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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 28068
Title: Anti-viral role of toll like receptor 4 in hepatitis B virus infection: An in vitro study
Reviewer's code: 03618521
Reviewer's country: India
Science editor: Jing Yu
Date sent for review: 2016-06-28 10:54
Date reviewed: 2016-08-18 16:00

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

In the manuscript entitled " antiviral role of TLR4 in hepatitis B virus (HBV) infection: an in vitro study" the authors have adopted a cell line model of HBV infection (HepG2.2.15), and have provided multiple experimental evidences that suggest the anti-viral role of TLR4 in HBV infection. The manuscript is well written; however, it needs certain major modifications/corrections. Major comments: 1. In figure-1B, the difference between LPS (1ug/ml) and LPS (2ug/ml) is very high compare to the difference observed in figure 1C & D. Please explain this discrepancy. 2. In figure legend 1C, the authors have mentioned that the effect is dose pendent; however, the data doesn't show a dose dependent effect between 1ug/ml and 2ug/ml doses. 3. In figure 2C the labelling for LPS-treated (72 HRS) group has to be corrected 4. Why results of MT assay (figure 2C) showed less viable cells at 72 hr LPS treatment group? Please explain 5. In figure 2D, the labelling should be LPS-treated not TLR4-treated 6. Throughout the manuscript, the p-value for different statistical analysis has to be provided 7. In figure 5B, what is the difference between the upper two and lower two panels? 8. In figure 6 B,C & D, all the inhibitors should be labelled with LPS 9. In figure 5A, the relative band intensity for p53 doesn't match with the images.

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Name of journal: World Journal of Gastroenterology
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COMMENTS TO AUTHORS

In the paper "Anti-viral role of TLR4 in hepatitis B virus (HBV) infection: an in vitro study", the authors study the role of TLR4 in HBV infection and the signaling pathways and mediators involved. Authors show changes in G1/S cell cycle, histone marks and various cell cycle regulators in HepG2.2.15 cells treated with LPS, an activator of TLR4. They show changes in the mRNA and protein expression of some of the downstream regulators of the TLR4 signaling pathway (NF-KB) and the inflammatory cytokines released. Authors conclude that TLR4 activation in HBV infection evokes changes in hepatocyte and can be used for developing therapeutic targets in the future. Major comments -In material and methods, the section of RT-PCR should be written with more detail: in which samples were performed the experiments? Which primers were used in the study? How was performed the quantification? -In material and methods, in the sections of western blotting and confocal imaging, the reference number of the antibodies and the concentrations used in the study should be indicated. -The technique used to determine the cytokines is not described in the material and methods. -In material and methods, in the section of Cell cycle analysis: How many cells were analysed for each sample in BD FACS Calibur platform? -In material and methods, in the section of

MTT assay. How many cells were seeded in each well? -In material and methods, authors should include a section explaining the Statistical analysis used for each technique. -The number of experiments performed for each technique is unknown. Please, clarify this point. -All the results presented in the paper are without a statistical analysis and that's why it is difficult to know if the changes described in the paper are true or not. Please add the statistical analysis for the data of each technique. -Figure 1. Why did the authors choose to do the experiments with the cell line HEPG2.2.15 instead of HEPG2? Authors described that HEPG2 has a higher level of TLR4 than HEPG2.2.15. Please explain the reasons. -Figure 1B ,C, D. How do the authors explain a decrease in the viral load but not in the viral proteins for the concentrations of 1 and 2 ug/ml of LPS? -Figure 1C, D. Authors should check if there is a concentration-dependent repression of viral protein by statistical analysis, because only the concentration of 4 ug/ml of LPS seems to reduce these proteins. -Figure 2 A, B. Authors should check if there are differences between control and LPS by statistical analysis, because the graphs of the FACS seem quite similar. -In Figure 5A, authors describe a downregulation of p53 and upregulation of NF-kB. However, in the picture of western blot, the levels of these proteins do not seem to be modified. Minor comments -There are some spelling mistakes in the manuscript. -In Material and Methods, section Cell cycle analysis, in the first line, remove Phosphate Buffered Saline, leave only PBS. -Graph bar of Figure 1B present a different style (letter, colour of bars) respect to graph bars of Figure 1C and 1D. -Figure 2D, 3A, 5A the titles on the Y axes are not present.

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COMMENTS TO AUTHORS

Background of the study: Chronic infection with hepatitis B (HBV) represents a leading cause of cirrhosis and Hepatocellular Carcinoma (HCC) worldwide. TLR4, one of the pattern recognition receptors, is a cell surface receptor which initiates key innate immune response pathways and previous studies have shown that TLR4 is crucial in initiating an innate immunity against HBV. Importance of the present work: The aim of the manuscript 28068 is to focus on the role of TLR4 in HBV infection to further investigate a new therapeutic approach against HBV by immune-targeting TLR4. The main results of the present study showed that TLR4 activation repressed HBV infection and several changes in host factors, such as release in G1/S cell cycle arrest and modifications in host epigenetic marks. It was also observed that anti-viral action of TLR4 took place through NF-kappaB pathway. 2) Specific comments The overall structure of the manuscript is complete. The Title is satisfactory. The abstract and the introduction are good, giving the main relevant data developed in the manuscript. The references cited are important and relevant. Major data: Interestingly, in the context of HBV infection, the authors observed that stimulating the TLR4 pathway in HepG2.2.15 cells reduced HBV DNA and protein titre in a dose dependent manner.

Stimulating TLR4 with LPS activated a MyD88-dependent and independent pathway. HBV suppression on TLR4 activation possibly developed through NF-kappaB thus blocking it. Moreover, HBV is admitted to upregulate the expression of p53, which was confirmed in liver biopsy samples and in HepG2.2.15 cells and p53 got repressed on viral elimination following TLR4 activation. At last, important host active signatures, H3K9Ac and H3K18Ac showed significant upregulation on pathway stimulation highlighting the ability of LPS to affect the cellular micro-environment. Expected complementary data to be produced by the authors: - Complementary data should be added in the Results on the calculation of the modifications in the quantitative markers - see below comment for the Figures. Thus, the figures are satisfactory but the calculation of the 'p' values, significant or not, is necessary in the legends (Fig 1-6). Examples: Figure 1 for HBV DNA, HBs Ag, HBe Ag; Figure 2 for Fow cytometry and Epigenetic signatures. Please add a systematic mention of the calculated 'p' value for the modulation in quantified parameters. - Concerning the Discussion/Conclusion section: The authors mention that after TLR4 activation, the acetylation signatures H3K9Ac and H3K18Ac, which are transcription activation marks increased. Their observation suggested that a combination therapy which can target multiple epigenetic factors can be important to limit HBV infection. It would be relevant for the authors to add here a paragraph devoted to the innovative therapeutic approaches which are in progress to fight against chronic HBV infection and how epigenetic modulation could help in allowing this research to improve.