

## **The impact of sterile inflammation in acute liver injury**

Benjamin L. Woolbright, Hartmut Jaeschke

*Corresponding author: Benjamin L. Woolbright, Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160, USA*

---

Handling editor:

Rowan van Golen

Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, the Netherlands

Review timeline:

Received: 18 December, 2016

Editorial decision: 1 February, 2017

Revision received: 10 February, 2017

Editorial decision: 12 February, 2017

Published online ahead of print: 12 February, 2017

---

1<sup>st</sup> Editorial decision

Date: 1 Feb, 2017

Ref.: Ms. No. JCTRes-D-16-00042

The Impact of Sterile Inflammation in Acute Liver Injury

Journal of Clinical and Translational Research

Dear Dr. Woolbright,

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 Jun 01, 2017.

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

Rowan van Golen  
Associate Editor  
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The liver has a number of functions in innate immunity. Sterile inflammation in the liver is a common outcome of a number of different clinical liver disorders which occurs in the absence of any infection. Liver sterile inflammation has been studied in a number of models for decades; but a great deal of controversy and many questions about the nature of sterile inflammation still exist.

In this review, the authors aim to provide a critical update on mechanisms of sterile inflammatory liver injury and further discuss the role of sterile inflammation in clinically relevant models of liver injury. The authors commence with a brief overview of sterile inflammatory liver injury using both a basic approach to what constitutes the inflammatory injury, and through examination of current models of liver injury and inflammation. And more information about sterile inflammation will be tied for both scientists and clinicians to develop rational treatments.

This is a well-designed and well written review. It fills an important knowledge gap and the work deserves to be published. There are several issues that needs to be further considered and clarified.

1. The review focused mainly on three types of immune cells, Kupper cells, monocytes-derived macrophages and neutrophils. Beside these cell types, are there any other cell types, such as dendritic cells, NK cells also involved in liver sterile inflammation and what are potential roles?
2. The role of neutrophils in liver I/R injury has been intensively studied. However, neutrophil extracellular traps have been recently found in various sterile pathophysiological conditions beside infection. Are they involved in liver sterile inflammation and what are potential roles of them?
3. Beside those highlighted DAMPs released after necrosis, any other DAMPs such as histones involved in liver sterile inflammation since it has shown as death mediators in sepsis model?

Minor issue:

Could not found reference: Woolbright and Jaeschke 2017, but in reference list: Woolbright and Jaeschke 2016

Reviewer #2: The current manuscript is a comprehensive review of the role of sterile inflammation in acute liver injury. The authors discuss the mechanisms of sterile inflammation, including the main cellular and molecular players, within the context of experimental models of acute liver injury with relevance to human disease, including ischemia/reperfusion, acetaminophen overdose, and obstructive cholestasis. I believe that this authoritative review article on a subject of great clinical relevance will meet the interest of a vast audience of readers. I only have a few minor issues, listed below.

1. Graphical abstract: I suggest that authors try to position the activated macrophage (presumably a Kupffer cell) closer to the damaged hepatocyte (like they did in the BDL model, Fig. 1), since close proximity is required for macrophage-derived ROS to act on the hepatocyte.
2. Page 4, lines 33-38: consider revising punctuation as follows: "While in some models there is near universal agreement that inflammation is a direct, pathological component of the injury process, there are other models wherein there is considerable debate over the role of sterile inflammation".
3. Page 4, line 50: I advice replacing "(...) in clinically relevant models of liver injury" with "(...) in clinically relevant models of acute liver injury", to stress the fact that only models of acute injury were addressed herein.
4. Page 6, line 28: "local productions" should read "local production".
5. Page 30, line 47: please spell out TGR5 and FXR.
6. Page 36, line 15: In the following sentence of the Conclusions, "A sterile inflammatory response is an important aspect of both acute and chronic liver injury", I suggest removing the reference to chronic injury, since this has not been discussed in the present work.
7. Whole text: The term "Kupffer cells" is either spelled out or abbreviated throughout the manuscript, and in many occasions both forms are employed within the same page (e.g. pages 7 and 8). Please choose one option only for consistency.

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

Reviewers' comments:

Reviewer #1: The liver has a number of functions in innate immunity. Sterile inflammation in the liver is a common outcome of a number of different clinical liver disorders which occurs in the absence of any infection. Liver sterile inflammation has been studied in a number of models for decades; but a great deal of controversy and many questions about the nature of sterile inflammation still exist.

In this review, the authors aim to provide a critical update on mechanisms of sterile inflammatory liver injury and further discuss the role of sterile inflammation in clinically relevant models of liver injury. The authors commence with a brief overview of sterile inflammatory liver injury using both a basic approach to what constitutes the inflammatory

injury, and through examination of current models of liver injury and inflammation. And more information about sterile inflammation will be tied for both scientists and clinicians to develop rational treatments.

This is a well-designed and well written review. It fills an important knowledge gap and the work deserves to be published. There are several issues that needs to be further considered and clarified.

**Thank you for the review.**

1. The review focused mainly on three types of immune cells, Kupper cells, monocytes-derived macrophages and neutrophils. Beside these cell types, are there any other cell types, such as dendritic cells, NK cells also involved in liver sterile inflammation and what are potential roles?

**Thank you for the question. We have added small sections on NK cells and dendritic cells as there may be minor roles for these cell types. See section “Other Inflammatory Cells”, p14. Also see discussion of Masson et al., 2008 and Liu et al., 2004 on pages 24-25.**

2. The role of neutrophils in liver I/R injury has been intensively studied. However, neutrophil extracellular traps have been recently found in various sterile pathophysiological conditions beside infection. Are they involved in liver sterile inflammation and what are potential roles of them?

**Thank you for the question. As of yet, we have not begun studying NETs in sterile inflammation after APAP overdose, hepatic IR or cholestatic liver injury. There is only minimal current information available on this topic, but we have added this information to this paper. See Neutrophils section page 13. Given the high degree of extracellular DNA present in patient serum from APAP overdose patients , it is entirely possible there is considerable NET deposition; however, it may be difficult to define this without intravital microscopy which our lab currently does not have available. Given the lack of information, we would prefer to avoid speculation outside the information presented.**

3. Beside those highlighted DAMPs released after necrosis, any other DAMPs such as histones involved in liver sterile inflammation since it has shown as death mediators in sepsis model?

**Thank you for the question. We have added some information on histone as a DAMP (page 5, page 18). Generally, we have tried to avoid discussing individual DAMPs excessively in this review, but rather we have focused on the pathophysiology.**

Minor issue:

Could not found reference: Woolbright and Jaeschke 2017, but in reference list: Woolbright and Jaeschke 2016.

**Thank you, we have resolved this and replaced 2017 with 2016. This was an error as we expected the references paper to be published at this time. See page 4.**

Reviewer #2: The current manuscript is a comprehensive review of the role of sterile inflammation in acute liver injury. The authors discuss the mechanisms of sterile inflammation, including the main cellular and molecular players, within the context of experimental models of acute liver injury with relevance to human disease, including ischemia/reperfusion, acetaminophen overdose, and obstructive cholestasis. I believe that this authoritative review article on a subject of great clinical relevance will meet the interest of a vast audience of readers. I only have a few minor issues, listed below.

**Thank you for the kind comments.**

1. Graphical abstract: I suggest that authors try to position the activated macrophage (presumably a Kupffer cell) closer to the damaged hepatocyte (like they did in the BDL model, Fig. 1), since close proximity is required for macrophage-derived ROS to act on the hepatocyte.

**Thank you for the comments, we have updated the graphical abstract.**

2. Page 4, lines 33-38: consider revising punctuation as follows: "While in some models there is near universal agreement that inflammation is a direct, pathological component of the injury process, there are other models wherein there is considerable debate over the role of sterile inflammation".

**Fixed as suggested. Page 4**

3. Page 4, line 50: I advice replacing "(...) in clinically relevant models of liver injury" with "(...) in clinically relevant models of acute liver injury", to stress the fact that only models of acute injury were addressed herein.

**Fixed as suggested. Page 4.**

4. Page 6, line 28: "local productions" should read "local production".

**Fixed as suggested. Page 6.**

5. Page 30, line 47: please spell out TGR5 and FXR.

**Fixed as suggested. Page 31.**

6. Page 36, line 15: In the following sentence of the Conclusions, "A sterile inflammatory response is an important aspect of both acute and chronic liver injury", I suggest removing the reference to chronic injury, since this has not been discussed in the present work.

**Fixed as suggested. Page 37**

7. Whole text: The term "Kupffer cells" is either spelled out or abbreviated throughout the manuscript, and in many occasions both forms are employed within the same page (e.g. pages 7 and 8). Please choose one option only for consistency.

**Fixed as suggested throughout manuscript.**

---

2<sup>nd</sup> Editorial decision

Date: 12 Feb, 2017

Ref.: Ms. No. JCTRes-D-16-00042R1

The Impact of Sterile Inflammation in Acute Liver Injury

Journal of Clinical and Translational Research

Dear Dr. Woolbright,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Thank you for submitting your work to JCTR.

Kindest regards,

Rowan van Golen

Associate Editor

Journal of Clinical and Translational Research