ORIGINAL ARTICLE

Hepatitis C virus structural proteins do not prevent human dendritic cell maturation

Les protéines de structure du virus de l’hépatite C n’empêchent pas la maturation des cellules dendritiques

C. Thumann\textsuperscript{a,*}, E. Schvoerera, J.-D. Abraham\textsuperscript{b}, A. Bohbot\textsuperscript{c}, F. Stoll-Kellera, A.-M. Aubertind, M.-P. Kienye\textsuperscript{e}

\textsuperscript{a} Inserm U748, institut de virologie, université Louis-Pasteur, 3, rue Koeberlé, 67000 Strasbourg, France
\textsuperscript{b} FRE3009 CNRS/BIO-RAD, 1682, rue de la Valsière, 34184 Montpellier, France
\textsuperscript{c} Service d’onco-hématologie, HUS Hautepierre, 67100 Strasbourg, France
\textsuperscript{d} Inserm U778, 3, rue Koeberlé, 67000 Strasbourg, France
\textsuperscript{e} World Health Organization, Geneva, Switzerland

Summary

Aim. — Infection with hepatitis C virus (HCV) results in chronic hepatitis in more than 70% of cases. Alterations in the maturation of dendritic cells (DC) might play a role in the immune system’s inability to eliminate the virus, although viral factors that could be involved have not been identified. This study in vitro investigated whether HCV structural proteins affect maturation of monocyte-derived DC.

Methods. — HCV proteins (core, E1, E2) were expressed by transduction with recombinant adenoviruses of immature DC. The ability of these transduced DC to respond to a maturation stimulus was evaluated by measuring cell surface markers, allogenic lymphocyte stimulation and interleukin (IL)-12 production.

Results. — Expression of HCV structural proteins did not modify DC maturation in the presence of lipopolysaccharide, as determined by their phenotype and stimulatory functioning. IL-12 secretion was not affected by HCV protein expression in mature DC.

Conclusion. — Our results suggest that HCV structural proteins do not affect maturation of monocyte-derived DC by lipopolysaccharide. These findings are important for further studies to clarify the pathogenesis of chronic HCV infection and towards the rational design of cellular vaccine approaches for immunotherapy against hepatitis C.

© 2007 Elsevier Masson SAS. All rights reserved.

\textsuperscript{*} Corresponding author.
E-mail address: christine.thumann@viro-ulp.u-strasbg.fr (C. Thumann).

0399-8320/$ — see front matter © 2007 Elsevier Masson SAS. All rights reserved.
Résumé

Objectifs. — L’infection par le virus de l’hépatite C évolue dans la majorité des cas vers l’hépatite chronique. La persistance du virus pourrait être favorisée par une altération de la maturation des cellules dendritiques (CD) qui jouent un rôle essentiel dans le déclenchement de la réponse immunitaire. Les facteurs viraux impliqués dans ce dysfonctionnement des cellules dendritiques n’ont pas encore été identifiés. Le but de cette étude in vitro était d’évaluer si les protéines structurales du virus de l’hépatite C pouvaient modifier la maturation des cellules dendritiques.

Méthodes. — Nous avons exprimé core E1 et E2 dans les cellules dendritiques avec des adénovirus recombinant. Nous avons étudié la maturation des cellules en présence de lipopolysaccharide en analysant les marqueurs de surface, l’induction de la lymphoprolifération allogénique et la production d’IL-12.

Résultats. — L’expression des protéines de structure du virus de l’hépatite C dans les cellules dendritiques n’a pas empêché leur maturation en présence de lipopolysaccharide ni leur capacité à induire une lymphoprolifération. La sécrétion d’IL-12 n’a pas été modifiée.

Conclusion. — Ces données sont importantes pour la compréhension de la pathogénie de l’hépatite C chronique par le virus de l’hépatite C et pour la conception d’une immunothérapie vaccinale de l’hépatite C.

© 2007 Elsevier Masson SAS. All rights reserved.

Introduction

Among hepatitis C virus (HCV)-infected individuals, more than 70% develop chronic infection with a high risk of complications of chronic liver disease, such as cirrhosis and hepatocellular carcinoma. The mechanisms involved in viral persistence are still not completely understood. It appears that humoral responses are, in some cases, associated with recovery from acute infection [1] and that cellular responses play a crucial role: strong Th1 cytokine responses have been reported to protect against chronic infection, while Th2 responses may favor HCV persistence [2,3].

HCV could interfere with innate and adaptive immunity by infecting and/or modifying the functions of cells involved in immune responses. Extrahepatic sites of HCV infection have been reported and, among them, dendritic cells (DC) have been found to be involved [4]. DC play a key role in the induction of immune responses [5]. Immature DC (iDC) in vivo act as sentinels for antigen detection and capture. Inflammatory stimuli or bacterial products, such as lipopolysaccharide (LPS) lead to a change in cell physiology described as "maturation". During maturation, the expression of CD83, one of the best markers for mature DC (mDC), is induced. The mDC process antigens into peptides during migration to lymphoid areas and present them to T cells. At least two subsets of DC are described in human blood: myeloid DC and plasmacytoid DC. Since they are both sparsely found, alternative methods have been devised to study them, such as generation of DC in vitro from monocytes after incubation with cytokines [6]. Conflicting results were obtained for monocyte-derived DC (Mo-DC) functioning in chronically HCV-infected patients: initially, DC from infected individuals were found to have an impaired capacity to stimulate allogenic T cells [7], but recent investigations revealed no such differences [8,9]. Interestingly, Anthony et al. discovered an impairment of blood DC function that differs between HCV and HIV infection [10].

The HCV genome encodes a polyprotein precursor cleaved into structural (core, E1, E2, p7) and nonstructural (NS) proteins. Structural HCV proteins have been selected for the present study because they are suspected to have immunomodulatory functions. The modulation of T-lymphocyte proliferation responses by core has been described [11]. Envelope proteins, E1 and E2, interact with host immunity and mediate virus entry through cellular receptors [12]. However, the data for the effects of HCV structural proteins on DC functions are controversial [13,14]. Examination of DC function in the context of HCV-derived antigens is essential for understanding the immune response and its regulation in HCV infection, as well as for better defining vaccine approaches. Overall, published results suggest that vaccine formulation capable of priming both anti-envelope antibodies and broad cellular immune responses to the virus may be effective [15].

In the present work, nonreplicating adenovirus was chosen as an effective vector to transduce DC and to investigate whether structural HCV proteins have an impact on Mo-DC maturation. As only mature DC can correctly prime naïve T cells, we focused our study on the effects of HCV proteins on induction of DC maturation.

DC maturation can be induced by several agents, including LPS, CD40 ligand and tumor necrosis factor (TNF)-α. LPS was chosen to induce maturation as the first results demonstrating an impaired DC allostimulatory function in chronic hepatitis C infection were obtained with DC treated with LPS [7]. We observed that the HCV structural proteins core, E1 and E2, expressed in human DC, did not alter the phenotype nor induce functional modifications, such as allogeneic lymphocytic stimulation or cytokine production in the presence of LPS.

Materials and methods

Generation of human DC

Dendritic cells were differentiated from monocytes (Mo) obtained from healthy donors (Établissement français du Sang, Strasbourg). Monocytes were purified by elutriation of peripheral blood mononuclear cells (PBMC). Immature DC...
(iDC) were generated as previously described [6]. Briefly, the monocytes were maintained for six days in RPMI 1640 medium (Invitrogen) supplemented with 5% fetal-calf serum (FCS; Invitrogen), 100 ng/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF; PeproTech, USA) and 20 ng/ml of interleukin-4 (IL-4; PeproTech). Cells were again fed with fresh cytokines on days 1 and 4. On day 6, nonadherent iDC were analyzed by fluorescence-activated cell sorting (FACS).

To induce maturation, iDC were maintained for 48 h in the presence of one volume of RPMI medium containing 5% FCS plus 1 μg/ml of LPS (Sigma Aldrich, L’Isle d’Abeau) and one volume of monocyte-conditioned medium (MCM) corresponding to cytokine-enriched medium from a 6-day culture of monocytes [16]; mature DC were identified by FACS.

Characterization of DC

The expression of DC surface molecules was analyzed by monoclonal antibodies (mAb) able to recognize iDC and mDC. Cells (2 × 10^5) were incubated with mAb against CD14, CD1a, HLA-ABC, HLA-DR, CD40, CD80, CD83 and CD86 at 20 μl/10^5 cells, as recommended by the manufacturer. All mAb and isotype controls were PE-labeled (Pharmingen, BD Biosciences, USA). Washed cells were fixed in 1.5% paraformaldehyde (Merck, Darmstadt, Germany). Data were acquired for 5000 cells using a FACScan (BD Biosciences) and expression was analyzed on the gated population using CELL Quest Software (BD Biosciences). Histograms were drawn from dot plots; the ratio of the geometric mean between the marker-specific antibody and the geometric mean of the isotype controls was determined.

Construction, production and titration of recombinant adenovirus

All recombinant adenoviral genomes were generated as previously described [17].

Dendritic-cell transduction with adenovirus

The iDC were transduced with purified recombinant adeno- viruses encoding HCV genotype 1b core or CE1E2, while Ad/EGFP (enhanced green fluorescent protein), Ad/βgal, Ad/empty and noninfected cells were used as controls. Briefly, 1.25 μg of lipofectamin (Invitrogen Life Technologies) was added to 2 × 10^5 adenovirus infectious units for 15 min [18]. One million cells per milliliter were incubated with lipofectamin-pretreated adenovirus at a multiplicity of infection (moi) of 200 for 2 h at 37°C. Nonadsorbed virus was eliminated.

Cells were maintained in culture at 37°C for 48 h at 0.5 × 10^6 cells/ml in a 1:1 ratio of RPMI plus 5% FCS plus 1 μg/ml of LPS and MCM. In some experiments, transduced cells were maintained either in RPMI plus 5% FCS and MCM or in RPMI plus 5% FCS plus IL-4 plus GM-CSF and MCM. In the latter experiment, LPS was added at 48 h after transduction and maintained for a further 24 h.

Heterologous expression in DC

Cytometry

Expression of EGFP was determined by FACS 48 h after transduction with Ad/EGFP. Intracellular core and E2 proteins were detected after permeabilization of DC transduced with Ad/core and Ad/CE1E2, respectively. One million cells were incubated for 1 h with mouse anticore (7G12A8; C. Jolivet, BioMérieux, France) or anti-E2 H52 (J. Dubuisson, Lille, France) diluted to 1/100 in phosphate-buffered saline (PBS; 0.3% saponin). Cells were washed with PBS (0.1% saponin), and fluorescein isothiocyanate (FITC)-labeled anti-mouse antibody was added (Sigma Aldrich; 1/100; 1 h).

Western-blot analysis

At 48 h post-transduction, DC (2 × 10^6) was treated with 100 μl of lysis buffer. After protein migration and transfer, saturated membranes were incubated for 1 h with the primary mAb: mouse anti-GFP (Chemicon International, CA; 1/1000), mouse anticore 7G12A8 (1/1000), rabbit anti-E1 (anti-aa1905AYEVRISSQYHVTNDCaa207; 1/100) and mouse anti-E2 H52 (1/100). The biotinylated antimouse (1/1000) or antirabbit (1/1000) antibody (Sigma) was detected using a streptavidin–horseradish peroxidase complex (1/1000; Amersham Life Science, UK) revealed by chemiluminescence.

Mixed leukocyte reaction

DC stimulatory capacity was determined using a mixed leukocyte reaction (MLR); 1.5 × 10^5 lymphocytes per well were mixed in decreasing DC concentrations (1 × 10^5 to 1 × 10^2 cells per well) and transduced with various adenoviruses. Coculture was maintained for five days at 37°C, and cells pulsed with 1 μCi per well of [3H]-thymidine (Amersham Biosciences, TRK120; specific activity: 25 Ci/mmol) for 18 h. After three cycles of freezing and thawing, radioactive DNA, collected on filters (Skatron, OSI, Paris), was quantified using a scintillation counter (Betaplate, Pharmacia, France). This was performed in triplicate. DC cultured in the absence of stimulator cells (DC−) were used to determine background signalling.

Cytokines in cell supernatants

Levels of bioactive IL-12 were estimated by IL-12 Quantikine (R&D Systems, UK) in supernatants 48 h after transduction. The IL-12 threshold was 5.0 pg/ml. For IL-18 detection, we used the human IL-18 ELISA kit (MBL; R&D Systems) with a sensitivity of 12.5 pg/ml.

Results

DC phenotype

Elutriated monocytes were treated in vitro with GM-CSF and IL-4 to differentiate iDC. Immature DC were characterized by flow cytometry using a panel of mAb (Fig. 1a): cells lost CD14 and expressed high levels of CD1a. Expression of HLA-ABC, HLA-DR, CD40, CD80 and CD86 was low, with no
Figure 1  Immunophenotyping of dendritic cells by flow cytometry: (a) immature dendritic cells (iDC); (b) mature dendritic cells (mDC) obtained after 48 h of treatment of iDC with lipopolysaccharide (LPS). Cells were stained with PE-conjugated antibodies specific for the markers indicated (shaded areas) or an isotype control antibody (gray line). The value indicated is the ratio between the geometric means of the marker-specific antibody and the isotype controls. The results shown were obtained from a single experiment with a single donor and are representative of 10 similar experiments showing similar results.

Phénotype des cellules dendritiques par cytométrie de flux : (a) phénotype des CD immatures (iDC) ; (b) phénotype des CD matures (mDC) obtenues après 48 heures de traitement des iDC avec du LPS. Les cellules sont marquées avec des anticorps conjugés à la PE spécifique du marqueur indiqué (aires pleines) ou avec un anticorps du même isotype (trait gris). La valeur indiquée sur l’histogramme est le rapport entre la moyenne géométrique de l’anticorps spécifique et la moyenne géométrique de l’isotype. Les résultats ont été obtenus avec un même donneur et sont représentatifs de dix expériences ayant donné les mêmes résultats.
expression of CD83. The mDC were generated from iDC in the presence of 1 µg/ml of LPS for 48 h (Fig. 1b): CD1a was down-regulated, as indicated by the ratio between the geometric means for specific staining and the isotype controls (12.4 versus 106); and higher expressions of HLA-ABC (9.8 versus four) and HLA-DR (20.9 versus 9.7) were observed. Expression of CD80 and CD86 was increased (15.3 versus 5.5 and 154.9 versus 2.7, respectively), while CD83 specific of mDC was induced (seven versus one). Similar results (data not shown) were obtained when mDC were generated by incubation for 24 or 72 h (instead of 48 h; Fig. 1b) in the presence of 1 µg/ml of LPS.

Efficient adenovirus-mediated gene expression in DC

To evaluate the efficiency of transduction, DC were infected with Ad/EGFP and their maturation induced by LPS. Expression of EGFP was shown by FACS (data not shown): 75% of cells expressed EGFP 48 h after transduction and similar results were obtained after 72 h. EGFP expression was lower when cells were maintained without LPS for the first 48 h, then incubated with LPS for the next 24 h. Intracellular core protein was detected in 70% of cells transduced with Ad/core and treated for 48 h with LPS (data not shown). E2 was also detected intracellularly after transduction with Ad/CE1E2 (not shown). Western blot analysis with anti-EGFP, anticore (7G12H8) and anti-E2 (H52) antibodies demonstrated that EGFP (27 kDa), HCV core (21 kDa) and HCV E2 (68 kDa) were efficiently expressed in the transduced DC and maintained with LPS for 48 h (Fig. 2).

Immunophenotyping of transduced DC

To validate our system, we checked if maturation could be induced by adenovirus vector itself by measuring CD86 and CD83 expression under different conditions. When cells were maintained for 48 h in a complete medium (RPMI + FCS + MCM) without adenovirus and without LPS (Fig. 3a), we observed partial expression of CD86 and no expression of CD83, similar to the expression seen in the control iDC (Fig. 1a). The values in the figure indicate the ratios between the geometric means for specific staining and the isotype controls — in this case, 6.7 for CD86 and 1.3 for CD83. When cells were transduced with Ad/empty for 48 h, but not treated with LPS (Fig. 3b), we observed higher expression of CD86 and weak expression of CD83, indicating slight maturation due to the adenovirus vector, confirmed by the ratio values: 39 for CD86, and 2.6 for CD83. To control for HCV protein-induced maturation in the absence of LPS, we checked for CD86 and CD83 after transduction of iDC with Ad/core and Ad/CE1E2 (Fig. 3c and d), and found results similar to those shown for Ad/empty. Ratios obtained were 40 and 2.2 for CD86 and CD83, respectively, in Ad/core transduced with the same cultures of DC, and 32.6 and 2.6 for CD86 and CD83, respectively, when DC were transduced with Ad/CE1E2. To control for the capacity of DC used in these experiments to respond to a maturation stimulus, we transduced the same cultures of DC with Ad/empty in the presence of LPS for 48 h and obtained complete maturation of DC, as shown in Fig. 3e by the ratios for CD86 (90) and CD83 (10.3). We can conclude that maturation was not induced by either adenovirus infection or by the HCV proteins in the absence of LPS.

The expression of CD86 and CD83 was then determined with Ad/core- and Ad/empty-transduced DC in the presence of LPS for 72 h (Fig. 4a) or only 24 h (Fig. 4b) after transduction. The ratios of the geometric means for specific staining and the isotype controls (Fig. 4a) show that expression of CD86 and CD83 was similar between DC transduced with Ad/core or Ad/empty, or in noninfected controls in the presence of LPS. In several experiments, the addition of LPS was delayed until 48 h after transduction and maintained for a period of 24 h (Fig. 4b), when recombinant protein (shown with EGFP) was expressed. This resulted in ratios that were equivalent or slightly lower for CD86 and CD83 (Fig. 4b), but which were not significantly different from the ratios obtained in the presence of LPS for 72 h (Fig. 4a).

For all other surface markers — CD1a, HLA-ABC, HLA-DR, CD40 and CD86 — no differences were found whichever recombinant proteins were expressed (core, CE1E2 or non-transduced controls) and whatever the LPS treatment (data not shown).

These results suggest that CE1E2 did not alter DC maturation (shown by phenotype analysis) induced by the addition of LPS either immediately after transduction (Fig. 4a) or 48 h after transduction (Fig. 4b).
Figure 3 Immunophenotyping of transduced DC. Histograms showing surface expression of CD86 and CD83 (shaded areas) are overlaid with the corresponding isotype (gray line). Values represent the ratio between the geometric means of the marker-specific antibody and the isotype: (a) iDC maintained in complete medium for 48 h without LPS; (b) iDC transduced with Ad/empty in the absence of LPS; (c) iDC transduced without LPS with Ad/core or (d) Ad/CE1E2; (e) iDC transduced with Ad/empty in the presence of LPS for 48 h.
**Figure 4** Immunophenotyping of transduced DC in the presence of LPS: (a) after transduction with recombinant adenoviruses, iDC were maintained for 72 h in the presence of LPS and subsequently stained with PE-conjugated antibodies against CD86 and CD83; (b) after transduction, cells were maintained in the presence of IL-4 and GM-CSF for 48 h, then LPS was added for the following 24 h. Values represent the ratio between the geometric means of the marker-specific antibody and the isotype. The results shown were obtained from a single experiment with a single donor, and are representative of three similar experiments that produced similar results.

**Phénotype des CD transduites en présence de LPS : (a) après transduction, les iCD sont maintenues pendant 72 heures en présence de LPS ; (b) après transduction, les cellules sont maintenues en présence d'IL4 et de GM-CSF pendant 48 heures et le LPS est ajouté pour les 24 heures suivantes. Les valeurs indiquées sur l’histogramme représentent le rapport entre la moyenne géométrique de l’anticorps dirigé contre le marqueur spécifique et la moyenne géométrique de l’isotype. Les résultats sont obtenus avec un même donneur et sont représentatifs de trois expériences similaires ayant donné les mêmes résultats.**

**Functioning of transduced DC: stimulation of lymphocytic proliferation**

To test the allostimulatory property of mDC-expressing HCV proteins, the cells were used in an MLR assay after transduction and culture in the presence of LPS for 48 h (Fig. 5a). Proliferation according to the four ratios between lymphocytes and transduced DC were similar, whichever recombinant adenovirus was used. Both HCV core- and CE1E2-expressing DC were able to induce lymphocytic proliferation as well as the DC transduced with Ad/empty or the nontransduced DC (Fig. 5a). Results obtained with DC-expressing HCV structural proteins and those expressing other proteins, such as EGFP were not significantly different (not shown). Values obtained with transduced DC in the absence of lymphocytes (DC−) confirmed that DC alone do not proliferate.

HCV proteins expressed in the absence of LPS did not induce maturation of DC (Fig. 3d) and therefore, we did not assess their lymphoproliferation capacity. However, to determine whether maturation induced immediately after adsorption could mask a potential modification in these cells due to HCV proteins, we compared the proliferative response of lymphocytes either maintained with LPS immediately after adsorption for 72 h or incubated with transduced DC stimulated only 48 h after adsorption for a period of 24 h (Fig. 5b). Representative results indicate that proliferation was similar under both conditions and that DC-expressing HCV structural proteins showed no impairment in the ability to stimulate allogenic lymphocytic proliferation. This was observed from a lymphocyte-to-DC ratio of 150 selected according to previous proliferation results.

**Cytokine production**

IL-12 and IL-18 produced after transduction in supernatants of mDC were quantified by ELISA tests. Levels of
Figure 5  Stimulation of allogenic lymphocytic proliferation. Mixed leukocyte reaction (MLR): (a) proliferative response of lymphocytes to allogenic mature DC transduced with recombinant adenoviruses. Counts per minute (cpm) reflect cell proliferation at different ratios (1.5—1500) and are expressed as means ± S.D. of triplicate wells. Results are representative of experiments for which the same source of responder lymphocytes was used. DC−: transduced DC maintained in the absence of responder lymphocytes; (b) proliferative response of lymphocytes to allogenic DC transduced with recombinant adenovirus and maintained under different conditions: in the presence of either IL-4 plus GM-CSF for the first 48h followed by the addition of LPS for 24h or LPS for 72h. Results obtained had a ratio of Lyc/DC of 150 and are expressed as means ± S.D. of triplicate wells.

IL-12 produced by DC-expressing HCV or control proteins were similar after 48 or 72h of culture in the presence of LPS. When transduced cells were maintained without LPS, incomplete maturation was seen and the production of IL-12 tended to be lower than that seen in transduced DC treated with LPS (not shown). The concentration of IL-18 measured in culture supernatants was always lower than threshold.
Discussion

Chronic hepatitis develops despite an immune response in nearly 80% of HCV-infected patients. The inability of the immune response to prevent persistent infection could be due to functional modification of the cells (DC) involved in the induction of immune responses when infected by HCV.

Previous studies focused on the putative interactions of HCV with DC. First, DC infection by HCV was demonstrated both in vivo and in vitro [19,20]. Second, several immunological properties of monocyte-derived DC (Mo-DC) were shown to be affected in infected patients [21]. Interestingly, an altered capacity of Mo-DC to stimulate lymphocytic proliferation, as reported in chronic infection, was not found in individuals who were cleared of HCV infection after antiviral treatment [7]. Finally, published data concerning circulating DC in HCV patients showed a reduction of myeloid and plasmacytoid DC numbers, disabling their ability to polarize a T-cell response, secrete cytokines or induce T-cell proliferation [22—25]. However, the published results are conflicting [9]. Longman et al. described a normal functional capacity in circulating DC from chronically HCV-infected patients [8]. Such contradictory findings could be due to the use of different cell populations in patients (Mo-derived or circulating DC), different inducers of maturation or different procedures for DC culture.

We chose to work with Mo-DC because circulating DC represent only 0.2% of leukocytes. Moreover, 25% of monocytes are found to migrate to T-cell areas of lymph nodes and to differentiate into lymph-node DC [26]. Finally, it is now established that Mo-DC infected with recombinant viruses are effective in inducing viral antigen-specific T-cell responses [27].

Lipopolysaccharide is easy to standardize and so was used as the maturation inducer in our experiments. The initial results demonstrating an impaired DC allostimulatory function in chronic hepatitis C infection were obtained with DC treated with LPS [7]. Recent data reported a significant role of LPS in the pathogenesis of HCV infection, suggesting that the maturation stimulus has physiological importance in vivo [28]. Endotoxemia is described in HCV patients and its origin appears to be multifactorial [29]. We tested several concentrations of LPS (1—0.01 μg/ml) and analyzed the phenotypes of the cells 48 h later by FACS; the DC specific markers were expressed with the same profile as for LPS at 1 μg/ml — from 0.5 to 0.05 μg/ml. At lower concentrations, several markers were not expressed similarly. The results suggesting HCV effects on the maturation of DC isolated from chronically infected patients were obtained in the presence of LPS at 1 μg/ml [7], whereas data obtained with lower concentrations of LPS were conflicting [9,13]. For this reason, we decided to use 1 μg/ml to induce complete phenotypic maturation and to detect any impairment of DC functioning. However, other agents, such as CD40 ligand and TNF-α, will be evaluated for their effect on maturation of DC in the presence of HCV.

We expressed HCV structural proteins intracellularly by transduction of Mo-DC with recombinant adenoviruses. Transduction efficiency was increased with lipofectamin [18]. Neither the phenotype nor the functioning of DC treated with LPS was modified by the expression of HCV core protein, with or without E1 and E2. The kinetics of CD83 appearance on nontransduced DC showed expression as early as three hours after treatment with LPS (not shown). To investigate whether concomitant LPS treatment of DC and transduction may mask potential effects of HCV proteins, we performed several assays in which DC maturation was induced by adding LPS 48 h after transduction (when EGFP was expressed). Similar results were obtained regardless of the delay in LPS treatment: expression of HCV core with or without E1 and E2 proteins did not interfere with LPS-induced DC maturation or mDC functioning.

We also tested the effect of HCV structural proteins on cell maturation without LPS after transduction. Under these conditions, maturation of DC did not proceed to completion, whichever recombinant adenovirus was tested (Ad/core or Ad/CE1E2), as shown by CD86 (Figs. 3c and d). Our results are in agreement with those of Dolganiuc et al., showing that LPS-induced DC maturation restored the reduced allostimulatory capacity of HCV core- and nonstructural protein-3 (NS3)-treated Mo-DC, unlike observations in HCV-infected patients [13].

Other authors have reported in vitro results in which DC were transduced with adenoviruses expressing proteins, but using different experimental approaches [14]. Impaired T-cell function was observed only when stimulated with DC transduced in their immature state with adenovirus CE1. The authors could find no modification when transducing DC in their mature state. These latter results are concordant with our findings. In addition, Li et al., recently, showed that DC-expressing HCV NS3 or core proteins had a normal phenotype and were able to effectively stimulate allogenic T cells [30].

Several authors reported impaired DC functioning with HCV infection in vivo, by using either patients’ monocyte-derived DC obtained by culture [7] or blood DC [10]. Moreover, Tsubouchi et al. [25] were the first to establish a relationship between the detection of viral RNA and DC functioning in chronically HCV-infected patients. In patients who cleared HCV RNA from circulating DC in response to antiviral treatment, DC functioning, such as allostimulatory capacity or IL-12 production were increased compared with DC in the same patients before antiviral treatment, when HCV RNA was detectable in those cells. Our results suggest that the impaired Mo-DC functions observed in HCV-infected patients are not related to a direct effect of HCV structural proteins. Impaired DC functioning might be related to the effects of nonstructural proteins, a combination of structural and NS proteins or some other, indirect mechanisms, and merits further investigation.

In conclusion, our results may contribute towards the elucidation of HCV pathogenesis and may help towards the design of cellular vaccine approaches based on the use of structural proteins.

Acknowledgements

We are grateful to Colette Jolivet (CNRS, BioMérieux, Lyon, France) for providing 7G12A8 (anticore) monoclonal antibody and to Jean Dubuisson (Biology Institute, Lille, France) for providing H52 (anti-E2) monoclonal antibody; we thank Transgene S.A. (Strasbourg, France) for supplying shuttle
plasmids to generate recombinant adenoviruses. This work was supported by the Association pour la recherche contre le cancer (ARC) and by grants from BioMérieux.

References


