosis that has been shown to correlate with liver collagen content (12). The scoring index included centrolobular vein, perisinusoidal collagen, portal tracts, and septa.

RESULTS

Generation of CRP/TGF-β1 transgenic mice. To control the basal level of hepatocellular TGF-β1 expression, we chose the highly inducible human CRP gene promoter, a human acute phase protein gene promoter. To bypass the not fully understood mechanism of activation of latent TGF-β1, we constructed a fusion gene consisting of a modified cDNA for simian TGF-β1 and modified portions of the human CRP gene (Fig. 1).
In this variant form of TGF-β1, two cysteines at positions 223 and 225 were replaced by serines using site-directed mutagenesis leading to expression of biologically active TGF-β1 without dimerization and cleavage of the proregion (9). As a consequence, transgene-derived TGF-β1 is recognized in Western blot analysis by antibodies to the mature region as well as to the proregion (see Fig. 6). Two founder lines were generated (lines 11 and 12). Screening for transgenic mice was done by Southern blot hybridization of DNA from tail tissue and by PCR.

Analysis of transgene expression. Transgene expression was reliably achieved by induction of the acute phase reaction by application of LPS. The kinetics of the appearance of simian TGF-β1 mRNA in the liver and TGF-β1 protein synthesis measured as TGF-β1 plasma levels were identical to the published kinetics of the utilized CRP gene promoter (15). As early as 6 h after induction with LPS, there was already a marked increase in transgene mRNA expression with peak values at 9–15 h after induction (Fig. 2A). Thereafter, transgene mRNA levels declined rapidly and returned to basal concentrations 24 h after induction. Uninduced transgenic animals showed only a weak basal transgene overexpression (Fig. 2A). Northern blot analysis of multiple organs 9 h after induction showed a strict liver-specific overexpression of simian TGF-β1 mRNA without any extrahepatic signal (Fig. 2B).

Additionally, we determined the kinetics of TGF-β1 plasma levels by ELISA. The highest TGF-β1 plasma levels were reached 15 h after induction with LPS with a decline to basal levels after 48 h (Fig. 3). Maximal TGF-β1 plasma concentrations reached >600 ng/ml in line 12, corresponding to ~100 times basal plasma concentrations of TGF-β1. Plasma levels of line 11 had the same kinetics but were considerably lower (50–200 ng/ml at peak values). Uninduced transgenic animals showed about two- to fivefold elevated plasma levels of TGF-β1 compared with control animals (20 vs. 5 ng/ml).

In a separate set of experiments, IL-6, as a downstream mediator of the acute phase response, has been tested to induce the expression of transgene-derived TGF-β1 in the liver. However, application of 20–40 mg ip of IL-6 did not lead to a significant induction of TGF-β1 expression (data not shown).

In situ hybridization for simian TGF-β1 mRNA in the liver confirmed massive hepatocellular accumulation after induction compared with basal levels (Fig. 4, B, B′, C, and C′). Specific signals were detected over hepatocytes in a patchy pattern. Induction of age- and sex-matched wild-type animals with LPS revealed no specific signal using the simian TGF-β1 antisense probe (Fig. 4, A and A′).

Three LPS injections per week as applied for long-term induction of the TGF-β1 transgene expression led to partial tolerance to LPS with considerably lower TGF-β1 mRNA levels after repeated inductions as shown in Fig. 5 in a representative Northern blot (n = 4). TGF-β1 plasma concentrations 15 h after the last application of LPS were ~50 ng/ml in these transgenic animals.

Western blot analysis of liver homogenates with two different TGF-β1 specific antibodies, one recognizing the proregion of TGF-β1 and the other recognizing the mature region of TGF-β1, was compatible with ELISA results showing TGF-β1 specific signals in LPS-induced transgenic animals 15 h after induction. TGF-β1 expression was undetectable both in uninduced transgenic animals and in induced wild-type animals (Fig. 6, lanes 3–6).

Bioassay for TGF-β1. To verify the biological activity of the transgene-derived simian TGF-β1, we tested plasma of uninduced and induced transgenic animals as well as wild-type animals in a bioassay for TGF-β on the basis of the growth inhibitory effect of TGF-β1 on mink lung cells. The concentration of biologically active TGF-β1 was determined by comparison with the standard curve (Fig. 7). Plasma of induced transgenic animals led to a significant growth inhibition of mink lung cells compared with plasma of induced wild-type animals. Uninduced transgenic animals exhibited only a weak growth inhibition.

Procollagen I and III mRNA expression after induction of the TGF-β1 transgene. After induction of TGF-β1 expression (36 h), corresponding to 21 h after the plasma peak of TGF-β1, we could demonstrate a marked increase of procollagen I and procollagen III mRNA expression in the liver (Fig. 8), which was spontaneously downregulated to baseline expression after 96 h. In contrast, uninduced transgenic animals and wild-type animals exhibited only a very low basal level of constitutive expression of procollagen I and procollagen III mRNA.

Liver histology. Liver histology 6 wk after repeated inductions of the acute phase by LPS resulted in a marked α-actin staining of hepatic stellate cells in
transgenic but not wild-type animals, indicative of an activated state of these cells (Fig. 9).

Collagen deposition around individual hepatocytes and a linear pattern of collagen in the space of Disse could be demonstrated by Sirius red staining (Fig. 10). The quantitative evaluation of hepatic fibrosis according to the scoring system of Chevallier gave a score of 0.1 for the wild type, 2.0 for transgenic line 11, and 2.6 for transgenic line 12 (P < 0.01). The scores were mainly due to centrolobular vein sclerosis and perisinusoidal fibrosis. Consistent with a higher TGF-β1 expression of line 12 (Northern blot, TGF-β ELISA), the fibrosis score was equally higher in line 12. This signifies a dose-effect relationship between the amount

Fig. 4. In situ hybridization experiments for transgene-derived TGF-β1. Brightfield (A, B, and C) and darkfield (A', B', and C') photomicrographs of in situ hybridization experiments using an antisense probe derived from the simian TGF-β1 cDNA on liver sections. After induction (9 h) of an acute phase response, wild-type animals showed no specific signal (A and A'). Uninduced TGF-β1 transgenic mice of line 12 exhibited only a weak signal (B and B'), whereas TGF-β1 transgenic animals (line 12) showed a strong transgene-specific signal over hepatocytes with a patchy pattern 9 h after induction of an acute phase response (C and C'). Bars, 200 µm.

Fig. 5. Northern blot analysis for transgene-derived TGF-β1 mRNA in the liver of transgenic animals (line 12) 9 h after application of 100 µg of LPS. A: TGF-β1 expression after the first LPS injection. B: TGF-β1 expression 6 wk after repeated LPS injections (3 times/wk). Note the increasing tolerance to repeated LPS injections leading to decreased expression of transgene-derived TGF-β1 mRNA.

Fig. 6. Western blot analysis for TGF-β1 in liver homogenates obtained 15 h after induction of an acute phase response or from uninduced animals. A: analysis with an antibody recognizing the mature form of TGF-β1. B: analysis with an antibody recognizing the proregion of TGF-β1 (LAP). Lanes 1 and 3, males; lanes 2 and 4, females; lanes 1 and 2, liver homogenates from transgenic animals (line 12) 15 h after LPS induction; lanes 3 and 4, liver homogenates from uninduced transgenic animals (line 12); lanes 5 and 6, liver homogenates from wild-type mice 15 h after LPS induction; lane 7, 20 ng recombinant TGF-β1.
Transiently induced TGF-β1 overexpression led to stellate cell activation and upregulation of procollagen I and procollagen III mRNA with subsequent collagen deposition in the liver, giving strong in vivo evidence for a stimulatory role of TGF-β1 in liver fibrogenesis. Although a significant amount of hepatic TGF-β1 is derived from stellate cells and Kupffer cells, there is now convincing evidence that, at least under pathological situations, hepatocytes are able to produce or secrete TGF-β1 (4, 6, 17, 29, 33). This was a further rationale for choosing the hepatocellular CRP gene promoter for TGF-β1 expression in our model.

Even though serum amyloid proteins rather than CRP are the major acute phase proteins in mice (43, 61), the expression of the human CRP gene is regulated in transgenic mice as it is regulated in humans (15). Corresponding to this finding, we could show by Northern blot hybridization and in situ hybridization a massive increase of transgene-derived TGF-β1 mRNA in the livers of transgenic animals after induction of an acute phase response by LPS. By Western blot analysis of liver homogenates with TGF-β1 antibodies or by ELISA for TGF-β1 in plasma, we could demonstrate a massive overexpression of TGF-β1 protein both in the liver and the plasma. Increased plasma levels result from TGF-β1 synthesized in the liver and released in the circulation, reaching >100 times basal plasma levels. The local concentrations of TGF-β1 in the liver are therefore probably much higher than the measured plasma levels. The kinetics of transgene-derived TGF-β1 mRNA induction and the kinetics of TGF-β1 plasma levels were identical to the CRP expression kinetics published by Ciliberto et al. (15).

After the peak of TGF-β1 expression, we could demonstrate a marked transient induction of procollagen I and procollagen III mRNA in the liver of transgenic but not of wild-type animals. To our knowledge, this demonstrates for the first time a direct causal relationship between TGF-β1 overexpression in the liver and consecutive procollagen I and procollagen III expression in vivo. With this inducible animal model, it will be possible to characterize factors or pharmaceuticals leading to downregulation of procollagen expression in the liver.

To achieve repetitive increased TGF-β1 expression in the liver, we treated transgenic and wild-type animals three times per week with LPS. Long-term induction of transgenic animals with LPS over 6 wk led to an attenuated transgene response, marking the development of partial tolerance to repeated LPS injections as reported previously (18, 20, 52, 58). Because TGF-β1 has been shown to be a possible mediator of LPS tolerance in vitro, the phenomenon of LPS tolerance might be aggravated by transgenic TGF-β1 in our animals (45). However, mRNA for transgene-derived TGF-β1 in the liver remained detectable. Future studies have to show if LPS dose escalation can maintain comparable levels of induction of transgene-derived TGF-β1, as had been proposed in a comparable transgenic study (50).
Six weeks after repeated treatments with LPS, wild-type animals showed no significant changes in liver morphology. Liver histology of uninduced, age-matched transgenic animals was equally normal. However, induced transgenic animals showed a marked activation of hepatic stellate cells. As the most important biological effect of TGF-β1 overexpression, transgenic mice showed beginning hepatic fibrosis after 6 wk of repeated inductions of TGF-β1 expression. The fibrotic process was characterized by increased perisinusoidal deposition of collagen. It is conceivable that LPS induced release of tumor necrosis factor (TNF), or other cytokines potentiate the profibrogenic effect of TGF-β1 in the liver of transgenic animals, even though LPS alone had no profibrinogenic effects in control mice. In this context, it has been shown that TGF-β1 and TNF have some synergistic effects on stellate cell activation in vitro (3). Furthermore, Greenwel and Rojkind (22) found an accelerated development of liver fibrosis in carbon tetrachloride-treated rats by the weekly induction of the acute phase response.

To avoid potential side effects and tolerance to LPS, we tested IL-6 as one of the downstream mediators of the acute phase response. However, IL-6 alone did not lead to a significant induction of transgene-derived TGF-β1, although the acute phase response was induced. This confirms recently published data showing that IL-6 is necessary, but not sufficient, to induce the human CRP gene in transgenic mice (59).

Interestingly, we did not observe significant extrahepatic pathology in kidneys, spleen, heart, lung, muscle, and intestine in long-term induced transgenic animals. This is in marked contrast to the albumin/TGF-β1 transgenic model of Sanderson and colleagues (53).
Transgenic mice with constitutive, hepatocellular over-expression of activated TGF-β1 under control of the albumin promoter also exhibited limited hepatic fibrosis but suffered from marked extrahepatic organ manifestations even though TGF-β1 plasma levels were relatively low compared with our model (peak values 40 ng/ml vs. >600 ng/ml). What are the reasons for this difference? First of all, the albumin promoter is a constitutive promoter leading to a constant TGF-β1 overexpression during embryogenesis and thus probably during a particularly vulnerable phase of organ differentiation. In addition, TGF-β1 plasma levels in the albumin/TGF-β1 transgenic animals were highest during the first 4 wk postpartum and might be even higher in utero. Furthermore, the albumin promoter is not liver specific during embryogenesis. Albumin mRNA and protein have been found prenatally in intestine, kidney, lung, and liver (44, 55). Postnatally, albumin promoter activity was demonstrated in kidneys until 3 wk postpartum, which could explain the high percentage of fatal glomerulonephritis in this model (53). The recently published PEPCK/TGF-β1 transgenic mouse model (16) has the same limitations as the albumin/TGF-β1 transgenic model. The promoter is postnatally constitutive and not liver specific, with high levels of expression in kidney, gut, adipose tissue, and a variety of other organs (57). In contrast, our inducible model gives us the opportunity to study effects of hepatic TGF-β1 overexpression in healthy adult animals. This is of pathophysiological significance, since liver fibrosis is mainly an acquired disease of adulthood.

The development of only mild fibrosis that we observed in repeatedly induced transgenic animals might have several reasons. LPS tolerance with significantly lower expression of transgene-derived TGF-β1 might be one important reason. Additionally, factors that abolish the effect of TGF-β1 could be upregulated. We have some preliminary evidence that antifibrogenic proteins are upregulated in LPS-stimulated transgenic animals compared with LPS-stimulated wild-type animals. Other antifibrotic mechanisms might also counteract the effects of TGF-β1. Finally, the limited fibrosis observed suggests that TGF-β1 is only one important piece in the puzzle of liver fibrogenesis. Additional factors such as necroinflammation and probably longer periods of continuous TGF-β1 exposure may be necessary for the development of full-blown fibrosis or cirrhosis. In consequence, future experiments should examine the effects of hepatotoxic chemicals like carbon tetrachloride and/or ethanol in these transgenic animals to study fibrogenesis in the setting of necroinflammation. Due to the high degree of inducibility, this animal model will also allow the study of the reversibility of liver fibrosis. Finally, it may serve as a model for the testing of antifibrogenic agents.

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REFERENCES


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