Interleukin-1 participates in the progression from liver injury to fibrosis

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Gieling RG, Wallace K, Han Y-P. Interleukin-1 participates in the progression from liver injury to fibrosis. Am J Physiol Gastrointest Liver Physiol 296: G1324–G1331, 2009. First published April 2, 2009; doi:10.1152/ajpgi.90564.2008.—Interleukin-1 (IL-1) is rapidly expressed in response to tissue damage; however, its role in coordinating the progression from injury to fibrogenesis is not fully understood. Liver fibrosis is a consequence of the activation of hepatic stellate cells (HSCs), which reside within the extracellular matrix (ECM) of subsinusoids. We have hypothesized that, among the hepatic inflammatory cytokines, IL-1 may directly activate HSCs through autocrine signaling and stimulate the matrix metalloproteinases (MMPs) produced by HSCs within the space of Disse, resulting in liver fibrogenesis. In this study, we first established a temporal relationship between IL-1, MMPs, HSC activation, and early activity. The roles of IL-1 and MMP-9 in HSC activation and fibrogenesis were determined by mice deficient of these genes. After liver injury, IL-1, MMP-9, and MMP-13 levels were found to be elevated before the onset of HSC activation and fibrogenesis. IL-1 receptor-deficient mice exhibited ameliorated liver damage and reduced fibrogenesis. Similarly, advanced fibrosis, as determined by type-I and -III collagen mRNA expression and fibrotic septa, was partially attenuated by the deficiency of IL-1. In the early phase of liver injury, the MMP-9, MMP-13, and TIMP-1 expression correlated well with IL-1 levels. In injured livers, MMP-9 was predominantly colocalized to desmin-positive cells, suggesting that HSCs are MMP-producing cells in vivo. MMP-9-deficient mice were partially protected from liver injury and HSC activation. Thus IL-1 is an important participant, along with other cytokines, and controls the progression from liver injury to fibrogenesis through activation of HSCs in vivo.

hepatic stellate cell; matrix metalloproteinase; carbon tetrachloride; thioacetamide; bile duct ligation

ACTIVATION OF HEPATIC STELLATE CELLS (HSCs) and deposition of interstitial extracellular matrix (ECM) are prominent features of liver fibrogenesis (10, 12). In normal liver, HSCs maintain quiescence by residing within the space of Disse, which is composed of loose ECM (2, 3, 11). Concomitant with the activation or transdifferentiation of HSCs is that the “normal” basement-membrane-like ECM in the Disse space is converted to interstitial matrix fibers, a key feature in liver fibrosis (1). Given such changes in hepatic infrastructure during wound repair and fibrogenesis, it is reasonable to assume that the degradation of the “beneficial” ECM may be a critical step to initiate HSC activation and fibrogenesis. Present evidence suggests that profibrogenic cytokines, such as transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor-BB, may promote the accumulation of ECM and cellular proliferation in response to tissue damage (5, 6, 8, 14, 19, 32). However, little is known about the factors involved in the transition from liver injury into a phase of repair or fibrogenesis. In an effort to understand the mechanism of liver fibrogenesis, we paid particular attention to the acute phase cytokines that can promote HSC activation in a matrix metalloproteinase (MMP)-dependent mode. Consequently, we identified IL-1, which induces multiple MMPs and provokes the phenotypic activation of HSCs in 3D ECM (17, 18).

As a key feature of innate immune action in response to inflammatory cytokines, a range of MMPs are upregulated at the onset of tissue damage and are believed to play a role in the mobilization of resting cells into the proliferative, migratory, and differentiation phases. Dynamic changes in MMP and tissue inhibitor of MMP (TIMP) levels have been associated with liver fibrogenesis and its regression (21, 37). Thus an altered balance between MMPs and their inhibitors has been revealed in experimental biliary fibrosis (24, 41). To define the function of a specific MMP in fibrosis, studies were carried out using mice lacking specific MMPs or overexpressing mutant MMPs (34, 39). Previously regarded as antifibrotic factors to resolve ECM deposition in advanced fibrotic tissues, MMPs have recently been viewed as profibrogenic mediators in the onset of fibrogenesis (15, 27). The hepatic source of MMPs in the liver is a topic of debate, relying on models, the nature of the injury, and markers used for examination (20). Despite diverse hepatic sources of MMP expression, the major substrates for MMPs, the extracellular matrices, are in close proximity to HSCs, which grant HSCs to a unique position in charge of the ECM levels in the space of Disse through ECM production and MMP activation. In this study, we examined the role of IL-1 as a possible participant in mediating the transition from liver injury to fibrogenesis and tested the role of MMP-9 in liver fibrogenesis.

MATERIALS AND METHODS

Animal work. Male C57BL/6, IL-1 receptor-deficient mice (B6.129S7-Ilr1tm1Imx/J), FVB, MMP-9 knockouts (KO) (FVB.Cg-Mmp9tm1Tvu/J), and BALB/c wild-type mice were purchased from Jackson Laboratory (Bar Harbor, ME). Animal care and use was in accordance with the Guide for Care and Use of Laboratory Animals from the National Institutes of Health and approved by the Institutional Animal Care and Research Advisory Committee at the University of Southern California. Mice were injected intraperitoneally with thioacetamide (TAA) (Sigma, St. Louis, MO) at 200 μg/g body wt or saline used as control (n = 6 for each experimental condition). The frequency of injection is indicated in the figures. MMP-9 KO and FVB wild-type mice were also subjected to bile duct ligation (BDL) for 4 wk. Small pieces of each lobe were snap frozen in liquid nitrogen, embedded in optimal cutting temperature compound, and stored at −80°C until analysis, or fixed in 10% phosphate saline-buffered neutral formalin.

Histochemical staining. Formalin-fixed liver specimens were dehydrated in alcohols, incubated in xylene, and embedded in paraffin. Five-micron-thick tissue sections were cut and stained with either hematoxylin-eosin (VWR Chemicals, Philadelphia, PA) or Sirius red Fast green FCF (Sigma), according to manufacturer’s protocols.

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Immunofluorescence staining. Sections were fixed in cold methanol and blocked within 5% nonfat milk (BD Bioscience, San Jose, CA). Slides were incubated overnight at 4°C with anti-desmin antibody (Chemicon, Temecula, CA) at 1:100, anti-α-smooth muscle actin (SMA) antibody (LabVision, Union City, CA) at 1:25, or anti-MMP-9 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:50 in 1% nonfat milk. To detect primary antibodies, sections were incubated in FITC-conjugated anti-mouse-IgG at a 1:200 dilution (for desmin, from Sigma), CY3-conjugated anti-rabbit-IgG at a 1:400 dilution (for α-SMA), or FITC-conjugated anti-goat-IgG (for MMP-9) at 1:200 in 1% skim milk. DAPI at 1 μg/ml in PBS was used to stain cell nuclei.

Immunoblotting and gelatin zymography. Liver homogenates were prepared by homogenizing 100 mg liver/ml NT-Triton on ice with a glass potter (NT-Triton: 20 mM NaCl, 50 mM Tris- HCl, pH 7.4, and 1% Triton-X 100). For Western blotting, 50 μg of total protein was separated on a 15% SDS-PAGE. PAGE. Thereafter, proteins were transferred to a PVDF-membrane (Millipore, Billerica, MA), and the membrane was incubated in NT-Tween-20 with 5% nonfat milk (100 mM NaCl, 50 mM Tris-HCl, pH 7.4, and 0.05% Tween-20). To visualize specific proteins, membranes were incubated in rabbit anti-α-SMA (LabVision) at 1:500 or mouse anti-GAPDH (Chemicon) at 1:3,000 followed by incubation in horseradish peroxidase-conjugated secondary antibody (Chemicon) anti-rabbit at 1:15,000 or anti-mouse at 1:25,000. The membrane was developed by using an ABI Prism 7900HT (Applied Biosystems, Foster City, CA). Ten-microliter reactions were set up in a 384-well PCR plate out using an ABI Prism 7900HT (Applied Biosystems, Foster City, CA). Two micrograms of total RNA were used for each reverse transcription reaction mixture (20 μl). Ten-microliter reactions were set up in a 384-well PCR plate using the following final concentrations: 1 μmol each of forward and reverse primers, 1× SYBR Green master mix (qPCR Mastermix Plus for SYBR Green I, Eurogentec, Seraing, Belgium) and 5 ng of cDNA. For each condition three duplicates were used to minimize the variation. Cycling conditions were as follows: initial step (50°C for 2 min), hot activation (95°C for 10 min), amplification (95°C for 15 s, 60°C for 1 min) repeated 40 times, and quantification with a single fluorescence measurement. The data were analyzed using the ABI Prism SDS 2.1 software. Values were calculated as n-fold differences in gene expression relative to GAPDH mRNA using the equation: n-fold = 2^(-ΔΔCtSample - ΔΔCtGAPDH), where ΔCt values of the sample and the calibrator were determined by subtracting the average Ct value of the transcript under investigation from the average Ct value of the GAPDH gene for each sample. The values were normalized to the lowest expression. Primers for mouse genes were as follows: IL-1α, 5′-CACAACCTGTGTCGAGCTGT-3′ and 5′-TTGGTTTTCTCTGGCAACTC-3′; IL-1β, 5′-ACTCTCTTAGTCTCCTGGCCA-3′ and 5′-TGTGTTTTCTGTGACCTGAGC-3′; MMP-9, 5′-CGTGTTCTGAGATTTGCTGTA-3′ and 5′-TGGGAAAGATGCAGTTGAGA-3′; MMP-13, 5′-ccctttctctgctgcaac-3′ and 5′-gctcttctctgctgcaac-3′; type-I collagen, 5′-GATGGCGCAAAGAGACATCC-3′ and 5′-CCTCGGTTTCTCACGTCCTC-3′; type-II collagen, 5′-aagttgcttgcttgcttgcttg-3′ and 5′-ggctgttgcttgcttgcttg-3′; type-α-SMA, 5′-AAACAGAATACGACGCAAA-3′ and 5′-CAGAATGATTGAGAGAAAGGA-3′; TIMP-1, 5′-gcacagctgtgcagctgc-3′ and 5′-gctgcagctgtgcagctgc-3′; GAPDH, 5′-GGCAGTCAGGGCCGCACTGAT-3′ and 5′-GCCTTCTCCAATGGTGTTGAAA-3′.

Alanine transaminase. Serum derived from mouse blood was mixed with Infinity alanine transaminase (ALT)-reagent (Thermo Electron, Louisville, CO) 1:10 (vol/vol), placed inside a spectrophotometer with a constant inner temperature of 37°C, and the absorbance was measured at 340 nm. The ALT concentration was obtained from the slope of the absorbance curve and was presented as units according to the manufacturer’s instruction.

Statistical analysis. The data are expressed as means ± SE. The differences between two groups were analyzed using the two-tailed Student’s t-test. Differences between multiple groups were tested with the Kruskal-Wallis nonparametric analysis of variance with multiple comparisons of groups. Values were regarded as significant for P < 0.05.

RESULTS

Chronological expression of IL-1, MMPs, TIMP, and TGF-β in acute liver injury and onset of liver fibrogenesis. Mice of three genetic backgrounds (FVB, C57BL/6, and BALB/c) were given TAA by intraperitoneal injection. In response to a single dosage of TAA in 24 h, the liver architecture, as measured by hematoxylin and eosin histological staining, displayed areas of centrilobular parenchymal hemorrhages; in concordance, ALT levels were sharply raised in the serum to more than 9,000 units/l (Fig. 1, A and B). MMP-9 expression, as measured by zymography, was clearly elevated and peaked at 24 h after TAA injection (Fig. 1C). Thereafter, MMP-9 levels declined to basal levels at day 7. In contrast, MMP-2 was induced to a lesser extent within 24 h of treatment. Double immunofluorescent staining revealed the colocalization of MMP-9 and desmin-positive cells with branched cellular bodies, the typical phenotypes of HSCs in the liver (Fig. 1D). Such a result is in line with our previous in vitro evidence, demonstrating HSCs as a hepatic source of MMP-9 in liver injury (13).

The mRNA levels of IL-1α and -β were elevated within 24 h after exposure to the toxin (Fig. 1E). In contrast, TGF-β mRNA was constitutively expressed and was not significantly upregulated during the early phase of injury response (Fig. 1F). The elevated level of MMP-9 mRNA (10–75-fold induction) was consistent with the zymographic data, depending on the particular mouse strain. Similarly, MMP-13, a major interstitial collagenase in rodents, was induced more than 50-fold in relation to the control. Up-regulation of α-SMA, a hallmark of tissue fibrogenesis, was used to evaluate HSC activation in vivo. As shown, α-SMA mRNA was initially observed at day 1, followed by its protein expression at day 3 (Fig. 1, E and F). TGF-1 was induced almost simultaneously with the MMP expression, suggesting a mode of inhibitory feedback to balance the proteolysis in the liver injury and early fibrogenesis. As a consequence of activation, HSCs produced interstitial ECM. Type-I collagen expression was upregulated, and it peaked at day 3, coinciding with HSC activation (α-SMA expression) (Fig. 1F). Our results underscore a possible chronological sequence of key events in tissue repair and the onset of liver fibrogenesis in the following order: hepatic injury > IL-1 in the space of Disse > HSC acute response > MMPs in the space of Disse > HSC activation (α-SMA) > expression of interstitial ECM (as outlined in Fig. 1G).

IL-1-dependent early fibrogenesis. To confirm the causative relationship of the outlined chronological events in liver injury
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**Fig. 1.** Early events in liver injury and fibrogenesis. A: hematoxylin and eosin (H and E) staining. FVB, C57BL/6, and BALB/c mice (n = 6 per experimental condition) were injected intraperitoneally with saline or thioacetamide (TAA) (0.2 mg/g body wt ip), and livers were harvested at the indicated time points. B: alanine transaminase (ALT) level in the serum. C: zymography of liver tissues. D: double immunofluorescent staining of liver tissue at day 3. Matrix metalloproteinase (MMP)-9 is expressed by desmin-positive cells with cytoplasmic extensions, the typical phenotypes of hepatic stellate cells (HSCs) in liver. E: real-time RT-PCR analysis of liver tissues. The gene expression levels are normalized by the mRNA of GAPDH. *P < 0.05 between groups indicated in graph. F: Western blot analysis of liver tissues. The positive control is the cell lysate from rat primary HSCs cultured on plastic for 5 days, by which the quiescent cells are converted to myofibroblasts. G: proposed schematic model of the chronological events in the liver injury, HSC activation, and fibrogenesis. TIMP, tissue inhibitor of MMP; SMA, smooth muscle actin; ECM, extracellular matrix.
and fibrogenesis, we examined mice with impaired IL-1 signaling. C57BL/6 wild-type and IL-1R-deficient mice were given a single dose of TAA. IL-1R KO mice had ameliorated tissue damage compared with that of the wild-type controls as indicated by hematoxylin and eosin staining and serum ALT levels (Fig. 2, A and B). IL-1R KO mice had lower levels of MMP-9 mRNA and protein than those detected in wild-type mice, and MMP-2 expression was unaffected, demonstrating an IL-1-dependent regulation of MMP-9 in liver injury (Fig. 2, C and D). Similarly, toxin-induced MMP-13 transcripts in the liver were abrogated in IL-1R-deficient mice, particularly in the early phase (≤3 days). Activation of HSCs, as characterized by the transcript and the protein levels of α-SMA expression in liver (Fig. 2, D and E), was suppressed in the IL-1R KO mice, which demonstrates a role for IL-1 in the activation of HSCs in vivo and liver fibrogenesis. Upregulation of type-I and -III collagen throughout the acute phase of fibrogenesis is not seemingly regulated by IL-1, but it is clearly controlled by IL-1 in the chronic fibrotic stage (detail in the following section).

The expression of the IL-1α, -β, and their receptors was not significantly affected by IL-1 signaling, as measured by real-time RT-PCR, whereas the expression of TIMP-1 is under the control of IL-1 (Fig. 2D).

**Development of advanced liver fibrosis is coordinated by IL-1 signaling.** We then addressed the problem of whether IL-1 signaling also coordinates the development of advanced fibrosis. Wild-type and IL-1R KO mice were injected with TAA twice a week for 8 wk. As shown in Fig. 3, A and B, in response to the chronic exposure to the hepatic toxin, the wild-type mice developed typical fibrotic features, as indicated by thick fibrotic septa bridging between the portal and central veins. In contrast, the IL-1R KO mice exhibited much thinner and diffuse fibrotic septa. α-SMA levels were also reduced in the IL-1R KO mice (Fig. 3C). Furthermore, after 8 wk of CCl₄ treatment, the IL-1R-deficient mice showed ameliorated fibrotic septa formation compared with that in the wild-type mice (data not shown). In line with the results of histological staining, the mRNA levels of type-I and type-III collagen were both significantly lower in IL-1R-deficient mice than those in the wild-type (Fig. 3D). In contrast to the acute injury model, in which MMP-9 is significantly induced, MMP-9 levels in the fibrotic livers are low (Fig. 3, D and E), in favoring of the ECM synthesis and accumulation in advanced fibrotic liver. The level of MMP-9 mRNA was 15 times lower in the chronic liver injury compared with the acute injury (Fig. 3F). Importantly, in contrast to the acute injury, the MMP-9 at the level of both mRNA and protein is no longer correlating with IL-1 in the advanced fibrotic liver. The overall expression level of MMP-13 in the fibrotic liver is significantly low (7-fold) in comparing to the acute response (56-fold) (Fig. 3D and Fig. 2E). Thus the loss of MMP response in fibrotic liver reflects well the shifted balance in favor of ECM accumulation.

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**Fig. 2.** IL-1 in liver injury and onset of fibrogenesis. A: H and E staining. C57BL/6 and IL-1R knockout (KO) (n = 6 per group) were subjected to TAA injection (intraperitoneally), and the livers were harvested at 24 h. B: time course of ALT levels in serum. C: zymography of liver tissue. D: real-time RT-PCR analysis of liver tissues. E: Western blot of liver tissue. *P < 0.05 between groups indicated in graph. WT, wild-type.
summary, IL-1 signaling is critical for the development of advanced fibrosis.

MMP-9 KO mice show ameliorated liver injury and moderate protection against early fibrosis. To examine the role of MMP-9 in liver injury and the onset of fibrosis, we used MMP-9 KO mice. MMP-9-deficient mice exhibited clear protection against the TAA-induced parenchymal necrosis and acute injury (Fig. 4A). Gelatin zymography confirmed the absence of functional MMP-9 in the deficient mice (Fig. 4B). ALT levels in the plasma of the MMP-9 KO mice were significantly lower than those in the wild-type mice (Fig. 4C).

The α-SMA mRNA level was significantly lower in MMP-9 KO mice, whereas the mRNA of type-I collagen was unaffected by the lack of MMP-9 (Fig. 4D). The transient expression of α-SMA protein in response to hepatic toxin was also significantly lower in the MMP-9 KO mice (Fig. 4E and F). The small window of protection against fibrosis by MMP-9-deficient mice could be partly attributed to the fibrotic resistance of the FVB strain. This becomes clear in Fig. 1E where, compared with the other strains, FVB shows a moderate response in terms of IL-1 expression after the injection of hepatic toxin.

MMP-9 KO mice in chronic injury and fibrosis. Finally, we examined the role of MMP-9 in chronic injury and fibrosis. Chronic injection of TAA into FVB wild-type mice failed to induce cirrhotic fibrosis although the mRNA level of α-SMA was enhanced (Fig. 5, A and B). Given such fibrotic resistance in the FVB genetic background, we could not confidently conclude, on the basis of the present data, the role of MMP-9 in the development of fibrosis using this animal model. Since a regular dosage of CCl₄ is sufficient to kill FVB mice, we performed BDL. After 4 wk of BDL, functional MMP-9 was still found in the wild-type (Fig. 5C). Histological staining revealed large areas of inflammatory cells around the portal veins in the wild-type livers (Fig. 5D). In contrast, the MMP-9-deficient mice were free of portal infiltration and hypertrophic compensation after BDL, indicating the involvement of MMP-9 in the recruitment of inflammatory cells and regeneration of bile ducts. Fibrosis, as shown by reticulin staining, exhibited fibrotic septa in wild-type, whereas MMP-9-deficient mice showed less fibrotic bundles (data not shown).

DISCUSSION

Little is known about the extracellular signals that coordinate the progression from tissue injury to repair and early fibrogenesis. The first wave of action following liver injury is the acute response, in which the innate immune system, in-
including Kupffer cells, participates. We recently showed that, as part of this acute response, intrahepatic levels of IL-1 are increased as early as 1 h after toxic liver injury (42). This causes acute liver failure, presumably through the HSC-produced MMPs that breakdown of ECM scaffolds in the space of Disse, leading to collapse of sinusoids (42). The involvement of IL-1 in fibrogenesis was also observed in experiments by Mancini et al. (29), showing that treatment with an IL-1 receptor antagonist protects rats from dimethylnitrosamine-induced liver fibrosis. Furthermore, IL-1 has been associated with fibrosis in other organs, such as lungs and kidney (25, 28, 30, 40). Similarly, increased TNF-α signaling, which shares many intracellular pathways with IL-1, is also associated with the onset of fibrosis (32).

Since IL-1 and Toll-like receptor 4 (TLR4) share many redundant intracellular signaling pathways, it is worth addressing the TLR4 in liver fibrogenesis. Recently, a finding on the basis of LPS-injured livers showed an important role for the TLR4 on HSCs in enhancing TGF-β signaling, leading to the activation of Kupffer cells and hepatic fibrosis (36). Although we did not observe significant changes in the mRNA of TGF-β levels in acute TAA-injured livers, compared with the nontreated wild-type, the regulation is likely at the protein level. Furthermore, studies in which Kupffer cells were depleted, either with gadolinium chloride or with CD11b-DTR transgenic mouse, have clearly revealed that Kupffer cells are vital for the activation of HSCs and the development of fibrosis (7, 33). In the TAA-injury model, it is more likely that the increased IL-1 levels (from activated Kupffer cells and dying hepatocytes trigger the activation of HSCs) (4). However, additional studies are required with the acute TAA model to produce more conclusive data on the involvement of the TLR4 receptor on HSCs, since this was beyond the scope of this study.

The data presented in this study explicitly demonstrate the importance of IL-1 in both the early fibrogenesis and the later phase of maintenance of fibrosis. However, little is known about how IL-1 exerts these fundamental changes. Our preliminary in vitro experiments with HSCs in 3D ECM indicated that MMP-9 may play an important role in liver fibrogenesis. First, acute liver damage induced by CCl₄ revealed that MMP-9 is a downstream target of IL-1 signaling, participating in the transition from liver injury to repair (42). Secondly, type-IV collagen among other ECM, which is abundantly available in the space of Disse of normal livers, is a favorite substrate for MMP-9 (31). Breakdown of the natural available ECM may allow the quiescent HSCs to activate or transdifferentiate. Thirdly, primary rat HSCs express, of about 16,000 genes, about 50% of the rat genome. In response to an IL-1 challenge in vitro, about 800

Fig. 4. MMP-9 in liver injury and early fibrogenesis. A: H and E staining. FVB and MMP-9 KO mice (n = 6 per group) were injected with TAA (0.2 mg/g body wt ip) and euthanized at the indicated time points. B: zymography analysis of liver tissues. C: ALT levels of serum. D: real-time RT-PCR analysis. E: Western blot analysis of liver tissues. F: quantification of α-SMA by densitometry scanning. *P < 0.05 as statistically significant.
genes appear to be upregulated, and among the most induced ones are MMP-9 and -13 (Han, unpublished data). Fourthly, MMPs in the space of Disse may indirectly activate HSCs through the proteolytic cleavage of TGF-β anchored to the ECM. Finally, MMPs may also directly bind to cell membrane “receptors”, provoking activation, migration and proliferation of the HSCs.

In this study, we showed in vivo evidence that HSCs are hepatic sources of MMP-9 during liver injury and early fibrogenesis, in correlation with IL-1 expression. We did not investigate whether other hepatic cell types produced MMP-9 since this was beyond the scope of this study. However, studies in chronic models of TAA-induced liver fibrosis revealed that neutrophils and macrophages served as major sources of MMP-9 (35). In line with our findings, the in vitro studies by others (38) have demonstrated that TNF-α, IL-1, and NF-κB are regulators of inducible MMPs such as MMP-9 and -13. Induction of the expression of several MMPs was also shown after an acute CCl₄ injury (24). In particular, MMP-2 activity, which overlaps with that of MMP-9, has been implicated as an important proteinase that is upregulated along with the progression of fibrosis (37, 43). We did not observe a significant difference in MMP-2 levels in the chronic injured liver, although it was slightly increased in acute liver injury (Figs. 2 and 4). In accordance with the evidence provided, we predicted that MMP-9 KO mice may be protected from liver fibrosis. However, MMP-9 KO mice were only marginally protected against early liver fibrosis and without clear protection against advanced fibrosis. The absence of a protective effect in MMP-9 KO mice against liver fibrosis may be due to inherited factors in the FVB strain, as well as to redundancy between various MMPs during the liver injury. Finally, the role of MMPs in fibrogenesis is complicated by their apparent role in both the resolution of liver fibrosis, in which MMPs promote ECM degradation, and their role in clearance of fibroblastic HSCs (9, 22, 23, 41). Thus the temporal and spatial presence of particular MMPs is crucial to fulfill their roles as “double-edged swords” in the phases of tissue injury, early fibrogenesis, and fibrosis regression. The role of MMP-9 in tissue fibrosis was demonstrated by studies in the lung under ovalbumin challenge, by which the MMP-9-deficient mice displayed reduced levels of peribronchial fibrosis and TGF-β1 (34). Finally, clinical studies revealed a link between the elevated MMPs, including MMP-9, and fragments of type-IV collagen in cirrhosis (13, 26). To clearly resolve these complexities it is necessary to have the MMP-9-deficient mice in fibrotic-sensitive strains and generate inducible KO mice to distinguish the roles of MMPs in fibrogenesis and fibrosis regression.

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Fig. 5. MMP-9 in advanced liver fibrosis. A: FVB and MMP-9 KO mice (n = 6 for each group) were injected with TAA (0.2 mg/g body wt ip) twice a week for 8 wk. Sirius red staining of the liver tissue. B: real-time RT-PCR analysis of the liver tissue of the mice subjected to TAA for 8 wk. C: zymography of liver tissue. FVB and MMP-9 KO mice were subjected to bile duct ligation for 4 wk (n = 8 per group). D: H and E staining of the liver tissues. *P < 0.05 as statistically significant.
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