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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 12678
Title: High Persistence Rate of Hepatitis B Virus in the Hydrodynamic Injection Based Transfection Model of C3H/HeN Mice
Reviewer code: 00503560
Science editor: Ya-Juan Ma
Date sent for review: 2014-07-21 18:57
Date reviewed: 2014-08-05 08:39

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

COMMENTS TO AUTHORS

General comments: The paper by Xiuhua Peng et al. showed HBV persisted longer in C3H/HeN(H-2k) mice after the hydrodynamic injection(HI) compared with C57BL/6(H-2b) mice, suggesting that host genetic background determines the rate of HBV clearance. The authors suggested that this could be a novel animal model for chronic hepatitis B infection to elucidate the disease pathogenesis and develop new antiviral treatments. Overall, the study showed a clear association between mouse genetic background and the rate of persistence. However, the authors should elucidate the mechanistic basis for the frequent persistence in the C3H/HeN (H-2k) mice. Major comments are as follows: 1. In this study, the authors demonstrated MHC haplotype H-2K, and young age of mice were related to the high rate of HBV persistence after hydrodynamic injections. The authors also claimed that the high persistence rate was associated with impaired HBsAg-specific T cell responses, but only three mice per group were examined with relatively large error bars. A larger number of mice should be examined to substantiate the authors' claim. In addition, the weak HBs-Ag specific T cell responses in H-2K mice could reflect the lack of positive signals required for the induction of HBsAg-specific T cell responses, or it could reflect the presence of negative signals that suppress HBsAg-specific T cell responses. The authors should distinguish these alternatives by hydrodynamically injecting HBV plasmid into F1 hybrid of C3H/HeN and C57BL/6. 2. As cited in this manuscript, Huang et al. have previously shown that HBV replication

persisted in FEV/N(H-2q) mice after hydrodynamic injection. Authors should explain why C3H/HeN mice are superior to FEV/N mice in studying the disease pathogenesis and HBV persistence. 3. In the Fig.4D, the authors showed three pictures depicting spots of IFN-γ after PMA and ionomysin stimulation. But it is impossible to assess the data because the differences between groups were very modest. The data should be numerically represented. Minor comments 1. In the Fig5, the label of horizontal axis should be changed to "Weeks after treatment " from "Weeks after hydrodynamic injection." 2. "mimcs the natural coarse" in the Introduction was a typo. 3. In the Materials and Methods, IFN-γELISPOT assay part reads "The splenocytes of C3H/HeN and C57BL/6 mice s.c. injected mice with 10μg OVA protein were stimulated with 10μg/ml OVA protein", but it was unclear why this should be described in this paper. 4. The title of Fig. 4, "Impaired HBsAg-specific T cell response were impaired in HI C3H/HeN mice." was incorrect. It should be either "Impaired HBsAg-specific T cell responses in HI C3H/HeN mice" or "HBsAg-specific T cell responses were impaired in HI C3H/HeN mice."

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

COMMENTS TO AUTHORS

The model of hydrodynamic injection of plasmids containing replication component of hepatitis B virus seems to be promissing. It appears that not only the way of HBV plasmid delivery, but also the right choice of mice with immunodefficient features are of importance for successful development of this model. I have no major problems with this manuscript. The minor problems are: 1. It is not quite clear why such reagimen of IFNa treatment is included: it is difficult to expect that the treatment as it is designed would clear the infection. 2. Few misspellings are in the text.

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ESPS PEER REVIEW REPORT

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[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
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		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

Hepatitis B virus infection is a major public health problem. The etiological mechanisms of the host immune responses that lead to HBV persistence or clearance are still to be elucidated integrally yet. Antiviral with IFN-alpha (IFN-α) and nucleoside/nucleotide analogs significantly improve the prognosis of patients with HBV infection, however the efficacy and safety of these drugs, as well as drug-resistances of nucleoside/nucleotide analogs remains unsettled tough issues in clinical practice. The ideal animal model is very important for playing research to resolve the issues above. In this manuscript, Peng et al. construct an ideal HBV infection model in C3H/HeN(H-2k) mice By hydrodynamic injection with pAAV/HBV1.2 plasmid DNA. Work is written correctly. The remaining description, discussion on the topic is clear and correct. And I think this manuscript can be accepted for published in WJG with a revision. Major comments; 1) The author state the hydrodynamic injection of HBV genome into C3H/HeN mice could impair the T cells' function in those mice, both globally and specifically (i.e. HBsAg-specific T-cell immunity). To demonstrate the issues, author evaluated the production of IFN-γ after HBsAg protein vaccination in HI C3H/HeN mice and HI C57BL/6 mice. However, the number of animals is low (n=3). And if the immune response (the production of IFN-γ) of HBsAg protein vaccination (S fragment of HBV DNA) in C3H/HeN mice and C57BL/6 mice after HI HBV genome can represent specific T-cell immunity? Please add comment in the revised manuscript. 2) Also, in Fig 4D, the differences in the pictures

depicting spots of IFN-γ after PMA and ionomysin stimulation were mild, please further present them with extensional numerous or with bar chart. 3) "Freshly isolated mouse splenocytes" in page 4, splenocytes including lymphocytes, antigen presenting cell, macrophages, dendritic cells, etc, in this manuscript, splenocyte is a mixed liquor of these cells or one of which? Minor comments 1) The references 2 is questionable, please check. 2) The format of the references should refer to WJG.