Published in final edited form as:

Hepatology. 2019 May ; 69(5): 1873–1884. doi:10.1002/hep.29843.

HEPATITIS C VIRUS E2 ENVELOPE GLYCOPROTEIN INDUCES AN IMMUNOREGULATORY PHENOTYPE IN MACROPHAGES

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Abstract

A comprehensive strategy to control HCV infection needs a vaccine. Our phase I study with recombinant HCV E1/E2 EnvGPs as a candidate vaccine did not induce a strong immune response in volunteers. We analyzed the interactions of HCV EnvGPs with human monocyte derived macrophages as antigen presenting cells. HCV E2 induced immune regulatory cytokine IL-10 and sCD163 protein expression in macrophages from 7 of 9 blood donors tested. Further, HCV E2 enhanced Stat3 and suppressed Stat1 activation, reflecting macrophage polarization towards M2 phenotype. E2 associated macrophage polarization appeared to be dependent of its interaction with CD81 leading EGFR activation. Additionally, E2 suppressed the expression of C3 complement, similar to HCV exposed dendritic cells, implying potential impairment of immune cell priming.

Conclusion : Our results suggest that E2 EnvGP may not be an ideal candidate for HCV vaccine development, and discrete domains within E2 may prove to be more capable of eliciting a protective immune response.

Keywords

Hepatitis C virus; E2 envelope glycoprotein; antigen presenting cells; transcription factors; immunity

INTRODUCTION

Chronic HCV infection often causes liver disease progression, and may lead to cirrhosis and hepatocellular carcinoma (HCC). The largely asymptomatic nature of HCV infection facilitates continued transmission in at-risk groups and resource constrained settings due to limited surveillance. Less than 5% of the world's HCV-infected population are aware that they are infected (1). A major global research effort has illuminated many aspects of HCV life cycle facilitating the development of direct-acting antivirals (DAA) with a sustained virological response (2). However, cure of viremia by treatment with DAA does not prevent

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Potential conflict of interest: Nothing to report.

reinfection with the same or different HCV genotypes. Even those cured of HCV viremia remain at a significantly elevated risk for HCC (3, 4). Consequently, a comprehensive strategy to control HCV infection must include a viable vaccine (1).

A phase I safety and immunogenicity trial of a dose escalation placebo-controlled recombinant HCV E1/E2 adjuvanted with MF59C.1 was conducted previously in healthy HCV-negative adults (5, 6). A detectable IL-12 induction was not observed in any vaccine dose group and a range of 25–91% among all groups of subjects produced IL-4. Vaccinated subjects developed a weak lymphocyte proliferation response (2–4 fold higher) to E1/E2. Only ten of 36 vaccinated sera had a neutralization titer of 1:20 against cell culture-grown HCV. These results suggested that vaccination impaired induction of a strong protective immune response. HCV E2 EnvGP has been associated with many intriguing functions, including the presence of cross-protective virus neutralizing epitopes for HCV genotypes (7). Virus neutralizing sera had increased affinity levels and displayed >2-fold higher specific activity to well-characterized epitopes, especially to the hypervariable region 1 of E2, and the already characterized multiple genotype specific neutralizing epitopes of E2 (7, 8).

We have previously reported that dendritic cells (DCs) exposed to cell culture-grown HCV (HCVcc) reduced HLA-DR surface expression; negatively regulated CIITA, GAS, and HLA-DR promoters, and inhibited DC-mediated antigen-specific T-cell activation (9). We investigated the effect of HCVcc on IL-12 (p70) and IL-10 secretion by the use of maturation cocktail (MC) induced human monocyte-derived DCs. Induced DCs did not produce IL-12 (p70) in the presence of HCV, while IL-10 secretion was significantly increased. DCs upon incubation with MC increased levels of maturation markers CD40, CD80, and CD83, in contrast, incubation with HCV led to lower expression levels of these markers, reflecting a less mature phenotype. These results suggested that HCV E2 EnvGP may inhibit antigen presentation function and T-cell-stimulatory capacity of DCs.

Macrophages are the frontier soldiers of innate immunity. Macrophages sense a wide spectrum of stimuli, spanning across microbial antigens (10). Macrophages are activated in response to a stimulus, which allows them to combat pathogens and exert an immunomodulatory role (10). A model has been developed which describes the complex mechanism of macrophage activation as a polarization towards two opposite states, the M1 (classical) and the M2 (alternative) activation. M1 macrophages produce IL-1 β , IL-12, IL-23 and TNF- α , as well as reactive oxygen and nitrogen intermediates, support Th1 responses, and mediate resistance to intracellular pathogens (11). While, M2 activation is induced by immune complexes, complements, apoptotic cells, M-CSF, IL-4, IL-13, IL-10, and TGF- β . They are widely considered as anti-inflammatory, and benign opposites of the M1 activated macrophages (12). In this study, we have examined the regulatory role of HCV EnvGPs as candidate vaccine antigens for polarization and functions of human monocyte derived macrophages. We show that HCV E2 EnvGP promotes IL-10 and sCD163 expression, regulates the Stat signaling pathway, and increases Akt activation in monocyte derived macrophages for M2 polarization.

MATERIALS AND METHODS

Isolation of human monocytes and differentiation into macrophages

Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coats of healthy blood donors (Gulf Coast Regional Blood Center, Houston, TX) by Ficoll-paque™ PLUS (GE healthcare) density gradient centrifugation. Cells were washed, suspended in RPMI containing penicillin/streptomycin (1%), glutamate (1%) and seeded onto 6 well plate at 5×10^6 cells/ml. Non-adherent cells, were removed by gentle pipette aspiration after 3 h of incubation at 37°C. Cells were incubated in 20% fetal bovine serum containing RPMI medium for 5–7 days to promote differentiation of monocyte-derived macrophages. Cells were incubated with HCV EnvGP for 24h (unless mentioned otherwise), harvested after rinsing, and lysed in appropriate buffer for use.

Purified protein

Clinical-grade, highly purified C-termini truncated recombinant HCV glycoproteins (E1 sequence from amino acids 192–326, procured from Innogenetics, Belgium; E2 sequence from amino acids 383–715, obtained from Chiron Corporation, USA) were used. The proteins were expressed in mammalian cells and purified by multiple distinct chromatographic steps (13, 14). E1 or E2 dose dependent effects were seen at 0.25, 1, and 4 µg/ml in some of the initial experiments. A 2ug/ml of E1 or E2 was determined from these experiments to offer optimum response and used in rest of the experiments. Macrophages did not show signs of toxicity even at a higher dose of protein (4µg/ml) tested.

Chemokine/cytokine array

A RT-PCR cytokine/chemokine array (84 genes) was performed to analyze markers associated with macrophage polarity. RNAs derived from macrophage (untreated or HCV E2 treated for 16 hr) culture were converted to cDNA using universal primers and analyzed for cytokine/chemokine array following supplier's protocol (Qiagen).

Antibodies

Antibodies to Stat1, phospho-Stat1 (Ser727), phospho-Stat3 (Y705, Ser727), Ikb, Stat3, phospho Akt, C3, Socs3 and phospho-Raf (Ser338) (Cell Signaling Technology); p21 HRP-conjugated antibody, and actin horseradish peroxidase (HRP)-conjugated antibody (Santa Cruz Biotechnology, CA); C3b (AbCam); phospho-ERK and CD81 (BD Pharmingen) were used.

Western blotting

Cells were lysed in sample-reducing buffer and subjected to SDS-PAGE. Separated proteins were transferred onto a nitrocellulose membrane (Bio-Rad Laboratories). The blot was blocked with 5% skim milk and incubated overnight with primary antibody, followed by treatment with a secondary antibody conjugated with HRP (Bio-Rad Laboratories). The protein bands were detected with SuperSignal West Pico ECL reagents (Pierce).

ELISA

Cytokines were quantified in culture supernatant of E2 treated monocyte-derived macrophages after 24 h. The IL-10 and IL-12 levels were determined by Microvue ELISA kit (Life Technology) following manufacturer's instructions. A CD163 ELISA (R&D Systems) and C3 ELISA (AbCam) were performed using culture supernatants from control and E2 stimulated monocytes/macrophages following the supplier's protocol.

Statistical Analysis

Results were expressed as the mean $\pm$ standard deviation (SD) from at least three independent experiments. Statistical analyses were performed using a two-tailed unpaired Student t test. A p value of <0.05 was considered significant.

RESULTS

HCV E2 EnvGP promotes cytokine/chemokine secretion in monocyte derived macrophages for polarization toward M2 phenotype

The microenvironment provides macrophages with diverse signals that divergently bias the macrophage's phenotype toward "classically activated" (M1) or "alternatively activated" (M2) (15). Several novel signals induce or impede M1 or M2 activation. A RT-PCR cytokine/chemokine array was performed to analyze markers associated with macrophage polarity after incubation with purified E2 EnvGP (Fig. 1). While IL-12 and TNF- α (associated with M1) expression did not increase significantly (8-fold and 3-fold, respectively), IL-10 expression was increased greatly (2,680-fold) upon incubation with E2 (Fig. 1, panel A) as compared to untreated control macrophages. M1 associated polarization markers (CCL8-2.5 fold, CCL3- did not significantly change, CxCL10-1.8 fold were weakly upregulated upon exposure to E2. CCL13 is a marker of human M2-phenotype (16), and was significantly upregulated (>90 fold). Recent findings support that CXCL12 functions as an anti-inflammatory chemokine and may suppress antigen specific immune responses (17). CXCL12 was found to be upregulated (~ 30 fold). Significant increase in other markers associated with the M2 phenotype were noted (CCL1- 29 fold, CCL18- 39 fold, CCL 13-30 fold) (Fig. 1, panel B). Together, these results indicated a polarization of HCV E2 exposed macrophages to the M2 phenotype.

CD163 is a Class B member of the scavenger receptor superfamily, and its soluble form, sCD163, is a marker of activated M2 macrophages (18). Culture supernatants from HCV E2 stimulated macrophages enhanced release of sCD163 by ELISA in 5 of 7 samples tested (Fig. 1, panel C).

HCV E2 EnvGP promotes signaling in monocyte derived macrophages toward M2 phenotype

IL-10 is an important cytokine for M2 polarization of macrophages, as well as in immune regulatory response. In our previous study, IL-10 induction, but not IL-12, was observed in individuals vaccinated with HCV E1/E2 glycoproteins (5). IL-10 has also been implicated as a co-factor responsible for the induction of regulatory T cell development. We have shown that IL-10 mRNA was highly induced in E2 treated macrophages (Fig. 1, panel A). Thus, we

examined IL-10 and IL-12 secretion by ELISA. A significant increase in IL-10 secretion was noted in E2 treated monocyte-derived macrophages, without an increase in IL-12 production in 7 of 9 subjects examined (Fig. 2, panels A and B).

Signaling pathways and transcription factors are important for the activation of macrophages (12). Several novel signals induce or impede M1 or M2 activation. IL-10 mediated M2 polarization is regulated by Stat3 (19), while expression of Socs3 is associated with M1 macrophages and pro-inflammatory responses (20). Without Socs3, macrophages have a reduced ability to develop pro-inflammatory features, but instead display immunoregulatory characteristics. We determined whether HCV E1 or E2 EnvGP regulates the Stat signaling pathway in monocyte-derived macrophages. Activation of Stat1 was inhibited in cells exhibiting increased IL-10 and sCD163 by impaired phosphorylation at the Ser727 site in human monocyte derived macrophages upon incubation with purified E2 EnvGP (Fig. 2, panel C). However, in cells not displaying increased IL-10 and sCD163 secretion, alteration of Stat1 phosphorylation was not observed (data not shown). The status of Socs3 and phospho-Stat3 (Y705) expression in E2 treated monocyte-derived macrophage were also examined. Socs3 expression was decreased and Stat3 was activated in E2 treated macrophages, while the total Stat3 level was not altered (Fig. 2, panels D and E). E2 induced p-Stat 3 (Y705) activation across a broad (0.25–4 µg/ml) concentration level (Fig. 2, panel F). The results suggest that E2 upregulates phospho-Stat3 in macrophages, which in turn is associated with increased IL-10 expression and M2 polarization. Six of the eight samples characterized exhibited Stat3 activation and also displayed a significant increase in IL-10 induction and increased the secretion of sCD163. While two other subjects displayed a modest level of IL-10 secretion, without Stat3 activation or a significant change in sCD163 expression.

Activation of CD81 mediated signaling does not mediate alterations in cells indicating M2 polarization

HCV E2 EnvGP binds with human CD81, a tetraspanin expressed on many cell types including hepatocytes, B-lymphocytes and macrophages and is known as one of the co-receptors of HCV (21). The binding between HCV E2 and CD81 inhibits host immune response including T cell, B cell or NK cells (22–25). CD81, tetraspanins are thought to exert their biological functions by coordinating the trafficking of associated ligands into tetraspanin-enriched microdomains. We have shown that CD81 specific antibody (JS-81) activates the EGFR mediated CD81 signaling pathway (26). Here, we examined whether the CD81 pathway is involved in E2 mediated M2 polarization of monocyte-derived macrophages using stimulation of cells by CD81 specific JS-81 antibody.

We treated monocyte derived macrophages with JS-81 antibody and determined the status of phospho-Stat1 (Ser727) and phospho-Stat3 (Tyr705). Total Stat1 level in cells treated with HCV E2 or JS-81 remained relatively unchanged. Activation of Stat1 was impaired weakly, while that of E2 treated macrophages was significant. As observed in Fig. 2, panels E and F, Stat3 activation was strongly observed in E2 treated macrophages, while those treated with antibody to CD81 exhibited no activation (Fig. 3, panel A). IL-10 secretion from subjects exhibiting prior enhancement due to HCV E2 incubation were used in paired samples with

those incubated in the presence of JS-81. Binding of JS81 by antibody was not able to induce IL-10 secretion in manner similar to HCV E2 (Fig. 3, panel B).

To further understand if E2-CD81 interaction is responsible for M2 polarization, two of the five subjects were further examined for sCD163 following incubation with CD81 binding JS-81 mAb. Specific activation of CD81 by the addition of monoclonal antibody JS-81 did not lead to increased shedding of sCD163 (Fig. 3, panel C). Similar experiments with E2 enhanced sCD163 even after 3h of incubation. Antibody to CD81 did not exhibit an effect towards sCD163 production, although antibody to CD81 is known to activate markers for altering normal cell proliferation. These data suggested that E2 mediated M2 polarization of macrophages follows through a different signaling pathway distinct from CD81 signaling from the binding of JS-81 antibody at a lower concentration (2ug/ml).

We have observed that treatment of primary macrophages with JS-81 antibody or with E2 induced tumor suppressor protein expression, p16INK4a and p21 (data not shown), suggesting a similar functional role via CD81. However, p-Stat3 or IL-10 secretion was not induced by JS-81, but with E2 alone, further suggesting this is independent of JS-81 and CD81 binding alone, though we cannot dismiss that a higher concentration of JS-81/CD81 binding helps in clustering and activation of EGFR. These results indicate that activation of Stat3/IL-10 may occur as a post-CD81 binding event via CD81 clustering due to its concurrent activation of EGFR.

HCV E2 EnvGP binds with CD81 and promotes EGFR activation

We asked whether CD81 binding to E2 induces the activation of EGFR regulatory pathways, by CD81 lateral diffusion and receptor clustering processes (27). This effect was observed by engagement of CD81 at a higher concentrations (10 or 30 µg/ml) of JS-81 antibody alone, but was negligible at a lower CD81 engaging JS-81 antibody concentration (<10 µg/ml). IL-10 and EGFR regulate each other through positive feedback (28) and IL-10 subsequently activates Stat3 signaling pathways. CBH-2 monoclonal antibody inhibits E2-CD81 binding. Multiple signaling effects may occur from E2 binding to CD81. Our results suggested treatment of macrophages with E2 promotes EGFR activation by phosphorylation at amino acid T1068 residue and is inhibited in the presence of CBH-2 at 2µg/ml (Fig. 4, panel A). CD81 binding to E2 induces the activation of EGFR regulatory pathways, by CD81 lateral diffusion and receptor clustering processes (27). This effect was observed by engagement of CD81 at higher concentrations (10 or 30 µg/ml) of JS-81 antibody alone, but was negligible at a lower CD81-engaging JS-81 antibody concentration (<10 µg/ml).

We subsequently examined for EGFR downstream Stat3 activation status. E2 treatment of macrophages markedly activated Stat3 phosphorylation of amino acid residue T705, and was inhibited by CBH-2 monoclonal antibody used at 2µg/ml concentration (Fig. 4, panel B). Macrophages treated with E2 also enhanced IL-10 expression and was reduced to about 34% in the presence of CBH-2 monoclonal antibody at a similar concentration of 2µg/ml (Fig. 4, panel C). These results suggested that HCV E2 mediated binding of CD81 activate EGFR, Stat3, and enhance IL-10 expression by macrophages.

HCV E2 EnvGP regulates Akt and polarization or proliferation markers of macrophages

Akt acts as a central regulator to integrate metabolic and inflammatory signals in macrophages to influence their activation phenotype, and control cytokine production (29, 30). EGFR activation promotes transient Akt activation (31). Inhibition of Akt is associated with a reduction in M2 associated gene expression (29), indicating that activation of Akt is involved in macrophage polarization to the M2 phenotype. IL-10 and EGFR regulate each other through positive feedback (28) and IL-10 subsequently activates Stat3 signaling pathways. Further, the induction of IL-10 in macrophages is associated with Akt activation (32). Here, activation of Akt was observed in all subjects previously identified as expressing an increased level of IL-10, sCD163 and phospho-Stat3 upon exposure to the HCV E2, but not with E1 EnvGP (Fig 5, panel A). While subjects which did not have these characteristics exhibited no change in Akt activation (data not shown). Further, we analyzed the potential for JS-81 to induce Akt activation, and only a mild decrease in Akt activation was observed.

P21 deficient mice exhibit impaired M2 polarization during *in vitro* endotoxin tolerance (33). Increased expression of p21 has been noted to arrest macrophage cell cycle progression (34) and M-CSF dependent macrophage proliferation via IL-4 induction of p21 (35). Incubation of monocyte derived macrophages with purified HCV E1 or E2 EnvGP (2 µg/ml) showed differential expression of the marker proteins (pRaf1, IκBα and p21) for proliferation (Fig. 5, panel B). In contrast, purified recombinant E1 EnvGP did not have a negative regulatory effect on these marker proteins.

HCV E2 EnvGP suppresses C3 in macrophage and dendritic cells

Complement component C3 is a key link between innate and adaptive immunity, and is an important mediator of antibody and T-cell immune responses (36, 37). C3 facilitates antigen uptake/presentation and immune cell priming. C3 is critically important for B cell activation and T cell-dependent antibody responses, and for optimal CD8⁺T cell expansion during a systemic viral infection. C3 suppresses FoxP3 and expression of Tregs may occur in HCV patients due to the loss of C3. We have previously observed that HCV proteins inhibit C3 complement production in hepatocytes primarily by downregulating C3 promoter activity (38). In this study, we observed HCV E1 induces C3 expression in human macrophages, while E2 significantly represses expression of the C3 complement component (Fig. 6, panel A). HCV E2 EnvGP treatment of monocytes/macrophages also suppresses C3b complement level. Macrophages treated with E2 exhibited a significant C3 reduction specifically in M2 marker positive subjects (Fig. 6, panel B). Dendritic cells (DCs) exposed to cell culture grown HCV (genotype 2a, JFH1 clone) also exhibited a decreased level of C3 expression (Fig. 6, panel C). Studies have demonstrated the importance of an intact complement classical pathway in the humoral immune response to both thymus-dependent and thymus-independent antigens (39). Therefore, the use of HCV full-length E2 as a candidate vaccine component may impair T-helper cell dependent humoral immune response by regulating C3 complement related components and associated immunoregulatory functions.

DISCUSSION

Identifying optimal candidate antigens from HCV is necessary for highly effective vaccine development. The initiation of an adaptive immune response depends on the coordinated interaction of antigen-presenting cells and antigen-specific lymphocytes. HCV E1 and E2 EnvGPs are the targets for inhibition of virus entry into hepatocytes and host immunity, and non-structural (NS) proteins are the primary targets for induction of T-cell responses. Our present study suggested that purified HCV E2 EnvGP biases human monocyte derived macrophages toward the M2 phenotype, and may impair DC/CD4⁺ T cell development. HCV E2 also modulates C3 complement component with the potential for impairment of T-helper cell function. IL-10 mediated M2 polarization is mediated by Stat3 (10). Socs1 and Socs3 are most widely characterized for their roles in shaping M1 and M2 macrophage polarization (40, 41). Expression of Socs3 is associated with M1 macrophages and pro-inflammatory responses. Without Socs3, both human and rodent macrophages have a reduced ability to develop pro-inflammatory features, but instead display immunoregulatory characteristics (40). Functional Stat1 is critical to host defense against microbial infection. Stat1 deficiency redirects the nature of IFN signaling from priming to suppression of TLR-induced macrophage activation via feedback regulation of Stat3 (42). Our observations from HCV E2 EnvGP suggest enhanced Stat3 and suppressed Stat1 activation, reflecting macrophage polarization towards M2 phenotype in 7 out of 9 blood donors tested. Our results suggested purified E2 exhibits a number of functional activities (cytokine/chemokine upregulation, s-CD163 production, Stat3 activation; and pRaf1, I κ B α , p21 regulation), which are not expected to be observed in the presence of contaminating detergent, chemicals or endotoxin. Further, 2 out of 8 donor macrophages behaved differently with the same purified E2 preparation, and some effects were initiated by JS81 and inhibited by the E2 specific antibody, CBH-2. Therefore, the observed effects appeared to be E2 specific. HCV E2 was also shown to inhibit the functions of T cells, B cells or NK cells by binding of E2 and CD81 (22–25).

HCV E1 EnvGP is relatively conserved. We and others have characterized linear epitopes from HCV E1 (43). In a Phase I study, a candidate E1 therapeutic vaccine delivered as virus like particles was well tolerated and immunogenic in healthy, adult males (44). Additionally, a strong cellular immune response towards E1 was elicited, which included a strong Th1 component. HCV E1 EnvGP vaccinated chimpanzees exposed to heterologous HCV clear infection, and induce neutralizing antibodies (45). Our current study suggested that HCV E2 possess immunoregulatory activities, and could be a reason for not inducing a strong immune response to E1 when used together (5, 6). We speculate that if E1 is taken up by the same cells for association with E2 as a heterodimer, dampening of antigen presenting cell functions and a robust immune responses are likely to be impaired.

In our earlier study, we used purified E1/E2 EnvGP together in a vaccine trial in humans and observed induction of a weak immune response (5, 6). MF59 was used in our vaccine trial, which generates a Th1 cytokine profile, CD4⁺ memory cells, and CTL. Therefore, MF59 should have override the effect of E2, but this did not happen as evident from IL-10 induction, and an undetectable level of IL-12, among the human volunteers. A similar induction of IL-10, but not IL-12, was observed using human macrophages in this study. A

number HCV E2 epitopes have already been identified (46). Amino acid residues 412–423 of E2 is highly conserved, contains residues critical for CD81 receptor binding, and is targeted by antibodies isolated from human and animal sera (43). Other E2 regions representing HLA restricted broadly reactive T-cell epitopes have been mapped (47). Future studies on identification of E2 specific region(s) as candidate vaccine for induction of broadly cross-neutralizing antibody (48) and T-helper cell responses will provide information for a rational vaccine design. Examination for regulatory role of these selected regions from E2 upon human antigen presenting cells will guide whether to incorporate as a candidate vaccine. This mechanistically guided approach will also hold promise for use of selected conserved antigenic targets those are cross-reactive among HCV genotypes and will be an effective component of a HCV vaccine. Thus, a careful selection of E2 associated immunogens, and other relatively conserved appropriate antigens (E1 and non-structural regions), will significantly contribute to the development of an effective vaccine against HCV infection.

HCV T-cell specificity may be distinct between genotypes with limited cross-reactivity (49). The use of E2 in its entirety for vaccine purpose may not be helpful for its negative effect on antigen presenting cells. As a result, it is difficult to study conformational epitopes generated from full-length E2 EnvGp, and even conformational epitopes of E1/E2 associated heterodimer. Peptides from E2 may also be tested in some MHC/HLA-restricted T-cell activation assays in future studies. The use of DNA prime, followed by a traditional vaccine boost in both animals and in humans recruit many more T-cells (50). We may use gene-based prime and boost vaccination with recombinant E1, non-structural proteins or peptides representing conserved T- and B- cell epitopes of diverse HCV genotypes to elicit a significant breadth of protection. Although HCV EnvGPs are expressed intracellularly, we anticipate the proposed immunization schedule will also generate strong cytotoxic T cells for recognition of hepatocytes expressing HCV E1 or E2 peptides in association with MHC/HLA.

Acknowledgments

We thank Michael Hoghton for providing purified HCV E2 GP.

Financial Support: This work was supported by research grant DK113645 and DK080812 from the National Institutes of Health, and from the Presidential and Liver Center Research Funds of Saint Louis University

Abbreviation

HCV	Hepatitis C virus
HCC	hepatocellular carcinoma
IL	Interleukin
DAA	direct-acting antivirals
DCs	dendritic cells
M-CSF	Macrophage colony-stimulating factor

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Fig. 1. Cytokine/chemokine array by real-time PCR for M1/M2 polarization of macrophages derived from a healthy blood donor (#468) incubated with purified HCV E2 EnvGP (panels A and B). SolubleCD163 was measured by ELISA from supernatants of macrophage from select blood donors after treatment with HCV E2 EnvGP for 24 hrs(panel C).

Fig. 2.

HCV E2 EnvGP promotes macrophages toward M2 polarization. Comparison of secretion of IL-10 (panel A) and IL-12 (panel B) in HCV E1 or E2 treated monocyte derived macrophages. A statistically significant change was not observed for panel B. HCV E2 glycoprotein modulates Stat1/Stat3 phosphorylation and Socs3 status in macrophages by Western blot analysis from representative donors (panels C – E). HCV E2 dose dependent response on phospho-Stat3 up-regulation is shown (panel F).

Fig. 3.

HCV E2 EnvGP mediated Stat1/Stat3 modulations and IL-10 induction occur independent of CD81 activation by JS-81 antibody. HCV E2 EnvGP suppressed phospho-Stat1 activation, while enhanced phospho-Stat3 activation in representative monocyte derived macrophages from 6 out of 8 donors (panel A). Densitometry scanning for relative differences in protein expression are shown on the right. IL-10 secretion in E2 or JS-81 antibody treated macrophages was analyzed by ELISA (panel B). Representative macrophages derived from two healthy blood donor (#100 and #161) were treated with E2 for two different time points (3hr or 24hr) or with JS-81 antibody for 24hr displaying sCD163 (panel C).

Fig. 4.**HCV E2 EnvGP promotes EGFR and Stat3 activation and increases IL-10 expression.**

EGFR activation by phosphorylation at amino acid T1068 residue following binding of E2 with CD81 on macrophages and inhibition by CBH-2 (2 μ g/ml) monoclonal antibody are shown (panel A). Results from densitometry scanning of the Western blot is shown on the right. Stat3 phosphorylation at amino acid T705 residue, and inhibition by CBH-2 monoclonal antibody are shown (panel B). Results from densitometry scanning of the Western blot is shown on the right. Macrophages treated with E2 also enhanced IL-10 expression and was reduced to about 34% in the presence of CBH-2 (2 μ g/ml) monoclonal antibody (panel C).

Fig. 5.

HCV E2 EnvGP mediated regulation of Akt and polarization/proliferation markers in macrophage culture. Western blot analysis for activation of Akt in monocyte derived macrophages from two representative subjects previously identified as expressing an increased level of sCD163 and exhibiting Stat3 activation upon exposure to the HCV E2 protein (panel A). Similar analyses with E1 EnvGP or antibody to CD81 (JS81) did not exhibit a change in Akt activation. Incubation of macrophages with purified HCV E1 or E2 (2μg/ml) suggested differential expression of the protein markers (pRaf1, IκBα and p21) for polarization and proliferation (panel B).

Fig. 6. Modulation of antigen presenting cell function correlates with a reduction in C3 complement. C3 and C3b is reduced in macrophages derived from PBMCs after exposure to purified E2, unlike E1 EnvGP of HCV (panel A). Analysis of human C3 in E2 treated primary macrophages exhibited a significant C3 reduction in M2 marker positive subjects (panel B). C3 expression is also reduced by HCV (genotype 2a, clone JFH1) exposed monocyte derived DCs (panel C). Results are shown from two representative healthy blood donors