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Title: Innate and adaptive immune escape mechanisms of hepatitis B virus

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Dear Editors,

We thank the reviewers and editors for their insightful comments and valuable suggestions, which have enabled us to greatly improve our manuscript. The manuscript has now been revised according to their comments, and the changes in the revised manuscript were underlined.

Please find enclosed, the point-by-point responses to the reviewer's comments and the revised version of the manuscript and figures. We have made our manuscript edited by professional English language editing company and address this in the revised manuscript.

We appreciate Editor/Reviewer's hard work earnestly and hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in *World Journal of Gastroenterology*.

Sincerely,

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EDITORIAL OFFICE'S COMMENTS

Answer: Thank you for insightful comments and valuable suggestions, and we have made modifications to our manuscript according to the Editorial Office's comments and suggestions, including performing further language polishing, keeping proper self-cited references, changing with "immune" instead of "immun", providing the aims and a figure of the review, and making a point-by-point response to the reviewer's comments as shown in the following **Responses to Reviewer' s Comments** and these changes in the revised manuscript were underlined.

Responses to Reviewer's Comments

Reviewer #1:

1. INTRODUCTION: Please describe the mechanism of HBV escape from immune surveillance. Such as neonatal immune tolerance and HLA-DP and -DQ antigen presenting system.

Answer: Thanks. In accordance with your suggestion, we have added the description on the mechanism of HBV escape from immune surveillance, including the neonatal immune tolerance and HLA-DP and -DQ antigen presenting system in revised **INTRODUCTION** as "A majority of HBV infections occur in newborn infants with the presence of immunological defects, characterised by a lower quality and quantity of HBV-specific T cells and B cells. In addition, maternal HBeAg can educate the Kupffer cells (KCs) of the offspring by upregulating PD-L1 to suppress the HBV-specific $CD8^+$ T cells response to support HBV persistence after birth. In addition, HBV circumvents endogenous IFN-I responses and inhibits the function of innate and adaptive immune cells. Prolonged exposure of T cells to large quantities of viral antigens, such as HBsAg and HBeAg, induces a defective T cell response with the loss of effector functions and increased inhibitory receptor expression, facilitating viral persistence. Moreover, HBV infection was found to be able to affect the expression of human leukocyte antigen (HLA)-II alleles, including HLA-DP, HLA-DQ, and HLA-DR, on APCs, which in turn impaired antigen presentation capacity with induction of inefficient T cell response, leading to persistent HBV infection."

2. HBV ESCAPES INNATE IMMUNE SURVEILLANCE: Reader will be appreciated to have a figure that demonstrate the interaction between HBV and innate immunity. Potential response with HBsAg, HBcAg, HBeAg and HBxAg.

Answer: Thank you for your insightful advice, we have added a figure to demonstrate the interaction between HBV and innate immunity, especially the potential response with HBsAg, HBcAg, HBeAg and HBxAg in our revised manuscript.

3. HBV infection and type-I interferon (IFN-I): It will be better to start with spontaneous

IFN secretion in resting stage of HBsAg carriers. IFN secretion is quite low at this stage that is compatible with the immune tolerance situation. The first paragraph seems to describe the response after interferon administration.

Answer: We appreciate your valuable suggestion. We have now started with the description of the impaired IFN-I secretion during CHB infection in revised **HBV infection and type-I interferon (IFN-I)** as "IFN-I, as a major component of the innate immune response, is critical for HBV clearance. However, HBV circumvents endogenous IFN-I responses through multiple pathways to sustain persistent HBV infection.

Chronic HBV infection downregulates the expression of TLR3, RIG-I, and MDA--5 in DCs and hepatocytes, leading to the reduction of responsiveness to PAMPs and impairment of IFN-I synthesis. Previous study found that HBV infection upregulated microRNA-146a (miR-146a) expression in hepatocytes, inhibiting the expression of RIG-I-like receptors and in turn suppressed IFN-I transcription. Additionally, HBsAg, HBeAg, HBx, and HBV virions themselves can inhibit IFN-β synthesis by downregulating MAVS and interfering with the interaction between MAVS and RIG-I.

Binding of IFN-I to the IFN receptor can induce the activation of IFN-stimulated genes (ISGs), thereby directly inhibiting HBV infection. However, HBV can extensively impair IFN-I-induced signal transduction and dampen IFN-I-mediated immune responses. Recent data showed that HBx was able to reduce the interferon-α receptor (IFNAR1) transcription and downregulated Tyk2, that is essential for cell-surface IFNAR1 expression. Additionally, matrix metalloproteinase 9, which is increased in peripheral blood mononuclear cells of patients with CHB, binds to IFNAR1 and facilitates its phosphorylation, ubiquitination, subcellular distribution, and degradation of IFNAR1. Previous results showed that HBV can inhibit the activities of interferon-stimulated response elements with lower ISG expression by disturbing the intracellular JAK–STAT1 signalling pathway. HBV downregulated cellular STAT1 levels *via* the inducing miR-146a and blocking STAT1-Tyr701 phosphorylation in hepatocytes. HBV polymerase was found to interfere with the binding of DEAD-box helicase 3 X-linked (DDX3) to the TBK1/IKKE complex and the induction of interferon-stimulated gene factor 3 (ISGF3) to inhibit IFN-β induction. In addition, HBV polymerase suppressed IL-1β production by inhibiting NF-κB signalling and the inflammasome–

caspase-1 pathway, resulting in IFN-α resistance and persistent HBV infection."

4. HBV infection and macrophages: Kupffer cells is a tissue-resident macrophages in the liver sinusoids. Please describe it function in liver during inflammation.

Answer: Thank you for raising this important point, we have added the description in revised **HBV infection and macrophages** as "Kupffer cells (KCs), localised in liver sinusoids, serve as the largest population of innate immune cells in the liver. They are stationary, and able to effectively phagocytose cellular debris, foreign material or pathogens, acting as critical sentinels for liver homeostasis. Chronic HBV infection induced the production of immunomodulatory mediators such as IL-10 and TGF-β, and the expression of PD-L1 and PD-L2 by KCs, suppressing anti-HBV T cell responses. Furthermore, upon HBV infection, elderly mice were found to have a significantly higher number of TNF-α-producing $Ly6C^+$ monocytes and a much lower number of IL-10-secreting KCs than younger mice, facilitating HBV clearance. However, KCs can play different roles in the presence of different HBV antigens. Boltjes *et al* found that KCs could interact with HBsAg, which induced secretion of the proinflammatory cytokines IL-6 and TNF that was substantially increased compared with that seen in healthy controls. *In vivo* experiments demonstrated that HBcAg interacted with KCs upon TLR2 activation, mediating humoral and cellular tolerance *via* IL-10 production during CHB infection, and TLR2 knockout or KC depletion led to accelerated HBV clearance and improved HBV-specific $CD8^+$ T cell responses. HBeAg suppressed lipopolysaccharide-induced NLRP3 activation and IL-1β maturation in KCs by inhibiting NF-κB phosphorylation and reactive oxygen species production. Nonetheless, HBeAg can play two distinct roles in macrophage function. Upon HBV infection, maternal HBeAg enhanced PD-L1 expression in KCs with an M2-like anti-inflammatory phenotype, which suppressed the HBV-specific cytotoxic T lymphocyte (CTL) response and led to HBV persistence; however, in control mice born to HBeAg-negative mothers, HBeAg promoted the M1 proinflammatory phenotype, contributing to HBV clearance."

5. HBV infection and NK cells: Please strengthen the main effector cells by "NK cells constitute up to 40–50% of human liver lymphocytes".

Answer: Thank you for this important comment. We have changed the sentence as "As the main effector cells of the innate immune system, NK cells constitute up to 40–50% of human liver lymphocytes, and serve as the first line of defence against pathogens. "

6. HBV ESCAPES ADAPTIVE IMMUNE SURVEILLANCE: Please give a brief description concerns about HLA-II on HBV antigen presentation and persistent infection.

Answer: Thank you for your valuable suggestion. We have added the description on **HBV infection and HLA-II** in revised **HBV ESCAPES ADAPTIVE IMMUNE SURVEILLANCE** as "HLA in APCs plays a critical role in initiating the host antiviral immune response against HBV infection due to its capacity to attract and bind peptides. HLA-I molecules can present HBV peptides to $CD8^+$ cytotoxic T lymphocytes, resulting in direct cytolysis of HBV-infected hepatocytes. HLA-II alleles, including HLA-DP, HLA-DQ, and HLA-DR, encode MHC-II molecules that present exogenous antigens to $CD4^+$ T cells. Different HLA-II alleles with particular amino acid polymorphisms determined which peptides can be presented to T cells. Moreover, these HLA alleles expressed on APCs contribute to presenting a broad range of peptides, thus determining the variability in host immune response to HBV. Compared to HLA-DP and HLA-DQ, HLA-DR allele containing an extra β-chain gene whose product can pair with the DRα chain on APCs, is more important for the induction of sustained HBV-specific immune response. Upon HBV infection, single nucleotide polymorphisms (SNPs) of HLA-II antigens may also contribute to the induction of immune tolerance, leading to persistent HBV infection. HBV infection reduced the expression of HLA-DP and HLA-DQ molecules on APCs, which in turn resulted in impaired antigen presentation capacity and an inefficient T cell response. Thus, polymorphisms of the HLA-II genes during HBV infection could alter the antigen-binding properties of HLA-II and affect the HBV-specific immune response, partly promoting the persistent HBV infection."