Research Article

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Hepatitis C virus induces the expression of CCL17 and CCL22 chemokines that attract regulatory T cells to the site of infection

José-Ignacio Riezu-Boj[1,†], Esther Larrea[1,†], Rafael Aldabe[1], Laura Guembe[2], Noelia Casares[1], Eva Galeano[1], Iciar Echeverria[1], Pablo Sarobe[1], Ignacio Herrero[1,3,4], Bruno Sangro[1,3,4], Jesús Prieto[1,3,4], Juan-José Lasarte[1,*]

[1]*Gene Therapy and Hepatology Area, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain;* [2]*Department of Morphology, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain;* [3]*CIBER-EHD, Pamplona, Spain;* [4]*Liver Unit, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain*

Background & Aims: The mechanisms by which Foxp3+ T regulatory cells (Treg) accumulate in HCV infected livers are not known. Here, we studied the role of chemokines CCL17 and CCL22 in this process.

Methods: Chemokine mRNA levels were determined by qPCR in liver biopsies from 26 HCV chronically infected patients (CHC), 11 patients with treatment-induced sustained virological response (SVR), 16 patients with other liver diseases unrelated to HCV, and 24 normal livers. Double-immunofluorescence Foxp3/CD3 or CD11c/CCL22 was performed in liver sections. Chemokine production by monocyte-derived dendritic cells (MDDC) co-cultured with uninfected or HCV-JFH1 infected Huh7 cells was measured by qPCR and ELISA. Chemotactic activity of culture supernatants was also tested.

Results: Foxp3+ Treg were increased in CHC livers as compared to controls. Patients with CHC showed elevated intrahepatic levels of *CCL17* mRNA compared to normal livers or livers from subjects with SVR or other forms of liver disease. Intrahepatic CCL22 expression was also higher in CHC than in healthy subjects or SVR patients but similar to that observed in other liver diseases. Dendritic cells producing CCL22 could be found inside the hepatic lobule in CHC patients. Contact between MDDC and HCV-JFH1-infected Huh7 cells induced the expression of CCL17 and CCL22 in a process partially dependent on ICAM-1. Transwell experiments showed that upregulation of these chemokines enhanced Treg migration.

Conclusions: Contact of HCV-infected cells with dendritic cells induces the production of Treg-attracting chemokines, an effect which may favour liver accumulation of Treg in CHC. Our findings contribute to explain the mechanism by which HCV escapes the immune response and thus reveals novel therapeutic targets.

Keywords: T regulatory cells, CCL17/CCL22 chemokine; Hepatitis C virus; Dendritic cells.

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*Corresponding author. Address: Centre for Applied Medical Research, CIMA, Gene Therapy and Hepatology Area, Avenida Pío XII, 55, 31008 Pamplona, Spain. Tel.: +34 948 194700; fax: +34 948 194717.

E-mail address: jjlasarte@unav.es (J.-J. Lasarte).

† These authors contributed equally to this work.

Abbreviations: HCV, hepatitis C virus; IFN, interferon; Treg, regulatory T cells; CHC, chronic hepatitis C; PBMC, peripheral blood mononuclear cells; MDDC, monocyte-derived dendritic cells; SVR, sustained virological response; RT-PCR, reverse transcription-polymerase chain reaction; qPCR, quantitative real time-PCR; DC, dendritic cells.

Introduction

Hepatitis C virus (HCV) is a major cause of liver disease worldwide [1]. More than 70% of infected patients develop chronic hepatitis with the risk of progression to liver cirrhosis and hepatocellular carcinoma [2]. Viral clearance after acute infection or after interferon (IFN) therapy is associated with the presence of a robust, polyclonal, and persistent T cell immune response against viral proteins. By contrast, the immune response is weak or absent in those patients who progress to chronicity (reviewed in [3]), Thus, the ability of HCV to evade the cellular immune response is believed to play a critical role in viral persistence.

Recent evidences indicate that CD4+ natural regulatory T cells (Treg) are important players in the establishment of peripheral dominant tolerance to self antigens and in the protection against organ-specific autoimmune diseases (reviewed in [4]). Treg exert a remarkably strong, cell-contact dependent suppressive activity on different components of the immune system, including lymphocytes, natural killer cells, and antigen presenting cells. Although Treg play a crucial role in physiological immune homeostasis, they may hinder the induction of immune responses against pathogens and tumors (reviewed in [5]). Thus, Treg may block protective cellular immune responses against viruses and favour the establishment of chronic infections by agents such as HIV, HBV, and HCV [6–12]. In particular, patients with chronic hepatitis C (CHC) have a higher number of peripheral CD4+CD25+ [8], and hepatic Foxp3+CD4+ Treg [11,12]. It has also been shown that CD4+CD25+ T cells from peripheral blood of patients with CHC suppress virus-specific CD8+ T-cell responses [7,10]. These evidences point to the important role of

Table 1. Patient characteristics.

Variable	Value for patient group			
	Normal Liver	Chronic Hepatitis C	Sustained virological response	Other Liver Diseases
AST (IU/L)				
Cohort 1	14.7 ± 4.6*	47.3 ± 40.0	10.9 ± 1.4	53.5 ± 46.7
Cohort 2		43.4 ± 24.8	13.5 ± 3.5	121 ± 236
ALT (IU/L)				
Cohort 1	19.3 ± 9.0	86.8 ± 84.9	13.6 ± 4.4	92.7 ± 56.9
Cohort 2		72.3 ± 35.0	14.6 ± 4.6	96.5 ± 144
Viral load (IU/ml)				
Cohort 1		4.7×10^7	0	
Cohort 2		9.3×10^6	0	
Viral genotype (1/non-1/not determined)				
Cohort 1		17/5/4		
Cohort 2		5/4/1		
Liver biopsy (Knodel's score) inflammatory activity				
Cohort 1		5.4 ± 2.2	2.3 ± 1.0	5.1 ± 2.5
Cohort 2		8.4 ± 2.4	2.2 ± 0.9	4.9 ± 2.0
Fibrosis score				
Cohort 1		0.7 ± 0.9	0.5 ± 0.5	1.7 ± 1.7
Cohort 2		2.0 ± 1.6	0.6 ± 0.6	1.2 ± 1.4

*Mean ± standard deviation.

Treg in HCV persistence [13,14]. However, the mechanisms by which HCV might induce proliferation of Treg or their recruitment to the sites of infection are not known. Herein, we report that contact of monocyte-derived dendritc cells (MDDC) with HCV-infected Huh7 cells strongly stimulates the expression of CCL17 and CCL22 chemokines, which act as attractants for Tregs. These results are paralleled by the findings of increased levels of both CCL17/CCL22 chemokines and Treg in the liver of HCV-infected patients. Finally, we identify new targets for the treatment of HCV chronic infection.

Materials and methods

Patients

Analysis of mRNA expression was carried out in: (a) liver biopsies from 26 patients with CHC (12 untreated patients, 14 non-responders to pegylated IFNα2 plus ribavirin) and 11 HCV patients with sustained virological response (SVR) after therapy; (b) 24 normal liver samples obtained at laparatomy (13 gastrointestinal neoplasm, 7 pancreatic tumors, 2 cholelitiasis, and 2 hydatidic cysts; these subjects had not received cytotoxic therapy previous to surgery, and in all cases, histological examination of biopsies showed normal liver architecture); (c) 16 liver samples from patients with miscellaneous liver disorders unrelated to HCV (8 chronic hepatitis B, 1 drugs, 7 steatohepatitis). Diagnosis of CHC was based on elevation of serum transaminases for more than 6 months, positivity for anti-HCV antibodies, presence of HCV-RNA by reverse transcription-polymerase chain reaction (RT-PCR), and histological evidence of chronic hepatitis. Alcohol consumption and other causes of liver disease were excluded. Immunofluorescence studies were performed in paraffin embedded liver biopsies from a second cohort of patients including 10 patients with CHC (6 untreated patients and 4 non-responders to pegylated IFNα2 plus ribavirin), 11 HCV patients with SVR, and 13 with miscellaneous liver disorders unrelated to HCV (8 patients with chronic hepatitis B, 1 alcoholic hepatitis, 2 steatohepatitis, 1 autoimmune hepatitis, 1 cryptogenetic hepatitis). In this miscellaneous group of patients, the extent of liver inflammatory infiltrate was comparable to that of HCV-infected patients. Clinical and virologic characteristics of the patients are indicated in Table 1. Written consent was obtained from each patient included in the study. The protocol was approved by the local ethical committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

CD4 and CD14 cell purification

CD4+ T lymphocytes and CD14+ monocytes were purified from PBMC of healthy donors using the automated Magnetic Cell Sorting autoMACS, the CD4+ T-cell Isolation Kit II, and the CD14 MicroBeads human kit (Miltenyi Biotec, Germany) according to manufacturer's instructions.

Monocyte-derived dendritic cells

MDDCs were obtained by cultivating peripheral blood monocytes in RPMI-1640 medium with 1000 U/ml of GM-CSF and 1000 U/ml of IL-4 for 5 days as described [15].

Cell cultures

Human hepatoma Huh7 cells were grown in DMEM (Invitrogen, New Zealand) supplemented with 10% of fetal bovine serum (Invitrogen). The pJFH1 plasmid containing the full-length cDNA of isolated JFH1-HCV was provided by Dr. T. Wakita. Genomic JFH1-HCV was transcribed *in vitro* and delivered to Huh7 cells by electroporation, as described [16]. Infectious supernatants were used in subsequent inoculations. JFH1-Huh7 infected cells were used at 25–45 days post infection, being about 60–70% of cells positive for HCV-core protein, as determined by immunofluorescence [17]. Under these conditions, HCV-infected cells have the same apoptotic stage as non-infected Huh7 cells (around 8% of Annexin V-positive cells). Viral titer in the supernatant of these cultures was 5×10^2–2×10^3 focus-forming U/ml.

Huh7 or JFH1-Huh7 cells were co-cultured with MDDC. Different ratios of cell co-cultures were maintained for three days. In some cases, MDDC were plated in the upper compartment of 0.4 μm pore size transwells (Becton Dickinson, NJ, USA) and co-cultured with Huh7 or JFH1-Huh7 cells placed in the lower