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Early viraemia clearance during antiviral therapy of chronic hepatitis C improves dendritic cell functions

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Abstract Plasma and cellular HCV RNA and core antigen were tested in monocyte-derived DC (MDDC) from chronic hepatitis C patients undergoing treatment with peg-interferon α2b/ribavirin. DC allostimulatory capacity, HCV-specific T-cell reactivity and IL-12 production were measured at baseline and treatment week (TW)12. Using DC and autologous $CD4^+$T-cells, obtained at baseline and TW12, we performed cross-over experiments to determine the relative role of DC and/or T-cells for impaired immune reactivity to HCV. HCV RNA and HCV core plasma levels had an impact on DC phenotype and allostimulatory capacity. In contrast, HCV genome/core protein, although detectable in DC from some patients had no effect on DC function. Antiviral immunity at TW12 was not improved in patients who remained HCV RNA positive, while early viraemia clearance (TW12) improved antiviral responses. The cross-over experiment revealed that changes in DC, rather than $CD4^+$T cells have a major role for enhanced anti-HCV responses.

Introduction

It is estimated that 170 million people worldwide are chronically infected with the hepatitis C virus (HCV), one of the principal causes of chronic hepatitis, cirrhosis and hepatocellular carcinoma [1,2]. Some individuals are able to clear HCV spontaneously during acute HCV infection by mounting vigorous cellular immune responses directed to multiple HCV antigens [1,3–6]. However in the great majority of patients viraemia persists, associated with impaired HCV-specific T-cell reactivity [6,7]. The mechanisms by which HCV establishes chronic infection are not fully understood.

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Table 1 Patients' characteristics for all patients included in the study (both treated and un-treated).

	$N=35$
Gender (males/females)	24/11
Age (years)	45.5 ± 10.2
Race (Caucasian/non-Caucasian *)	34/1
Genotype (1/3)	16/19
Pre-treatment plasma HCV RNA (log_{10} IU/ml)	−6.07 ± 6.14
Pre-treatment ALT levels (IU/L)	83 ± 52.7
Inflammation (grade **)	3.4 ± 1.4
Fibrosis (stage **)	2.03 ± 1.07
	Stages 0–3: $n=18$, stages 4–6: $n=17$

Scale data are presented as mean ± standard deviation.
* Asian.
** Modified Ishak scale.

Dendritic cells (DC), the most potent antigen presenting cells, have a critical role in the initiation of adaptive immune responses [8–10]. Consequently, DC functions are targeted by numerous viruses in order to disrupt the generation of efficient antiviral cellular immune responses [11–17]. In HCV infection, several studies, using monocyte-derived DC (MDDC), have suggested that dysfunctional DC are responsible for the impaired Th1 response [18–24]. However, other authors found no significant defects in the phenotype and function of MDDC generated from patients with chronic hepatitis C (CHC) [25–28]. Similarly, controversial findings have been reported when using circulating myeloid or plasmacytoid dendritic cells, directly isolated from peripheral blood [27,29–32].

In the present study we investigated viraemia levels and MDDC functions and phenotype before treatment and early during antiviral therapy, trying to understand the role of DC in CHC. Our working hypothesis was that 'early' (by week 12 of treatment, TW12) clearance of viraemia and subsequent viral antigen loss leads to improvement of dendritic cell functions, promoting HCV-specific Th1 responses in the host. We further investigated whether improved adaptive immune response after 'early' viraemia clearance is due to restored DC function, or to improvement of $CD4^+$ T-cell reactivity. In addition, we tested the relation between the presence of HCV genomic sequences and proteins (in particular HCV core) in patients' plasma and in DC lysates, and DC functions and phenotype.

Materials and methods

Patients

Thirty-five consecutive ($n=35$), treatment-naïve patients with chronic hepatitis C, monitored at the Hepatitis Clinic, University College Hospital, London, were enrolled in the study (Table 1) after giving informed consent. All patients were positive for anti-HCV and HCV RNA (Amplicor HCV v2.0; Roche Molecular Systems, Pleasanton, CA). The HCV genotype was determined by a restriction–fragment–length–polymorphism method [33]. All patients were tested negative for HBsAg and antibodies against HIV1,2 by commercially available assays (Abbott Diagnostics, Maidenhead, UK). Patients underwent a liver biopsy with evaluation of the grade of liver inflammation and stage of fibrosis according to established criteria [34].

Twenty-two ($n=22$) (Table 2) of the thirty-five enrolled patients received antiviral treatment with pegylated Interferon-α2b (1.5 μg/kg) once weekly and daily Ribavirin (Viraferon-Peg® and Rebetol®, both from Schering-Plough, Welwyn Garden City, UK) for 24 to 48 weeks, according to HCV genotype. The study was approved by the University College Hospital Ethics Committee.

Generation of monocyte-derived DC (MDDC)

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation with Lymphoprep 1077

Table 2 Patients' characteristics.

	$N=22$
Gender (males/females)	13/9
Age (years)	47.3 ± 9.7
Race (Caucasian/non-Caucasian *)	21/1
Genotype (1/3)	8/14
Pre-treatment plasma HCV RNA (log_{10} IU/ml)	−5.95 ± 5.98
Pre-treatment ALT levels (IU/L)	83 ± 49.6
Inflammation (grade **)	4.3 ± 1.2
Viral load decline at TW12 ('log_{10} drop'), 'good responders' *** ($n=17$)	5.48 ± 0.59
Viral load decline at TW12 ('log_{10} drop'), 'poor responders' **** ($n=5$)	1.12 ± 0.94
Viral load at TW12, 'poor responders' **** (log_{10} IU/ml)	−5.07 ± 0.79
Fibrosis (stage **)	2.7 ± 1.7
	Stages 0–3: $n=17$, stages 4–6: $n=5$

Patients who received antiviral treatment with Pegylated-IFNα2b and Ribavirin. Scale data are presented as mean ± standard deviation.
* Asian.
** Modified Ishak scale.
*** 'good responders': undetectable HCV RNA by TaqMan real-time PCR at TW12.
**** 'poor responders': still detectable HCV RNA by TaqMan real-time PCR at TW12.