January 14, 2014

Dear Editor,

Please find enclosed the reviewed manuscript in Word format (5906-reviewed.docx).

Title: Immunologic, metabolic and genetic factors in hepatitis C virus infection

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

Reviewer 00502973
The major concern is that many references in the review are outdated and new information should be incorporated in this manuscript.
Response:
According to reviewer's comment, new information was incorporated and references were updated.

(1) In the section 1.1. Innate and adaptive immune response to HCV: CD4+ T cell is a heterogeneous population, and is consisted of a variety of cell types, e.g. Th1, Th2, Th17, Treg cells. These cells play different roles in vivo. The authors should discriminate the effects of these distinct cells rather than mention them as a whole
Response: The effects of distinct T cell populations on HCV outcome are included in the new version. Briefly, CD4+ T cells are critical for the elimination of viruses through mechanisms tightly regulated. CD4+ T-helper cells are divided into four subsets (Th1, Th2, T regulatory, Th17) based on their expression profile of transcription factors and secreted cytokines. An effective Th1 cellular function is crucial for an adequate immune response against HCV, given the specific production of IFN-γ by this cellular subtype whereas unbalanced Th1/Th2 T-cell responses in the liver are a characteristic of hepatic inflammation and subsequent liver fibrosis as a result of HCV infection (Rehermann B. *Nat Med* 2013). Clinical data reveals increased IL-17F levels in HCV-infected patients that had increased ALT values suggesting that in chronic hepatitis C infection, Th17 cells might be associated with the control of liver

injury. However, determinations are preliminar and are not fully conclusive at present (Hammerich L, *Clin Dev Immunol* 2011 and Sousa GM,. *Cytokine* 2012). The role of T regulatory cells in HCV seems to range from suppressing T-cell responses directed against type C hepatitis virus to down-regulating the immune responses causing the liver damage (Alatrakchi N, *J Gastroenterol Hepatol* 2010)

(2) In the section 1.2. Neutralizing antibodies: the abbreviation should be spelt out at the site where it is first present in the manuscript, e.g. HVR.

Response: Abbreviations are spelt out at the site where they are first present in the new version, e.g hypervariable region 1 (HVR).

(3) In the section 1.3. Mechanisms of immune evasion by HCV: the author wrote -genetic analysis reveals a positive correlation between distinct HCV quasiespecies, viral clearance and slowly adapating viral population- I think using -close correlation- is better than -positive correlation- as not all these factors are positively corrected.

Response: Correction was conducted.

(4) In the section 2.1. HCV entry: the first sentence is -after this discovery, HCV was found associated to liporpoteins- I cannot understand what is -this discovery-. The author should indicate what the discovery is before this statement. Also in this paragraph, the author wrote - LVPs contain HCV-RNA and the totality of the structural viral proteins, mainly E1 and E2 responsible for assembly of the virus with the hepatocyte, LVPs are rich in triglycerides- I cannot understand this sentence. I also don't think E1 and E2 can assemble HCV virus with hepatocytes. -LVPs are also rich in triglycerides- is a ducplicate statement of the previous sentence -these particles also recognized as LVPs are rich in triglycerides- and should be deleted.

Response: Paragraph was rewritten. After its discovery, HCV was found associated to lipoproteins. Thompssen *et al* demonstrated the existence of distinct HCV particles categorized as high and low density particles. These particles also recognized as lipoviroparticles (LVP) are rich in triglycerides (TG) and they can be almost completely precipitated by anti-Apo lipoprotein B and E (apoB and apoE). LVPs contain HCV-RNA and the totality of the structural viral proteins, mainly E1 y E2 that attach the virus with its receptors on hepatocyte surface.

(5) In the section 2.2. HCV replication, virion assembly and secretion: role of ApoB and MTP: the last sentence of the first paragraph, the author started -understanding the components involved in this process will allow the design of specific therapeutic targets- I am not sure about this statement. I suggest this sentence change to -will allow the possible design of-.

Response: correction was conducted.

In the second paragraph of this section, the author stated -according to clinical data and experimental models, the HCV core protein has been shown to inhibit MTP protein. The authors should provide references to support this statement.

Response: Reference was incorporated in the new version.

(6) In the section 2.3. Mechanism of HCV-associated steatosis: the knowledge in this section is generally outdated. New discoveries should be included.

Response: New information and updated references are included in the new version. Briefly, microRNAs (miRNAs) exert regulatory control through modulation of many targets. In the liver MiRNA-122 is important for regulating lipid metabolism (Elmen J, Nature, 2008, Lanford RE. Science, 2010 and Bartel DP. Cell 2009). It has been recently reported that HCV replication induces the expression of miR-27 *in vitro* and *in vivo*. This results in a larger and more abundant lipid droplets and coincides with repression of regulators of triglyceride homestasis including PPAR*a*, revealing HCV's up-regulation of miR-27 as a novel mechanism that might to contribute to the devolpment of steatosis (Singaravelu R, Hepatology 2013. In press).

PPARα, δ and γ are differentially involved in HCV infection. A clear effect of PPARα in HCV-RNA replication has been described and a recent report has shown that PPARδ-selective antagonists inhibit HCV replication whereas PPARγ is not involved in this process (Shintaro B, 2013. Bioorganic).

In contrast to the anti-inflammatory role of adiponectin, leptin is a pro-inflammatory adipocytokine identified as one of the best markers of total body fat whose elevated expression can result in the stimulation of cellular lipolysis and fatty oxidation promoting a negative energy balance. Both, adiponectin and leptin are crucial for hepatic steatosis (Janeckova R. Physiol Res. 2001). However, data concerning adiponectin and leptin role on HCV-related steatosis remains divergent. This is mainly due to the multiple functions where adipocytokines are involved. By using a HCV core-transgenic mice model, a recent report reveals that HCV core-induced nonobese hepatic steatosis is associated with a down regulation of the *leptin* gene in visceral fat and hypoadiponectinemia (Chang ML, 2012. Obesity,). A better understanding of this process might be valuable in the design of new therapeutic interventions, particularly in the cases of non obese hepatic steatosis involving HCV infection.

The anti-HCV activity of 2-octynoic acid (2-OA), used in perfumes, lipstick, and many food avorings has been recently revealed *in vitro*. This activity seems to be associated with the activation of AMPK by 2-OA that regulates ISGs and suppress miRNA122 expression, inhibiting HCV infection. This represents a novel mechanism to explain inhibition of infection by AMPK (Yang D, Plos One. 2013).

(7) In the last paragraph of page 9: the author stated –recent reports demonstrate that HCV infection enhances the proteolytic cleavage of SREBP precursors in hepatic cells- the only references in this paragraph are Ref. 89 and 90. These 2 reports were published in 2008 and 2005 respectively. I don't think these two publications are recent reports.

Response: References were updated.

Also in page 11, the second paragraph, the author stated –recent studies have demonstrated the presence of- yet the reference was published 10 years ago, and cannot be regarded as recent study. And the last paragraph of page 11, -a recent analysis reveals that pathologies associated to high levels.

Response: Information is updated in new version. Briefly, the host subtilisin/kexin/isozyme/1 (SKI-1) or site 1 (S1P) plays a crucial role in the proteolitic activation of SREBP. The use of a SKI-1/S1P-specific protein-based inhibitor has recently demonstrated that SKI-1/S1P inhibition blocks HCV infection in hepatoma cells following a mechanism associated with a dramatic reduction of lipid droplets and adipose differentiation-related protein (ADRP)/perilipin 2. This result in the inhibition of virus assembly from infected cells and identifies SKI-1/S1P as both: a regulator of the HCV lifecycle and a potential host-directed therapeutic target against HCV infection (Olmstead AD, Plos pathogens. 2012)

Reviewer 02446172

(1) The abbreviations should be spelt out at the site where it is first present in the article. For example, in the page 6.2.2 MTP, page 9, line 2, upre-regulating.

Response: Abbreviations are spelt out at the site where they are first present .

(2) To correct page 9, line 2, upre-regulating.

Response: Correction was conducted.

(3) Page 11, line 7: recent studies; which studies? References should be written.

Response: References were incorporated in the new version.

(4) In references, spaces should be corrected.

Response: Correction was conducted.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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