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The hepatitis C virus (HCV) induces a long-term increase in interleukin-10 production by human CD4+ T cells (H9).

Abstract

Patients with chronic hepatitis C present an imbalance of Th1/Th2 cytokine production. Therefore, we investigated whether the exposure of the CD4+ T cell line H9 to HCV could induce activation of cells through synthesis of IL-10. Three infection protocols were performed to enhance HCV propagation. Viral particles were prepared by ultracentrifugation of serum from patients. From 3 to 81 days

post-infection (p.i.), HCV-RNA was monitored both in supernatants and cells by nested RT-PCR, IL-10 protein in medium by ELISA, and IL-10 mRNA in cells by semi-quantitative RT-PCR. The expression of tetraspanins was analyzed by flow cytometry. The PKC signal pathway was studied using specific inhibitors. The H9 cells express CD81. HCV-RNA (+) was detected in cells until 21 days p.i, and in culture media over 39 days p.i. Up to day 81 p.i., HCV exposure induced a specific, 2-fold increase of IL-10 production by H9 cells. IL-10 production was inhibited by a PKC inhibitor (Calphostin C). This study shows that even if the infection of H9 T cells did not result in any viral progeny, HCV induced the activation of IL-10 secretion, which supports the role of IL-10 in HCV pathogenesis.

Full text

INTRODUCTION

The hepatitis C virus (HCV) is recognized as a member of the *Flaviviridae*, together with the pestiviruses and flaviviruses [1, 2]. Its genome is a positive, single strand RNA molecule with a length of 9.4 kb [3]. It includes two untranslated regions at the 5' and 3' ends, and a large open reading frame encoding a 3,000-amino acid polyprotein. This polyprotein is then cleaved into structural proteins (including the core protein and two envelope proteins, E1 and E2) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). HCV is the major aetiologic cause of non-A non-B hepatitis [4, 5] after transfusion. HCV infection frequently (60 to 80%) runs a chronic course with viral persistence and continued low-grade liver cell injury, which may lead to cirrhosis and hepatocellular carcinoma (HCC). The pathogenesis of HCV infection is not fully understood. It is most likely that both virus-related and immune-mediated mechanisms may play a pivotal role and contribute to HCV persistence. The virus may have found devices that can disturb immunoregulation in infected patients. Resolution of acute hepatitis C is generally associated with detection of neutralizing antibodies, a high frequency of circulating HCV-specific T cells with a predominant production of Th1 cytokines. In contrast, virus-specific T cell responses in the peripheral blood are usually undetectable during the acute phase of the disease and tend towards a predominant production of Th2 cytokines when HCV infection becomes chronic [6, 7]. Human $CD4^+$ T helper (Th) lymphocytes have predominantly immunoregulatory functions. Activation of this cell subset induces secretion of various cytokines which promote growth

and differentiation of antigen specific B cells and cytotoxic $CD8^+$ T lymphocytes and recruit inflammatory cells.

Among HCV components, core protein has been reported to have a pleiotropic function in the replication cycle of the virus, besides being a component of viral nucleocapsids. Several core-binding proteins were identified. Among them, cytoplasmic tails of cytokine receptors (TNF-alpha and lymphotoxin beta-receptors) [8-10] have been reported, suggesting the possibility that this viral protein has an immunomodulating function and may be involved in the mechanism of persistence and pathogenesis of HCV. The liver is the major organ for HCV replication and cellular injury. However, it has been suggested that peripheral blood mononuclear cells (PBMC) may be a possible site of extrahepatic replication of HCV [11-14]. PBMC could be a reservoir for HCV [15, 16].

Evidence of *in vitro* infection of three human T cell lines has been shown: MOLT-4 cells [17], HPB-Ma cells [18] and H9 cells [19]. H9 cells constitutively produce interleukin-10 (IL-10). Human IL-10 is produced by $CD4^+$ T helper cells (Th2-type cells) and regulates both lymphoid and myeloid cells. IL-10 inhibits Th1 (INF-gamma) production [20], T cell proliferation [21], and induces B cell activation [22]. Therefore, we studied the effects of H9 cell exposure to natural HCV particles on the production of IL-10. Even if no prolonged active viral replication was obtained, the induction of a specific long-term increased production of IL-10 by HCV was evident.

MATERIALS AND METHODS

Cell culture. The human lymphoma H9 T cell line consisted of 100% $CD4^+$ T cells as determined by FACS analysis after labeling with monoclonal specific-CD4 antibody (Immunotech SA). H9 T cells were grown in the presence of 5% CO_2 in complete medium. The complete medium consisted of RPMI 1640 medium (Life Technologies, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 1mM sodium pyruvate, 2 mM L-glutamine, 1X non-essential amino acids, and 100 U/ml penicillin, 100 mug/ml streptomycin, 1.5 mg/ml amphotericin B (Gibco BRL and Biological Industries). Cells were seeded at 0.3×10^6 cells/ml and subcultured every 3 to 4 days when they reached 1×10^6 cells/ml. Cell viability was assessed at each subculturing by measurement of blue trypan (0.4%, Sigma) exclusion.

HCV isolates. The HCV isolates used for the inoculation of H9 T cells were obtained from serum of 4 HCV chronically-infected patients: one *(Ver.)* was selected before transplantation, and three *(Cha., Le., Mal.)* showed recurrent HCV infection after liver transplantation (LT). All serum samples were genotype 1b, and contained > 10^6 copies HCV-RNA per ml, as determined by using the Amplicor HCV Monitor assay (Roche Diagnostics). They were clarified by low-speed centrifugation (3,000 x *g* for 20 min at 4° C). HCV-associated particles were concentrated (10-fold) by ultracentrifugation (210,000 x *g* for 4 hours). The final, viral particle-enriched pellet was resuspended in TN buffer (10 mM-Tris-HCl, pH7.2, 140 mM-NaCl) and immediately stored at - 80° C as aliquots of 100 mul. Two normal human sera (N1, N2) and one serum from an HCV-negative liver transplanted patient were used as specificity controls and were subjected to same centrifugation procedures. All the selected sera were negative for Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

HCV infection protocols. Three infection protocols (No. 1, 2 or 3) of H9 cells, which differ from each other as regards the medium changing conditions, were performed with the HCV inoculum *Cha.* in order to enhance viral propagation. For that, cells were seeded in 6-well plates with 6 x 10^6 cells/ml of complete medium (CM) per well. One hundred microliters of HCV inoculum *(Cha.)*, corresponding to about 10^5 copies of HCV-RNA, or 100 mul of CM as negative control, were added per well and first incubated for 2 hours at 37° C. The cells were then diluted 4-fold, and further incubated overnight. On day 0 (d0) post-infection (p.i.), they were again diluted 2-fold. On d1, three washings were done, and the cells were seeded at 0.3 x 10^6 cells/ml in 75 cm^3 flasks. The medium was changed twice a week (t.a.w.) for all protocols. All the medium was removed and replaced with fresh medium at each subculturing for protocol 1 (infection 1, control 1). From d4 p.i. for protocol 2 (infection 2, control 2), or from d11 p.i. for protocol 3 (infection 3, control 3), only half the medium was removed and replaced by fresh medium (50:50) at each subculturing. To confirm the specificity of effects observed after HCV exposure of H9 T cells, we performed additional experiments with three other HCV isolates *(Le, Mal, Ver)* and 4 different negative controls: complete medium (CM), 2 normal human sera (N1, N2), one serum from a liver transplanted patient (LT), 2 heat-inactivated (I) (10 min at 100° C) HCV isolates *(Le., Mal.)*. The infection protocol 2 was selected. Supernatants and cells were harvested every 3 or 4 days p.i. (at each subculturing) for HCV-RNA (+) detection by nested RT-PCR, and for IL-10 protein and mRNA analysis. HCV-

associated particles were pelleted from supernatants by ultracentrifugation (210,000 x *g* for 4 hours, 4° C).

HCV-RNA nested RT-PCR. RNA was extracted from 140 mul of supernatant or 100 mul of pelleted supernatant by using the QIAamp viral RNA kit (Qiagen GmBH, Germany). Total RNAs were extracted from 5 to 10 x 10^6 cells using the RNeasy mini-kit (Qiagen). Fifty microliters of RNA samples were stored at - 80° C. The Ready-To-Go RT-PCR Beads (Pharmacia Biotech, Uppsala, Sweden) were used to perform the reverse transcription (RT) and the first-round PCR. For that, 5 mul of total RNA and outer primers were used. Reverse transcription was performed for 30 min at 42° C, and then for 5 min at 92° C. Thirty five cycles, with an annealing temperature of 55° C were performed in an MJ Research Mini Cycler (from Prolabo). Three microliters of the first PCR product and inner primers were subjected to a second-round PCR using the Ready-To-Go PCR Beads (Pharmacia Biotech). All the primers were derived from the HCV core region. Outer primers were 5' CGC GCG ACT AGG AAG ACT TC 3' (480-499) and 5' ATG TAC CCC ATG AGG TCG GC 3' (732-751), and inner primers 5' AGG AAG ACT TCC GAG CGG TC 3' (489-508) and 5' CCG CAC GTA GGG TAT CGA TGA C 3' (705-727). The expected size of the amplification product was 238 bp. Amplification products (10 mul) were revealed by ethidium bromide (Gibco) staining after 2% agarose gel (Bioprobe) electrophoresis.

Single strand conformation polymorphism analysis (SSCP). SSCP was performed using the Multiphor II electrophoresis unit (Pharmacia/Biotech). Three microliters of the core PCR product were mixed with 7 mul of formamide (92%), denatured at 95° C for 10 min, and immediately chilled on ice. Electrophoresis was done for 1 hour (500 V) at 5° C. DNA bands were subsequently visualized by silver staining (Pharmacia). The SSCP analysis was applied to HCV isolates before inoculation, and to several samples collected at different times p.i. for the three infection protocols.

Sequencing of PCR products. Core PCR amplification products from samples collected at days 1 and 32 p.i. for the infection protocols 2 and 3, were subjected to direct sequencing by ESGS (Euro Sequence Gene Service, Groupe CYBERGEN, Les Ulis, France).

Interleukin-10 detection. Supernatants collected at different times p.i. from the three protocols were conserved at - 80° C, and then analysed for IL-10

protein using specific enzyme-linked immunosorbent assay (ELISA) from Immunotech. All samples were assayed in duplicate. Results are expressed as pg or ng/ml. The lower detection limit of ELISA for IL-10 was 20 pg/ml. The IL-10 gene expression was detected using semi-quantitative Human Multiplex PCR (MPCR) application kit from Genzyme (Maxim Biotech, Inc., San Francisco, USA). Th1/2 switch 2 kit included IL-2, IL-4, IFN-gamma and GAPDH gene measurement. The inclusion of GAPDH as a housekeeper gene allowed normalisation of gene expression therefore relative quantities of product could be deduced. The assay was performed according to the manufacturer's recommendations.

Signal transduction experiments. H9 cells exposed to HCV *(Le* or *Ver)* as well as control cells (N1, N2, LT and CM) were seeded at 0.9 x 10^6 cells in 3 ml of supplemented RPMI per well (6-well plate) and treated separately with signal transduction inhibitors from Calbiochem. These were W-7 (28 muM, calmodulin antagonist), EGTA (1 mM), H89 (100 nM, protein kinase A inhibitor), Calphostin C (100 nM, PKC inhibitor). After 40 hours of incubation, supernatants were tested for IL-10 protein.

Monoclonal antibodies and flow-cytometry analysis. The anti-tetraspanin mAbs Z81 (CD81) and Z53 (CD53) were kindly provided by Dr. F. Lanza, and the CD37 mAb (BL14) by Dr. J. Brochier. The CD9 mAb (SYB-1) and the anti-VLA5 mAb have been previously described [23]. The anti-VLA-4 mAb (HP2/1) was purchased from Coulter-Immunotech (Marseille, France). The cells were stained by indirect immunofluorescence and analyzed using a FACScalibur (Becton-Dickinson, San Jose, CA, USA).

RESULTS

Phenotype of cultured H9 T cells. Phenotyping of human H9 T cells revealed that they consisted of a clonal population (labelled cells = 100%) which expressed several molecules of the tetraspanin superfamily, including CD81 (HCV E2-binding protein, 24), CD82 and CD53 with a high MFI (331, 267 and 151, respectively, Table 1). A lower, but detectable expression of CD37 was observed, whereas CD9 was not expressed by these cells. The integrin alpha4 subunit (VLA-4), that strongly associates with CD81 [23] was also expressed, as well as VLA-5 which to date has not been demonstrated to associate with CD81. This finding supports a possible HCV-E2 ← › CD81 interaction required for viral binding and penetration. The expression of all these cell surface markers was

not found to be significantly modified in HCV-exposed H9 T cells (results not shown).

Detection of HCV-RNA in cell extracts and supernatants from H9 T cells exposed to HCV. *In vitro* HCV exposure of H9 T cells was performed according to three protocols (1, 2 and 3, respectively A, B and C in Figure 1), which differ from each other in terms of complete or partial (50:50) medium changing at each subculturing, to enhance virus contact with the cells and viral propagation. The medium was completely removed 2 t.a.w. throughout the protocol 1 (Figure 1A). Half the medium was removed as from day 4 (d4) for protocol 2 (Figure 1B) and as from day 11 (d11) for protocol 3 (Figure 1C). Culture medium and cells were harvested at days - 1 (before HCV exposure), 1+ (before washings after HCV exposure), 1- (after extensive washings), and thereafter every 3 or 4 days at each subculturing until day 81. Large volumes (> 5 ml) of each supernatant collected were subjected to ultracentrifugation for pelleting HCV-RNA-associated particles. RNAs were extracted from cells, supernatants and pellets from supernatants. HCV-RNA (+) was monitored by nested-PCR (core) in all samples. Adequate positive and negative controls were included.

In the case of the protocol 1 (Figure 1A, complete changing of medium at each subculturing), HCV-RNA(+) was not detected after day 4 (d4) post-infection (p.i.) in supernatants (Figure 1A, a) and intermittently until day 11 (d11) in cell extracts (Figure 1A, b). In the case of protocol 2 (Figure 1B, replacement of half the medium at each subculturing from d4 p.i.), HCV-RNA(+) was detected both in supernatants (Figure 1B, a) and in cell extracts (Figure 1B, b) until d14 p.i. In the case of the protocol 3 (Figure 1C, replacement of half medium at each subculturing from d11 p.i.), HCV-RNA(+) was detected intermittently until to d14 p.i. in supernatants (Figure 1C, a) and until d21 in cell extracts (Figure 1C, b). When supernatants collected at days 32 and 39 in protocols 2 and 3, were subjected to ultracentrifugation, HCV-RNA(+) could be detected in the resulting pellet. We observed that partial replacement of medium (from d4 or d11) seems to result in better HCV propagation. However, it was not possible to reliably detect HCV-RNA negative strand (-) as a replication intermediate.

SSCP and sequence analysis from PCR products. Since the negative strand PCR was not detected, SSCP and sequence analysis from PCR products was performed upon the amplified core region in order to test whether the major species of HCV differed in inoculum and in *in vitro* HCV-exposed H9 T cells. SSCP

analysis (Figure 2B) showed a different profile of core sequences in pelleted supernatants of HCV-exposed H9 T cells at d1 (lane 1) and d32 p.i. (lane 2). This difference was confirmed by direct sequencing of PCR products from inoculum (Cha.) (1, Figure 2A), and from sample collected at d32 from protocol 2 (2, Figure 2A). Sequence analysis showed that the core sequence from the HCV genome present in particles secreted by HCV-exposed H9 T cells differed in three nucleotides (A → G, G → A, C → A). Two mutations between the inoculum (Cha. d1) and sample at d39 from protocol 3 were also identified (data not shown). Sequence analysis was done in triplicate to avoid potential Taq polymerase errors, and all these mutations were observed in three, independent PCR experiments. It is worth noting that the core region is one of the most conserved region of HCV genome, in contrast to HVR1 sequences. So, these data from sequence analysis suggest that HCV could replicate at a very low level in the H9 T cells.

IL-10 production is persistently increased by HCV in H9 T cells. Control (no HCV exposure) from protocol 1 (all the medium replaced) produced about 19 ng of IL-10 per 10^6 cells (Figure 3A). Controls from protocols 2 and 3 (replacement of half the medium from d4 or d11, respectively), after 2 weeks for adaptation to different culture conditions, produced about 29 ng of IL-10/10^6 cells (Figure 3B and C), which corresponded to expected theoretical values (19 + 19:2 = 28.5 ng/10^6 cells). In the case of protocol 1 (Figure 3A), a non-significant transient increase of approximately 60% in IL-10 production was observed between d7 and d14. In contrast, in the case of protocols 2 and 3 (Figure 3B and C, respectively), a significant, 2-fold, stable increase in IL-10 protein secretion was found from d32 to d49 p.i. Between d49 and d81 p.i., the supernatants from HCV-exposed H9 cell cultures (protocols 2 and 3) and from their corresponding controls were harvested at each subculturing (2 t.i.w.) and assayed for IL-10 protein by ELISA. For both protocols (2 and 3), a significant ($p < 0.001$) increase in IL-10 production by the cells exposed to HCV was observed (58 ± 4 ng/10^6 cells instead of 30 ± 2 ng/10^6 cells for the corresponding controls). Throughout the experiments and whatever the conditions used, no changes were observed in cell viability that always exceeded 95 ± 3%, or in cell growth rate (0.3 x 10^6 cells/ml/day). This supports the significance of the increased IL-10 production in HCV-exposed cultures.

Using a semi-quantitative RT-PCR kit (from Genzyme) for the determination of Th1/Th2 cytokine mRNA levels, we did not find any significant modification in the

IL-10 mRNA expression between d1 and d49 in HCV-exposed cells (+) compared to their corresponding controls (-) for protocols 2 and 3. The Figure 4 shows the results obtained at d49, but similar results were obtained earlier.

To confirm the specificity of the effect of HCV on IL-10 protein production by H9 T cells, we performed 4 additional experiments with three distinct HCV inocula *(Mal, Ver, Le)* according to protocol 2. We included several kinds of negative controls: heat-inactivated (I) HCV inocula *(Le, Mal)*, two human normal sera (N1, N2), one serum from a liver transplanted patient negative for HCV (LT) and the complete medium (M). All the controls (M, N1, N2, LT and I corresponding to 2 out of 3 HCV inoculums) did not show any significant increase in IL-10 secretion by H9 T cells from day 7 to day 39 post-inoculation. In contrast, the three HCV (+) inocula tested, induced an enhancement of IL-10 secretion compared to their respective controls. In Figure 5, we have represented the results obtained for the *Mal* inoculum (HCV^+), the inactivated *Mal* inoculum (I), N1 (called N), LT and M, analyzed together in the same IL-10 assay. We observed that HCV specifically induced IL-10 production in H9 T cells up to 35 days post-exposure.

IL-10 protein production by H9 T cells involves the protein kinase C (PKC) signal transduction pathway. To evaluate the signal transduction pathway involved in IL-10 production by H9 T cells, the control and HCV-exposed cells were incubated with several signal transduction inhibitors in three separate experiments. As shown in the Figure 6, Calphostin C, a PKC inhibitor-like staurosporine, significantly inhibited IL-10 protein production by control and HCV + H9 T cells. The inhibition involved both basal and HCV-enhanced IL-10 secretion. W-7, an inhibitor of calcium/calmodulin-dependent phosphodiesterase, H89, a protein kinase A inhibitor, and the calcium-chelating agent EGTA had no effect on IL-10 protein production by H9 T cells exposed or not to HCV (data not shown). These findings suggest that the production of IL-10 by the $CD4^+$ H9 T cell line involves the PKC-dependent signal transduction pathway, in the absence of exogenous activation, as well as after activation by HCV.

DISCUSSION

In this study, we demonstrate that exposure of $CD4^+$ H9 T cells to HCV resulted in increased IL-10 production even under circumstances that did not result in detectable, productive infection. To enhance viral propagation in this culture

system, we used different infection protocols, with the aim of prolonging the contact of virus with the cells. For that, we partially replaced the medium at each subculturing from day 4 (protocol 2) or day 11 (protocol 3) after HCV exposure. We observed that under such experimental conditions, HCV-RNA (+) was detected for a longer period (up to day 21), both in supernatants and cells, than under classical conditions (up to day 7) where the medium was completely removed at each subculturing throughout the experiment (protocol 1). However, even optimum conditions (protocols 2 and 3), it was necessary to concentrate by ultracentrifugation, the viral particles present in the culture medium in order to detect HCV-RNA (+) by a sensitive method of nested-PCR from day 25 to day 39 post-inoculation. So, we were unable to detect an HCV-RNA (-) replicative intermediate. SSCP analysis and sequencing results revealed the presence of three mutations within the most conserved core region of the HCV genome from the pelleted viral particles recovered at day 32 after the contact of cells with the virus. In addition, the H9 T cells were subcultured every 3 or 4 days and thus were subjected to between 7 to 11 passages. This suggests that the H9 T cell line could transiently replicate HCV at a very low level. Infection becoming abortive with time, and negative strand PCR being undetectable within the cells, we could not confirm efficient HCV replication in the human lymphoid T cell line H9, as previously claimed by Nissen *et al.* [19]. Thus, this culture system cannot be used for studying the HCV life cycle.

However, we now report that HCV potently induces IL-10 production by H9 T cells in a specific and reproducible manner. The virus-induced enhancement of IL-10 secretion is specific since it disappeared after heat-inactivation of the inoculum. In addition, when HCV-negative preparations (material pelleted by ultracentrifugation) from sera of different origin (healthy donors or transplanted patients) were used as inocula, no positive effect was observed (Figure 5). The production of IL-10 remains enhanced (2-fold) for 2.5 months only after prolonged exposure (protocols 2 and 3) of H9 T cells to HCV. The effect exerted by HCV seems not to act on the transcription level, since no significant changes in IL-10 mRNA expression could be observed either immediately after virus exposure or later. This could correlate with the role played by the HCV core protein, known as a regulator of the host gene expression through its RNA-binding activity [24], at the translational level due to its location in the cytoplasm [25].

After all, the question remains: how could HCV up-regulate the release of IL-10 by the $CD4^+$ H9 T cells in the absence of efficient viral replication? HCV tropism is controversial, and the mechanisms of cell entry remain unknown. Nevertheless, the HCV envelope protein E2 was recently shown to bind human CD81 [26], a tetraspanin expressed on various cell types including hepatocytes, B and T lymphocytes [27]. A major feature of the tetraspanins is that they are engaged in multimolecular complexes containing other proteins such as beta1 integrins and MHC antigens at the cell surface. Here, we demonstrate that H9 T cells naturally express CD81 molecules, as well as other tetrapanins such as CD82, CD53 and CD37, with integrins (VLA-4 and VLA-5). It has been shown that CD81 is crucial to the induction of IL-4 and the development of Th2 responses *in vivo* [28]. It has been reported that stimulation of CD81 with anti-CD81 antibody caused cell adhesion, and that CD81 is a molecule involved in signal transduction [27, 29]. It could be thus expected that the E2 envelope protein of HCV may exert similar effects and that E2 interaction with CD81 could modulate T cell function through an increased IL-10 secretion. We did not observe visible changes by light microscopy. E2-CD81 interaction, if it took place, induced neither anti-proliferative effects nor cell aggregation. In this matter, over a 3-4 day period, both control and HCV-exposed cells proliferated 3-4-fold. The viability of cells exposed or not to HCV always exceeded 95% throughout the culture. This supports a biological significance of the increased IL-10 production in HCV-exposed cultures. One hypothesis is that binding of CD81 to E2 of HCV may stimulate endogenous production of IL-10. Unfortunately, it was not possible to test the effect of CD81-specific antibodies on the HCV-induced IL-10 secretion, since antibody binding itself resulted in activation of cells. We did however notice that heat-denatured $E2_{661}$ glycoprotein failed to bind CD81 [30], in addition heat-denaturation of the HCV inoculum suppressed the activation of H9 T cells. The cross-linking of CD81 by monoclonal antibodies has been reported to lead to calcium signalling. We therefore studied here the signalling pathway involved in the IL-10 production. Treatment of H9 cells with different specific inhibitors showed only significant inhibition after treatment with Calphostin-C. This inhibition indicates the PKC-dependent nature of the IL-10 secretion by H9 T cells. Finally, it could be suggested that E2 HCV interaction with CD81 might modulate T cell function. But, CD81 alone or with additional factors at the surface of H9 T cells are not sufficient to allow efficient entry and propagation of HCV *in vitro* in these cells. This confirms that other factors, truly specific to human hepatocytes *in vivo*, are required for productive virus replication [31].

IL-10, a cytokine produced by monocytes/macrophages, B lymphocytes, and mainly T helper type 2 (Th2) lymphocytes, is a powerful suppressor of cell-mediated immunity [21]. A shift to a Th-2 predominant profile has been suggested to occur in chronic hepatitis C [6, 7, 32]. Increased IL-10 serum levels were observed [33]. Patients with persistent viremia and chronic liver disease showed IL-10-specific responses to HCV core-derived peptides [34] or NS3 antigen [35]. Although greater numbers of type 2 cytokine-producing cells have been reported in patients with chronic hepatitis [7], the phenotype of cells in the peripheral blood has not been clearly determined. The results of our study using an *in vitro* cell culture system, support the notion that HCV could achieve IL-10-mediated immunoregulation by inducing endogenous production of the cytokine by human $CD4^+$ $CD81^+$ T cells. Interestingly, it has been recently shown that the therapeutic benefit of ribavirine in the treatment of chronic HCV infection lies in enhancing a type 1 and suppressing a type 2 cytokine response [36]. It has also been reported that the greater efficacy of combination therapy with interferon plus ribavirin may be related to its ability to suppress HCV-specific IL-10 production by T helper cells [37]. Finally, other findings support the importance and complexity of the role of IL-10 in chronic inflammation and fibrogenesis during HCV infections [38]. However, recombinant human IL-10 administration in patients with chronic hepatitis C infection showed that IL-10 has no antiviral effect (no change in serum HCV-RNA burden) [38, 39], indicating that IL-10 does not exert an inhibitory effect on HCV replication as previously reported for HIV replication [40]. In this context, the H9 T cell line may represent a useful experimental cell culture model for studying the effect of new therapies on T cell reactivity in terms of production of IL-10.

ABBREVIATIONS	
HCV : hepatitis C virus	CMV : cytomegalovirus
IL-10 : interleukin-10	HIV : human immunodeficiency virus
PBMC : peripheral blood mononuclear cell	HBV : hepatitis B virus
Th : T helper	d : day

HCC : hepatocellular carcinoma E : envelope NS : non structural TNF-alpha : tumor necrosis factor alpha IFN-gamma : interferon gamma LTbeta-R : lymphotoxin beta-receptor LT : liver transplantation TNB : tris-NaCl buffer EBV : Epstein-Barr virus	p.i. : post-infection t.a.w. : time a week CM : complete medium I : inactivated N : normal human serum RT-PCR : reverse transcription-polymerase chain reaction SSCP : single strand conformational polymorphism PKC : protein kinase C mAb : monoclonal antibody

CONCLUSION

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References

1. Miller R H, Purcell R H. 1990. Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as a members of two plant

virus supergroups. *Proc. Natl. Acad. Sci. USA* 87: 2057.

2. Major M E, Feinstone S M. 1997. The molecular virology of hepatitis C. (Review) *Hepatology* 25: 1527.

3. Choo Q L, Kuo G, Weiner A J, Overby L R, Bradley L W, Houghton M. 1989. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. *Science* 244: 359.

4. Alter H J, Purcell R H, Shih J W, Melpolder J C, Houghton M, Choo Q L, Kuo G. 1989. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N. Engl. J. Med.* 321: 1494.

5. Kuo G, Choo Q L, Alter H J, Gitnick G L, Redeker A G, Purcell R H, Miyamura T, Dienstag J L, Alter M J, Stevens C E, *et al.* 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 244: 362.

6. Cacciarelli T V, Martinez O M, Gish R G, Villanueva J C, Krams S M. 1996. Immunoregulatory cytokines in chronic hepatitis C virus infection: pre- and post-treatment with interferon-alfa. *Hepatology* 24: 6.

7. Tsai S L, Liaw Y F, Chen M H, Huang C Y, Kuo G C. 1997. Detection of type 2-like T helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 25: 449.

8. Chen C M, You L R, Hwang L H, Lee Y H. 1997. Direct interaction of hepatitis C virus core protein with the cellular lymphotoxin-beta receptor modulates the signal pathway of the lymphotoxin-beta receptor. *J. Virol.* 71: 9417.

9. Matsumoto M, Hsieh T Y, Zhu N, Van Arsdale T, Hwang S B, Jeng K S, Gorbalenya A E, Lo S Y, Ou J H, Ware C F, Lai M M. 1997. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin-beta receptor. *J. Virol.* 71: 1301.

10. Zhu N, Khoshnan A, Shneider R, Matsumoto M, Dennert G, Ware C, Lai M M. 1998. Hepatitis C virus core protein binds to the cytoplasmic domain of tumor

necrosis factor (TNF) receptor 1 and enhances TNF-induced apoptosis. *J. Virol.* 72: 3691.

11. Bouffard P, Hayashi P H, Acevedo R, Levy N, Zeldis J B. 1992. Hepatitis C virus is detected in a monocyte/macrophage subpopulation of peripheral blood mononuclear cells of infected patients. *J. Infect. Dis.* 166: 1276.

12. Müller H M, Pfaff E, Goeser T, Kallinowski B, Solbach C, Theilmann L. 1993. Peripheral blood leukocytes serve as a possible extrahepatic site for hepatitis virus replication. *J. Gen. Virol.* 74: 669.

13. Lerat H, Berby F, Trabaud M A, Vidalin O, Major M, Trépo C, Inchauspé G. 1996. Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. *J. Clin. Invest.* 97: 845.

14. Wang J T, Sheu J C, Lin J T, Wang T H, Chen D S. 1992. Detection of replicative form of hepatitis C virus RNA in
peripheral blood mononuclear cells. *J. Infect. Dis.* 166: 1167.

15. Féray C, Samuel D, Thiers V, Gigou M, Pichon F, Bismuth A, Reynes M, Maisonneuve P, Bismuth H, Bréchot C. 1992. Reinfection of liver graft by hepatitis C virus after liver transplantation. *J. Clin. Invest.* 89: 1361.

16. Brind A, Jiang J, Samuel D, Gigou M, Féray C, Bréchot C, Kremsdorf D. 1997. Evidence for selection of hepatitis B mutants after liver transplantation through peripheral blood mononuclear cell infection. *J. Hepatology* 26: 228.

17. Shimizu Y K, Iwamoto A, Hijikata M, Purcell R H, Yoshikura H. 1992. Evidence for *in vitro* replication of hepatitis C virus genome in a human T cell line. *Proc. Natl. Acad. Sci. USA* 89: 5477.

18. Shimizu Y K, Yoshikura H. 1995. *In vitro* systems for the detection of hepatitis C virus infection. *Viral hepatitis* 1: 59.

19. Nissen E, Höhne M, Schreier E. 1994. *In vitro* replication of hepatitis C virus in a human lymphoid cell line (H9). *J. Hepatol.* 20: 437.

20. Fiorentino D F, Zlotnick A, Vieira P, Mosmann T R, Howard M, Moore K W, O'Garra A. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.* 146: 3444.

21. de Waal Malefyt R, Haanen J, Spits H, Roncarolo M G, te Velde A T, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries J E. 1991. Interleukin-10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminushing the antigen-presenting capacity of monocytes *via* downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* 174: 915.

22. Itoh K, Hirohata S. 1995. The role of IL-10 in human B cell activation, proliferation, and differentiation. *J. Immunol.* 154: 4341.

23. Serru V, Le Naour F, Billard M, Azorsa D, Lanza F, Boucheix C, Rubinstein E. 1999. Selective tetraspan/integrin complexes (CD81/alpha4beta1, CD151/alpha3beta1) under conditions disrupting tetraspan interactions. *Biochem. J.* 340: 103.

24. Santolini E, Migliaccio G, La Monica N. 1994. Biosynthesis and biochemical properties of the hepatitis C virus core protein. *J. Virol.* 68: 3631.

25. Kim D W, Suzuki R, Harada T, Saito I, Miyamura T. 1994. *Trans*-suppression of gene expression by hepatitis C viral core protein. *Jpn J. Med. Sci. Biol.* 47: 211.

26. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner A J, Houghton M, Rosa D, Grandi G, Abrignani S. 1998. Binding of hepatitis C virus to CD81. *Science* 282: 938.

27. Levy S, Todd S C, Maecker H T. 1998. CD81 (TAPA-1): a molecule involved in signal transduction and cell adhesion in the immune system. (Review) *Annu. Rev. Immunol.* 16: 89.

28. Maecker H T, Do M S, Levy S. 1998. CD81 on B cells promotes interleukin 4 secretion and antibody production during T helper type 2 immune responses. *Proc. Natl. Acad. Sci. USA* 95: 2458.

29. Lagaudrière-Gesbert C, Le Naour F, Lebel-Binay S, Billard M, Lemichez E, Boquet P, Boucheix C, Conjeaud H, Rubinstein E. 1997. Functional analysis of four

tetraspans, CD9, CD53, CD81, and CD82, suggests a common role in costimulation, cell adhesion, and migration: only CD9 upregulates HB-EGF activity. *Cell Immunol.* 182: 105.

30. Flint M, Maidens C, Loomis-Price L D, Shotton C, Dubuisson J, Monk P, Higginbottom A, Levy S, McKeating J A. 1999. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. *J. Virol.* 73: 6235.

31. Rice C M. 1999. Is CD81 the key to hepatitis C virus entry? (Review) *Hepatology* 29: 990.

32. Kakumu S, Okumura A, Ishikawa T, Iwata K, Yano M, Yoshioka K. 1997. Production of interleukins-10 and 12 by peripheral blood mononuclear cells (PBMC) in chronic hepatitis C virus (HCV) infection. *Clin. Exp. Immunol.* 108: 138.

33. Piazzolla G, Tottorella C, Schiraldi O, Antonaci S. 2000. Relationship between interferon-gamma, interleukin-10, and interleukin-12 production in chronic hepatitis C and *in vitro* effects of interferon-alpha. *J. Clin. Immunol.* 20: 54.

34. Lechmann M, Woitas R P, Langhans B, Kaiser R, Ihlenfeldt H G, Jung G, Sauerbruch T, Spengler U. 1999. Decreased frequency of HCV core-specific peripheral blood mononuclear cells with type 1 cytokine secretion in chronic hepatitis C. *J. Hepatol.* 31: 971.

35. Eckels D D, Tabatabail N, Bian T H, Wang H, Muheisen S S, Rice C M, Yoshizawa K, Gill J. 1999. *In vitro* human Th cell responses to a recombinant hepatitis C virus antigen: failure in IL-2 production despite proliferation. *Hum. Immunol.* 60: 187.

36. Tam R C, Pai B, Bard J, Lim C, Averett D R, Phan U T, Milovanovic T. 1999. Ribavirin polarizes human T cell responses towards a type 1 cytokine profile. *J. Hepatol.* 30: 376.

37. Cramp M E, Rossol S, Chokshi S, Carucci P, Williams R, Naoumov N V. 2000. Hepatitis C virus-specific T cell reactivity during interferon and ribavirin treatment in chronic hepatitis C. *Gastroenterology* 118: 346.

38. Nelson D R, Lauwers G Y, Lau J Y, Davis G L. 2000. Interleukin-10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders. *Gastroenterology* 118: 655.

39. McHutchinson J G, Giannelli G, Nyberg L, Blatt L M, Waite K, Mischkot P, Pianko S, Conrad A, Grint P. 1999. A pilot study of daily subcutaneous interleukin-10 in patients with chronic hepatitis C infection. *J. Interferon Cytokine Res* 19: 1265.

40. Masood R, Lunardi-Iskandar Y, Moudgil T, Zhang Y, Law R E, Huang C L, Puri R K, Levine A M, Gill P S. 1994. IL-10 inhibits HIV-1 replication and is induced by *tat*. *Biochem. Biophys. Res. Comm.* 202: 374.

Figures

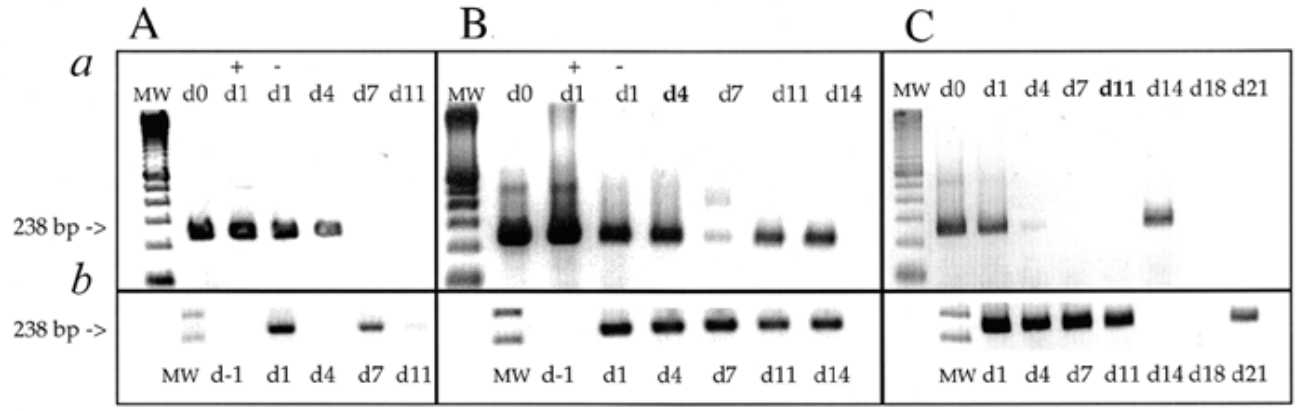


Figure 1

Detection of HCV-RNA positive-strand.

a: in supernatants and *b:* in cell extracts harvested from *in vitro* HCV-exposed H9 T cells at various times of culture, according to infection protocol 1 (A), 2 (B), or 3 (C). Day (d) - 1 = before HCV exposure, d1+ = after HCV exposure and before extensive washings, d1- = after HCV exposure and extensive washings (3-4 times). Nested RT-PCR and gel electrophoresis in 2% agarose. Arrows and the numbers on the left indicate the expected size of the amplification core PCR product. MW, molecular weight markers.

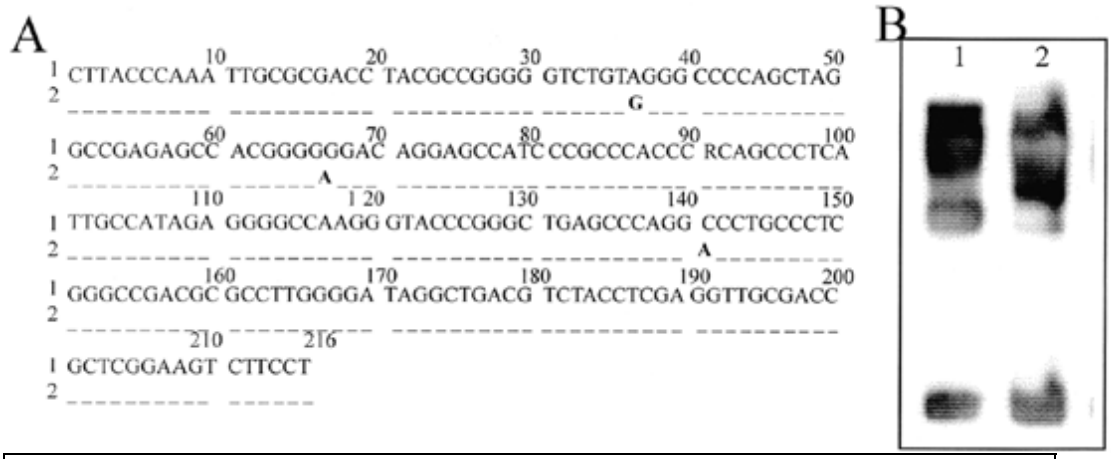


Figure 2

Sequence and SSCP analysis.

A: direct sequencing of the core region from the HCV genome in the pellet from supernatant harvested at day 1 (d1, sequence 1) and day 32 (d32, sequence 2) after *in vitro* HCV exposure according to protocol 2. This corresponds to three independent PCR experiments. **B:**

SSCP analysis of the pelleted supernatant at d1 (lane 1) and at d32 (lane 2).

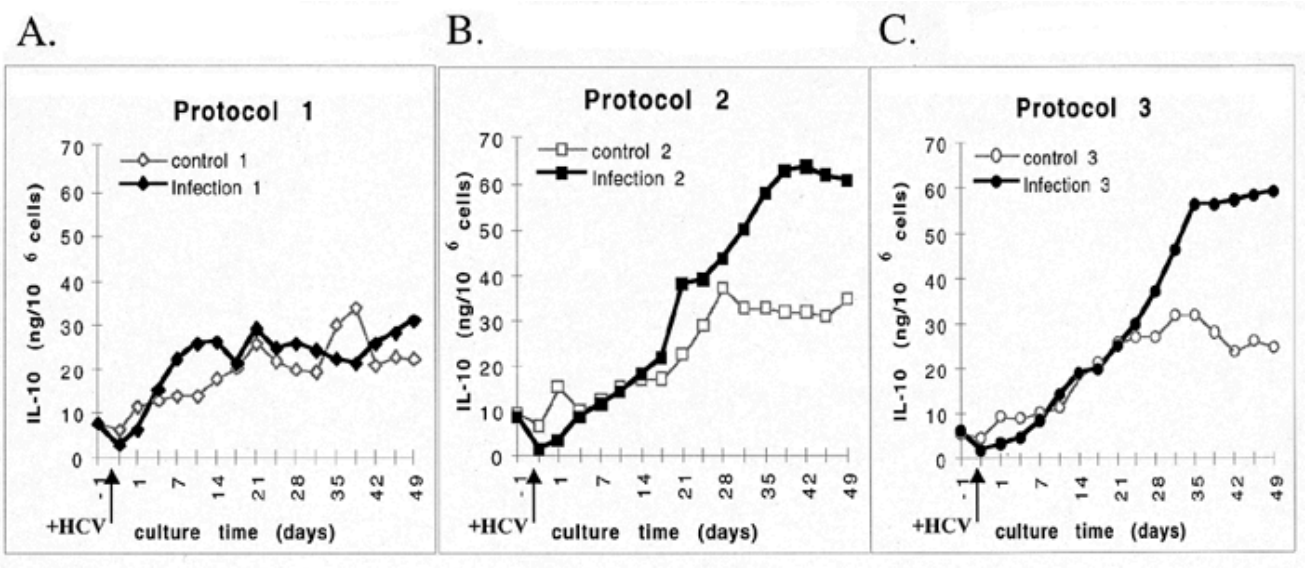


Figure 3

Kinetics of IL-10 secretion by control and *in vitro* HCV-exposed H9 T cells.

A: protocol 1. **B:** protocol 2 and **C:** protocol 3. All the medium was removed and replaced by fresh medium twice a week at each subculturing from d1 to the end of the protocol 1. Half the medium was removed and replaced by fresh medium twice a week at each subculturing from d4 for the protocol 2, and from d11 for the protocol 3 up to the end of experiments. ELISA assay of IL-10 protein was performed at each time, *i.e.* every 3 or 4 days. Representative data were obtained from 2 independent experiments.

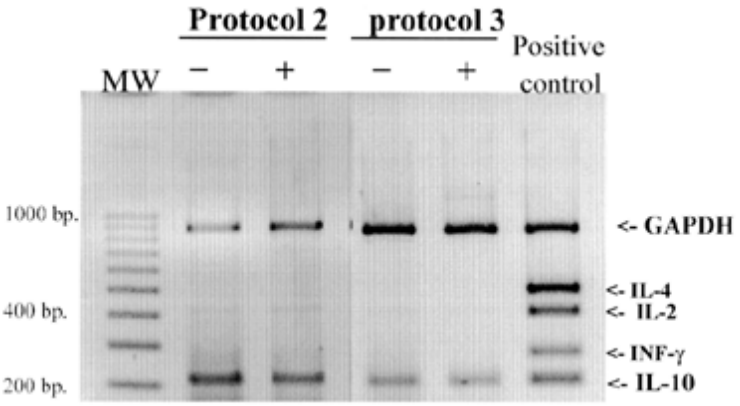


Figure 4

Detection of intracellular Th1/Th2 cytokine mRNA.

Results from semi-quantitative RT-PCR (Genzyme). (-) H9 cells not exposed to HCV, (+) *in vitro* HCV-exposed cells at d49 after protocols 2 and 3. MW, molecular weight markers.

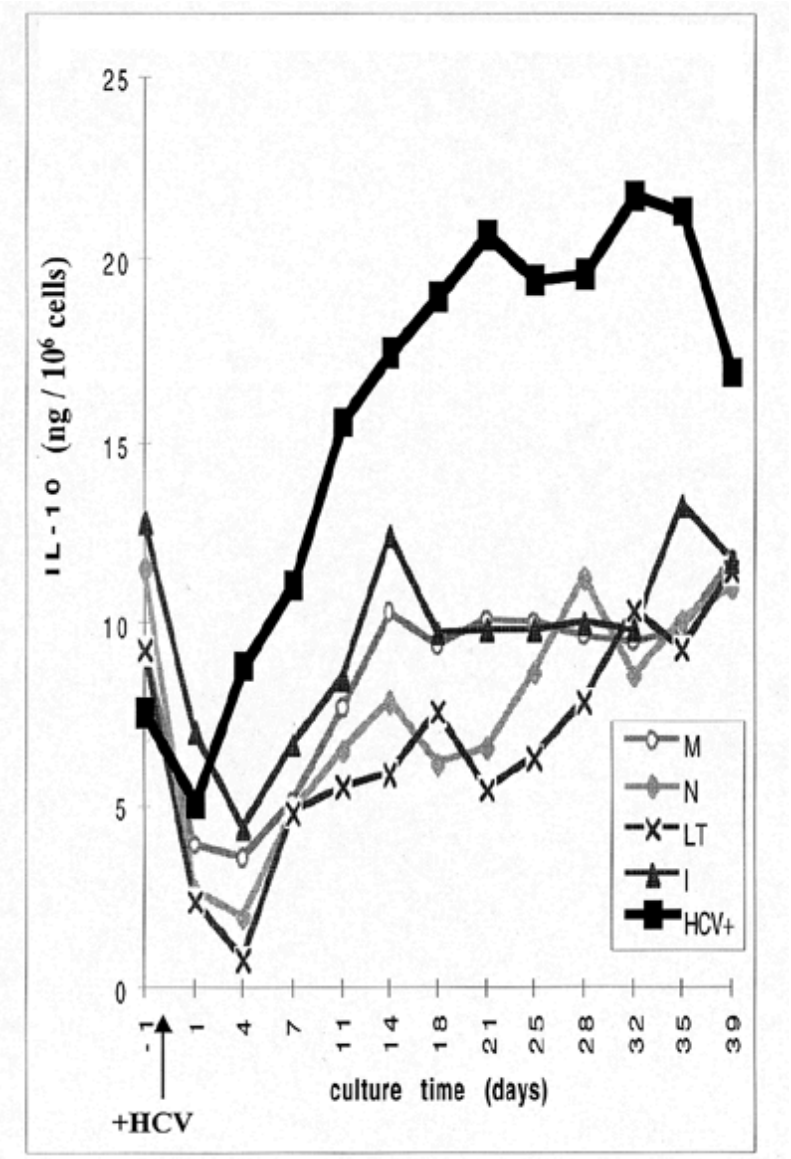

IL-10 (ng / 10^6 cells)
25
20
15
10
5
0
M
N
LT
I
HCV+
-1
1
4
7
11
14
18
21
25
28
32
35
39
culture time (days)
+HCV

Figure 5

Specific activation of H9 T cells by HCV.

The results presented here correspond to experiments performed according to protocol 2, using the *Mal* inoculum (HCV^+, heavy line), and four different negative controls: the inactivated *Mal* inoculum (I), one pelleted normal human serum (N1, called N), one pelleted serum from a liver transplanted patient negative for HCV (LT), and complete medium (M). The supernatants were collected at each subculturing, *i.e.* twice a week. Aliquots were performed and stored at - 80° C until the IL-10 assay. All the samples were analyzed in the same test.

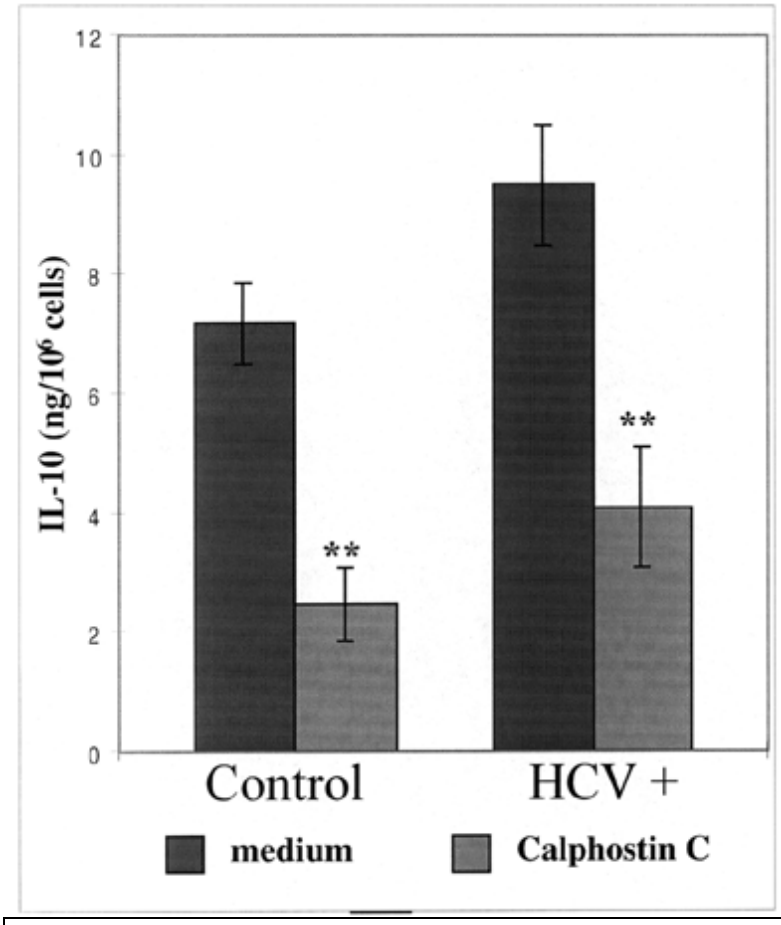


Figure 6

Signal transduction experiments.

These were performed between d49 and d76 p.i. using several inhibitors. Control and HCV exposed-H9 T cells were treated for 2 hours with medium or inhibitor alone. Inhibitors used were 100 nM H89, 28 muM W7, 1 mM EGTA, and 100 nM Calphostin C. IL-10 production was assayed by ELISA 40 hours later in the supernatants of H9 T cells (Control and HCV^+) from three different expriments. Statistical significance between samples not treated (medium) and treated with Calphostin C. ** $p < 0.01$.

Table 1
Detection of tetraspanins and integrins on the surface of H9 $CD4^+$ T cells by FACS analysis

Cell surface antigens	Mean fluorescence intensity (MFI)
CD81	331
CD82	267
CD53 tetraspanins	151
CD37	21
VLA-4 (alpha4 chain) integrins	87
VLA-5 (alpha5 chain)	23
Control	2.5

Authors

O. Delpuech, D. Buffello-Le Guillou, E. Rubinstein, C. Féray, M.A. Petit

INSERM E. 99-41, Centre Hépato-Biliaire, Hôpital Paul-Brousse, 12, avenue Paul-Vaillant Couturier, 94800 Villejuif, France.

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IL-10, recombinant, rat, cytokines.

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