Kinetics of MMP-1 and MMP-3 Produced by Mast Cells and Macrophages in Liver Fibrogenesis of Rat

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Abstract. *Background: The involvement of whether macrophages and mast cells in the kinetics of matrix metalloproteinases-1 (MMP-1), MMP-3 and MMP-9, tissue inhibitors of metalloproteinases-1 (TIMP-1) and TIMP-2 in hepatic fibrosis/cirrhosis was examined. Materials and Methods: The MMPs and TIMPs were examined by histopathology, immunohistochemistry and immunoblotting using carbon tetrachloride-induced fibrotic rat liver. Results: MMP-1 increased in proportion to the development of fibrosis and reached maximum at week 14. In the first four weeks, MMP-3 expression was mainly observed in many hepatocytes. At week 8, the macrophages in the fibrous septa, as well as hepatocytes, expressed MMP-3. TIMP-1 and -2 progressively increased throughout the experimental periods. The MMP-1 expression in the mast cells, however, did not decrease the degree of liver cirrhosis. Conclusion: MMP-1, TIMP-1 and -2 expressions increased in the late stages of fibrosis and cirrhosis. During recovery, the MMP-3 expression of macrophages greatly increased in the unresolved fibrous septa.*

Liver fibrosis results from an imbalance of an extracellular matrix (ECM) deposition and degradation by matrix metalloproteinases (MMPs) and a tissue inhibitor of metalloproteinases (TIMPs). It is characterized by the accumulation of collagen I, III and IV and other ECM proteins that disrupt normal hepatic architecture and impair liver functions (1-6). The regulation of the enzymatic system involved in ECM degradation may be an important factor in liver fibrogenesis.

MMPs fall into three main groups: collagenases, gelatinases and stromelysins (3). Collagenases have the most specific substrate profile and they specifically degrade the native forms of fibrillar collagen types I and III. Gelatinases cleave denatured collagen, type IV (basement membrane) collagen, type V collagen and elastin. Stromelysins have a broad substrate profile and degrade proteoglycans, glycoproteins and have some activity on type IV collagen and elastin. In addition, stromelysins are involved in the activation of procollagenase and progelatinase-B. The activity of MMPs can be regulated at the level of transcription, pro-enzyme activation, or the binding of pro-enzymes or active enzymes to specific inhibitors, such as TIMP-1 and -2 (7-9).

Until now, several authors have reported that activated hepatic stellate cells (HSCs), macrophages and mast cells are known to participate in liver fibrosis and play an important role in the accumulation of ECM (6, 10). HSCs are known to be major producers of MMPs and TIMPs *in vitro* and *in vivo* (3, 11-14). It is not well-known, however, whether mast cells and macrophages could produce MMPs or TIMPs in liver fibrogenesis. Knittel *et al.* reported that ED1 (monocyte/macrophage/dendtritic cells) overlap with the MMP-9 immunoreactivity, however, only a subpopulation of ED1-positive cells could be MMP-9-positive, as noted in normal and acutely, as well as chronically injured livers (15). Galis *et al.* reported that the MMP-1 (interstitial collagenase), MMP-3 (stromelysin-1) and MMP-9 (gelatinase B) expressions were observed in macrophages of atherosclerotic plaques (16, 17). Other reports also described the production of TIMP-1 and -2 by human alveolar macrophages (18, 19). Mast cells, containing the tryptase and chymase enzymes that are capable of stimulating the MMP cascade, were identified in the endometrium, rheumatoid lesions and atherosclerotic

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Table I. *Hepatic lesions, number of mast cells and the grade of liver fibrosis.*

Week	Hepatic lobular lesions	No. of mast cells / x400	Grade of liver fibrosis[b]
0	Normal	8.2±1.0	Grade 0
4	Fibrosis (mild)	15.3±2.7[a]	Grade 2
8	Fibrosis (moderate)	21.8±2.3[a]	Grade 3
12	Fibrosis and Cirrhosis	28.3±1.0[a]	Grade 3 ~ 4
14C[c]	Cirrhosis	35.7±1.4[a]	Grade 4
14R[d]	Fibrosis (moderate)	19.3±2.5[a]	Grade 2-3

[a]$p<0.01$, significant difference from the values of the control and previous week.
[b]Literature cited: (21). Grade 0, none; Grade 1, short collagenous septa extended from central veins; Grade 2, slender septa link the central veins, but lobular architecture is preserved; Grade 3, pseudolobuli are formed by thin septa; Grade 4, parenchyma is subdivided into smaller pseudolobuli by thin septa.
[c]C, cirrhotic group with CCl_4 treatment for 14 weeks.
[d]R, recovery group with CCl_4 treatment for 12 weeks was allowed to recover during the last 2 weeks (between weeks 13 and 14).

Table II. *MMPs and TIMPs producing cells during fibrogenesis and recovery.*

Week	MMP-1	MMP-3	MMP-9	TIMP-1	TIMP-2
0	MC	Hep	ND[a]	ND	ND
4	MC	Hep	KC, Mac	Mac, aHSC	ND
8	MC, Mac	Hep, Mac	KC, Mac	Mac, aHSC	Mac
12	MC, Mac	Mac	Mac	Mac, aHSC	Mac
14C[b]	MC, Mac	Mac	Mac	Mac, aHSC	Mac
14R[c]	MC, Mac	Mac	Mac	Mac, aHSC	Mac

[a]ND: not detected.
[b]C: cirrhotic group with CCl_4 treatment for 14 weeks.
[c]R: recovery group with CCl_4 treatment for 12 weeks was allowed to recover during the last 2 weeks (from week 13 to 14).
MC: mast cell; Hep: hepatocyte; KC: Kupffer cel; Mac: macrophage.

plaques (20-22). In addition, recent studies suggested that the mast cell expressed MMP-1 in the endometrium and gingiva (23, 24).

Accordingly, the present study aimed to demonstrate the expression of MMPs by mast cells and macrophages by immunohistochemistry and to identify the cells and MMPs playing a role in both the progressive and recovery phases of CCl_4-induced experimental liver fibrogenesis in rats.

Materials and Methods

Experimental design. Male Wistar rats (n=48), weighing 200-220 g, were housed at a 22±2˚C room temperature and 12-h light-dark cycle. The experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals. Fibrosis/cirrhosis was induced by an intraperitoneal injection of 1.0 ml/kg body weight of 10% CCl_4 in olive oil three times a week for 14 weeks. Rats were divided into two groups: Group 1 (n=40) was the cirrhotic group with CCl_4 treatment for 14 weeks and every eight rats were sacrificed at weeks 0, 4, 8, 12 and 14, respectively; Group 2 (n=8) was the recovery group with CCl_4 treatment for 12 weeks after which the rats were allowed to recover for the last 2 weeks (between weeks 13 and 14). These eight rats were sacrificed in week 14.

Histopathology and immunohistochemistry. Livers were rapidly removed at random and fixed in 10% neutral-buffered formalin, processed routinely and embedded in a paraffin wax. The sections were cut into 4 μm and stained with hematoxylin and eosin (H-E) and with a special stain including Azan for collagen fibers and a Toluidine blue stain for mast cells. To quantify hepatic fibrosis, a grading system between 0 and 4 was employed (6). For immunohistochemistry, sections were deparaffinized in xylene, rehydrated in a graded alcohol series, incubated in a solution of 3% H_2O_2 in methanol for 30 min and microwaved at 750W for 10 min in a 10 mmol/L citrate buffer (pH 6.0). The sections were washed with PBS and then immunostained with antibodies of α-smooth muscle actin (α-SMA) (Sigma, St. Louise, MO, USA), ED1 (Chemicon, Temecula, CA, USA), MMP-1 (Chemicon), MMP-3 (C-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA), MMP-9 (H-129, Santa Cruz Biotechnology), TIMP-1 (H-150, Santa Cruz Biotechnology) and TIMP-2 (H-140, Santa Cruz Biotechnology). The antigen-antibody complex was visualized by an avidin-biotin-peroxidase complex solution using an ABC kit (Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (Zymed Laboratories Inc., San Francisco, CA, USA). The sections were then rinsed in distilled water and counterstained with Mayer's hematoxylin or methyl green. For negative control, the primary antibody was replaced by a PBS. As a negative control, normal mouse or rabbit IgG was used instead of primary antibody.

The expression levels of MMPs and TIMPs were graded between 0 to +++ as either no, weak, slight, moderate or severe. Two different pathologists examined the histological slides.

Immunoblotting. Protein samples (50 ~ 100 μg per lane) were separated by 8 ~ 12% SDS-polyacrylamide gel electrophoresis. For immunoblotting, proteins were electro-transferred to a PVDF membrane (Schleicher & Schuell, Dassel, Germany). After blocking with a 1.5% bovine serum albumin in a Tris-buffered saline (TBS), the primary antibody was used; rabbit polyclonal antibody against MMP-1, MMP-9, TIMP-1, TIMP-2 and goat polyclonal antibody against MMP-3. After washing in a TBS, the blots were incubated with anti-rabbit or goat IgG HRP conjugated secondary antibody (Promega, Madison, WI, USA). Specific binding was detected using the Super Signal West Dura Extended Duration Substrate (PIERCE, Rockford, IL, USA) and the blots were exposed to Medical X-ray Film (Kodak, Tokyo, Japan).

Cell counting and statistics. The numbers of mast cells were counted in three areas: the perivenular, midzonal and periportal regions of the three specimens from each rat liver at a magnification of χ400. For statistical analysis, the Student's *t*-test was employed. Values of $p<0.05$ were considered significant.

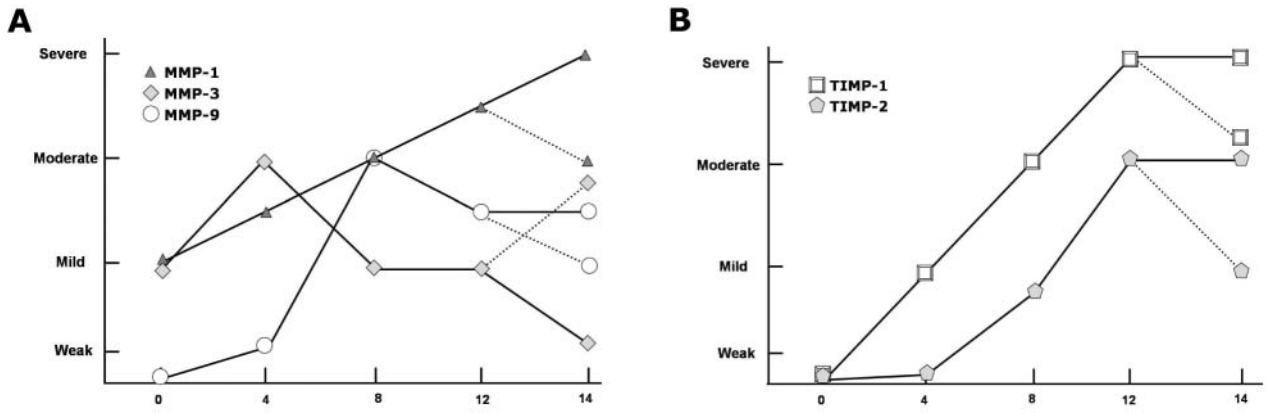

Figure 1. *(A) Extent of MMP expression during fibrogenesis and recovery. (B) Extent of TIMP expression during fibrogenesis and recovery. The solid line represents the cirrhotic group with CCl_4 treatment for 14 weeks. The dotted line represents the recovery group with CCl_4 treatment for 12 weeks followed by recovery during the last 2 weeks (from week 13 to 14). Eight animals were sacrificed at each time point, respectively.*

Results

Liver fibrosis and mast cells. The grade of the liver fibrosis changed between 0 and 4 (Table I). At week 0, collagen fibers were normally observed in the areas of the portal and central veins. Four weeks after the administration of CCl_4, collagen formed bridging fibrosis. By week 8, hepatic tissues were divided by collagen septa into pseudolobules of different sizes. From week 12, the level of fibrosis progressed much more severely and the parenchyma was subdivided into smaller pseudolobules. At week 14, remarkable liver cirrhosis with dense fibrous septa occurred in the cirrhotic group while, in the recovery group, there was a prominent decrease of collagen fibers. Pseudolobules or bridging fibrosis, formed by thin fibrous septa, were also detected. From our previous results (6, 10), the number of mast cells increased significantly in proportion to the development of liver fibrosis and this amount peaked at week 14 (Table I). In the recovery livers, the number significantly decreased compared to those of cirrhotic liver at week 14.

Detection of positive cells for MMPs and TIMPs. The cells positive for MMPs and TIMPs during liver fibrogenesis and recovery are described in Table II. The extent of MMP and TIMP expression produced by several type cells is presented in Figure 1.

At week 0, MMP-1 was detected strongly in the mast cells of the portal areas. The expressions of MMP-1 increased in proportion to the increase of mast cells and the development of fibrosis (Figure 2). These results paralleled those of the significant accumulation of mast cells in portal areas from the Toluidine blue staining (data not shown). Between weeks 8 and 14 macrophages and mast cells also expressed MMP-1, only at the fibrous septa (Figure 2F). Between weeks 0 and 4, MMP-3 only was expressed in many hepatocytes. At week 0, hepatocytes in pericentral lesion showed a stronger positive reaction for MMP-3 than those of other areas (Figure 3A). At week 8, the macrophages in fibrous septa produced MMP-3, as well as hepatocytes (Figure 3B). The MMP-3 expressions in both cell types decreased between weeks 8 and 14. Furthermore, the hepatocytes did not express MMP-3 from week 12 (Figure 3C). Moreover, MMP-3 was expressed only in some of the detectable macrophages (ED1-positive monocytes) within the fibrous septa, as seen when comparing Figure 3C and D.

MMP-9 was detected in Kupffer cells and macrophages starting from week 4 (Figure 4A) and peaked at week 8. MMP-9 expression, however, decreased from week 12 and was detected only in macrophages within the fibrous septa between weeks 12 and 14 (Figure 4B). It is interesting to note that the macrophages within the fibrous septa of pseudolobules expressed all three MMPs between weeks 8 and 14. In recovery, the expressions of MMP-1 and -9 decreased at week 14. The expression of MMP-3, however, increased strongly with the infiltrating macrophages in the unresolved fibrous septa (Figure 5), whereas the activated HSCs disappeared in the fibrous septa (Figure 5B), indicating that hepatic macrophages were involved in fibrillar ECM degradation.

The expressions of TIMP-1 and TIMP-2 progressively increased throughout the experimental periods. From week 4, the TIMP-1 expression was detected in the macrophages and activated HSCs in the fibrous septa (Figure 6A, B), but the expression of TIMP-1 in the macrophages was more prominent than that of the myofibroblasts in the cirrhotic livers (Figure 6C). The TIMP-2 expression increased markedly at weeks 12 and 14 compared to week 0 (Figure

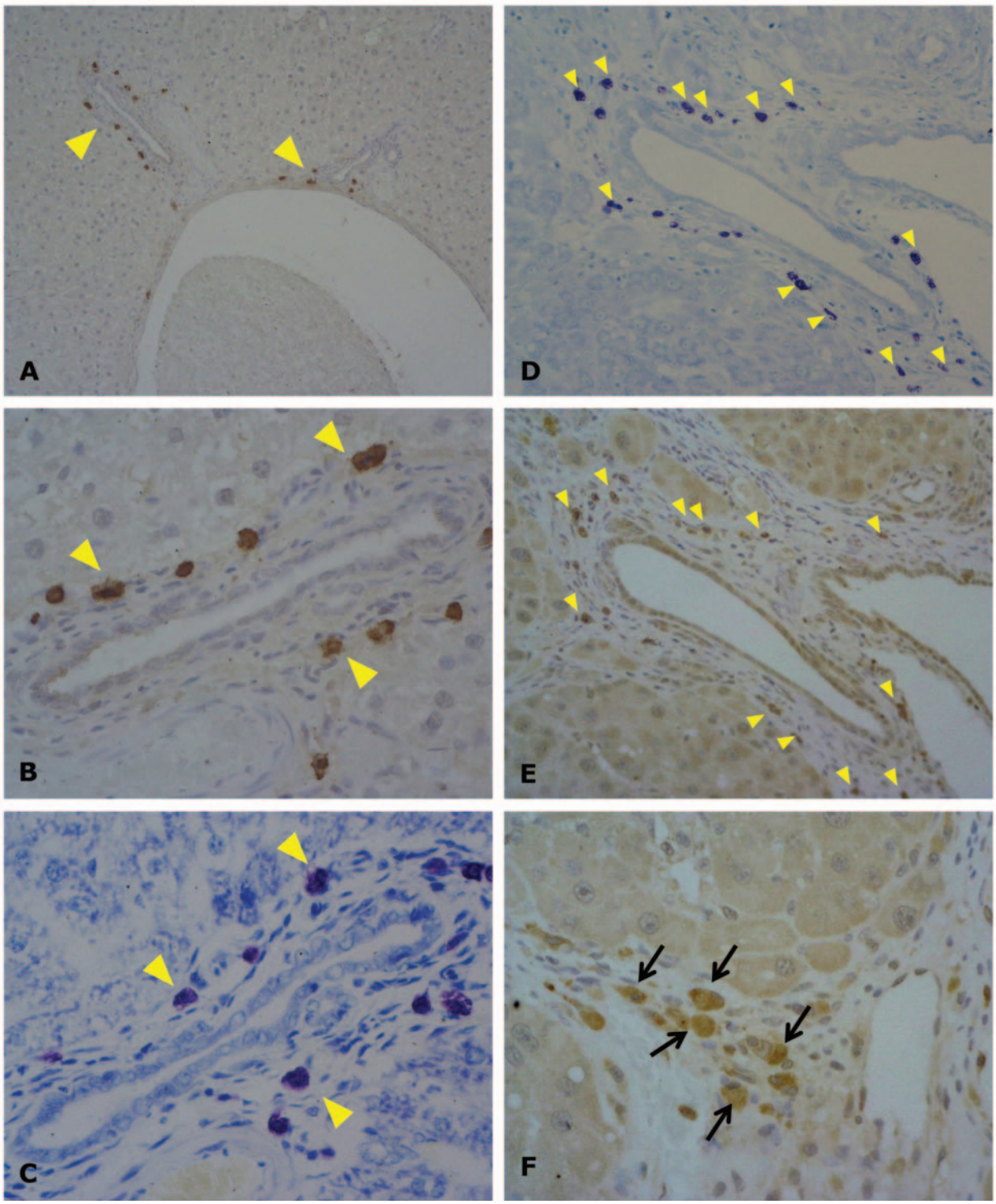

Figure 2. *MMP-1 expression in the mast cells and macrophages. (A) At week 0, MMP-1 expression was strongly detected in the mast cells (arrowhead) of the portal areas. (B) and (C) The larger magnification of (A). Co-localization of MMP-1 and mast cell granules at week 0. Mast cells expressing MMP-1 are located around the bile duct. (D) and (E) Co-localization of MMP-1 and mast cell granules at week 14. Expressions of MMP-1 increased in proportion to the increase of the mast cells. (F) Infiltrated macrophages (arrow) were positive for MMP-1 within the fibrous septa. (A, B, E, F, immunostain for MMP-1; C, D, Toluidine blue stain. Magnification. A, x33; B, C, F, x132; D, E, x66).*

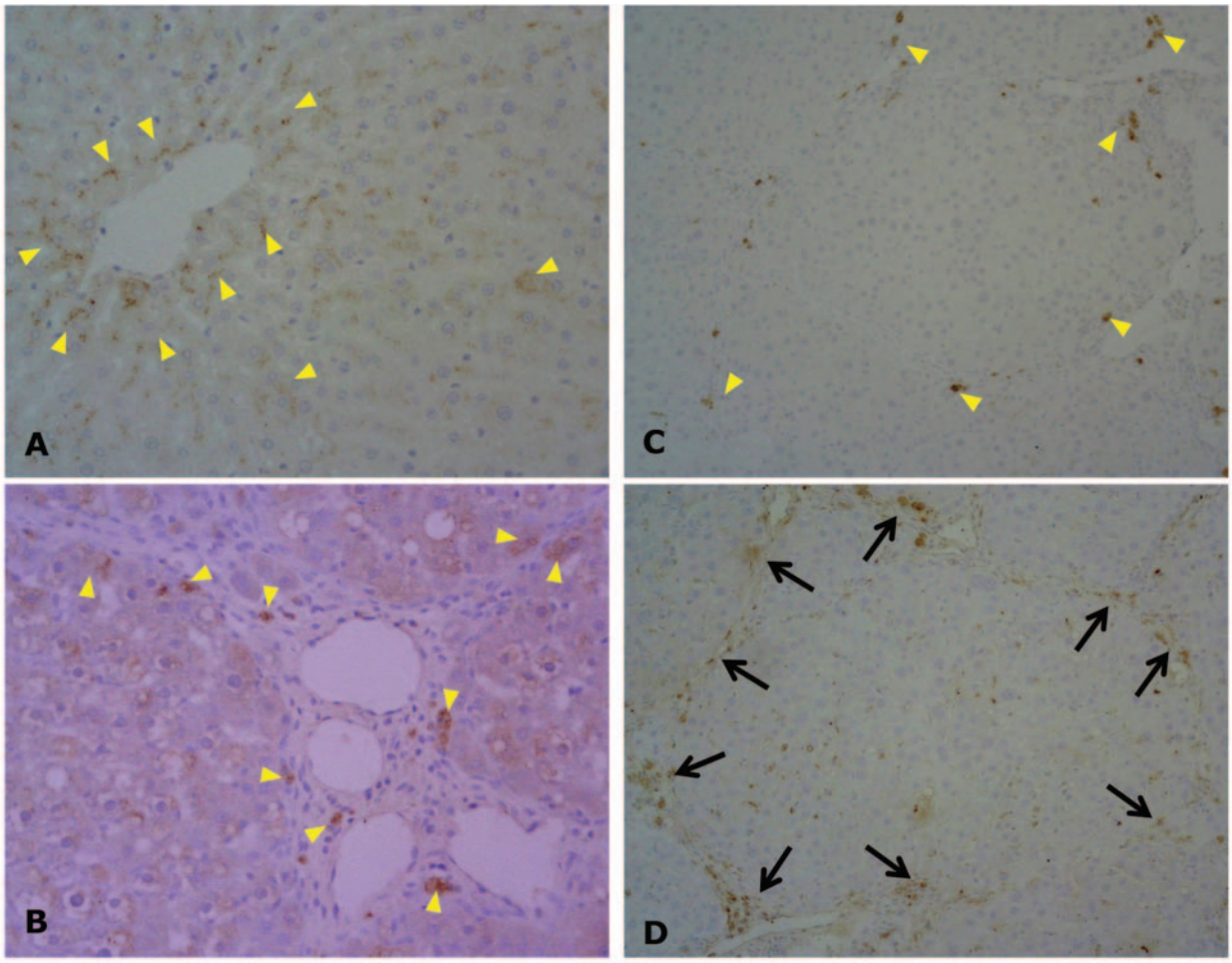

Figure 3. *MMP-3 expression in hepatocytes and macrophages. (A) At week 0, MMP-3 only was expressed in hepatocytes (arrowhead). The hepatocytes in zone 3 showed a strong positive reaction for MMP-3 compared to those of other areas. (B) At week 8, macrophages and hepatocytes in the fibrous septa produced MMP-3. (C) Hepatocytes stopped expressing MMP-3 from week 12. (D) MMP-3 was expressed in some of the detectable macrophages (ED1-positive macrophages; arrow) within the fibrous septa compared with (C). (Immunostain for MMP-3. Magnification. A, B, x66; C, D, x33).*

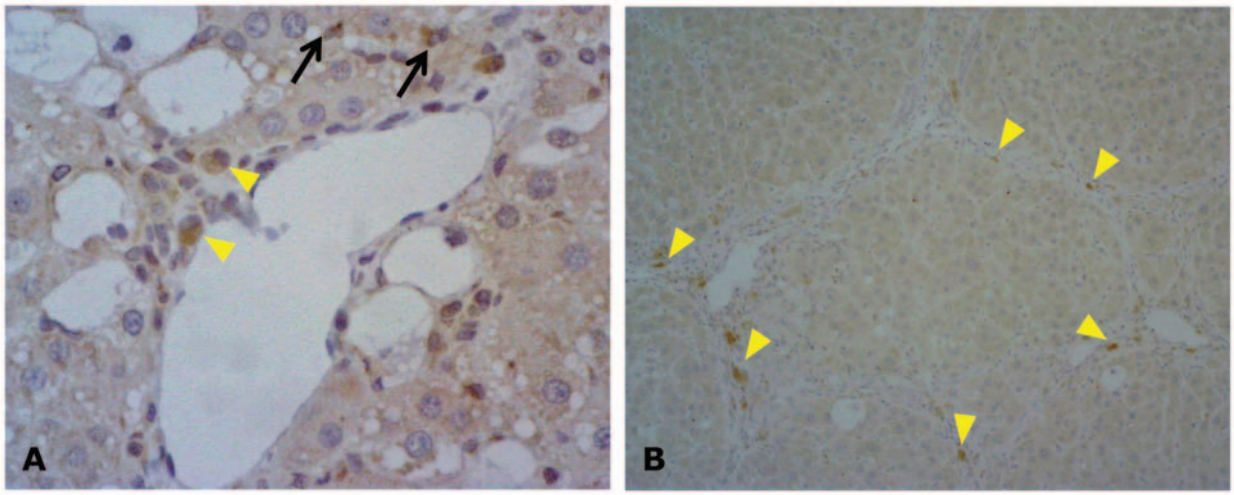

Figure 4. *MMP-9 expression in Kupffer cells and macrophages. (A) MMP-9 expression was detected in Kupffer cells (arrow) and macrophages (arrowhead) at week 4. (B) At week 8, the expression of MMP-9 was detected only in the macrophages within the fibrous septa. (Immunostain for MMP-9. Magnification. A, x132; B, x33).*

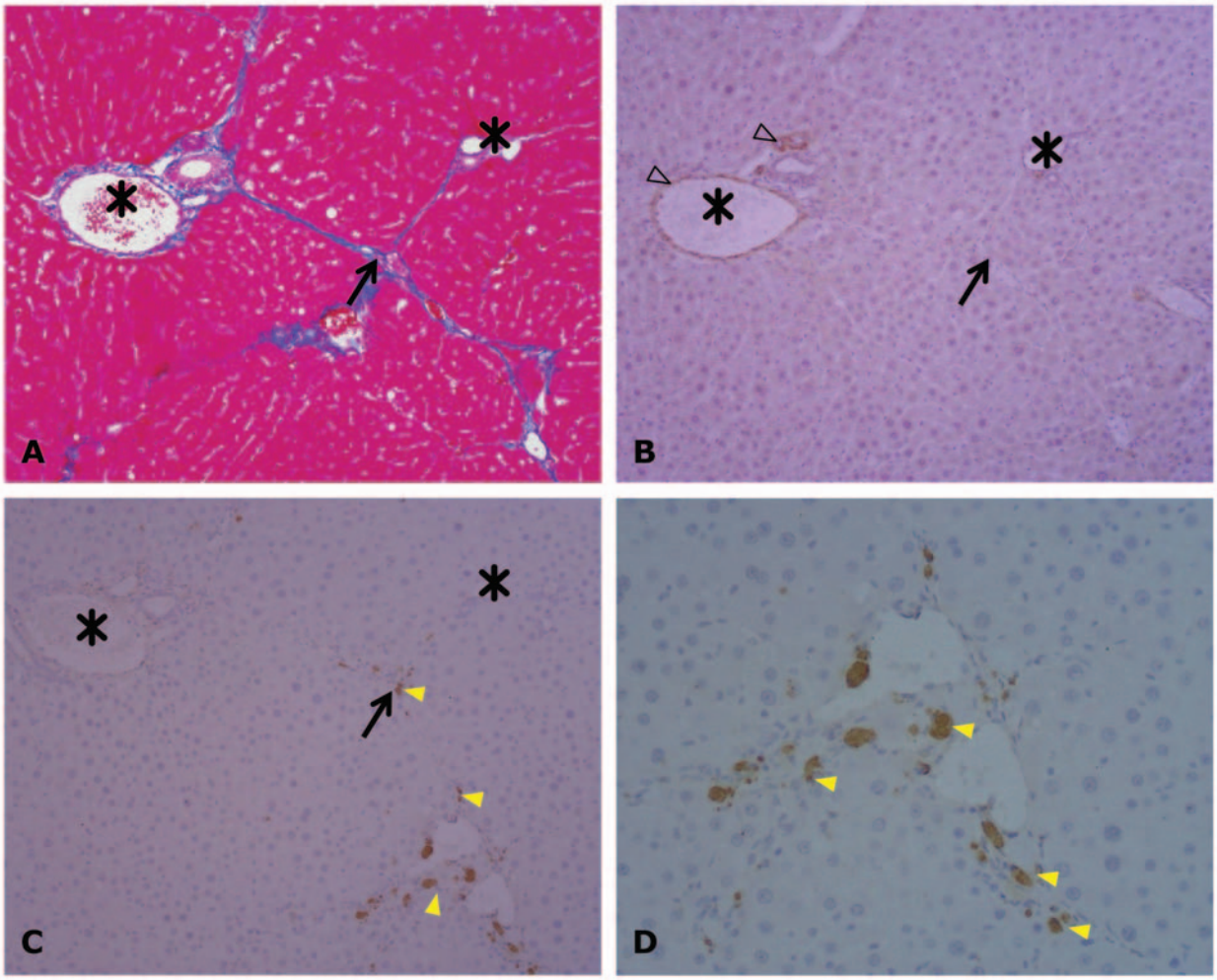

Figure 5. *MMP-3 expression in macrophages in a recovering liver. (A) Collagen fibers form thin fibrous septa (bridging fibrosis, arrow) linking portal areas (asterisk) and central vein in recovering liver. (B) Positive cells (blood vessels, open arrow head) for α-SMA in portal areas were detected, while activated HSCs were not detected within the fibrous septa. (C) At the same region of (A), MMP-3 expression was observed in macrophages (arrowhead) within the unresolved fibrous septa. (D) The larger magnification of (C). (A, Azan stain; B, immnostain for α-SMA; C, D, immunostain for MMP-3. Magnification. A, B, C, x33; D, x66).*

6D). The expression of TIMP-1 and TIMP-2 decreased in the recovery livers.

Immunoblots for MMPs and TIMPs. The results of the immunoblot for MMPs and TIMPs are shown in Figure 7. MMP-1 expression increased progressively and peaked at weeks 12 and 14. MMP-3 expression increased at week 4 but decreased between weeks 8 and 14. MMP-9 expression increased markedly at week 8, but decreased at week 12. In recovery, there was a reduction in all MMP and TIMP expressions compared with those of the cirrhotic livers. Interestingly, MMP-3 expression increased again, especially with respect to the decrease in TIMPs in the recovery livers.

Discussion

The MMPs play a pivotal role in ECM turnover, but TIMPs protect the ECM from degradation by inhibiting the MMPs. Until now, many studies have reported that ECM degradation and accumulation might be regulated by HSCs (2, 3). The HSCs are known to be a major producer of collagens, MMPs and TIMPs *in vitro* and *in vivo* (2, 3, 11-14, 25, 26). In early fibrosis, quiescent and activated HSCs mainly produced MMPs to degrade ECM, but fully activated HSCs remarkably expressed TIMPs instead of MMPs in late fibrosis, thereby providing an explanation for the matrix accumulation co-localized with these cells during chronic liver injury (2, 3, 11, 13, 25).

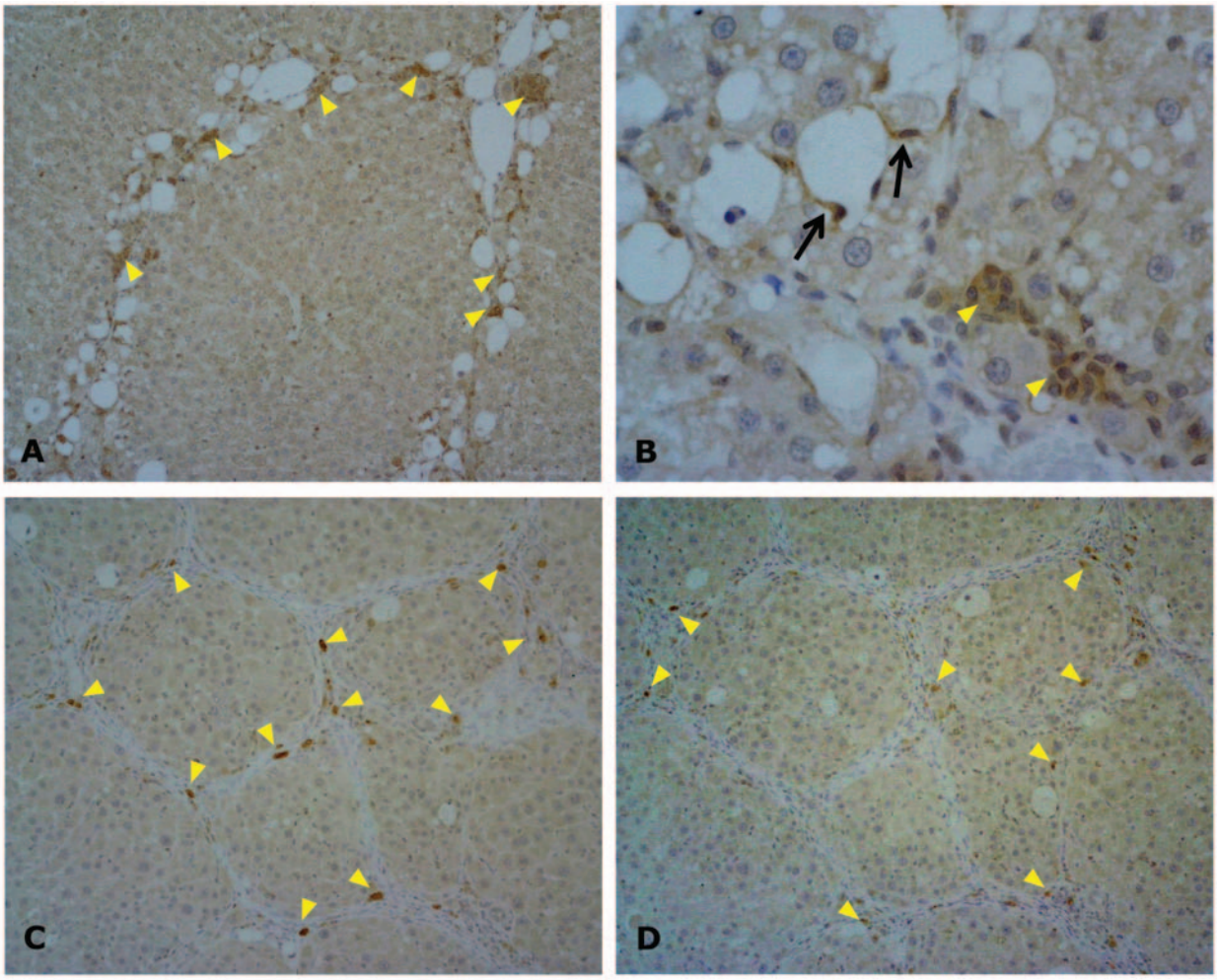

Figure 6. *TIMP-1 and TIMP-2 expressions in macrophages and activated HSCs. (A) At week 4, TIMP-1 expression was detected strongly in macrophages (arrowhead) within the fibrous septa. (B)TIMP-1 expression was detected also in activated HSCs (arrow), as well as macrophages in the fibrous septa. (C) The expression of TIMP-1 in macrophages was more prominent than that of the activated HSCs in a cirrhotic liver. (D) TIMP-2 expression was also detected in macrophages within the fibrous septa in a cirrhotic liver. (A, B, C, immunostain for TIMP-1; D, immunostain for TIMP-2. Magnification. A, C, D, x33; B, x132).*

Recently, activated HSCs, macrophages and mast cells were considered to play an important role in the accumulation of ECM with an increase of their population in liver fibrosis (27, 28). In our previous study, it was demonstrated that the number of macrophages and myofibroblasts increased with the development of fibrosis, but decreased when cirrhosis occurred (6). Mast cells, however, increased in both fibrotic and cirrhotic conditions. Unfortunately, we did not clearly define the role of mast cells during liver fibrosis.

Mast cells have been reported to participate in the development of liver fibrosis in rodents and humans (29, 30). Fibrosis was induced by CCl_4, porcine serum, bile duct resection and secondary biliary cirrhosis (30-33). In previous studies of hepatic mast cells, Umezuk *et al.* showed an increase of mast cells in the proliferated collagen fiber septa. There was also a good correlation between the number of mast cells and the progression of fibrosis in CCl_4-treated rat liver (34). However, it is not yet known how the mast cells participate in the liver fibrosis. In the present study, we found that MMP-1 was expressed strongly in the mast cells throughout hepatic fibrogenesis. The MMP-1 expression in the mast cells did not decrease, although TIMP-1 and -2 were still expressed in cirrhotic liver. In addition, immunoblotting revealed that the total MMP-1 level did not decrease in the cirrhotic condition, in spite of a decline of mast cells and a decreased degree of fibrosis in recovery. Interstitial collagenases, such as MMP-1, MMP-8 and MMP-13, were considered essential enzymes for

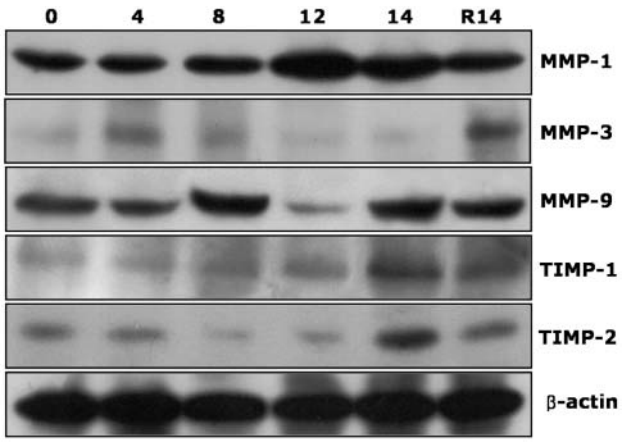

Figure 7. *Immunoblot for MMPs and TIMPs. MMP-1 expression increased progressively and peaked at weeks 12 and 14. MMP-3 expression increased at week 4 but decreased between weeks 8 and 14. MMP-9 expression increased markedly at week 8, but decreased starting at week 12. At week 14, MMP-9 expression increased again did not reach the control value. TIMP-1 and TIMP-2 expression increased gradually and peaked at week 14. In recovery, there was a reduction in all MMP and TIMP expressions compared with those of the cirrhotic liver. However, MMP-3 expression increased only in recovery.*

collagenolysis in liver fibrosis, since type I collagen, which increases predominantly in liver fibrosis (35, 36), cannot be degraded by other MMPs, *e.g.,* MMP-9 (37) or MMP-3 (38). Hence, MMP-1 plays a major role in pathologies where the collagen turnover is aberrant (2, 39).

The results of a recent study using mast cell-deficient Ws/Ws rats suggested that an increase of mast cells in the liver may be associated with fibrosis, but was not a cause of fibrosis (40). Another study proposed that mast cells were not involved in the development of liver fibrosis induced by the intraperitoneal injection of CCl_4 in mast cell-deficient Ws/Ws rats or W/Wv mice (41). In the latter study, the authors suggested that the CCl_4-induced liver fibrosis in mast cell-deficient Ws/Ws rats was similar to or of a higher degree than those in the controls. The data are contradictory since our studies clearly presented mast cells involved in fibrotic and cirrhotic conditions. A higher level of fibrosis in mast cell-deficient Ws/Ws rats than in the controls was undisputed results because there were no mast cells expressing MMP-1 that could degrade ECM. This was already described in the case of uterine and gingival cells. Recently, two groups reported that mast cells expressed MMP-1 in the endometrium and gingiva, using the double immunostaining method (23, 24). In inflamed gingiva, the mast cells have the potential to degrade ECM (24). Mast cells in the myometrium of uterine tissue co-expressed MMP-1 and tryptase in the same intracellular granules (23). These studies might also explain the possibility of the MMP-1 expression in liver mast cells.

As reported previously (16-19), macrophages expressed MMP-1, MMP-3 and MMP-9, as well as TIMP-1 and TIMP-2 in atherosclerotic and alveolar lesions. Apart from the activity of HSC, the activities of other inflammatory agents might be important for the regulation of MMP and TIMP. Furthermore, the results of the present study indicate that MMP-1, MMP-3, MMP-9, TIMP-1 and TIMP-2 could be detectable in macrophages from the mid stage of fibrosis to cirrhosis.

In addition, it was remarkable that MMP-3 expression was high in the macrophages in the recovery livers with the decreased TIMP expression while the expressions of MMP-1 and MMP-9 were low in the macrophages. In contrast to a previous study (3), where the authors suggested that HSC identified a major cellular source of MMPs and TIMPs *in vitro*, MMP-3 expression in our study was detected not in HSCs, but in macrophages in the unresolved septa of the rat livers. Immunoblotting revealed that an increase of MMP-3 was detected, whereas a decrease of both MMP-1 and MMP-9 were observed in the recovery livers at week 14. Therefore, each macrophage seemed to have several unique and divergent roles concerning MMP production in the liver fibrogenesis and recovery stage. Duffield *et al.* also reported that functionally distinct subpopulations of macrophages exist in the same tissue. In the present study, MMP-3 expression was only detected in hepatocytes at weeks 0. The extent of MMP-3 expression in the hepatocytes, however, gradually decreased between weeks 4 and 8. Finally, the hepatocytes no longer expressed MMP-3 upon pseudolobule formation, leading to hepatocyte hypoxic status starting at week 12. We considered that an unknown functional change of hepatocytes must have occurred at that time. In our previous study (42), we demonstrated the hypoxia-potentiated TGF-β expression of hepatocytes during cirrhotic conditions. TGF-β is known to stimulate TIMP expression and to diminish MMP expression (3). Therefore, we suggest that there is an unknown relationship between the increase of TGF-β expression and the decrease of MMP-3 expression in hypoxic hepatocytes. Furthermore, in addition to HSCs, hepatocytes could also represent an important cellular source of MMP-3 at the onset of fibrosis.

The presence of MMPs/TIMPs within the macrophages could possibly be related to phagocytosis and degradation rather than inflammatory reactions. Antibodies, which could not distinguish the precursor from the proteolytically processed form of MMPs, are presently available. Ubiquitous TIMPs could also prevent matrix degradation by MMPs even if the enzymes were in an active form. The MMP-1 and -3, expressions were not detected in macrophages, although their levels were high at weeks 0 and 4. We, therefore, postulated that there was little possibility that the MMP/TIMP expressions within the macrophages were related to phagocytosis.

In the present study, we defined the pattern of MMP/TIMP expression, as well as the cell types demonstrating such expression during liver fibrosis/cirrhosis and recovery. Furthermore, the present results suggest that the role and regulation of MMP and TIMP expression in macrophages might have played an important part in the fibrosis/cirrhosis and recovery of the livers of rats, indicating a potential involvement of hepatic macrophages. We showed that the expressions of MMP-1, TIMP-1 and TIMP-2 increased, while those of MMP-3 and MMP-9 however, significantly decreased during the later stages of fibrosis and cirrhosis. During recovery, however, the expression of MMP-3 in macrophages greatly increased in the unresolved fibrous septa. In addition, we demonstrated that mast cells might participate in fibrolysis by producing MMP-1 throughout the experimental periods. MMP-1 expression did not decrease although TIMP-1 and TIMP-2 were highly expressed in cirrhosis. In the later stages of fibrosis and cirrhosis, the expressions of MMP-3 and -9 in macrophages decreased. The immunohistochemical results were in excellent agreement with the immunoblotting of the MMP/TIMP expression patterns in the liver fibrosis/cirrhosis and recovery *in vivo*. MMP-1 and MMP-3 in the mast cells and macrophages might, thus, contribute to determination of liver fibrosis and its management.

Additional *in vivo* studies are necessary to elucidate the mechanisms of matrix degradation and accumulation in liver fibrosis/cirrhosis with regard to MMP/TIMP expression in liver cells.

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