Kupffer cell-dependent TNF-α signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse

Yinghua Tian*, Wolfram Jochum†, Panco Georgiev*, Wolfgang Moritz*, Rolf Graf*, and Pierre-Alain Clavien*‡

Swiss Hepato-Pancreatico Biliary Center, Departments of *Visceral and Transplantation Surgery and †Pathology, University Hospital of Zurich, Raemistrasse 100, CH-8091 Zurich, Switzerland

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Implantation of small liver grafts causes liver injury and defective regeneration leading to graft failure. We investigated whether Kupffer cell-dependent TNF-α signaling contributes to this poor outcome. Partial 30% liver transplantation was performed in C57BL/6 wild-type mice (control group), and in three groups with down-regulation of the TNF-α pathway: (*i*) TNF receptor 1 knockout [TNFR-1(−/−)] mice, and mice pretreated with (*ii*) gadolinium chloride or (*iii*) pentoxifylline (PTX). Fifty-percent partial liver transplantation, a model associated with full recovery, and transplantation in IL-6 knockout [IL-6(−/−)] mice were performed in some experiments. Graft injury, regeneration, portal flow, liver microcirculation, leukocyte adhesion, and animal survival were assessed. Animal survival rates were 14% in the control group vs. 43% in the gadolinium chloride group, 57% for the TNFR-1(−/−) group, and 86% in the PTX group ($P < 0.001$). Markers of liver injury were reduced in all treated groups when compared with controls. Each treated group disclosed better portal flow and sinusoid perfusion, decreased leukocyte adherence, particularly in the PTX group. Liver regeneration occurred only in the treated groups. IL-6 and IL-10 levels were dramatically up-regulated (50×) in the PTX group, and at lower levels in other experimental groups. The protective effect of PTX was lost in IL-6(−/−) mice and protection was restored by a single dose of r-IL-6. In conclusion, interruption of TNF-α signaling or depletion of Kupffer cells improves survival after 30% liver transplantation, reduces liver injury, and enhances regeneration. The superior effects of PTX are mediated by IL-6.

The shrinking organ pool of liver grafts to treat patients with end-stage liver disease has led to alternative approaches aiming at increasing the number of grafts available for transplantation. In particular, living donor liver transplantation (LDLT) is a promising procedure with many advantages for the recipient (1–3). Currently, the right hemiliver is preferentially used, because the left hemiliver is usually too small for successful transplantation in an adult recipient (4). For the safety of living donors, a left hemihepatectomy (segments II–IV) would be optimal. The observation of a critical mass of the liver graft in relationship to the body size of the patient led to the concept of small-for-size liver transplantation (5). The critical mass for safe LDLT is ≈40% of the standard liver volume. An intriguing observation is that small residual liver volume is sufficient to sustain life after liver resection, but not after LDLT. Thus, understanding the mechanisms of liver failure in small-for-size liver transplantation may promote new strategies to improve the outcome of the left hemiliver or segmental transplantation.

LDLT combines several stresses that are potentially harmful for the graft including a short period (≈1 h) of cold ischemia, a rewarming time during implantation, and reperfusion (6). These events initially cause sinusoidal endothelial damage and Kupffer cell (KC) activation, and later hepatocyte injury with enhanced regeneration (7–9). TNF-α, mostly released from activated KC, plays a critical role in this type of injury ranging from endothelial and hepatocyte cell death after transplantation (10–12) to cell proliferation (13). TNF-α may also prompt the expression of adhesion molecules and accumulation of neutrophils (14–16), a critical mechanism of injury following reperfusion and transplantation (17).

We have previously shown in a mouse model of arterialized small-for-size orthotopic liver transplantation (OLT) which mimics the human situation, that liver graft injury is volume-dependent. Implantation of a 50% liver graft is tolerated whereas 30% is not. Therefore, the 30% liver graft in the mouse is a suitable model of small-for-size liver grafts (18, 19).

In this study, we aimed to test the hypothesis that TNF-α signaling plays a central role in the development of small-for-size syndrome. We designed experiments to modulate the release and the signaling of TNF-α in small-for-size OLT.

Results

Are Gadolinium Chloride ($GdCl_3$) and Pentoxifylline (PTX) Able to Inhibit TNF-α Signaling and/or KC Function? The first set of experiments evaluated the effects of GdCl3 and PTX on TNF-α levels after 30% OLT. Serum TNF-α levels 3 h after reperfusion in animals receiving a 30% graft were 9.07 ± 4.1 (mean ± SD) pg/ml in the control group, 12.86 ± 5.31 pg/ml in the TNF receptor 1 knockout [TNFR-1(−/−)] group, 2.98 ± 1.76 pg/ml in the GdCl3 group, and 2.66 ± 1.87 pg/ml in the PTX group. These data indicate an inhibition of the TNF signaling pathway shortly after reperfusion.

Next we asked whether treatment with $GdCl_3$, and PTX or absence of TNFR-1, in TNFR-1(−/−) mice may reduce phagocytic activity of KC. Latex particles were injected, and the retention in KC was determined. Similar to sham-operated animals, untreated 30% OLT controls exhibited a high activity with >95% of the particles immobilized by phagocytic uptake (Fig. 1). In $GdCl_3$-treated grafts, the phagocytic activity was reduced by 50%. In animals pretreated with PTX, the phagocytic activity was ≈70%. As expected, KC activity in the TNFR-1(−/−) group was comparable with the untreated group, suggesting that KC activity was not affected by the lack of TNFR-1 (Fig. 1).

Does Disruption of TNF-α Signaling or Impaired KC Function Decrease Reperfusion Injury? Studies investigating the mechanism of ischemic injury indicated that KC-derived TNF-α plays a crucial role in the induction of tissue injury (10, 20). Therefore, we tested whether silencing or removal of KC, or disruption of the TNF-α

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Abbreviations: AST, aspartate aminotransferase; $GdCl_3$, gadolinium chloride; IL-6(−/−), IL-6 knockout; IVFM, intravital fluorescence microscopy; KC, Kupffer cell; LDLT, living donor liver transplantation; OLT, orthotopic liver transplantation; PTX, pentoxifylline; TNFR-1(−/−), TNF receptor 1 knockout.

‡To whom correspondence should be addressed. E-mail: clavien@chir.unizh.ch.

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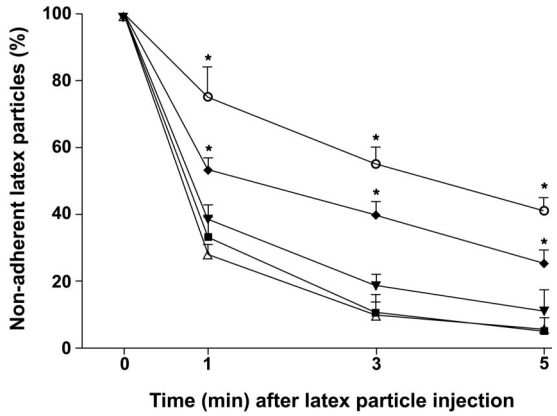

Fig. 1. Phagocytic activity after 30% OLT. To assess KC activity, the nonadherent latex particles were observed for 5 min in sinusoidal vein after injection and expressed as percentage of total latex particle. Untreated controls (■) showed very high phagocytic activities comparable with sham-operated animals (△). $GdCl_3$ (○) and PTX (◆) pretreated animals had significantly reduced phagocytic activities compared with untreated controls (*, $P = 0.034$ and $P = 0.043$, respectively). TNFR-1(−/−) (▼) mice showed comparable phagocytic activity as untreated controls (mean ± SD, $n = 5$ in each group).

signaling pathway, would minimize reperfusion injury of partial liver grafts.

Serum aspartate aminotransferase (AST) levels, reflecting hepatic injury, were 4-fold higher after 30% than after 50% OLT (Fig. 2). Disruption of TNF-α release by PTX or TNF-α signaling in TNFR-1(−/−) mice resulted in a significant protection of 30% grafts as indicated by AST levels in the range of 50% OLT. AST levels in the $GdCl_3$ group were reduced to ≈50% of the untreated controls, supporting the role of KCs in the ischemic tissue damage.

Histological analysis of grafts was performed 2 days after transplantation to substantiate that tissue injury was alleviated in each treatment group. We observed diffuse microvesicular steatosis after 30% OLT (Fig. 3) (18). $GdCl_3$ and PTX treatment reduced steatosis to a similar degree observed after 50% OLT. In TNFR-1(−/−) mice, some macrovesicular steatosis was observed, but the hepatocytes were less affected than in the untreated 30% partial OLT group. These results suggest that

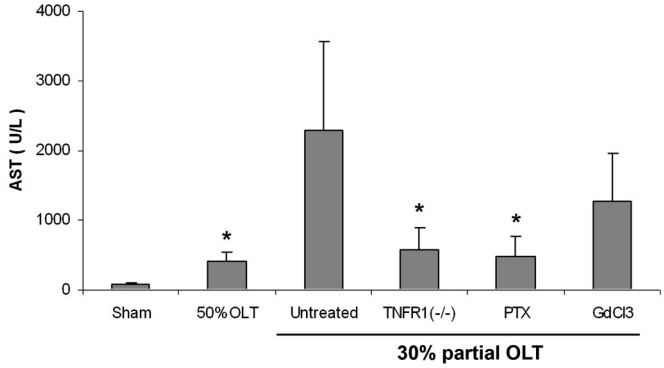

Fig. 2. Liver injury after transplantation. Serum AST levels were determined as markers of liver injury 2 days after surgery. Levels reached almost 2,500 units/liter in recipients of a 30% partial liver graft (untreated). In contrast, each strategy targeting on TNF-α signaling [PTX, TNFR-1(−/−)] or impairment of KC ($GdCl_3$) disclosed lower AST levels, $P < 0.02$, for PTX and TNFR-1(−/−). Values for PTX and TNFR-1(−/−) were comparable with those observed after 50% partial OLT (mean ± SD, $n = 5$ in each group; *, $P < 0.05$ compared with untreated controls).

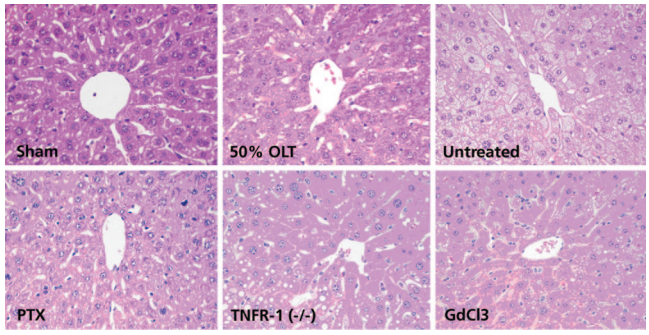

Fig. 3. Histological analysis of graft tissue after transplantation. Hematoxylin/eosin staining of partial OLT grafts was performed 2 days after transplantation. In contrast to untreated 30% partial OLT grafts, which revealed diffuse microvesicular steatosis, only mild macrovesicular steatosis was found in 30% partial OLT grafts after treatment with PTX and $GaCl_3$ and in TNFR-1(−/−) grafts. (Original magnification: ×200.)

hepatocyte integrity is directly affected by the TNF-α pathway and KCs.

Does Disruption of TNF-α Signaling or Impaired KC Function Lead to Improved Survival in Small-for-Size Liver OLT? Assessment of survival after a therapeutic intervention in an animal model with a lethal outcome is a solid proof of principle. Therefore, livers were orthotopically transplanted, and the animals were observed for a maximum of 30 days. Fig. 4 shows a Kaplan–Meier plot demonstrating the effect of the various treatments on animal survival. The 30% OLT untreated controls had a very low survival of 14% at 30 days (1 of 7) whereas PTX pretreatment significantly improved recipient survival to 86% (6 of 7), a figure comparable with the 50% OLT group. Chemical inactivation of KCs by $GdCl_3$ resulted in an improvement to 43% (3 of 7), and the specific disruption of TNF-α signaling using a TNFR-1(−/−) mouse improved survival to 57% (4 of 7). A Kaplan–Meier log-rank survival analysis showed a statistically significant ($P = 0.0004$) improved survival in the treatment groups.

From this set of experiments we conclude that TNF-α signal-

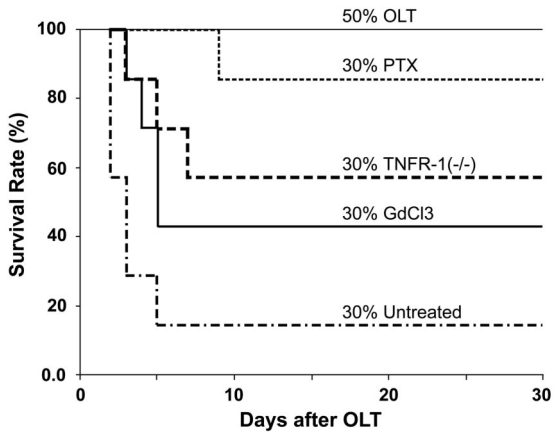

Fig. 4. Animal survival after transplantation. Kaplan–Meier analysis showed that recipients transplanted with a 30% partial graft (30% untreated controls) had a dramatically poorer survival (14%) compared with those receiving a 50% partial OLT graft (100% survival, $P = 0.003$). The strategies modulating TNF-α signals and KC activity had different effects on animal survival. Animals transplanted with a 30% partial graft and pretreated with PTX had a 86% long-term survival ($P = 0.005$ versus untreated controls), whereas TNFR-1(−/−) and $GdCl_3$ pretreated animals had improved survival of 57% and 43% ($P = 0.025$ and $P = 0.049$ versus untreated controls, respectively; $n = 7$ in each group).

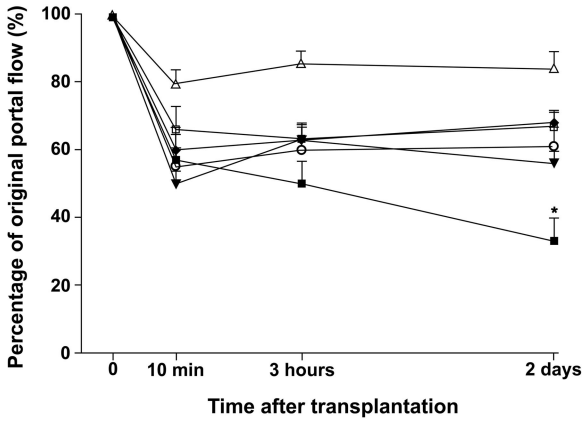

Fig. 5. Portal flow after transplantation. Portal flow was measured 10 min, 3 h, and 2 days after partial OLT. In recipients transplanted with 30% partial grafts (■), portal flow decreased to 60% of baseline value by 10 min of reperfusion, and subsequently to 33% of baseline value by 2 days. Comparable initial post transplantation drops occurred in PTX (◆), $GdCl_3$ (○), and TNFR-1(−/−) (▼) groups, but then values remained stable or slightly improved over the subsequent 2 days. Fifty-percent OLT (□) showed a similar pattern. Sham-operated mice (△) showed an initial drop in portal flow to ≈80% of baseline values without further changes (mean ± SD, $n = 5$ in each group; *, $P < 0.05$ when compared with the three protective strategies).

ing and KC function are important factors mediating injury in small-for-size liver transplantation.

Does Disruption of TNF-α Signaling or KC Function Improve Microcirculation and Portal Flow? Clinical (21) and experimental (22) evidence suggests that portal flow may play a central role in the poor outcome after small-for-size LDLT. The improved survival in the treatment groups led us to investigate portal flow. Therefore, we determined portal flow in recipient animals before transplantation as well as 10 min, 3 h, and 2 days after liver transplantation (Fig. 5). At 10 min of reperfusion, portal flow dropped to ≈60% in all four groups. However, on day 2 portal flow further decreased in untreated animals whereas it remained stable in all treatment groups with values comparable with those observed in 50% partial OLT. Sham-operated mice exhibited an initial drop in portal flow to ≈80% of baseline values without further changes.

Based on the improved hepatic function and portal flow, particularly by PTX, a phosphodiesterase inhibitor affecting vascular physiology, we asked whether microcirculation was improved after targeting KC function with PTX, $GdCl_3$, and TNFR-1 disruption. Using intravital fluorescence microscopy (IVFM), grafts were observed 3 h after transplantation (Fig. 6). The sinusoidal perfusion rate is an indicator of patency of sinusoids. In untreated 30% OLT controls, the nonperfused area was 37% indicating that more than one-third of the liver had some degree of persistent sinusoidal defect (Fig. 6*A*). Of interest, all experimental groups showed a high protection in sinusoidal perfusion with <10% of nonperfused sinusoids ($P = 0.009$) and only slightly inferior to those in sham-operated animals.

Next, we determined leukocyte adhesion to the endothelium of the post sinusoidal venules (Fig. 6*B*). In the untreated 30% OLT controls, ≈900 adherent leukocytes per mm^2 were observed, compared with fewer than 10 per mm^2 adherent leukocytes in sham-operated animals. Each treatment group was associated with a significantly lower degree of leukocyte adhesion (<400 per mm^2) with the lowest observed in the PTX group (250 ± 35 per mm^2).

Taken together, these results suggest that a strategy targeting

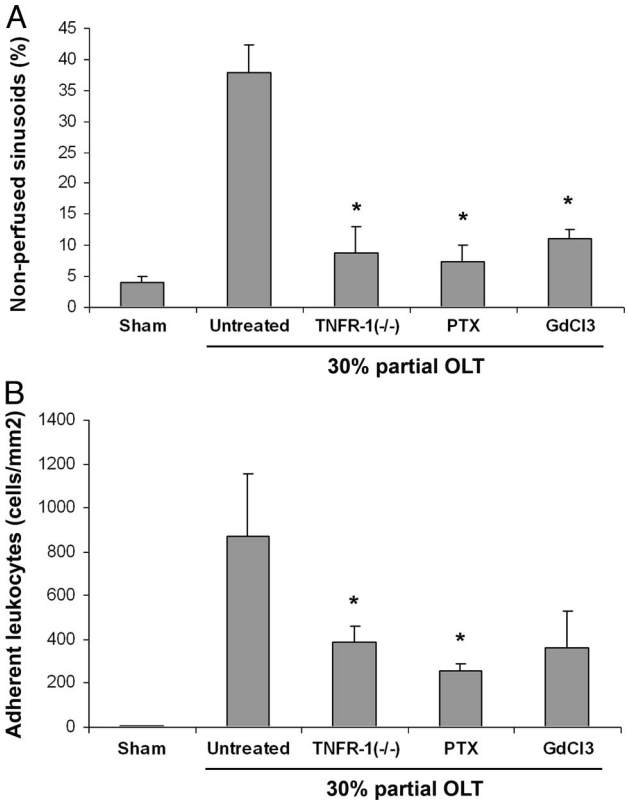

Fig. 6. IVFM after 30% OLT. Microcirculatory changes 3 h after transplantation indicating nonperfused sinusoids (*A*) and leukocyte adhesion (*B*). In TNFR-1(−/−) mice, PTX and GdCl3 pretreated animals disclosed only slightly higher values than sham-operated animals (*, $P < 0.01$ compared with untreated controls). Approximately 900 adherent leukocytes per mm^2 were observed in untreated 30% grafts (*B*), whereas this number was halved in PTX, TNFR-1(−/−), and $GdCl_3$ groups. Sham-operated animals presented fewer than 10 adherent leukocytes per mm^2 (mean ± SD, $n = 5$ in each group).

KC and TNF-α signaling improves microcirculation, which may contribute to the protection against small-for-size injury.

Do the Protective Strategies Correct the Failure of Regeneration in Small-for-Size OLT? In a previous report (18), we observed a complete failure of liver regeneration in small-for size liver grafts. Thirty-percent partial grafts disclosed no sign of hepatocyte proliferation at 48 h after OLT whereas 50% partial grafts exhibited significant hepatocellular proliferation. Hence, the improved survival of animals treated with $GdCl_3$, PTX, or in TNFR-1(−/−) animals suggested that regeneration of the graft might be a decisive factor influencing the outcome of transplantation. Livers were harvested 2 days after OLT and stained for the expression of Ki-67 protein, a marker for liver regeneration. In untreated 30% OLT grafts, hepatocyte proliferation was almost absent [one to two Ki-67-positive hepatocytes per high-power field (HPF)], consistent with our prior observation (Fig. 7). In contrast, 50% partial OLT triggered significant hepatocyte proliferation. $GdCl_3$-treated animals showed a moderately increased Ki-67 labeling index in two animals (8 positive hepatocytes per HPF), and three animals did not differ from untreated controls. In the TNFR-1(−/−) group, significantly increased Ki-67 labeling index (20 hepatocytes per HPF) was observed in three animals, and two animals exhibited only mild proliferation slightly above controls. Finally, the PTX-treated group disclosed increased regeneration (20 Ki-67-positive hepatocytes per HPF) in all but one animal. These results suggest that early after transplantation, regeneration in small-for-size liver grafts is

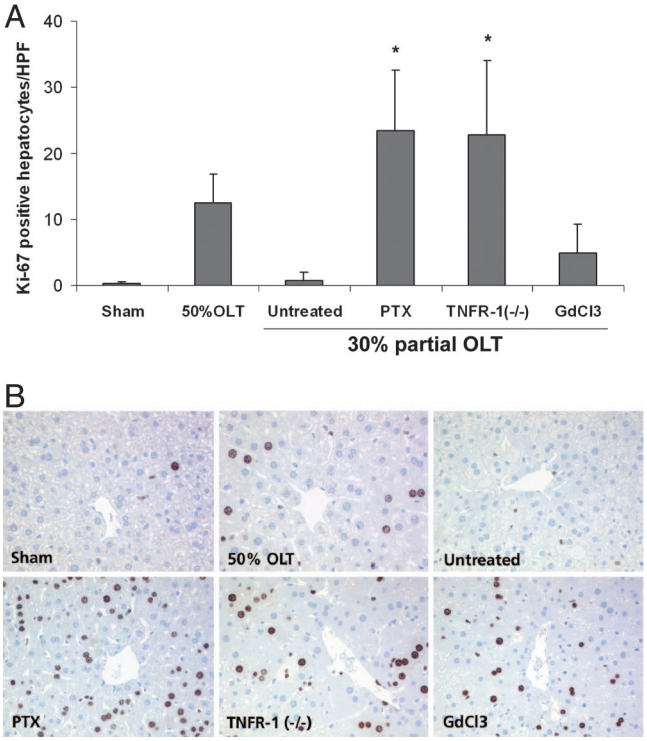

Fig. 7. Liver regeneration in partial OLT grafts. Quantitation (*A*) of hepatocyte proliferation analyzed in partial OLT grafts 2 days after transplantation using immunostaining for Ki-67 protein (*B*). Ki-67-positive hepatocytes were absent in untreated 30% partial OLT grafts but were numerous in 30% partial OLT grafts after treatment with PTX or $GdCl_3$, and in TNFR-1(−/−) grafts, suggesting rescue of impaired regeneration (original magnification: ×200) (*, $P < 0.01$).

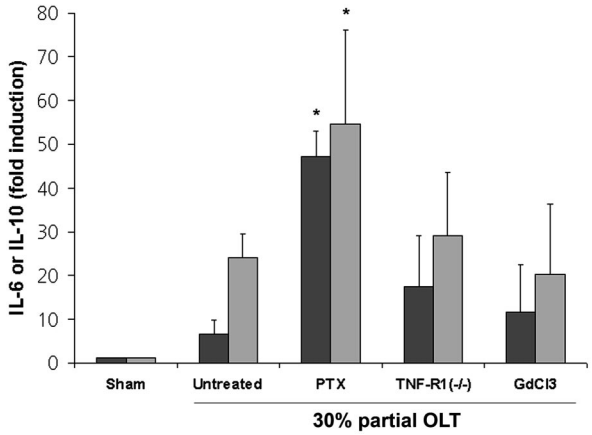

Fig. 8. Cytokine expression levels after 30% OLT. Transcript levels of protective cytokines IL-6 (dark-gray bars) and IL-10 (light-gray bars) 1 h after transplantation. PTX-treated animals showed dramatically increased IL-6 transcript compared with untreated controls (*, $P < 0.01$). IL-10 mRNA levels in PTX-pretreated animals were significantly elevated compared with untreated controls (*, $P < 0.047$; mean ± SD, $n = 5$ in each group).

rescued in a subpopulation of animals possibly correlating with survival.

Do the Protective Strategies Impact on Protective Cytokines Such as IL-6 and IL-10 After Small-for-Size OLT? From the previous set of experiments, PTX appears to be the most effective protective strategy against small-for-size injury, also promoting regeneration. IL-6 and IL-10 have both been shown to protect against ischemic injury (23) and promote regeneration (24, 25) *in vivo*. Therefore, we measured transcript levels of these cytokines in liver tissue 1 h after 30% partial OLT in all groups (Fig. 8). Low values were measured in sham-operated animals. IL-6 mRNA levels in untreated 30% partial OLT mice were about five times higher than in sham-operated animals, with only a slight nonsignificant increase over this value in TNFR-1(−/−) mice or those treated with $GdCl_3$. In contrast, a 50-fold increase was recorded in animals treated with PTX ($P < 0.01$ compared with all other groups). Similar patterns were noted in IL-10 mRNA levels, although values among untreated 30% partial OLT, $GdCl_3$, and TNFR-1(−/−) groups were similar (about 20 times increase to baseline).

These results indicate that PTX treatment leads to a significant induction of IL-6 and IL-10 production. To further evaluate a possible protective pathway linking PTX to IL-6 production, we performed 30% partial OLT in IL-6 knockout [IL-6(−/−)] mice pretreated with PTX. Animal survival was used as an endpoint with an observation extending up to 14 days. The protective effects of PTX resulting in a high rate of long-term animal survival as shown in Fig. 4 were lost as all animals died within 3 days (median, 2.5 days). To confirm the central role of IL-6, we pretreated in the same set of experiments IL-6(−/−)-recipient mice with a single dose of recombinant IL-6, as reported in ref. 25. These animals then received the standard PTX regimen and were subjected to 30% OLT. rIL-6 treatment restored long-term animal survival in 60% (3 of 5) of the animals (median, 15 days; range, 5–15 days).

Discussion

To our knowledge, this is the first attempt to investigate mechanisms of liver failure in a mouse model of arterialized small-for-size liver transplantation. We found a central role of the TNF-α signaling pathway and KC in mediating injury related to small-for-size OLT. PTX, however, offered the best protection with conversion of nonsurvival to survival conditions, possibly related to improved microcirculation after reperfusion and production of antiinflammatory and growth promoting cytokines such as IL-6 and IL-10.

Such study is possible only with the availability of a suitable model mimicking the clinical situation. Partial liver transplantation in humans is successful when a liver mass of >40% is available. Smaller grafts are associated with signs of liver failure causing death of the recipient within a few days in the absence of retransplantation (26). Therefore, we first developed a mouse model of liver transplantation demonstrating the need to reestablish hepatic artery blood flow to prevent biliary injury and graft loss (19). Then, we developed a model of small-for-size OLT consistent with the clinical situation, where a graft of 50% of the initial liver could consistently sustain long-term survival and a 30% volume graft corresponds to nonsurvival conditions (18). Of interest, a 30% partial graft resulted in massive microvesicular steatosis in hepatocytes after transplantation as described in the clinical setting. The availability of such a model in mice enabled us to apply genetically modified animals such as TNFR-1(−/−) and IL-6(−/−) mice, and open the door for further studies investigating other pathways and protective strategies.

We first tested the hypothesis that the TNF-α signaling pathway and KCs play a crucial role in graft damage after 30% OLT. We targeted this pathway using three different strategies [TNFR-1(−/−), PTX, and $GdCl_3$] having all in common an interruption of TNF signaling including two strategies with modulation of KC activity (PTX and $GdCl_3$). Lower TNF-α levels were documented in the PTX and $GdCl_3$ groups, and reduction in KC activity after reperfusion by $GdCl_3$ and PTX was established in this model by latex particle uptake using IVFM.

All strategies conferred protection in terms of postreperfusion injury (serum AST levels and histologic changes), portal flow, microcirculation, and hepatocyte proliferation, and therefore suggest a key role for the postulated TNF-α signaling pathway and KC in this model. Of importance, each treatment group was associated with significant improvement in animal survival, the most relevant parameter to ascertain protection.

PTX offered superior protection converting nonsurvival conditions after 30% partial OLT to near 100% animal survival. Postreperfusion injury, microcirculation, and regeneration were all improved to a high degree despite lesser inhibition of KC compared with $GdCl_3$-treated mice. Several mechanisms might be responsible for the beneficial effects of PTX treatment. PTX is a methylxanthine phosphodiesterase inhibitor that has potent vasodilatating properties for peripheral blood vessels including the hepatic vasculature (27). Using IVFM and direct portal flow measurement, we found better preservation of portal flow, sinusoidal perfusion, and reduced leukocyte adhesion. PTX has well established hemorheologic effects on red blood cells, leucocytes, and platelets, resulting in increased deformability and lower blood viscosity, possibly because of increased concentrations of cellular adenosine triphosphate, stabilizing the cellular membrane (28). Also, PTX improved the survival from 0% to 60% in a non-heart-beating rat liver transplantation model with 60 min warm ischemia plus 9 h cold ischemia (29). It is tempting to correlate these findings with a number of clinical observations showing increased portal pressure and possibly flow in small-for-size OLT, with a beneficial effects of a variety of strategies decreasing portal pressure such as porto-systemic shunt (30). We would postulate here that high portal pressure/flow induces arterial vasoconstriction as shown in many studies looking at the respective role of portal and arterial flow to the liver (31–33). Poor arterial flow is possibly one of the most significant factor for graft survival after OLT in humans and mice (19, 34). Thus, the vasodilator effects of PTX might play a significant role in graft function.

The most striking results relate to the dramatic increase in synthesis of the antiinflammatory and growth-promoting cytokines IL-6 and IL-10, which were observed only in all treatment groups with the highest values in the PTX group. We have previously shown that IL-6 protects against ischemia-reperfusion injury in a rat model of prolonged normothermic ischemia (23). IL-6 also plays a central role in regeneration, possibly acting downstream of TNF-α (24, 25). In a human study (35) TNF-α and IL-6 were both significantly elevated during endotoxin stress. In line with our findings, application of PTX reduced TNF-α to normal levels but IL-6 remained at high levels, suggesting that PTX blocks the endotoxin-induced synthesis of TNF-α in man (35). The increase of IL-6 production (5-fold) in TNFR-1($-/-$) mice may also explain the high regeneration observed in these animals after partial OLT. We also previously demonstrated the protective effects of IL-6 in restoring regeneration in liver subjected to warm (36) and cold (37) ischemia. Similar data are emerging for IL-10 (38), and in cold ischemia-reperfusion injury pretreatment of the donor with IL-10 decreased liver injury and the release of T cell and macrophage-dependent cytokines (39). To test whether the additional benefit of PTX might be mediated by IL-6, we performed small-for-size OLT in IL-6($-/-$) mice with the same PTX treatment as in wild-type mice. The protective effects of PTX were completely lost, unless rIL-6 was injected in these animals. These data demonstrate that the up-regulation of IL-6 is crucial for the effects of PTX in addition to the inhibition of TNF-α. Future studies are needed to evaluate the respective involvement of IL-6 and IL-10 in small-for-size OLT.

Immunosuppression is an additional parameter that has to be taken into consideration. In contrast to the clinical situation, transplantation between mice was always syngeneic and did not require immunosuppressive treatment. Because our study focused on the role of TNF-α and protective approaches, additional effects of immunosuppressive drugs were not included.

The failure of regeneration in the small-for-size model may be the single most important factor associated with a poor outcome. Interestingly, despite overall improved regeneration in each experimental group, some animals disclose absent or poor regenerative ability. The number of animals with a lack of regeneration in each group matched the data on animal survival. Unfortunately, a direct link could not be established as the experimental set-up does not allow monitoring of survival and liver regeneration in the same animal. But it is tempting to speculate that PTX also exerts protection through induction of proliferative and antiinflammatory cytokines such as IL-6 and IL-10, resulting in improved regeneration. We were surprised to find TNFR-1($-/-$) mice subjected to 30% partial OLT disclosed excellent regenerative activity. This supports the finding of Fausto and his group (24) that the failure of liver regeneration in this strain can be restored by high IL-6 levels.

Taken together, our study demonstrates that interruption of TNF-α signaling by KC inactivation through administration of PTX and GdCl3 or the use of TNFR-1($-/-$) mice improves animal survival after small for size OLT. Inhibition of TNF-α minimized hepatic ischemic injury, improved portal flow and microcirculation, and enhanced liver regeneration. The superiority of PTX is related to additional improvement in the microcirculation and induction of the protective cytokines IL-6 and IL-10. These data open the door for new strategies to improve the outcome of LDLT in patients receiving a small graft and eventually may enable the use of small graft volume from split cadaveric liver graft or living donation of only two segments associated with minimal risk for the donor.

Methods

Animals. Male inbred C57BL/6 mice (Harlan), TNFR-1 knockout [TNFR-1($-/-$)] mice, and IL-6($-/-$) mice, both with C57BL/6 background, were used as syngeneic transplant donors and recipients. Animals were kept in accordance with University of Zurich Animal Care Committee guidelines.

Surgical Procedure. Syngeneic arterialized orthotopic 50% and small-for-size (30%) partial liver transplantations were performed in mice according to our recently reported techniques (18). After the operation, the animals were closely supervised to detect surgical complications. In pilot experiments, we established the cause of death after transplantation: if the animals died within the first 24 h after transplantation it was always because of technical problems (19). Therefore, animals dying within the first day were excluded irrespective of the experimental regime.

Experimental Groups. Small-for-size liver transplantation was performed in wild-type mice and three treatment groups with appropriate controls. For the PTX group, PTX (100 mg/kg) was dissolved in saline and injected s.c. into the donor and recipient animal 1 h before the operation, and then twice a day in the recipient for 5 days postoperatively (10, 15, 29). For the $GdCl_3$ group, $GdCl_3$ (10 mg/kg) was injected i.p. to the donor 24 h before the operation. For the TNFR-1($-/-$) group, the animals were used for both the donor and recipient operation. Recipients were killed at 1 h, 3 h, and 2 days. For the animal survival experiments, seven small-for-size OLTs were performed in each treatment group. Survival of >30 days was considered permanent. Fifty-percent partial OLT was added as a control in some experiments, and the effects of PTX were tested in IL-6($-/-$) mice.

Histology, Immunohistochemistry, and AST. Tissues were immersion-fixed in 4% buffered formalin, embedded in paraffin wax, sectioned, and stained with hematoxylin/eosin. Ki-67 staining was used to quantify hepatic regeneration 2 days after transplantation (18, 19). The percentage of Ki-67-positive hepatocytes was determined in eight representative high-power fields. Serum levels of AST as markers of hepatocyte injury were performed as described.

Portal Flow Measurement. A transit-time vascular flow meter (Transonic Systems, Ithaca, NY) was used to measure portal flow (ml/min) before and after reperfusion during the transplant surgery procedure and 2 days after transplantation in the recipients.

IVFM. Recipient mice of 30% OLT were prepared for IVFM 3 h after transplantation (40). A Leica CLS 150X microscope was used for IVFM with epiillumination, using blue and green filter systems (excitation/emission wavelength: 450–490 nm/greater than 520 nm and 530 to 560 nm/greater than 580 nm, respectively). The microscopy sequences were recorded by a video system for offline evaluation.

Quantitative Real-Time PCR (RT-PCR). Total RNA was extracted from 50 mg of liver tissue by using TRIzol reagent (Invitrogen). Five micrograms of RNA were reverse transcribed by using the ThermoScript RT-PCR system (Invitrogen). Quantitative RT-PCR was performed by using an ABI PRISM 7000 sequence detector system (PE Applied Biosystems). TaqMan gene expression assays (PE Applied Biosystems) for IL-6 (assay ID Mm00446190_m1) and IL-10 (assay ID Mm00439616_m1) were used for quantification of mRNA expression of the respective genes. To normalize, amplification of 18S RNA (TaqMan ribosomal RNA control reagents) was performed as an endogenous control.

Statistical Analysis. Values are expressed as means ± SD. The data were analyzed by using SPSS software (Version 9.0.0, SPSS, Chicago). Differences between groups were evaluated by using the Student *t* test and Mann–Whitney test. Animal survival was evaluated by using the Kaplan–Meier method and compared by using the log-rank test. Differences were considered statistically significant if $P < 0.05$.

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