ELSEVIER

Journal of Hepatology 42 (2005) 61–67

Journal of Hepatology

www.elsevier.com/locate/jhep

Comparable functions of plasmacytoid and monocyte-derived dendritic cells in chronic hepatitis C patients and healthy donors

Diego Piccioli[1], Simona Tavarini[1], Sandra Nuti[1], Piero Colombatto[2], Maurizia Brunetto[2], Ferruccio Bonino[2], Pietro Ciccorossi[2], Francesca Zorat[3], Gabriele Pozzato[3], Consuelo Comar[3], Sergio Abrignani[1,*], Andreas Wack[1]

[1]*Immunology and Virology Department, Chiron Vaccines Research Center, via Fiorentina 1, 53100 Siena, Italy*
[2]*Gastroenterology and Hepatology Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy*
[3]*Institute of Internal Medicine, School of Medicine, University of Trieste, Trieste, Italy*

***Background/Aims*: Dendritic cells (DCs) play a key role in immune responses through antigen presentation and cytokine secretion. Hepatitis C virus (HCV) is able to escape elimination by the immune system and often establishes a chronic infection. To investigate whether DC dysfunction is involved in this process, we have studied monoycte-derived DCs (Mo-DCs) and plasmacytoid DCs (pDCs), which produce large amounts of IFN-α, from chronic HCV patients and healthy donors.**

***Methods*: We have assessed TNF-α and IFN-α production by pDCs using intracellular staining after total PBMCs stimulation with unmethylated CG dinucleotides (CpGs). The induction of allogeneic T cell proliferation by immature Mo-DCs was measured using the MLR assay. The up-regulation of maturation markers and the production of TNF-α in response to LPS were analyzed using flow cytometry and ELISA, respectively.**

***Results*: We have detected comparable frequencies of pDCs producing TNF-α and IFN-α in both chronic HCV patients and healthy donors and we have found that immature Mo-DCs from both patients and donors similarly induce allogeneic T cell proliferation and mature and secrete TNF-α in response to LPS.**

***Conclusions*: Our results demonstrate that both pDC and Mo-DCs are not impaired in HCV infected patients.**

***Keywords*: Dendritic cell; HCV; Maturation; TNF-α; IFN-α**

1. Introduction

Successful elimination of a pathogen requires a robust innate immune response and efficient priming of adaptive immunity. Dendritic cells (DCs) are central in both these processes through two functions, induced by the recognition of pathogen-associated molecular patterns (PAMPs) in peripheral tissues at the immature stage: (1) secretion of pro-inflammatory cytokines; (2) maturation, characterized by migration to the lymph nodes, processing and presentation of antigens engulfed in the periphery, and up-regulation of co-stimulatory molecules such as B7.1 (CD80) and B7.2 (CD86), leading to efficient priming of naïve T cells [1,2].

DCs can be of lymphoid and myeloid origin [3]. Myeloid DCs respond to activation producing large amounts of TNF-α and IL-6 and, to a lesser extent, IL-12 [2]. Blood monocytes can be considered precursors of myeloid DCs [3–5]. Plasmacytoid dendritic cells (pDCs) are lymphoid DCs that produce high amounts of TNF-α and IFN-α when stimulated with endogenous or pathogen stimuli and for this reason are proposed to have a central role in the elimination of intracellular infection, such as viral infection [6].

Hepatitis C Virus (HCV) is a positive strand RNA virus of the flaviviridae family infecting about 200 million people worldwide and leading to chronic infection in a high

Received 20 July 2004; received in revised form 14 September 2004; accepted 21 September 2004; available online 8 October 2004
* Corresponding author. Tel.: +39 577 243032; fax: +39 577 243564.
E-mail address: sergio_abrignani@chiron.it (S. Abrignani).

0168-8278/$30.00
doi:10.1016/j.jhep.2004.09.014

percentage of individuals [7,8]. A sizeable fraction of chronically infected people develops liver diseases such as hepatitis and cirrhosis or hepatocellular carcinoma (HCC) [7,8]. Apparently, the immune response against HCV is often not sufficient to lead to the elimination of the virus. A number of causes for this impairment have been proposed, such as hypervariability of the virus [9,10], inhibition of Natural Killer (NK) cell function [11,12], inefficient induction of T cell responses [13–15] or dysfunction of DCs.

The latter aspect has been addressed in previous studies which assessed the function of monocyte-derived DCs (Mo-DCs) from chronic HCV infected patients [16–20]. Some of these studies indicate that Mo-DCs have impaired allo-stimulatory capacity [17–20], suggesting a variety of mechanisms such as the reduction of basal IL-12 transcription [20], the loss of maturation capacity [18], infection of Mo-DCs by the virus [19] or increased IL-10 production due to interaction with HCV Core or NS3 [17]. It was also shown that DCs transfected with Core and E1 HCV proteins are poor stimulators for allogeneic and autologous T cells [21]. However, the viral infection and replication in PBMCs including monocytes from HCV patients, investigated in many studies during the past decade, is still controversial [22–24]. Moreover, there is no evidence for the expression of HCV proteins in Mo-DCs from chronic HCV patients.

A recent report shows that Mo-DCs from chronic HCV patients mature in response to TNF-α and stimulate allogeneic T cells normally [16]. In addition, it was previously shown that DC dysfunction is restricted to HCC conditions in both HCV and Hepatitis B Virus (HBV) infected patients, independently of which type of viral infection determines the HCC [25]. Clinical observation of chronic HCV infected patients does not reveal signs of impaired immune responses like opportunistic infections [26], even in end stage liver disease, indicating that chronic infection is not caused by a general block of immune responses, but rather by a failure to respond specifically to HCV.

Two recent studies show largely similar frequencies of pDCs in untreated chronic HCV patients versus healthy donors but discrepant results regarding IFN-α production by pDCs. One of these studies concludes that the antiviral therapy may be responsible for reduced IFN-α production [27,28].

In the light of these contradictory observations, we decided to compare the function of Mo-DCs and pDCs from healthy donors and chronic HCV patients. We found that HCV Mo-DCs show normal ability regarding maturation, allo-stimulation and cytokine secretion compared to those of healthy donors. Similarly, pDCs from healthy donors and chronic HCV patients show comparable cytokine production in response to unmethylated CG dinucleotide (CpG) stimulation. We conclude that both myeloid and plasmacytoid DCs are normal in chronic HCV patients, suggesting that other reasons have to be considered to explain the frequent failure of the immune response to eliminate HCV.

2. Materials and methods

2.1. Patients and healthy donors

Table 1 shows the principal clinical features of the patients involved in this study, followed at the Gastroenterology and Hepatology Unit, Azienda

Table 1
Principal features of patients chronically infected with HCV

Patient.	Sex	Age (year)	Genotype	Viral load (IU/ml)	ALT (GPT) (U/l)	Histology grade	Histology stage
1	M	58	1b	4.32×10^5	185	A7	F1
2	F	36	1b	1.14×10^6	53	A11	F3
3	M	40	1a	2.53×10^5	32	A6	F0
4	M	51	1b	8.24×10^5	100	A9	F6
5	F	61	1b	4.54×10^5	72	A5	F0
6	M	42	1b	4.54×10^5	51	A6	F1
7	M	40	1b	4.76×10^5	73	A7	F2
8	M	67	2a/c	4.90×10^5	144	A12	F1
9	F	54	1b	2.50×10	35	A11	F5
10	F	46	Unknown	5.00×10	67	Unknown	Unknown
11	M	71	1b	8.50×10^5	63	A2	F2
12	M	59	1b	5.00×10^6	63	A2	F2
13	M	70	1b	4.30×10^5	348	A3	F2
14	M	51	3a	9.60×10^5	70	A3	F3
15	M	41	3a	5.00×10^5	207	A3	F3
16	M	36	3a	Unknown	158	A1	F2
17	M	61	1b	2.90×10^5	48	A3	F4
18	M	36	4c	1.07×10^5	285	A2	F1
19	M	29	3a	2.65×10^6	124	A2	F1
20	M	60	1b	2.50×10^5	84	A3	F3

Patients 1–10 are used to obtain Mo-DCs. Patients 11–20 are used to analyse pDC function. None of the patients had undergone antiviral treatment. ALT (GPT) indicates alanine amino transferase. A indicates inflammation activity score (maximum 18). F indicates stage of fibrosis (maximum 6).

Ospedaliera Universitaria Pisana, Pisa and at the Institute of Internal Medicine, University of Trieste, School of Medicine, Trieste. All patients are HCV positive (anti-HCV Ab and HCV-RNA positive), HIV/HBV negative with chronic hepatitis and not previously treated with interferon therapy. Buffy coats from healthy donors tested serologically negative for HIV, HBV, HCV, were obtained courtesy of Blood Transfusion Section, Alta Val D'Elsa Hospital, Poggibonsi. Informed consent was obtained prior to all blood donations. The study protocol was approved by the institute's ethical committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Cell preparations and cultures

PBMCs were obtained from patient blood and healthy donor buffy coats by Ficoll Hypaque (Amersham Biosciences, Uppsala, Sweden) density gradient centrifugation, according to standard protocols. Immature Mo-DCs were generated as described [5]. Briefly, highly purified (>98%) monocyte population was obtained from PBMCs by MACS® (Miltenyi Biotec, Bergisch Gladbach, Germany), using a one step positive selection of CD14+ cells with anti-CD14 coated magnetic microbeads (Miltenyi Biotec). Monocytes were then cultured using six well cell culture cluster (Costar®, Corning, New York, USA) for 6 days in RPMI-1640 medium (Invitrogen Life Technologies, Carlsbad, California, USA), supplemented with 10% of Fetal Calf Serum (FCS) (Hyclone, Logan, Utah, USA), 1% Penicillin–Streptomycin–Glutamine solution (PSG) (Invitrogen Life technologies), IL-4 (10% supernatant from IL-4 secreting cell line, kindly provided by A. Lanzavecchia, Institute for Research in Biomedicine, Bellinzona, Switzerland) and 50 ng/ml of GM-CSF (Leucomax®, Novartis, Basel, Switzerland). Immature DCs were washed and cultured for the experiments in RPMI-1640 medium supplemented with 10% FCS, 1% PSG, using 96 well U-bottom cell culture cluster (Costar®). To analyse pDC function, total PBMCs were cultured as described for immature Mo-DCs, and pDCs were identified by BDCA-2 expression.

2.3. Flow cytometry

Surface and intracellular staining of cells was performed according to standard protocols using mouse monoclonal anti-CD86-FITC or -PE, anti-CD83-FITC or -PE, anti-HLA-DR-FITC or -PE (Becton Dickinson, San Jose, California, USA), anti-BDCA-2-PE (Miltenyi Biotec), anti-TNF-α-APC (Becton Dickinson), and anti-IFN-α-FITC (ImmunoKontact, Abingdon Oxon, UK). Flow cytometric analyses were performed using a FACSCalibur™ cytometer (Becton Dickinson).

2.4. MLR

Allo-stimulatory capacity of immature DCs was evaluated through a standard MLR. Briefly, irradiated and washed DCs (Stimulator cells) were mixed with allogeneic PBMCs from healthy donors (Responder cells) in an increasing Stimulator/Responder ratio and cultured for 5–7 days. The cell cultures were pulsed with 1–2 μCi of [^{3}H] thymidine (Amersham Biosciences) during the last 16–18 h. The incorporation of [^{3}H] thymidine in proliferating cells was measured by a liquid scintillation counter (Packard, Palo Alto, California, USA).

2.5. Measurement of cytokine production

TNF-α production in culture supernatants was quantified by specific standard sandwich ELISA, using capture B154.9 and biotinylated B154.7 mouse monoclonal antibodies, kindly provided by G. Trinchieri, Schering Plough Corporation, Lyon, France.

3. Results

Mo-DCs were derived from CD14+ monocytes purified from PBMCs of healthy donors and chronic HCV infected individuals as described in Section 2. Mo-DCs from the two groups of people were routinely controlled for differentiation and were similarly positive for CD1a, negative for CD14 and displayed the same morphology in light microscopy (data not shown).

A hallmark of DC maturation is the up-regulation of maturation markers in response to a variety of endogenous or pathogen-derived stimuli. Therefore, we assessed the expression of HLA-DR, B7.2 (CD86) and CD83 in response to LPS. After culture with LPS for 48 h, Mo-DCs from chronic HCV patients up-regulate B7.2 (CD86), CD83 and HLA-DR similarly to Mo-DCs from healthy donors (Fig. 1). Fig. 1(A) shows the average of mean fluorescence intensity (MFI) values of Mo-DC maturation markers before and after LPS stimulation, from two groups of 10 healthy donors and 10 chronic HCV infected patients. In Fig. 1(B), representative results from an individual healthy donor and a chronic HCV patient are shown. Flow cytometry histograms show expression levels of the indicated molecules before (thin line) and after (bold line) LPS stimulation. A principal feature of DCs is to prime T cell activation. To assess directly this function, we used immature Mo-DCs as stimulators in a MLR assay. Fig. 2(A) compares the average stimulation indices (SIs) of Mo-DCs from 10 healthy donors (white bar) and 10 HCV infected patients (black bar), at a Stimulator/Responder (S/R) ratio of 1/20. In Fig. 2(B), a representative experiment is shown, performed with Mo-DCs from a healthy donor and an HCV infected patient at the indicated S/R ratios. In Fig. 2(C), the thymidine incorporation in MLRs using donor or patient DCs are compared. Our data clearly indicate that Mo-DCs from HCV infected patients stimulate allogeneic T cells as well as Mo-DCs from healthy donors.

TNF-α is a pro-inflammatory cytokine with pleiotropic activity and a central role in the innate immune response. Therefore, we measured by ELISA the secretion of this cytokine by Mo-DCs in response to LPS. As shown in Fig. 3, HCV Mo-DCs produce similar amounts of TNF-α as compared with Mo-DCs from healthy individuals, and both groups display a similar degree of variability in the TNF-α response to pathogen stimulation.

Another important DC subset involved in innate immune responses are pDCs, identified by the specific expression of BDCA-2 [29] which secrete high amounts of TNF-α and are the principal producer of the antiviral cytokine IFN-α. To assess the function of pDCs in HCV patients, total PBMCs were stimulated with CpGs and production of TNF-α and IFN-α by BDCA-2+ pDCs was analyzed by intracellular staining. Fig. 4 shows the TNF-α and IFN-α production by pDCs from healthy donors and chronic HCV patients. In Fig. 4(A), the average percentage of TNF-α and IFN-α positive pDCs from groups of 10 patients and 24 donors is shown, while in Fig. 4(B) and (C), production of TNF-α and IFN-α by pDCs from a healthy donor (B) and a chronic HCV patient (C) are compared. Our data demonstrate that cytokine production by pDCs from chronic HCV patients is similar to that of healthy donors.

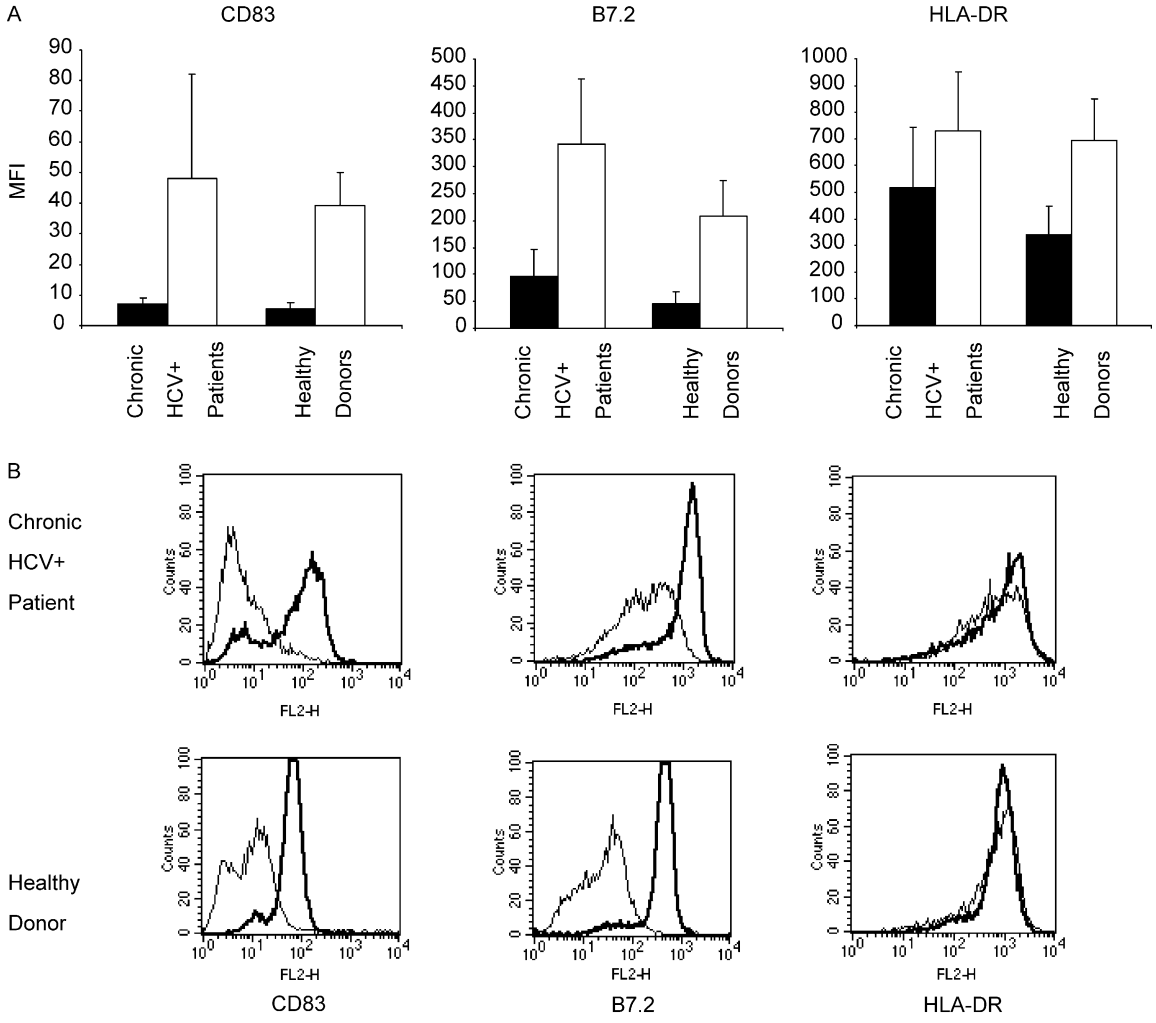

Fig. 1. Chronic HCV patient and healthy donor Mo-DCs respond similarly to LPS by up-regulation of maturation markers. A, average of MFI ± SD of B7.2 (CD86), CD83, HLA-DR maturation markers with (white bar) or without (black bar) LPS stimulation in HCV patient and healthy donor Mo-DCs. B, comparison, as representative example, of B7.2 (CD86), CD83, HLA-DR fluorescence histograms before (thin line) and after (bold line) LPS stimulation, between a healthy donor and a HCV patient Mo-DCs. Cells from 10 different healthy donors and 10 HCV patients were assessed. LPS was used at 100 ng/ml.

4. Discussion

HCV frequently establishes a chronic infection, clearly indicating the inefficiency of the immune response to eliminate the virus. Among the studies focused on the causes of this failure, some groups proposed defective functions of HCV patient Mo-DCs [17–20]. In order to re-evaluate and extend these findings, we compared the function of both Mo-DCs and pDCs from chronic HCV patients and healthy donors. Our study clearly demonstrates that DCs from the two groups of individuals display similar capacity to induce innate and adaptive immune responses.

Our results are in agreement with a recent study which showed that Mo-DCs from chronic HCV patients undergo normal TNF-α dependent maturation and that immature and mature Mo-DCs from healthy donors and chronic HCV patients display a similar ability to stimulate allogeneic and influenza-specific autologous T cells [16]. We extend these findings to LPS induced DC maturation, showing comparable T cell allo-stimulation and up-regulation of all maturation markers studied. In addition, we evaluated the ability of Mo-DCs to induce the innate immune response, using the production of TNF-α in response to LPS as functional readout. TNF-α is the major cytokine produced by Mo-DCs and has a pro-inflammatory activity with a central role in innate immune response [30]. Our results show that Mo-DCs from patients and donors respond to the pathogen stimulus with a comparable TNF-α secretion and similar degree of variability. A number of studies showed that Mo-DCs from HCV patients have impaired ability to induce T cell responses compared to that of healthy donor DCs, in terms of proliferation or IFN-γ secretion by allogeneic PBMCs or by autologous or allogeneic CD4+ T cells [17–20]. These divergences cannot be accounted for

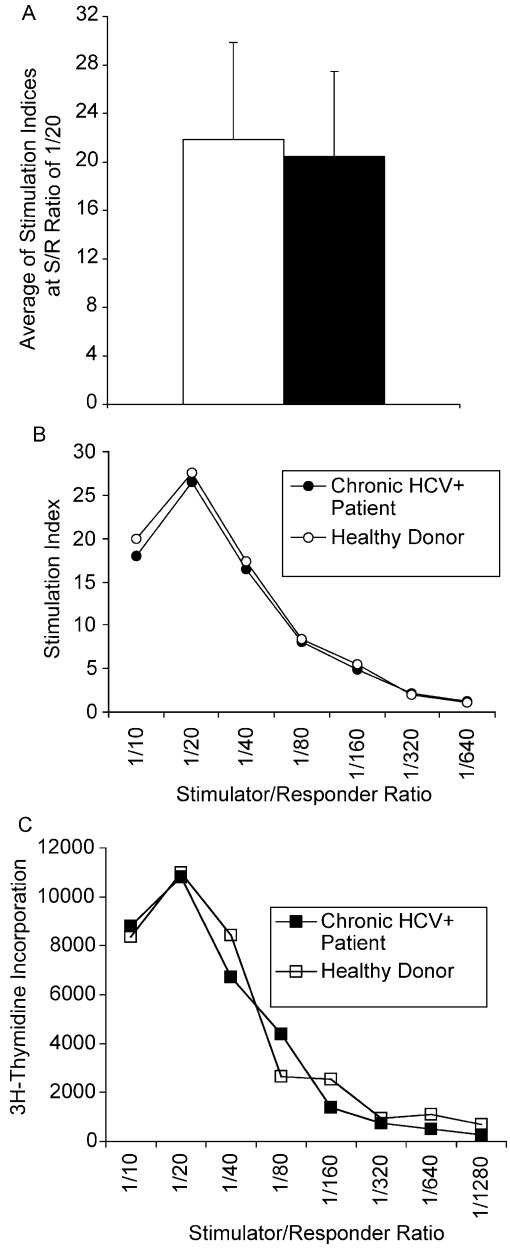

Fig. 2. Chronic HCV patient and healthy donor Mo-DCs have comparable allostimulatory capacity. (A) Average of stimulation indices (SIs) ± SD in MLR at the Stimulator/Responder ratio of 1/20, using as stimulator cells immature Mo-DCs from healthy donors (white bar) or from HCV chronic patients (black bar). Allogeneic PBMCs are used as responder cells. 10 different healthy donors and HCV patients were analyzed. (B) Representative example of SIs at different Stimulator/Responder ratios, in two MLRs performed using healthy donor (white circle) or HCV patient (black circle) Mo-DCs as stimulator cells. (C) Thymidine incorporation in two MLRs with donor (white square) and HCV patient (black square) Mo-DCs as stimulator cells. SI is the ratio of cpm between samples with stimulator plus responder cells and samples with responder cells alone.

by any apparent difference between the patient groups (Table 1). As suggested by Longman et al., slight differences in the culture conditions may affect the efficiency of DC differentiation and effector function.

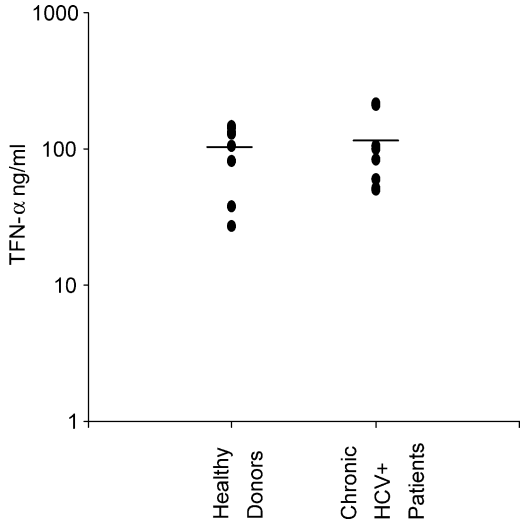

Fig. 3. Chronic HCV patient and healthy donor Mo-DCs produce TNF-α with similar efficiency. TNF-α quantified by sandwich ELISA in culture supernatant of healthy donor and chronic HCV patient Mo-DCs stimulated with LPS at 100 ng/ml. We used 10 different healthy donors and HCV patients. $P > 0.3$ (Student's *t*-test).

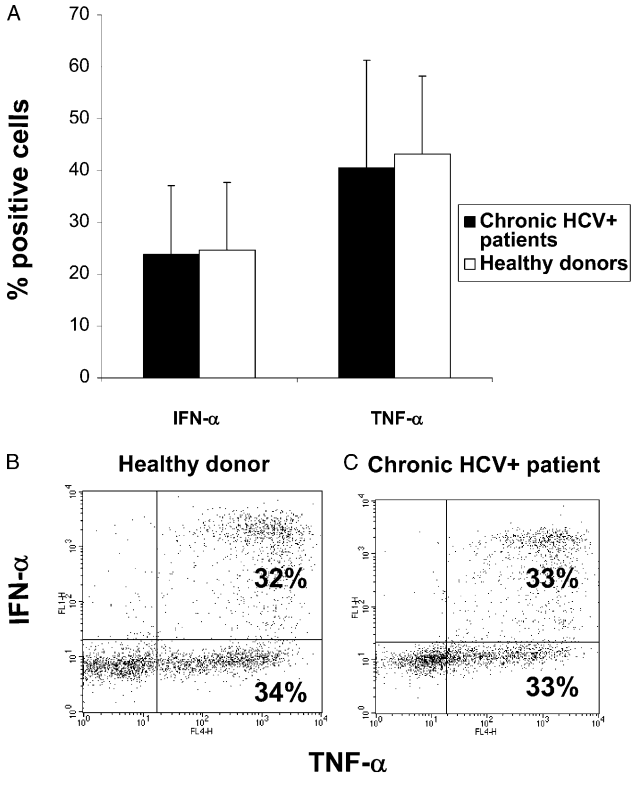

Fig. 4. pDCs from chronic HCV patients and healthy donors show comparable TNF-α and IFN-α production. (A) Average ± SD of percentage of TNF-α and IFN-α positive pDCs, as revealed by intracellular staining, in 24 healthy donors (white bar) and 10 HCV chronic patients (black bar). (B) and (C) Dot plots of BDCA-2+ gated pDCs stained for TNF-α and IFN-α from a healthy donor (B) or a chronic HCV patient (C). PBMCs were incubated overnight with CpG 2216 at 10 μg/ml. Brefeldin was added after 4–6 h of stimulation at final concentration of 2 μg/ml.

While the experimental procedures in the above mentioned studies are very similar, different effects were observed and therefore, different explanations for the observed immuno-stimulatory defect of the HCV patient DCs were put forward. In fact, two studies concluded that immature DCs from HCV patients lost the co-stimulation capacity because of the reduced IL-12 basal transcription and CD86 expression [20], or the inability to mature in response to TNF-α [18]. However, examining carefully a number of maturation markers, neither we nor Longman et al., observed defects in maturation in response to either LPS or TNF-α [16]. Another study showed that patient and healthy donor DCs had comparable maturation capacity in response to TNF-α and suggested that the viral infection of DCs could be the principal cause of the DC impairment, finding HCV positive strand RNA of unique sequence variants in patient DC cultures [19]. However, discordant results about HCV infection and replication in PBMCs and consequently in monocytes were obtained in the past decade [23,24]. An explanation for the discrepancies could be that the findings of HCV RNA in PBMCs from HCV patients is due to passive absorption of the circulating virus to the cells [22]. In addition, no study reveals HCV protein expression in Mo-DCs from patients. Interestingly, Sarobe et al. showed that HCV patient Mo-DCs have normal capacity to stimulate T cells but become functionally impaired only upon expression of HCV core and E1 proteins induced by in vitro transfection with adenovirus. These results indicate that even if DCs or their precursors are infected by HCV in vivo, this infection is not able to induce intracellular expression of HCV structural proteins sufficient to cause DC dysfunction [21]. A more recent study proposed that the reduced immuno-stimulatory abilities of immature DCs are caused by autocrine action of IL-10 induced by the presence in culture of Core and NS3 HCV proteins [17]. It is unclear whether this encounter between maturing DCs and HCV proteins occurs in vivo, given the relatively high amounts of protein employed and the rather narrow time window during which modulation of DC function appears possible. In addition, the inhibitory effects of the HCV proteins are overcome upon DC maturation. It could be that DC dysfunction observed in HCV patients is only indirectly linked to the viral infection. In fact, a previous study found impaired DC allo-stimulatory capacity in the clinical state of HCC, in HCV or HBV patients, regardless of the type of viral infection [25]. However, a definitive explanation for the divergence between the results shown here or by Longman et al. and previously published data still has to be found.

In the light of the normal functional activity of Mo-DCs found in this study, we decided to investigate also cytokine production by pDCs. These cells have the unique feature to produce high amounts of IFN-α in response to endogenous and pathogen stimuli such as CpG [6]. IFN-α is the major antiviral cytokine with a primary role in the modulation of immune response [31]. Our results indicate for the first time that HCV patient pDCs have similar ability to produce IFN-α and TNF-α compared to healthy donor pDCs in response to CpGs, clearly indicating that the antiviral functions of pDCs are unaffected in HCV infected patients. Recently, a study that directly correlated the blood pDC count with IFN-α secreted by total PBMCs stimulated with Herpes Simplex Virus 1 (HSV-1), observed a comparable frequency of pDCs in PBMCs from healthy donors and untreated HCV patients [28]. Because HSV-1 is a potent stimulus for pDCs, these results reinforce our data, even if it is not possible to exclude indirect effects on IFN-α secretion due to HSV-1 stimulation of other cell populations. Another recently published work shows a minimal reduction in pDC frequency in PBMCs from untreated HCV patients, while a significant decrease in IFN-α production by pDCs stimulated with CpGs is observed [27]. At present, we have no explanation for this discrepancy, and in particular, the type of CpG used in the two studies is the same.

In conclusion, our study shows that both DC subsets display normal effector functions during chronic HCV infection. These and published [16] results are consistent with the observation that HCV infected patients have no signs of generally impaired immune response [26], which would be expected in the case of DC dysfunction.

Acknowledgements

Thanks to Nick Valiante and Ugo D'Oro for critical reading of the manuscript.

References

[1] Gordon S. Pattern recognition receptors: doubling up for the innate immune response. Cell 2002;111:927–930.
[2] Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18: 767–811.
[3] Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002;2:151–161.
[4] Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 1998;282:480–483.
[5] Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and down-regulated by tumor necrosis factor alpha. J Exp Med 1994;179: 1109–1118.
[6] Liu YJ, Kanzler H, Soumelis V, Gilliet M. Dendritic cell lineage, plasticity and cross-regulation. Nat Immunol 2001;2:585–589.
[7] Houghton M. Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM, editors. Virology. Philadelphia, PA: Lippincott; 1996. p. 1035–1058.
[8] Racanelli V, Rehermann B. Hepatitis C virus infection: when silence is deception. Trends Immunol 2003;24:456–464.
[9] Farci P, Shimoda A, Coiana A, Diaz G, Peddis G, Melpolder JC, et al. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science 2000;288:339–344.

[10] Erickson AL, Kimura Y, Igarashi S, Eichelberger J, Houghton M, Sidney J, et al. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity 2001;15:883–895.
[11] Crotta S, Stilla A, Wack A, D'Andrea A, Nuti S, D'Oro U, et al. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. J Exp Med 2002;195:35–41.
[12] Tseng CT, Klimpel GR. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. J Exp Med 2002;195:43–49.
[13] Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000;191: 1499–1512.
[14] Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395–1406.
[15] Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 2002;169:3447–3458.
[16] Longman RS, Talal AH, Jacobson IM, Albert ML, Rice CM. Presence of functional dendritic cells in patients chronically infected with hepatitis C virus. Blood 2004;103:1026–1029.
[17] Dolganiuc A, Kodys K, Kopasz A, Marshall C, Do T, Romics LJ, et al. Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. J Immunol 2003;170:5615–5624.
[18] Auffermann-Gretzinger S, Keeffe EB, Levy S. Impaired dendritic cell maturation in patients with chronic, but not resolved, hepatitis C virus infection. Blood 2001;97:3171–3176.
[19] Bain C, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspe G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C virus infection. Gastroenterology 2001;120:512–524.
[20] Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, et al. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol 1999;162:5584–5591.
[21] Sarobe P, Lasarte JJ, Casares N, Lopez-Diaz de Cerio A, Baixeras E, Labarga P, et al. Abnormal priming of CD4(+) T cells by dendritic cells expressing hepatitis C virus Core and E1 proteins. J Virol 2002; 76:5062–5070.
[22] Meier V, Mihm S, Braun Wietzke P, Ramadori G. HCV-RNA positivity in peripheral blood mononuclear cells of patients with chronic HCV infection: does it really mean viral replication? World J Gastroenterol 2001;7:228–234.
[23] Boisvert J, He XS, Cheung R, Keeffe EB, Wright T, Greenberg HB. Quantitative analysis of hepatitis C virus in peripheral blood and liver: replication detected only in liver. J Infect Dis 2001;184:827–835.
[24] Gong GZ, Lai LY, Jiang YF, He Y, Su XS. HCV replication in PBMC and its influence on interferon therapy. World J Gastroenterol 2003;9: 291–294.
[25] Kakumu S, Ito S, Ishikawa T, Mita Y, Tagaya T, Fukuzawa Y, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. J Gastroenterol Hepatol 2000;15:431–436.
[26] Abrignani S. Immune responses throughout hepatitis C virus (HCV) infection: HCV from the immune system point of view. Springer Semin Immunopathol 1997;19:47–55.
[27] Anthony DD, Yonkers NL, Post AB, Asaad R, Heinzel FP, Lederman MM, et al. Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. J Immunol 2004;172:4907–4916.
[28] Goutagny N, Vieux C, Decullier E, Ligeoix B, Epstein A, Trepo C, et al. Quantification and functional analysis of plasmacytoid dendritic cells in patients with chronic hepatitis C virus infection. J Infect Dis 2004;189:1646–1655.
[29] Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. J Exp Med 2001;194:1823–1834.
[30] Tracey KJ, Cerami A. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. Annu Rev Med 1994;45:491–503.
[31] Biron CA. Interferons alpha and beta as immune regulators—a new look. Immunity 2001;14:661–664.