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Immune mechanisms of idiosyncratic drug-induced liver injury

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Dear Dr. Uetrecht,

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Dec 15, 2016.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

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Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This is an excellent review of the clinical and experimental evidence supporting the role of immune responses in idiosyncratic drug-induced liver injury (IDILI). I commend the great examples supporting the idea of immune mechanisms being the reason behind IDILI. I like how the paper is organized into 5 parts (introduction, evidence that IDILI is immune mediated, mechanistic hypotheses, impaired immune tolerance animal models, and conclusion). I think this is a good way to ease into the animal models because it was emphasized in the introduction that the mechanisms remain unclear because of the lack of reliable animal models that reflect what happens in humans. I also believe the figures were a great way to summarize for the reader the vast amount of information in this review.

Recommendations

1. The main points of this paper were organized really well but some of the sub sections could use some reordering.
a) For part 2 (Evidence that IDILI is immune mediated), I think you could separate (using sub-heading) it into subsections, including delayed onset of liver injury, rapid onset on re-challenge, HLA associations, positive lymphocyte transformation tests ect. This would keep it consistent with the format of the rest of the review and is easier for the readers to see the major clinical characteristics supporting that IDILI is immune mediated.
b) For part 3 (Mechanistic hypotheses), the authors only mentioned the hapten and danger hypotheses in the introduction of this part. Although these are the two major hypotheses, it would be beneficial to mention the other 3 hypotheses briefly in the introduction.
c) Part 4 (Impaired immune tolerance animal models), I think it would be better to labeled the sections "Depletion of Myeloid-Derived Suppressor Cells" and "Inhibition of Immune Check-Point Molecules".
d) Bigger font is recommended in some places of the figures (e.g. Figs. 2 and 3).
e) Color Contrast (e.g. Fig 2 (CD28 on the stressed hepatocyte is black font on dark purple background).
f) In Fig. 4, it would be beneficial to indicate which part is explaining which animal model.

Reviewer #2: The manuscript provides a detailed and high quality overview of the mechanistic basis of idiosyncratic adverse drug reactions. The content will be of great interest to readers of the Journal of Clinical and Translational Research. I feel the authors should address one important point. Near the end of the manuscript, the authors focus on

immune checkpoint inhibitors and the development of potentially exciting animal models. The receptors these molecules bind to have been studied widely in the clinical setting. A paragraph should be included to discuss differences in their expression/activity in various disease states including (if possible) idiosyncratic drug reactions. If not the latter is not possible, could the authors propose a future clinical strategy that would confirm their observations in experimental animals.

********Authors' rebuttal********

Re: revision JCTRes-D-16-00035

We would like to thank the reviewers for their comments. Both reviewers appeared to enjoy and approve of the content in the article. We have addressed all the reviewer comments. Changes in the actual document can be viewed by the track changes function. Additionally, we have responded to each reviewer comment below.

Reviewer #1: This is an excellent review of the clinical and experimental evidence supporting the role of immune responses in idiosyncratic drug-induced liver injury (IDILI). I commend the great examples supporting the idea of immune mechanisms being the reason behind IDILI. I like how the paper is organized into 5 parts (introduction, evidence that IDILI is immune mediated, mechanistic hypotheses, impaired immune tolerance animal models, and conclusion). I think this is a good way to ease into the animal models because it was emphasized in the introduction that the mechanisms remain unclear because of the lack of reliable animal models that reflect what happens in humans. I also believe the figures were a great way to summarize for the reader the vast amount of information in this review.

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Subheadings have been added.

b) For part 3 (Mechanistic hypotheses), the authors only mentioned the hapten and danger hypotheses in the introduction of this part. Although these are the two major hypotheses, it would be beneficial to mention the other 3 hypotheses briefly in the introduction.

The other hypotheses have been added to the introduction of the section “Mechanistic Hypotheses”.

c) Part 4 (Impaired immune tolerance animal models), I think it would be better to labeled the sections "Depletion of Myeloid-Derived Suppressor Cells" and "Inhibition of Immune Check-Point Molecules".

The subheadings for the section “Impaired immune tolerance animal models” have been changed to "Depletion of Myeloid-Derived Suppressor Cells" and "Inhibition of Immune Check-Point Molecules".

d) Bigger font is recommended in some places of the figures (e.g. Figs. 2 and 3).

Font has been increased.

e) Color Contrast (e.g. Fig 2 (CD28 on the stressed hepatocyte is black font on dark purple background).

Colour has been changed to aid in clarity.

f) In Fig. 4, it would be beneficial to indicate which part is explaining which animal model.

Additional labels in Figure 4, as well as the description of Figure 4 have been added.

Reviewer #2: The manuscript provides a detailed and high quality overview of the mechanistic basis of idiosyncratic adverse drug reactions. The content will be of great interest to readers of the Journal of Clinical and Translational Research. I feel the authors should address one important point. Near the end of the manuscript, the authors focus on immune checkpoint inhibitors and the development of potentially exciting animal models. The receptors these molecules bind to have been studied widely in the clinical setting. A paragraph should be included to discuss differences in their expression/activity in various disease states including (if possible) idiosyncratic drug reactions. If not the latter is not possible, could the authors propose a future clinical strategy that would confirm their observations in experimental animals.

A paragraph addressing immune tolerance in various disease states, as well as IDILI has been added.

PD-1 and CTLA-4 are expressed on a large proportion of tumour infiltrating lymphocytes in many different cancers (Montler et al., 2016, Sfanos et al., 2009, Ahmadzadeh et al., 2009). The expression of these molecules promote immune tolerance

and protect the tumours from attack by the immune system. Additionally, tumour cells from many types of cancers also express high levels of the major PD-1 ligand, PD-L1 (Dong et al., 2002, Konishi et al., 2004). Aside from cancer, PD-1 and CTLA-4 expression was elevated in other immune mediated diseases such as acute hepatitis A infection (Cho et al., 2016), hepatitis C infection (Nakamoto et al., 2009), and HIV infection (Day et al., 2006). This may seem ironic, but the immune system must keep a balance between an immune response that can destroy pathogens and an excessive reaction that causes tissue damage. In terms of IDRs, although most reactions are believed to be immune mediated, immune tolerance has not received much attention, and there is nothing published on the expression levels of PD-1 and CTLA-4 in these adverse reactions. In a study by Metushi et al., 2014 mentioned previously, patients taking INH as a precaution with no active tuberculosis were recruited. Blood samples were taken from these patients over time and their peripheral blood mononuclear cells (PBMCs) were phenotyped for changes over time. Although it was unlikely that a patient in this experiment would develop IDILI, 6 out of 16 patients did develop a small increase in ALT during INH treatment. Although in this experiment PD-1 and CTLA-4 expression was not evaluated, the patients that developed a small increase in ALT showed a significant increase in in T cells producing IL-10. IL-10 is considered an anti-inflammatory cytokine and is involved in immune tolerance. Follow-up studies to assess PD-1 and CTLA-4 expression in the same category of patients have been attempted in our lab; however, patient recruitment levels have been low and therefore there is no complete data as of now.

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