Gelatinases in chronic liver disease. The clinical relevance of MMP-2 and MMP-9 in orthotopic liver transplantation

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Chapter 1

Introduction

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Abbreviations:
BIA, biochemical immunosorbent activity assay; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; I/R, ischemia and reperfusion; MMP, matrix metalloproteinase; OLT, orthotopic liver transplantation; PA, plasminogen activator; TIMP, tissue inhibitor of metalloproteinases; TNF, tumour necrosis factor.

1. Matrix metalloproteinases and liver disease: an overview.

1.1 Matrix metalloproteinases.

Matrix metalloproteinases (MMPs) encompass a family of enzymes whose main function is degradation of the extracellular matrix. MMPs are important for many normal processes requiring matrix turnover, e.g., embryonic development, wound healing and angiogenesis. In addition to matrix degradation for normal tissue remodeling and repair, MMPs have also been implicated in a variety of pathological processes, including tumour metastasis, periodontal disease, atherosclerosis, and pulmonary emphysema.(1)

The family of MMPs can be loosely divided into four subgroups, identified by their structure and substrate preference, i.e., interstitial collagenases, gelatinases, stromelysins and membrane-type MMPs. Currently, more than 25 different MMPs have been described in the mammalian systems (Table 1). All MMPs have been assigned an MMP number and most also have a common name.

Table 1. List of major MMP-subgroups with some of their main substrates.(17;64)

Group	**Descriptive name**	**Number**	**Principal substrate**
Collagenases	Interstitial collagenase	MMP-1	All: fibrillar collagens I, II and III
	Neutrophil	MMP-8	
	collagenase	MMP-13	
	Collagenase-3	MMP-18	Not determined
Stromelysins	Stromelysin-1	MMP-3	All: proteolycans, ECM glycoproteins
	Stromelysin-2	MMP-10	and nonfibrillar collagens
	Stromelysin-3	MMP-11	Serine protease inhibitor
Gelatinases	Gelatinase A (72 kDa)	MMP-2	Both: collagens IV and V, gelatins,
	Gelatinase B (92 kDa)	MMP-9	elastin, laminin
Membrane-type	MT1-MMP	MMP-14	Activates MMP-2 and MMP-13, collagens I, II and III
	MT2-MMP	MMP-15	Activates MMP-2, laminin
	MT3-MMP	MMP-16	Collagen III, fibronectin
	MT4-MMP	MMP-17	Fibrinogen, fibrin, activates TNF-α
	MT5-MMP	MMP-24	Activates MMP-2, proteoglycans
	MT6-MMP	MMP-25	Collagen IV, fibronectin, fibrin
Others	Matrilysin	MMP-7	Both: elastin, nonfibrillar collagen
	Metalloelastase	MMP-12	

The main characteristics of these MMPs are (2-4):
(1) the catalytic activity depends on a metal ion (i.e., Zn^{2+} at the active site);
(2) most are secreted in a latent form;
(3) the zymogen forms can be activated by other proteinases or organo-mercurials;
(4) they are inhibited by tissue inhibitors of metalloproteinases (TIMPs);
(5) they share common amino-acid sequences;
(6) the enzymes cleave at least one component of the extracellular matrix (ECM).
MMPs contain at least three domains: a signal peptide that directs the translational product to the endoplasmic reticulum for secretion; a propeptide domain that is removed when the enzyme is activated and a catalytic domain. Most MMPs also possess a C-terminal domain with sequence homology to hemopexin. The gelatinases differ from the other MMPs in that, the catalytic domain is separated from the hemopexin-like domain by a fibronectin-like domain. The latter is required to bind and cleave collagen and elastin.(2;5-7)

The regulation of MMPs is stringent and occurs at several levels.(3)
(1) Gene expression. Normal gene expression of MMPs is characterized by tightly controlled regulation to maintain normal tissue function. The expression is transcriptionally regulated by numerous stimulatory and suppressive factors such as growth factors (e.g., transforming growth factor-beta), hormones, cytokines [e.g., tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1], and cellular transformation. Not only soluble factors but also cell-matrix and cell-cell interactions play a role in the expression of MMPs.(1)
Single nucleotide polymorphisms in gene promoters of MMPs have been shown to affect transcriptional activity. The –1306 C/T transition in the MMP-2 promoter sequence disrupts an Sp1 site and thus results in strikingly lower promoter activity.(8) In contrast, the –1562 C/T transition in the promoter of MMP-9 results in higher promoter activity, which appeared to be due to preferential binding of a putative transcription repressor protein to the C allelic promoter.(9)
(2) Secretion in latent form. MMPs are secreted in a latent proenzyme form, with the exception of MT-MMPs which are membrane-bound, and so they require activation in order to have any effect on the extracellular matrix. Several enzyme activators, which include the plasminogen activator (PA) / plasmin system, plasma kallikrein, neutrophil elastase, and trypsin have been implicated in the activation of MMPs.(5) Unlike other MMPs, proMMP-2 is constitutively expressed by many cell types and activation occurs at the cell surface.(10) Since the activation occurs in the immediate pericellular milieu, the local degradation of the matrix can be closely regulated, e.g., by an invasive cell. The major proMMP-2 activation pathway appears to be through MT1-MMP and is regulated by TIMP-2 in a trimolecular interaction.(7) The involvement of the PA / plasmin system in the activation of proMMP-2 is controversial.(7;11) Yet, activation of proMMP-2 involves cleavage by MT1-MMP, yielding an intermediate form that may be activated by plasmin.(12)

In contrast, proMMP-9 is not constitutively expressed but the secretion can be induced and is controlled by other factors. For example, proMMP-9 is synthesized by differentiating neutrophils in the bone marrow, stored in specific granules of circulating neutrophils, and released following neutrophil activation by inflammatory cytokines.(7;13) ProMMP-9 activation can occur directly via plasmin dependent mechanisms.(10;11) Plasmin is a protease which is generated by the activation of the proenzyme plasminogen by the action of tissue-type plasminogen activator (PA) and urokinase-type PA. PA is active upon secretion and can also directly interact with ECM components. Activation of proMMP-9 can also be plasmin-independent, e.g., mediated by a second MMP (MMP-2 or matrilysin).(10;13)

(3) Inhibition by TIMPs. Four naturally occurring specific inhibitors of MMPs have been described, namely tissue inhibitors of matrix metalloproteinases (TIMP)-1,-2 ,-3, and -4. The TIMPs reversibly inhibit active MMPs by forming a strong 1:1 stoichiometric non-covalent complex, resulting in loss of proteolytic activity. In addition to binding to the active enzyme, TIMP-1 forms preferentially complexes with proMMP-9, while TIMP-2 binds to proMMP-2.(4) TIMPs may also exert direct growth-promoting activity independent of their metalloproteinase inhibitory activity.(7;10) Because α_2-macroglobulin is an abundant plasma protein, it represent the major inhibitor of MMPs in tissue fluids, whereas TIMPs act more locally.(7)

(4) MMP catabolism and clearance. Little is known about autoproteolysis of active MMPs but some cleavages seem to inactivate MMPs.(7) The degradation and excretion pathways of MMPs and TIMPs in the body have not been examined to date.(14) Therefore, it is not known whether hepatic or renal dysfunction influences the clearance of MMPs.

The gelatinases MMP-2 and MMP-9.

The gelatinases, which are also known as type IV collagenases, degrade gelatin (denatured collagen) and type I, IV, V, VII, and X collagen. Type IV collagen is particularly abundant in basement membranes, which separate organ parenchyma from the underlying stroma. These enzymes also cleave the noncollagenous proteins elastin, fibronectin, and laminin. This subgroup of MMPs has 2 distinct members, known as gelatinase A (MMP-2) and gelatinase B (MMP-9). Generally, these 2 gelatinases are thought to have a similar substrate specificity.(4) Some characteristics of these enzymes are shown in Table 2.

Table 2. Characteristics of the gelatinases.(6)

Common names	**Gelatinase A**	**Gelatinase B**
Nomenclature	**MMP-2**	**MMP-9**
Substrate specificity	Gelatin, collagen type IV and V, elastin, laminin	Gelatin, collagen type IV and V, elastin, laminin
Molecular mass	72 kD	92 kD
Molecular mass of active species	64 kD, 62 kD	82 kD, 67 kD
Physiological activators	MT1-MMP, type I collagen, hepatocyte growth factor	Serine proteases, MMP-2 and MMP-7
Latent form binds to TIMP	TIMP-2	TIMP-1
Synthesis by cells	Constitutive	Inducible

MMP-9 is released predominantly from neutrophils and macrophages, but the principal source in the liver is the Kupffer cell, the resident macrophage of the liver.(15) In addition, one study demonstrated some MMP-9 labeling of hepatocytes close to the portal areas.(16) Neutrophils do not produce MMP-2 or TIMP, whereas most other cell types

do.(13) Monocytes, lymphocytes, dendritic cells, fibroblasts, and tumor cell lines produce MMP-2 constitutively, albeit sometimes in small quantities. MMP-9 is an inducible enzyme in most of these cell types. In the liver, the hepatic stellate cell is suggested to be the main cellular source of MMP-2. Following liver injury, these cells become activated and can express a wide range of MMPs and TIMPs, but in particular MMP-2.(17) From other nonparenchymal liver cells, MMP-2 and MMP-9 synthesis has only been demonstrated in sinusoidal endothelial cells.(18)

Methods of detection.

MMPs can be detected by a variety of techniques, each with its own advantages and disadvantages. Immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), mRNA detection, gelatin-zymography and Western blotting are the main techniques used. Immunohistochemistry and mRNA detection can be used to localize the MMPs, thus determining their site of production, but they do not detect enzyme activity. Gelatin-zymography has the advantage of measuring enzymatic activity quantitatively and of distinguishing the active from the inactive enzyme. However, only MMP-2 and MMP-9 can be measured easily with this technique.(3) Our group applied highly specific ELISAs for determining MMP-2 and MMP-9, which measure the grand total of pro-enzyme, active- and inhibitor complexed forms of the respective MMP. (19-21) MMP-2 and MMP-9 enzymatic activities can also be determined in blood samples by using specific biochemical immunosorbent activity assays (BIA).(20-22) Free, active MMPs, however, cannot be detected in most blood samples because of rapid complex formation with specific inhibitors in the circulation.

1.2 A Liver fibrosis.

Longstanding alcohol abuse, viral hepatitis (especially hepatitis B and hepatitis C), autoimmune hepatitis and cholestatic liver diseases, such as primary biliary cirrhosis, are important causes of chronic liver disease in Western countries. In chronic liver disease, a process of tissue remodeling develops, in which destruction of hepatic cells is followed by repair mechanisms characterized by increased collagen production, accumulation of ECM and progressive destruction of the organ architecture. Ultimately, hepatic fibrosis leads to cirrhosis, characterized by nodule formation and organ contraction, which can lead to life-threatening complications such as portal hypertension, hepatic failure and hepatocellular carcinoma. The pathological accumulation of ECM can be a result of an increase in synthesis or a decrease in degradation of ECM, or a combination of both, since hepatic fibrosis is a dynamic process.(17;24-26) Activated hepatic stellate cells are central to the process of fibrosis as the major source of fibrillar matrix components.(27;28)

Like other parenchyma, the normal liver contains an epithelial component (hepatocytes), an endothelial lining (which in the liver is distinguished by fenestrae), tissue macrophages (Kupffer cells), and a perivascular mesenchymal cell called the stellate cell (Figure 1). The cellular elements of the liver are organized within sinusoids, with the subendothelial space of Disse separating the hepatocytes from the sinusoidal endothelium. The space of Disse contains a basement membrane-like matrix, allowing maximized passage of molecules from the fenestrated sinusoidal endothelium to hepatocytes and providing structural integrity of the liver parenchyma.(27;29) The main components of the ECM in normal liver are collagen type I, III, IV, V, and VI, although other types of collagen are present in smaller proportions. There are also many noncollagenous matrix components, including fibronectin, laminin, elastin and proteoglycans.(24;30) MMPs, e.g., synthesized by

hepatic stellate cells within the space of Disse, are the main degrading enzymes of the ECM proteins, such as collagen, gelatin and laminin, and therefore play an important role in the process of tissue remodeling.(2;5)

In the liver, stellate cells express virtually all key components required for matrix degeneration.(24;26;28) In particular, they are the key source of MMP-2, which is responsible for the degradation of type IV collagen, a major component of the basement membrane. Conversely, active MMP-2 has been shown to promote proliferation of activated stellate cells.(32) An increased mRNA expression of MMP-2, but also TIMP-1 and TIMP-2, was reported in liver biopsy samples of patients with cirrhosis.(32-36) In patients with chronic hepatitis and cirrhosis there is evidence of an increased expression in hepatic stellate cells of MMP-2 and MT1-MMP mRNA, which is able to activate proMMP-2.(37) The presence of MMP gene transcripts does not per se provide clues as to the presence of the corresponding enzymatic activity in the tissue. Albeit, as assessed by gelatin-zymography, MMP-2 could also be detected in human fibrotic liver but not in normal liver tissue.(38;39)

In accordance to increased production of MMPs in liver tissue samples, *serum* MMP-2, and also TIMP-1 and TIMP-2 concentrations, were markedly increased in patients with liver cirrhosis, and showed a good correlation with the degree of liver fibrosis.(39-43) However, in one study no correlation was observed between plasma MMP-2 and both liver fibrosis and grade of inflammation in patients with chronic hepatitis C.(44)

Kupffer cells can influence stellate cells through the secretion of MMP-9.(15) MMP-9 can activate latent transforming growth factor-beta, which is the dominant stimulus to ECM production by stellate cells.(45;46) In human liver, MMP-9 mRNA expression was increased in patients with chronic hepatitis and cirrhosis, as compared to controls, but there was no correlation with the grade of inflammation in liver biopsies or serum AST.(47) In addition, both cirrhotic and normal liver samples displayed a 92-kDa gelatinolytic band corresponding to Kupffer cell-derived MMP-9.(38) Data on *serum* MMP-9 in patients with chronic liver disease are conflicting. Both lower (48;49) and similar (50) levels are reported compared to controls.

In summary, the available evidence suggests that stellate cells express a combination of MMPs and TIMPs that have the ability to degrade normal matrix, while inhibiting degradation of fibrillar collagens that accumulate in liver fibrosis. This pattern is characterized by MMP-2 and MT1-MMP expression, which seems to lead to localized pericellular degradation of normal matrix. In addition, there is a relative increase in expression of TIMPs leading to a more global inhibition of degradation of fibrillar liver collagens.(17;25) Kupffer cells, on the other hand, seem to be involved in the regulation of the inducible MMP-9.

1.2 B Hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) is an epithelial cancer originating from hepatocytes and is one of the most common cancers worldwide, especially in Asia. The annual incidence in patients with cirrhosis range between 3-10 %.(51) The most important etiologic factors implicated in HCC are the hepatitis B and C viruses, abuse of alcohol, and hemochromatosis.(52) Although many improvements have been made in terms of diagnosis and treatment, the prognosis of HCC is still very poor.(53) HCC frequently shows early invasion to blood vessels as well as intra- and extrahepatic metastasis. In western countries HCC develops multifocally in the chronically injured liver in approximately 90% of the cases, where altered turn-over and increased deposition of extracellular matrix proteins has been reported. In malignancy, MMPs seem to be induced and used by invasive tumours to remodel

the local environment, allowing tumour growth, neo-angiogenesis, and metastasis.(54;55) The source of MMPs in human cancer was originally assumed to be the carcinoma cells. However, it seems that the expression of several MMPs can also be induced in stromal tissue, with the highest levels of induction in the invasive tumour margins.(55)

The expression of MMPs in HCC is poorly understood and the evidence from various researches is contradictory. High levels of MMP-2 mRNA and MMP-2 activation have been reported in HCC.(36;57;58) By contrast, another study reported a lower expression of MMP-2 in tumour tissue than in non-tumour tissue.(58) In a study by Määtä *et al*., MMP-2 mRNA was expressed predominantly by cells of the tumour stroma (59), whereas others demonstrated that MMP-2 was preferentially located at the invading border of tumour tissue.(60) Increased amounts of MMP-2 were found by zymography in tissue samples of HCC, as compared with adjacent non-tumourous liver tissue.(59) In addition, MMP-2 activity was significantly higher in tumours arising in cirrhotic livers than in those arising in non-cirrhotic livers, and it associated with the stage of fibrosis.(61) In contrast to tumour samples, *serum* MMP-2 levels were not significantly different between patients with liver cirrhosis and those with HCC.(39;41;62) Moreover, these studies found no correlation between MMP-2 and alpha-fetoprotein, tumour size or tumour differentiation. In these studies, all patients with HCC had underlying cirrhosis (39) or severe active hepatitis.(41) Therefore, these studies suggest that the increased serum MMP-2 levels in patients with HCC is derived from the non-tumourous part of the liver rather than from the carcinoma.

In HCC, MMP-9 mRNA was found to be expressed mainly by neoplastic epithelial cells and to a lesser extent in stromal cells.(59) Increased MMP-9 mRNA expression, as measured by Northern hybridization, was associated with capsular infiltration.(63) Zymography showed almost equal amounts of the latent form of MMP-9 in both tumour and adjacent liver tissue, while its active form was present only in HCC.(59) Hayasaka *et al.*(50), even suggested that MMP-9 could be a diagnostic marker for HCC, because the mean plasma levels of MMP-9 in patients with HCC were significantly elevated compared to controls, patients with chronic hepatitis and patients with cirrhosis.

The important role of MMPs in the process of tumour growth and metastasis has led to the development of specific MMP inhibitors (e.g., batimastat), which have been used in the treatment of several malignancies.(55;64) Up to now MMP inhibitors have not been used, however, in clinical trials including patients with HCC.

1.2 C Ischemia and reperfusion injury after OLT.

Orthotopic liver transplantation (OLT) has become an established therapy for patients with end-stage liver disease and acute liver failure. Interruption and subsequent restoration of the blood flow is unavoidable in transplantation of organs. Organ injury caused by this transient ischemia followed by reperfusion is one of the main causes of initial poor function after OLT.(65) The spectrum of clinical manifestations of ischemia and reperfusion (I/R) injury can range from asymptomatic elevation of liver enzymes to primary non-function of the liver. Hypothermia and specific preservation solutions are used to limit the injury to the graft during and immediately after preservation.(66;67)

Hepatic I/R injury is a complex, multifactorial pathophysiologic process that affects all types of hepatic cells, such as Kupffer cells, endothelial cells, hepatocytes and neutrophils.(67-73) The histopathological characteristics of ischemic preservation include hepatic vacuolisation and swelling of endothelial cells. Within minutes after reperfusion with oxygenated blood, endothelial cells round up and eventually detach from the connective tissue matrix, consisting of cords of collagen linked to the cells through intermediate molecules such

as fibronectin.(67) This process of detachment appears to be mediated by proteases.(70) Hepatocytes retain their viability and initially seem minimally affected.(67) Soon thereafter, Kupffer cells become activated, as indicated by degranulation, increased phagocytosis, and release of oxygen free radicals and inflammatory mediators, such as TNF-α, IL-1, and platelet activating factor. Together, Kupffer cell activation and endothelial cell injury lead to profound microcirculatory disturbances and sinusoidal accumulation of leukocytes and platelets.(67;68) In the late phase of injury, neutrophils infiltrate the liver in response to chemoattractants released by activated Kupffer cells and expression of intercellular adhesion molecules on endothelial cells.(71) Accumulation of activated neutrophils within the hepatic parenchyma causes further hepatocyte damage several hours after reperfusion through the release of oxidants and proteases.(70;74) Microcirculatory changes appear to reach a maximum within 48 hours after reperfusion.(67)

In the liver, MMPs-secreted by lipocytes, Kupffer cells, endothelial cells or neutrophils are capable of digesting connective tissue matrix that, for example, anchors sinusoidal lining cells to underlying cords of collagen in the space of Disse, and have an important role in exposing the matrix to neutrophils and platelets upon reperfusion.(13;71;74-76) Upadhya *et al*.(18;77;78), have shown that the gelatinases MMP-2 and MMP-9 may play an important role in hepatic I/R injury. Liver effluents, collected after various periods of preservation, contained MMP-2 and MMP-9, and their gelatinolytic activity increased with time of preservation.(18) Furthermore, cultured endothelial cells produce these gelatinases when stored in the cold.(18) In addition, several preservation solutions were found or even constructed to inhibit MMP activity.(77) Recently, hepatic MMP expression was evaluated by Northern blot analysis using a model of partial liver I/R in rats.(79) The transcripts of MMP-9 in liver lobes were rapidly induced after reperfusion, and returned to basal levels after 24 hours. A second phase was noted after 48 hours. MMP-2 expression was low after reperfusion and showed a peak 2-3 days after I/R, followed by a slow decline. The gelatinolytic activity of MMP-9 in liver tissue increased rapidly after reperfusion with a biphasic profile, reflecting the accumulation of MMP-9 mRNA. Pre-treatment of the animals with the MMP inhibitor RXPO3 significantly reduced markers of parenchyma injury and apoptosis.(79)

MMPs are also involved in I/R injury in other organs and diseases. Increased MMP-9 activity and mRNA expression was found after reperfusion using a rat lung transplantation model.(80) In myocardial ischemia and reperfusion, increased levels of MMP-9 were reported as well.(81) Increased levels of MMP-9 were also found in rats after cardiac transplantation as compared to normal rat hearts and compared to rats who were treated with an MMP inhibitor after reperfusion.(82) Studies on the role of MMP-2 in I/R injury are conflicting. An acute release of MMP-2 was noted in coronary effluents of rats during reperfusion after ischemia of isolated, perfused hearts.(83) However, MMP-2 activity and mRNA expression did not change during ischemia and after reperfusion in the rat lung transplantation model.(80) Also, in the kidney, MMPs did not seem to play a role during the early phase of experimental I/R injury in the rat.(84)

1.2 D Acute rejection after OLT.

Acute cellular rejection of the liver allograft remains an important problem following OLT and is the major reason that immunosuppressive therapy must be administered. Complications related to the administration of immunosuppressive therapy remain a predominant cause of mortality in the liver transplantation recipient.(85) Most commonly, the onset of rejection occurs between the fourth and fourteenth postoperative day. There are few typical symptoms of hepatic rejection, although fever and malaise are not uncommon. The

first signs of rejection are elevation of liver function tests. Leucocytosis and eosinophilia are also frequently present. The bile, if available for inspection, will be lighter and less viscous. In most cases acute rejection responds well to additional immunosuppression. The rejection incidence varies between 20-70 %, depending on the immunosuppressive regimen and the diagnostic criteria used in the different studies.(86;87) Other factors that influence the incidence of acute rejection include the indication for OLT, age, race of the recipient, and preservation injury.(86)

Many centers perform routine biopsy on approximately the seventh postoperative day because of the frequency of rejection at this time point. The typical histopathological findings include a mixed portal inflammation, containing activated lymphocytes, mononuclear cells, and frequently eosinophils. Polymorphonuclear leucocytes may also be present. Other critical findings include bile duct inflammation and venous endotheliitis.(88) Acute rejection is usually graded into three categories: mild, moderate and severe. A 0- to 9-point scale, the Rejection Activity Index, can be used as a numerical score with the option to further characterize rejection histologically.(87)

Acute rejection is characterized by an immunological attack on the allograft, which is mediated by cellular components of the immune system. T lymphocytes are the predominant cellular components. CD4+ cells appear to have a dominant role in initiating and amplifying the immune response, and CD8+ cells have a central effector role. The macrophages are pivotal in the immune response as the antigen-presenting cells. The other cells, identified in the portal tract, such as polymorphonuclear leukocytes and eosinophils, most likely reflect the influence of local cytokine production.(89;90) The interaction between the donor antigen and recipient antigen-presenting cell provide the signals stimulating responder CD4+ cells. These cells then produce a number of cytokines, of which IL-2 seems to be the most important. These cytokines can change endothelial behaviour, causing an increase in vascular permeability and the upregulation of adhesion molecule expression on endothelial cells.(91) Adhesion molecules not only provide important cellular interactions of T cells with antigen presenting cells, but the integrins also promote leukocyte migration and extravasation, adhesion to endothelial cells and extracellular matrix.(92)

In addition to the effects of cytokines on adhesion molecule expression, cytokines such as TNF-α, IL-1 and IFN-γ have also been demonstrated to influence the secretion of MMPs by infiltrating lymphocytes.(93) MMP promote matrix degradation and facilitate lymphocyte trafficking through the gelatinous extracellular matrix of the allograft. Tissue injury and immune responses thus induce a complex of tissue repair and remodelling processes.(91)

Degradation of extracellular matrix, as evidenced by the increase of laminin, hyaluronic acid and fibronectin receptor, was found to be a prominent feature in acute hepatic allograft rejection.(94;95) T-cells, that infiltrate the allograft, secrete predominantly the gelatinases MMP-2 and MMP-9, after β1-integrin and adhesion molecule dependent stimulation by cytokines and inflammatory mediators.(76) Also in a rat model of small bowel rejection, upregulated MMP-2 and MMP-9 expression was demonstrated in crypt epithelium and submucosal areas, as well as in T cell areas of the small bowel during rejection.(96)

2. Outline of the studies described in this thesis.

In this thesis several studies are described on the putative role of the gelatinase-type of matrix metalloproteinases MMP-2 and MMP-9 in chronic liver diseases, with emphasis on orthotopic liver transplantation (OLT). MMPs are the main degrading enzymes of extracellular matrix proteins and basement membrane, and therefore may play an important role in tissue remodeling and repair in many physiological and pathological processes. As introduction**,** a short overview is given on MMPs in general and especially their potential role in liver fibrosis, hepatocellular carcinoma, ischemia/reperfusion injury and acute rejection after OLT.

Chapter 2 reports on the clinical significance of serum MMP-2 and MMP-9 as assessed in patients with several chronic liver diseases and in patients with hepatocellular carcinoma. Serum MMP levels were assessed in relation to liver function and disease activity. Furthermore, we studied whether these enzymes could be of diagnostic value in the detection of hepatocellular carcinoma, since this serious complication of end-stage liver disease is often identified when it is beyond cure.

Patients with end-stage liver disease can be eligible for OLT. Previous studies demonstrated an interaction between the fibrinolytic system and MMPs, and OLT was found to be associated with increased fibrinolytic activity. However, little is known of MMPs during OLT. In **chapter 3**, the evolution of plasma MMP-2 and MMP-9, and their inhibitors TIMP-1 and TIMP-2, is described in 24 patients during OLT. In addition, the effect of serine protease inhibition by aprotinin on the level and the activation of these MMPs was assessed in these patients. MMPs have also been suggested to play an important role in ischemia/reperfusion injury during OLT. In the same group of patients, the changes of plasma MMP-2 and MMP-9 were investigated with respect to early ischemia/reperfusion injury during OLT.

A subgroup of the patients described in chapter 3 underwent an OLT in the Leiden University Medical Center because of severe liver dysfunction and/or hepatocellular carcinoma. In this group of 33 patients the changes in serum MMP-2 and MMP-9, and their composition, were assessed with respect to the late phase of ischemia/reperfusion injury after OLT, with a follow-up of one week (**chapter 4**).

A further exploration of MMPs in chronic liver disease and during OLT is reported in **chapter 5**. In order to determine whether the cirrhotic liver is the production site of the high serum MMP-2 level in end-stage liver disease, MMP-2 was also determined in specimens of cirrhotic liver and control liver tissue. In addition, the time course of serum MMP-2 and MMP-9 during one year after OLT was studied, with emphasis on acute allograft rejection. In order to determine the cellular source of MMP-9, immunohistochemical studies were performed on liver biopsies taken at one week after OLT.

Due to disputed results from chapter 5, the significance of blood collection as a preanalytical determinant for MMP measurements is described in **chapter 6**. MMP-2 and MMP-9 were determined in simultaneously collected serum, sodium-heparin and EDTA plasma samples from OLT patients, in order to assess the influence of the preanalyte used.

Single nucleotide polymorphisms in gene promoters of MMP-2 and MMP-9 have been shown to affect their transcriptional activity. The potential contribution of these functional gene polymorphisms and their respective protein levels in the serum was investigated in patients with various chronic liver diseases. Furthermore, it was evaluated whether the MMP-2 and MMP-9 gene polymorphisms are involved in the late phase ischemia/reperfusion injury and allograft rejection after OLT (**chapter 7**).

The different studies are finally compiled in a summarizing discussion (**chapter 8**).

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