ELSEVIER

Journal of Hepatology 48 (2008) 208–215

Journal of Hepatology

www.elsevier.com/locate/jhep

The fractalkine receptor CX3CR1 is involved in liver fibrosis due to chronic hepatitis C infection☆

Hermann E. Wasmuth[1,*], Mirko Moreno Zaldivar[1], Marie-Luise Berres[1], Alexa Werth[1], David Scholten[1], Sonja Hillebrandt[1,2], Frank Tacke[1], Petra Schmitz[1], Edgar Dahl[3], Tonio Wiederholt[4], Claus Hellerbrand[5], Thomas Berg[6], Ralf Weiskirchen[7], Christian Trautwein[1], Frank Lammert[1,2]

[1]*Medical Department III, University Hospital Aachen, Pauwelsstrasse 30, D-52057 Aachen, Germany*
[2]*Department of Internal Medicine I, University Hospital Bonn, Germany*
[3]*Department of Pathology, University Hospital Aachen, Germany*
[4]*Department of Dermatology, University Hospital Aachen, Germany*
[5]*Department of Medicine I, University of Regensburg, Germany*
[6]*Department of Gastroenterology and Hepatology, Charité University Hospital Berlin, Germany*
[7]*Institute of Clinical Chemistry and Pathobiochemistry, University Hospital Aachen, Germany*

***Background/Aims*: The chemokine receptor CX3CR1 and its specific ligand fractalkine (CX3CL1) are known to modulate inflammatory and fibroproliferative diseases. Here we investigate the role of CX3CR1/fractalkine in HCV-induced liver fibrosis.**

***Methods*: A genotype analysis of *CX3CR1* variants was performed in 211 HCV-infected patients. Hepatic expression of CX3CR1 was studied in HCV-infected livers and isolated liver cell populations by RT-PCR and immunohistochemistry. The effects of fractalkine on mRNA expression of profibrogenic genes were determined in isolated hepatic stellate cells (HSC) and *CX3CR1* genotypes were related to intrahepatic *TIMP-1* mRNA levels.**

***Results*: The intrahepatic mRNA expression of *CX3CR1* correlates with the stage of HCV-induced liver fibrosis ($P = 0.03$). The *CX3CR1* coding variant V249I is associated with advanced liver fibrosis, independent of the T280M variant ($P = 0.009$). CX3CR1 is present on primary HSC and fractalkine leads to a suppression of tissue inhibitor of metalloproteinase (*TIMP*)-*1* mRNA in HSC ($P = 0.03$). Furthermore, *CX3CR1* genotypes are associated with *TIMP-1* mRNA expression in HCV-infected liver ($P = 0.03$).**

***Conclusions*: The results identify the fractalkine receptor CX3CR1 as susceptibility a gene for hepatic fibrosis in HCV infection. The modulation of *TIMP-1* expression by fractalkine and *CX3CR1* genotypes provides functional support for the observed genotype–phenotype association.**

***Keywords*: Liver fibrosis; CX3CR1; Fractalkine; Genetic predisposition; Immunology; Hepatic stellate cells; Hepatitis C; Chemokines**

1. Introduction

Liver fibrosis is the hallmark of all chronic liver diseases and is associated with high mortality and morbidity. Chronic hepatitis C virus (HCV) infection is a leading cause of end stage liver disease worldwide and represents the major indication for liver transplantation

Received 18 April 2007; received in revised form 24 August 2007; accepted 14 September 2007; available online 20 November 2007
Associate Editor: A. Geerts

☆ The authors declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

* Corresponding author. Tel.: +49 241 8080861; fax: +49 241 8082455.
E-mail address: hwasmuth@ukaachen.de (H.E. Wasmuth).

0168-8278/$32.00
doi:10.1016/j.jhep.2007.09.008

[1]. However, whereas some patients infected with HCV rapidly progress, a significant number of individuals remain stable over time and develop only minor liver damage [2]. These large inter-individual differences in fibrosis progression are considered to be due to age at infection, gender and multiple exogenous (e.g. alcohol intake, co-infections) and endogenous, i.e. genetic, factors [3–5].

In recent years it has become apparent that the genetic basis of liver fibrosis is complex and that multiple genes are involved in the distinct pathobiological processes of fibrogenesis. Although some risk loci for liver fibrosis have been validated, most genes that contribute to the progression of liver scaring have yet to be identified [4,6]. It is important to note that different intrahepatic cell populations are involved in liver fibrogenesis. Whereas immune cells, like Kupffer cells and recruited lymphocytes, are important for the inflammatory phase of the fibrogenic response, hepatic stellate cells (HSCs) and portal myofibroblasts have been implicated in the later stages of fibrogenesis [7,8]. Therefore, genes that are involved in both phases are suitable candidates that could determine the progression of liver damage due to HCV.

Chemokines are a class of small chemoattractant cytokines that are synthesised at sites of inflammation and are known to be major regulatory proteins for leukocyte recruitment and activation [9]. Chemokines are expressed in chronic HCV infection and constitute important chemoattractants for the recruitment of lymphocytes to inflamed liver [10–12]. Interestingly, some chemokines have also been shown to activate HSCs, thereby directly contributing to liver fibrogenesis [13,14].

The CX3C subfamily differs from other chemokines as only one receptor (CX3CR1) and one specific ligand (CX3CL1 or fractalkine) have been identified so far. CX3CR1 is a highly selective chemokine receptor and surface marker for NK cells, T lymphocytes and $\gamma\delta$ T cells, as well as monocytes [15]. On $CD4^+$ T cells, CX3CR1 is preferentially expressed on Th1 compared to Th2 cells, and Th1, but not Th2, cells respond to the CX3CR1 ligand fractalkine [16,17].

In acute liver injury, CX3CR1 is present in areas of necrosis and inflammation [18], whereas it is expressed on biliary epithelial cells, in portal tracts and in fibrotic septa of chronically injured liver [19]. Serum levels of fractalkine, the specific ligand for CX3CR1, are significantly higher in patients with HCV and other chronic liver diseases compared to healthy controls [19] and activated HSCs are involved in the expression and activation of fractalkine [20].

There are two single nucleotide polymorphisms (SNPs) encoding non-synonymous amino acid substitutions at positions 249 (valine to isoleucine) and 280 (threonine to methionine) within the *CX3CR1* gene [21]. These SNPs are functionally relevant as they influence the binding of fractalkine to CX3CR1 [22]. Specifically, the 249I and the 280M alleles result in fewer receptor binding sites and decreased ligand affinity [23,24]. These SNPs have already been shown to be associated with the progression of extrahepatic inflammatory [24,25] and fibrotic diseases [26–28], and might therefore also be relevant to liver fibrosis due to HCV.

We therefore conducted a systematic study of *CX3CR1* SNPs with regard to HCV-induced liver fibrosis and analysed the hepatic CX3CR1 expression. Furthermore, we show a potential functional consequence of *CX3CR1* variants on *TIMP-1* expression.

2. Patients and methods

2.1. Patient and control cohorts

The study comprised 211 patients chronically infected with HCV. The diagnosis was based on a positive anti-HCV test (Abbott), a positive HCV-RNA (Cobas Taqman) and elevated aminotransferase activities for at least six months. None of the study subjects had detectable HBsAg (Abbott) or was anti-HIV positive. Other chronic liver diseases were excluded by appropriate serological tests and none of the patients had an alcohol intake of more than 40 g/day. All patients underwent percutaneous liver biopsy for histological examination of inflammation and fibrosis prior to antiviral therapy. All liver biopsies were evaluated for grade of necroinflammatory activity and stage of fibrosis according to the Desmet and Scheuer score [29]. In further analyses, we chose stage of fibrosis, corrected for the age at liver biopsy and gender, as the main outcome variable. However, the exact duration of infection was available in a subgroup of study subjects ($n = 121$). In this subgroup, we also analysed the association of *CX3CR1* genotypes with the rate of fibrosis progression (fibrosis stage/duration in years). The scoring pathologist was unaware of the study protocol.

In 54 separate patients with chronic HCV infection quantitative *CX3CR1*, tissue inhibitor of metalloproteinases (*TIMP*)-*1*, *GATA-3* and T-box expressed in T cells (*T-bet*) mRNA levels were determined in liver samples. Total liver RNA was isolated from paraffin embedded sections of these patients as described [30].

Furthermore, 153 control individuals without chronic liver diseases were included for validation of genotyping results. This cohort has been described previously [31].

The study protocol was approved by the Ethics Committee at the University of Aachen and Berlin. Patients and controls gave informed consent for participation in the study.

2.2. Genotyping of CX3CR1 SNPs and genetic analysis

Genotyping of the functional *CX3CR1* SNPs V249I (*rs3732378* G > A) and T280M (*rs3732379* C > T) was performed with 5′-endonuclease (Taqman) assays with an ABI PRISM 7000 sequence detection system (Applera). Genomic DNA was extracted from EDTA blood using the DNeasy kit (Qiagen). Primers and probes are available from the website www.appliedbiosystems.com.

2.3. Immunohistochemistry of CX3CR1 in HCV-infected liver and on isolated myofibroblasts

Immunohistochmistry for CX3CR1 was performed on paraffin embedded sections of HCV-infected liver with a rabbit anti-human CX3CR1 antibody (1:200, Torrey Pines Biolabs, USA) and Dako REAL detection system with alkaline phosphatase/RED (Dako Chemicals, Germany). To validate the expression of CX3CR1 on HSCs, immunocytochemistry for CX3CR1 was also performed on isolated stellate cells/activated myofibroblasts [32] with the same primary