Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

# The hepatitis C virus core protein indirectly induces alpha-smooth muscle actin expression in hepatic stellate cells via interleukin-8

Sophie Clément[1,*], Stéphanie Pascarella[1], Stéphanie Conzelmann[1], Carmen Gonelle-Gispert[3], Kévin Guilloux[1], Francesco Negro[1,2]

[1]*Division of Clinical Pathology, Geneva University Hospital, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland;* [2]*Division of Gastroenterology and Hepatology, Geneva University Hospital, 4 rue Gabrielle-Perret-Gentil, 1211 Geneva 14, Switzerland;* [3]*Surgical Research Unit, Department of Surgery, Geneva University Hospital, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland*

**Background & Aims**: Progressive deposition of liver fibrosis is a common feature of chronic hepatitis associated with hepatitis C virus (HCV) infection, and it may eventually lead to cirrhosis and liver failure. Although this fibrogenic process appears to be linked to HCV protein expression and replication *via* indirect mechanisms, i.e., to be mediated by virally-driven inflammation, a direct role of HCV in inducing fibrosis deposition has never been entirely excluded.

**Methods**: We established an *in vitro* system in which the human hepatic stellate cell line LX-2 was cultured in the presence of conditioned medium from human hepatoma Huh-7 cells transduced with a lentiviral vector expressing HCV core proteins of different genotypes.

**Results**: Treatment of LX-2 cells, with conditioned medium from Huh-7 cells expressing HCV core protein, led to the activation of α-smooth muscle actin expression. Among the chemokines secreted by cells transduced with HCV core, interleukin-8 was identified as the strongest inducer of α-smooth muscle actin expression in LX-2 and primary hepatic stellate cells. This effect was accompanied by a decrease in cell migration and increased focal contact organisation.

**Conclusions**: The expression of the HCV core in hepatocytes may contribute to the establishment of a profibrogenic microenvironment.



Keywords: Liver fibrosis; Cytokines; Hepatitis C.

*Received 22 June 2009; received in revised form 8 October 2009; accepted 11 October 2009; available online 5 March 2010*

* Corresponding author. Tel.: +41 22 3795060; fax: +41 22 3724920.

*E-mail address:* sophie.clement@unige.ch (S. Clément).

*Abbreviations:* HCV, hepatitis C virus; HSC, hepatic stellate cells; IL-8, interleukin-8; PCR, polymerase chain reaction; EF1, elongation factor 1; EGFP, empty and green fluorescent protein; SMA, smooth muscle actin; FCS, foetal calf serum; CM, conditioned medium; SD, standard deviation; MMP2, matrix metalloproteinase 2; TIMP1, tissue inhibitor of metalloproteinase 1; CTGF, connective tissue growth factor; EMT, epithelial to mesenchymal transition.

## Introduction

Hepatitis C virus (HCV) infects approximately 170 million people worldwide, and a proportion of them develop cirrhosis and hepatocellular carcinoma. Chronic hepatitis C is characterised by the relentless deposition of liver fibrosis, a process that may take decades before the cirrhotic stage is reached.

Hepatic fibrosis occurring in chronic hepatitis C is a wound-healing response to persistent liver injury [1]. Damaged hepatocytes infected by HCV release various mediators that recruit inflammatory cells [2]. These, in turn, activate hepatic stellate cells (HSC) to secrete collagen [3]. Since the latter are a major source of proinflammatory chemokines, a vicious circle ensues, with inflammation and fibrogenesis amplifying each other [1]. Thus, in chronic hepatitis C, the fibrogenic process seems to be mostly mediated by virally-driven inflammation.

The liver fibrosis progression rate, however, varies considerably among chronically HCV-infected patients. Some patients present with no fibrosis even after decades of infection while others rapidly develop cirrhosis in a few years. Host cofactors like age at infection, insulin resistance, gender, alcohol consumption, coinfections, and genetic polymorphisms influence the progression of hepatitis C [4,5]. Conversely, the impact of viral factors is debated, although recent reports suggest a direct fibrogenic effect of HCV [4,6]. Several viral proteins may induce oxidative stress, steatosis, and apoptosis, leading to direct HSC activation without the participation of the inflammatory response. Thus, some patients may present with significant liver fibrosis despite minimal inflammation.

In the present work, we show that HCV core protein-expressing hepatocytes secrete interleukin-8 (IL-8), and that this cytokine participates in the activation of HSCs, suggesting a fibrogenic effect of HCV via paracrine mechanisms.

## Materials and methods

### *Antibodies and chemicals*

Anti-α-SMA (αsm-1, [7]) and anti-β-cytoplasmic actin antibodies were obtained from C. Chaponnier (Geneva). Monoclonal anti-core (C7–50) antibody was from Axxora Europe (Lausen, Switzerland). Monoclonal anti-vinculin antibody (clone

# Research Article

hVin-1) was from Sigma (St Louis, MO). Blocking anti-IL-8 and human recombinant IL-8 were from R&D Systems Europe (Abingdon, UK). The chemokine (C–X–C motif) receptor 2 (CXCR2) antagonist (SB 265610) and TGF-β1 were from Tocris (Ellisville, MO) and ABD Serotech (Düsseldorf, Germany), respectively.

*Cloning of HCV core in a lentiviral vector*

Core-encoding sequences were amplified by PCR from pIRES2-EGFP plasmids [8] and inserted into pENTR4 (Gateway lentiviral system, Invitrogen) after digestion with XhoI and BamHI. With respect to the originally reported sequence, the genotype 1b core variant used in the present study has a threonine replacing an alanine at position 131. This plasmid was recombined with pENTR-EF1-α containing the ubiquitous Elongation Factor 1 (EF1) promoter into the destination vector. The lentivector particles were produced by transient transfection in HEK 293T cells, collected after 72 h and concentrated 120-fold by ultracentrifugation. Viral titre was estimated by quantitative RT-PCR [9] and immunofluorescence (see below). Empty and green fluorescent protein (EGFP)-encoding lentivectors were used as controls.

*Cell culture, transduction, and treatment*

Human hepatoma Huh-7 cells were cultured in DMEM (Invitrogen Life Technologies, Basel, Switzerland, Ref. 31885–023) supplemented with 10% foetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen Life Technologies). Twenty-four hours before transduction with lentiviral vectors, the growth medium was replaced with serum-free medium consisting of

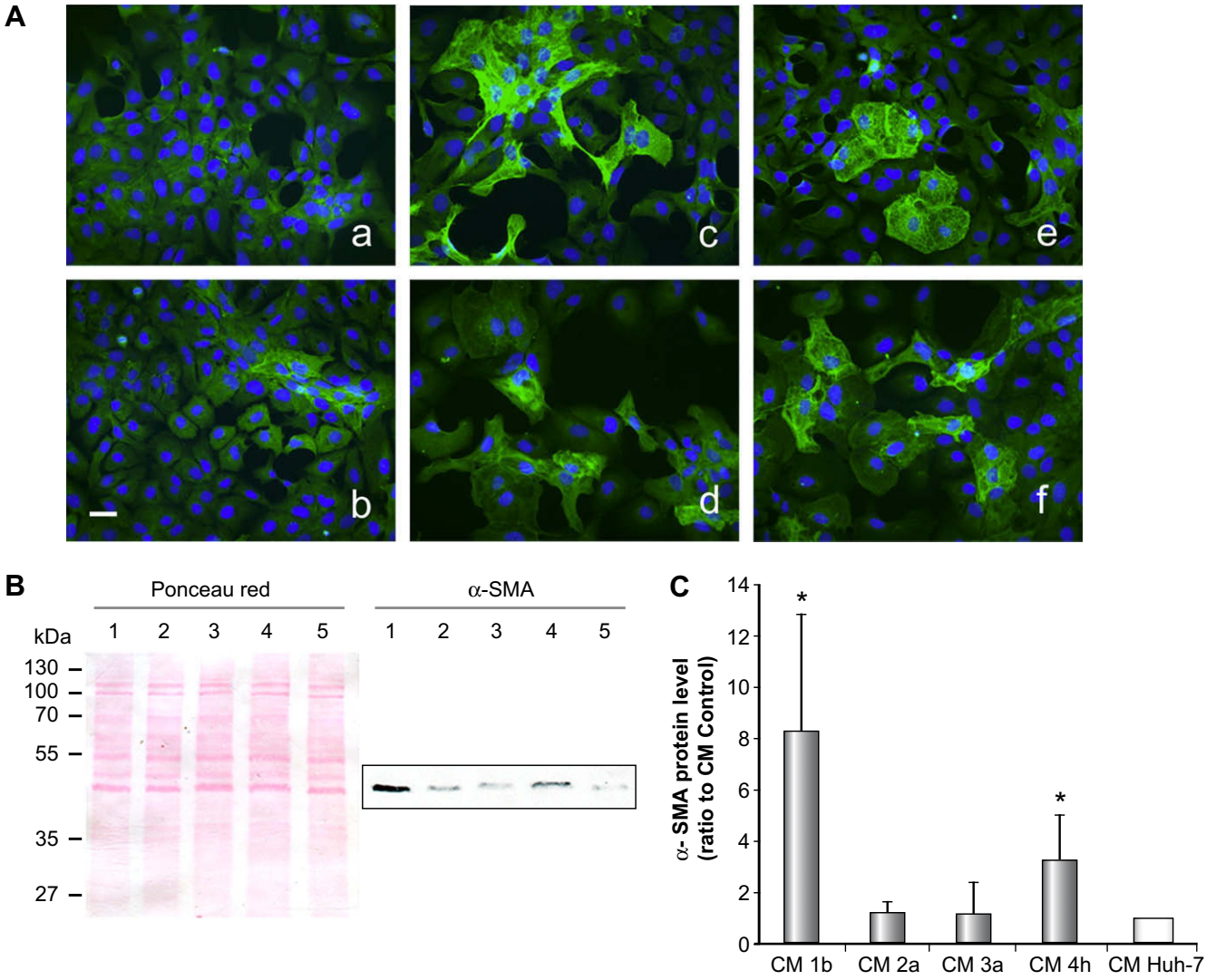


**Fig. 1. Effect of CM from Huh-7 cells expressing HCV core protein on LX-2 activation.** CM obtained from Huh-7 cells expressing HCV core 1b, 2a, 3a, and 4h was applied to LX-2 cells for 72 h. Activation of LX-2 cells was estimated by α-SMA expression levels. CM control (CM cont) corresponds to the mean of two controls used in these experiments: cells untransduced or transduced with an EGFP-encoding lentivector. (A) α-SMA was stained by immunofluorescence and nuclei by DAPI. *(a: CM Huh-7, b: CM GFP, c: CM 1b, d: CM 2a, e: CM 3a, f: CM 4h)* Scale bar: 20 μm. (B and C) α-SMA expression was also estimated by immunoblot. (B) Total protein extracts from LX-2 cells treated with the different CM were subjected to SDS–PAGE, transferred to nitrocellulose (first 5 lanes, Ponceau red staining) and blotted with anti-α-SMA. (*1: CM 1b, 2: CM 2a, 3: CM 3a, 4: CM 4h, 5: cont CM*) (C) α-SMA signals were quantified by densitometry and normalised using ponceau red staining as a loading control. Bars represent the SD of three independent experiments (*$p \leqslant 0.05$ compared to control).