We read with interest the manuscript by Huang and co-workers [1]. The authors reported that peripheral CD14+ monocytes of patients chronically infected with HBV presented reduced Toll-like receptor (TLR)9 protein expression as determined by flow cytometry in comparison to healthy controls. Interestingly, the study further showed that effective therapy with either IFN-α-based therapy or entecavir restored TLR9 protein levels on monocytes to normal levels. In-line with the observations in the periphery, reduced intrahepatic expression of TLR9 mRNA was observed in the same patients [1].

The crucial role of monocytes in immunity to a broad range of pathogens and of TLRs in triggering the cells highlight the importance of the topic addressed in this study. Reduced TLR expression on monocytes in patients chronically infected with HBV may have serious consequences for their susceptibility to pathogens. Also, as described in the manuscript, a better understanding of TLR9 alterations in HBV patients and the association between its expression on peripheral monocytes and treatment effectiveness provides a potential predictor for therapeutic response of chronic HBV patients.

We read the paper with great interest because it relates to a study we recently completed in which monocytes purified from patients chronically infected with HCV were examined. We previously reported that peripheral CD14+ monocytes of patients chronically infected with HCV presented reduced TLR4 and TLR8 mRNA expression as compared with healthy controls [2]. We extended these studies by also assessing TLR9 mRNA expression levels by quantitative PCR. As shown in Figure 1A, we observed that TLR9 mRNA expression was comparable between chronic HCV patients and healthy individuals [2].

That TLR9 mRNA expression by monocytes from healthy individuals is undetectable and that monocytes do not respond to TLR9 agonists by producing TNF or IL-6 [3,4].

Furthermore, we examined TLR9 mRNA expression in purified monocytes before and during IFN-α-based therapy in chronic HCV. The patients enrolled in our study are those who achieved an early viral response to IFN-α-based therapy at week 12. Monocytes were phenotyped and transcriptomically sequenced. RNA sequencing showed that TLR9 was not expressed in peripheral monocytes of chronic HCV patients and was not enhanced at week 12 during successful IFN-α-based therapy (<1 FPKM) (Figure 1B). In the same experiment, we found that during IFN-α-based therapy, the absolute number of peripheral monocytes was decreased, but monocytes became more active as evidenced by up-regulated expression of the activation markers CD69 and CD83 on monocytes (2.5-fold and 1.8-fold, respectively). Combined, we believe that TLR9 triggering of monocytes in chronic HCV patients is of limited biological relevance, since the receptor is expressed at low or undetectable levels, and therefore it is not possible to use TLR9 mRNA expression in peripheral monocytes as a biomarker to predict response to IFN-α-based therapy in chronic HCV.

The study by Huang and our study cannot simply be compared, since both studies have their limitations. Our study did not perform functional assays to examine whether monocytes of chronic HCV patients are unresponsive to stimulation with the TLR9 agonist CpG and we did not assess TLR9 protein by flow cytometry. Huang et al. [1] also did not perform functional assays and did not perform TLR9 mRNA expression levels of purified monocytes from chronic HBV patients. Regarding the flow cytometric TLR9 analysis, it would be extremely informative to have comparative data of the mean fluorescence intensity of the TLR9 staining on monocytes with TLR9 staining...
J Hou & A Boonstra

The study by Huang et al. [1] on the importance of TLR9 in chronic HBV as well as our findings in chronic HCV demonstrate that more studies need to be performed to be able to evaluate the importance of the TLR9 responses in monocytes. In these assessments it is important to appreciate that in humans especially plasmacytoid DC and B-cells of healthy individuals express high levels of TLR9 mRNA, while other leukocyte subpopulations in blood express relatively low TLR9 levels and are virtually unresponsive to triggering with TLR9 agonists.

Disclosure statement

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References


Figure 1. TLR9 mRNA expression in peripheral monocytes of chronic HCV patients

(A) Toll-like receptor (TLR9) mRNA expression in CD14+ monocytes isolated from HCV patients and healthy individuals was quantified by real-time PCR, and normalized to the house-keeping gene GAPDH. TLR9 mRNA expression in PBMCs and B-cells were included as controls. All leukocytes were purified using Miltenyi Biotec microbeads (Bergisch Gladbach, Germany).

(B) TLR9 mRNA expression determined from RNA-seq data of individual HCV patients. Paired data of TLR9 mRNA expression by purified monocytes obtained at baseline and at week 12 during therapy are presented. The expression values (FPKM) of TLR9 mRNA were calculated from the abundance of RNA-seq reads mapped to the corresponding genes, then normalized by kilobase of transcript per million mapped reads, or normalized to GAPDH.

on B-cells and plasmacytoid DC, as well as isotype control stainings. These data will allow the reader to get insight into the relative contribution of TLR9 on monocytes in the overall response to its agonist as compared with other leukocyte populations.
Reply to:

Modulation of Toll-like receptor 9 expression on monocytes of viral hepatitis patients

Yi-Wen Huang1,2,3, Sien-Sing Yang1,4, Jia-Horng Kao2,5,6*

1Liver Center, Cathay General Hospital Medical Center, Taipei, Taiwan
2Division of Gastroenterology, Department of Internal Medicine, National Taiwan University College of Medicine and Hospital, Taipei, Taiwan
3School of Medicine, Taipei Medical University College of Medicine, Taipei, Taiwan
4School of Medicine, Fu Jen Catholic University College of Medicine, Taipei, Taiwan
5Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan
6Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

*Corresponding author e-mail: kajoj@ntu.edu.tw

We thank Hou et al. [1] for their interest in our work [2]. They showed comparable Toll-like receptor (TLR)9 mRNA expression on monocytes between chronic hepatitis C patients and controls. Furthermore, they concluded that TLR9 mRNA expression on monocytes was not a biomarker for prediction of interferon (IFN) treatment response [1]. As we found in our study, there were many variables affecting TLR9 expression on monocytes, such as age, gender and serum ALT levels, which should be adjusted or stratified by regression analysis. Hou et al. [1] did not provide the sample size of their study, nor did they consider the effects of the above variables, thus, the results of their study should be interpreted with caution.

We had reported the reduced expression of TLR3 and TLR9 expression on monocytes in chronic hepatitis B patients that were both confirmed on liver tissues [2,3]. The liver rather than monocytes, total PBMCs or B-cells is the main organ infected by hepatitis viruses, thus, the liver should be targeted for study. Our data lent strong support to the hypothesis that innate immunity is inhibited by HBV infection, which may contribute to persistent HBV infection. The ability of HBV to suppress innate immunity could be reasoned by the lack of induction of type I IFN response as well as blocking of its antiviral activity with subsequent inhibition of IFN-α or -β produced by non-infected parenchymal cells, such as dendritic cells and Kupffer cells [4,5]. In addition, HBV polymerase might interfere IFN regulatory factor (IRF) signalling to modulate IFN induction [6].

Innate immune recognition of the virus involves sensing of cytoplasmic, extracellular, nuclear DNA with subsequent activation of transcription factors (such as IRF3, IRF7, NF-kB) to induce IFNs and proinflammatory cytokines (such as IL18) [5]. We explored the mRNA expression of cytoplasmic DNA receptors (DAI, RIG-1, helicase DHX9, AIM2, immune interferon-16 [IFI16]), extracellular DNA receptors (receptor for advanced glycated end products [RAGE]), Myd88, caspase, IRF3, IRF7, NF-kB, type I interferon (IFNα1, IFNα2, IFNβ), IL18 and IL1β in the liver of chronic hepatitis B patients whom had reduced expression of TLR3 and TLR9. Among them, we found that the mRNA expression of DAI, helicase DHX9, AIM2, IRF3, IFNα1, IFNα2 and IL1β in chronic hepatitis B patients was reduced as compared with non-HBV non-HCV controls (Figure 1). Therefore, reduced expression of TLR3 and TLR9 mRNA might inhibit IFNα1, IFNα2 and IL1β through suppression of DAI, helicase DHX9, AIM2 receptors and transcription factor IRF3 (Figure 2).

Disclosure statement

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References

Figure 1. Intrahepatic mRNA expression of TLR3, TLR9, cytosolic DNA receptors, IRF3, IFNα1, IFNα2 and IL1β between non-HBV non-HCV controls and chronic hepatitis B patients

*Relative quantification, fold change. IFN, interferon; IRF, interferon regulatory factor; TLR, Toll-like receptor.

Figure 2. Intrahepatic downstream signalling of TLR3 and TLR9 inhibition in chronic hepatitis B patients

IFN, interferon; IRF, interferon regulatory factor; TLR, Toll-like receptor.