Liver transplantation : chimerism, complications and matrix metalloproteinases

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Chapter 8

Summarizing discussion

Introduction

Chimerism after orthotopic liver transplantation (OLT) is the main focus of the studies described in this thesis. The first study showed that chimerism of different cell lineages within the liver graft does occur after OLT. Subsequently, in allogeneic blood stem cell recipients, chimerism was demonstrated in liver tissue, providing evidence that circulating progenitor cells can indeed differentiate into parenchymal liver cells. The secondary focus of this thesis is on matrix metalloproteinases (MMPs). These proteolytic enzymes are involved in a wide variety of physiological and disease-related matrix remodeling processes. MMP-2 and MMP-9 gene promoter polymorphisms were assessed of OLT donors and recipients in relation to ischemia/reperfusion injury, acute rejection and non-anastomotic biliary strictures after transplantation. We performed a side study, in which we evaluated the value of serum liver chemistry profile and abdominal ultrasound for detecting clinically relevant biliary strictures using time-dependent multivariate regression analysis.

The two main themes, chimerism and MMPs, merge in the final chapter of this thesis. In liver transplant recipients with donor/recipient mismatches for MMP gene polymorphisms chimerism was studied, in both liver biopsies as well as peripheral blood after OLT.

Chimerism

Since the early days of organ transplantation the interaction between cells of donor and recipient origin has been studied by many in an attempt to understand rejection, graft tolerance and graft-versus-host disease.[1-3] Cell migration from the graft to the recipient results in systemic chimerism and cell migration from the host to the transplanted organ results in intragraft chimerism. Methods used to detect chimeric cells in transplanted organs are based on mismatches between donor and recipient.[4,5]

Liver chimerism was evaluated in sex-mismatched donor/recipient combinations, as described in **chapter 2**. This chimerism within the transplanted liver was studied after transplantation of a female donor liver into a male recipient. Cells of recipient (male) origin were identified with the use of a Y-chromosome specific in-situ hybridization technique. In the same study HLA-mismatched liver transplants were studied using immunohisto-chemistry with HLA class I-specific antibodies. Double staining techniques were used with antibodies against specific cell types

and subsets to differentiate between different cell lineages. Endothelial cell chimerism was found to be quite common. Chimerism for biliary epithelial cells and hepatocytes could be shown only in a minority of cases. HLA staining was found to be not adequate for differentiating hepatocytes from inflammatory cells with certainty. Findings were also limited by the (sometimes poor) quality of liver biopsy samples for immunohistochemical analysis. From these findings we postulated that circulating progenitor cells of recipient origin are probably capable of differentiating into endothelial and even epithelial cells within the transplanted liver.

In **chapter 3** a study is described in which the theory of blood stem cells developing into liver cells was further explored. Liver tissue specimens were examined from female patients who had received allogeneic blood stem cells from a male donor. All patients had been diagnosed with a hematologic malignancy, and treated with high-dose chemotherapy followed by allogeneic bone marrow transplantation or allogeneic peripheral blood stem cell transplantation. Fine-needle liver biopsy specimens were obtained because graft-versus-host-disease was suspected or from autopsy liver tissue. In this case, Y-chromosome identification was used to identify cells of donor origin. For consecutive staining an antibody against all known isotypes of the CD45 leucocyte common antigen family, present on lymphocytes, monocytes, granulocytes, and other inflammatory cells, was used. Y-chromosome-positive cells, indicating male (donor) origin, were found in all studied liver tissue samples. Many of these were likely to be of hematopoietic origin, representing infiltrating leukocytes but in some cases, donor-derived cells clearly appeared to be of non-lymphohematopoietic origin, supporting the presence of true tissue chimerism.

Matrix metalloproteinases and transplantation complications

Matrix metalloproteinases are proteolytic enzymes, involved in a wide variety of physiopathological tissue matrix remodeling processes.[6-9] We focused on MMP-2 and MMP-9 gene promotor polymorphisms in relation to clinical outcome of liver transplantation. **Chapter 4** describes the relationship of these polymorphisms with ischemia-reperfusion damage and rejection after OLT. Serum MMP-2 and MMP-9 levels were determined to study potential phenotypical consequences. No statistically significant differences between MMP gene promotor polymorphisms were found in relation to the development of I/R injury and to rejection after liver transplantation. Serum levels of MMP-2 were

found to be increased in patients with chronic liver disease and therefore a drop of MMP-2 levels was expected after OLT.[10,11] This decrease was indeed seen, but it occurred independent of the genotype. Serum levels of MMP-9 were found to peak at 1 week after OLT, which was associated with acute allograft rejection. Irrespective of a genotype mismatch between the donor and the recipient we found a comparable serum MMP-9 peak and pattern over time.

After liver transplantation the development of biliary strictures is a major clinical problem, due to its relatively high frequency, complications, morbidity and even mortality.[12-15] We were able to assess predictive factors of biliary strictures in a large cohort of liver transplant recipients, as described in **chapter 5**. This study focused on routine clinically diagnostic tests and their predictive value for the development of bile duct strictures, anastomotic as well as non-anastomotic (3 to 12 months after OLT). These common tests include liver chemistry profile and abdominal ultrasound (US) that are ubiquitously used to assess liver transplant recipient. The relationship between these tests and biliary complications is obvious, but very little is known on the predictive value of sequential profiling of these parameters. [16-18] We performed a time-dependent Cox regression analysis to identify predictive factors for the development of biliary strictures and found an elevated gamma-glutamyltranspeptidase and dilated bile ducts on ultrasound to have an independent predictive value for the development of biliary strictures requiring intervention. We advocate that dilated bile ducts on ultrasound and elevated gamma-glutamyltranspeptidase should prompt cholangiography for early diagnosis and therapeutic intervention of biliary strictures.

Given the fact that stricture formation in the liver bile ducts is accompanied by tissue remodeling and since MMP-2 and MMP-9 are considered to play a key role in connective tissue remodeling processes in the liver, we decided to study the relation of MMP-2 and 9 gene polymorphisms with non-anastomotic biliary strictures (NAS) after liver transplantation, as well. Specifically NAS was studied, because these strictures are a common and troublesome complication after OLT, leading to graft dysfunction, septic complications, secondary cirrhosis and even graft loss. The results of this study are described in **chapter 6**. The MMP-2 polymorphism was significantly associated with NAS, and donor and recipient genotypes had additive effects. Moreover, primary sclerosing cholangitis (PSC) and MMP-2 genotype polymorphism were independent risk factors for the development of NAS after OLT. No association was found with MMP-9 genotype. A higher risk for

developing (late) NAS was recently also described for graft recipients carrying specific *CCR5* alleles (encoding a chemokine receptor), especially in patients transplanted for PSC.[19] These and our findings strongly suggest that innate pathways contribute to NAS, the etiology of which involves multiple other mechanisms, such as ischemia, viral infection and bile salt-related injury.[15,20]

Matrix Metalloproteinase Chimerism

The last study of this thesis, **chapter 7**, merged our main focus areas, i.e., chimerism and MMPs. Donor/recipient mismatches for MMP gene polymorphisms were selected and evidence of chimerism was found in liver biopsy specimens for MMP-2 and MMP-9 polymorphism mismatches. An association between rejection and a specific MMP-2 genotype, in donors as well as in recipients, was found, but its role in pathophysiology remains to be established. In addition, evidence of donor chimerism was found in peripheral blood samples of liver transplant recipients in a few selected cases, in accordance with other recent studies.[21]

Discussion

In the chimerism studies described in this thesis a variety of techniques was used to study this intriguing phenomenon. Convincing evidence of chimerism is found in all three studies, but these studies also illustrate the difficulties encountered when studying this phenomenon. The selection of donor/recipient combinations depends on the technique which is chosen, often cutting down the number of cases that can be included (e.g., only the combinations of a female donor and a male recipient as in **chapter 2**). The next challenge was to further identify the chimeric cells. Circulating blood cells within the donor liver are without question from recipient origin and should be excluded since we are interested in true intragraft chimerism. To establish the functional relevance of chimerism it is essential to differentiate between different cell lineages. With a combination of techniques (determination of Y-chromosomes, HLA immunohistochemistry and double staining techniques with antibodies against specific cell types) the determination of chimeric cells in the liver graft was sometimes possible and at times even convincing, but often remained questionable. Poor quality of liver tissue samples made this an even more hazardous challenge, especially in autopsy specimens (**chapter 3**).

Donor/recipient mismatches for MMP gene polymorphisms (**chapter 7**) provided a larger study sample. In this study, MMP gene polymorphisms were solely used as a genetic association marker to explore chimerism and not as a functional parameter for disease progression as such. The disadvantage of this technique is that DNA is extracted from study specimens (whole liver tissue or peripheral blood samples), thus making differentiation of cell type impossible. However, the larger study sample made it possible to study the relation of chimerism in liver tissue to clinical outcome variables (I/R injury and rejection) and a statistically significant association was found between chimerism for MMP-2 (but not MMP-9) and the occurrence of rejection. This observation was consistent with earlier studies. However, the limitations in studying chimerism after liver transplantations with the described techniques are obvious. Indeed, we did find compelling evidence of chimerism, but quantification of chimerism could not be assessed and association of chimerism to some clinically important variables was difficult, due to lack of power. The relationship between the presence of chimerism and clinical outcome of organ transplantation thus remains largely unresolved. To establish this, we need techniques with a high(er) specificity to additionally distinguish between different cell types and the possibility to include a much larger cohort of patients for association studies with clinical outcome parameters.

Specific MMP-2 and MMP-9 gene promoter polymorphisms were studied in relation to clinical outcome parameters after liver transplantation. We found a strong association of a specific MMP-2 genotype with the development non-anastomotic biliary strictures (**chapter 6**). To assess changes in functional activity of MMPs, we studied changes in serum levels in relation to MMP gene polymorphisms. Before transplantation, we did not find an association between the genotype and the respective serum levels. After transplantation, however, MMP-2 levels tended to be lower in patients that developed NAS and in association with MMP-2 CT genotype. MMP-2 levels are increased in patients with chronic liver disease and therefore were expected to drop after OLT, as reported previously.[10] This decrease was indeed observed, but this occurred independent of the genotype or a MMP genotypic mismatch between donor and recipient. In addition, no association of the MMP polymorphisms and mismatches was observed with ischemia/reperfusion or rejection after OLT (**chapter 4**). Similarly, a peak in MMP-9 levels was found after OLT, as previously described by our group again independent of genotype and donor-recipient mismatch.

Concluding remarks

The studies described in this thesis provide compelling evidence of chimerism after orthotopic liver transplantation. Cells of recipient origin can indeed replace endothelial cells, biliary epithelial cells and hepatocytes. After blood stem cell transplantation, donor-derived cells can be found in liver tissue specimens. These findings strongly support the theory of blood stem cells developing into liver cells of mesenchymal origin. We found an association between MMP-2 chimerism and acute rejection, but many questions on the clinical relevance of chimerism remain unanswered, due to limitations of available techniques. Matrix metalloproteinases are important in connective tissue processes after liver transplantation. In our liver transplant series, we did not find a relationship between MMP genotype polymorphisms and ischemia/reperfusion damage or rejection after OLT. We did find a strong relationship, however, between MMP-2 CT genotype in donor and recipient and the development of non-anastomotic biliary strictures. In fact, in predicting the development of biliary strictures after OLT, we found MMP-2 CT genotype and dilated bile ducts on abdominal ultrasound to have a high and comparable hazard ratio. An elevated gamma-glutamyltranspeptidase appeared to be a much weaker, but nevertheless independent and statistically significant, predictor of these biliary strictures.

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