



HAL
open science

Intrahepatocytic necroptosis is dispensable for hepatocyte death in murine immune-mediated hepatitis

Annaïg Hamon, Claire Piquet-Pellorce, Marie-Thérèse Dimanche-Boitrel,
Michel Samson, Jacques Le Seyec

► **To cite this version:**

Annaïg Hamon, Claire Piquet-Pellorce, Marie-Thérèse Dimanche-Boitrel, Michel Samson, Jacques Le Seyec. Intrahepatocytic necroptosis is dispensable for hepatocyte death in murine immune-mediated hepatitis. *Journal of Hepatology*, 2020, 73 (3), pp.699-701. 10.1016/j.jhep.2020.05.016 . hal-02888815

HAL Id: hal-02888815

<https://hal.science/hal-02888815>

Submitted on 7 Oct 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Intra-hepatocytic necroptosis is dispensable for hepatocyte death in murine immune-mediated hepatitis

Hamon Annaïg¹, Piquet-Pellorce Claire¹, Dimanche-Boitrel Marie-Thérèse¹, Samson Michel^{1*}, Le Seyec Jacques¹

¹ Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France.

* **Corresponding author:** Michel Samson, INSERM U1085, Irset, Université de Rennes 1, 2 av Prof Leon Bernard, 35043 Rennes cedex, France ; Tel: (+33) (0)2 23 23 69 11 ; Fax : (+33) (0)2 23 23 50 55 ; michel.samson@univ-rennes1.fr

Keywords: Hepatitis; Inflammation; Cell Death; Disease Models, Animal.

Electronic word count: 696

Number of figure: 1

Conflict of interest statement: The authors have no conflicts of interest to declare.

Financial support statement: This work was supported by the “Contrat de Plan Etat-Région” (CPER) grant named “Infectio”; the “Ligue Contre le Cancer, Comités du Grand Ouest”; the Biology and Health Federative Research Structure of Rennes (Biosit, UMS CNRS 3480 / US INSERM 018); the “Agence Nationale de la Recherche” (ANR Labcom Oncotrial) and the “Fondation pour la Recherche Médicale” (FRM # DEQ20180339216). A.H. was supported by a postdoctoral fellowship from the “Fondation pour la Recherche Médicale”.

Authors' contributions: Conceptualization, A.H., C.P.P., M.T.D.B., M.S. and J.L.S.; Methodology, A.H., C.P.P., M.T.D.B., M.S. and J.L.S.; Validation, A.H., C.P.P., M.T.D.B., M.S. and J.L.S.; Formal Analysis, A.H.; Investigation, A.H., C.P.P. and J.L.S.; Data Curation, A.H.; Writing – Original Draft Preparation, A.H.; Writing – Review & Editing, M.S. and J.L.S.; Visualization, A.H.; Supervision, M.S. and J.L.S.; Project Administration, A.H., C.P.P., M.T.D.B., M.S. and J.L.S.; Funding Acquisition, M.T.D.B., M.S. and J.L.S.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Letter to the editor

Worldwide hundreds of millions of people are affected by acute or chronic hepatitis, without effective treatments. Hepatocyte death is a key element in both initiation and progression of these liver diseases. Necroptosis emerged recently as one of the possible cell death programs involved in liver damage. This pathway is a regulated modality of necrosis that occurs in the presence of caspase inhibition. It involves RIP1/3 (receptor interacting protein) kinases and a final effector, the pseudokinase MLKL (mixed lineage kinase domain-like protein) [1]. Many studies have investigated the role of these necroptosis-associated proteins in different liver injury models showing sometimes conflicting results [2]. Thus, the involvement of necroptosis in different liver diseases still remains controversial and a hot topic in hepatology research.

In 2016, Günther *et al.* have reported that necroptosis mediates hepatocyte death in a murine model of immune-mediated hepatitis [3]. Indeed, using mice with a total ablation of *mlkl* (Mkl^{-/-} mice) challenged by Concanavalin A (ConA), a lectin triggering severe inflammation and T-cell mediated liver injury, they found that Mkl^{-/-} mice are completely protected from ConA-induced damage compare to wild-type mice [4]. The authors concluded that the pseudokinase MLKL is an essential mediator of hepatocellular necrosis in ConA-induced hepatitis in mice.

To deepen the understanding of the role of MLKL in ConA-induced hepatitis and particularly in hepatocyte it-self, we have generated a new mouse line with conditional ablation of *mlkl* specifically in liver parenchymal cells (MLKL^{LPC-KO} mice). As expected, these Mkl^{LPC-KO} mice present reduced level of MLKL in the whole liver and total absence of MLKL in isolated hepatocytes as shown by western blotting analyses (Fig. 1A). Importantly, intravenous treatment with ConA at a dose of 12 mg/kg for 6h, 11h and 16h did not reveal any differences in levels of serum transaminases and degree of necrotic damage of the liver between Mkl^{LPC-KO} mice and Mkl^{fl/fl} littermate controls (Fig. 1B and 1C). These results show that Mkl^{LPC-KO} mice are not protected from ConA-induced hepatitis demonstrating that hepatocyte-intrinsic MLKL is dispensable for ConA-induced hepatolysis in contrary to what has been advanced by Günther *et al* [5].

The opposed results obtained with the Mkl^{-/-} and the Mkl^{LPC-KO} mice, explained by the use of a constitute knockout model on the one hand and specific knockout model on the other hand, suggest that necroptosis occurring in other cell population(s) than in hepatocytes themselves is involved in hepatolysis in response to ConA injection, in which immunity is essential to hepatocyte damage. Günther *et al.* excluded the involvement of immune cells performing bone marrow graft experiments [3]. However, they conclude for an essential role of the hepatocyte-intrinsic MLKL excluding the possibility of a role of necroptosis in other intra-hepatic cell populations. Further investigations studying the effect of ConA in mice lacking MLKL in other cell populations than parenchymal cells are needed to better understand liver damage process. Liver sinusoidal endothelial cells (LSEC) are potential interesting candidates to investigate since necroptosis could be induced in endothelial cells [5].

Moreover, our new data show that other cell death types than necroptosis can lead to hepatocyte death during ConA-induced hepatitis. As Günther *et al.*, we did not observe apoptotic cells following ConA injection either in Mkl^{fl/fl} or in Mkl^{LPC-KO} mice (data not shown), in contrast to what we already observed in immune-mediated hepatitis in Ripk1^{LPC-KO} mice [6,7]. Other cell death pathways have now to be considered. Luan *et al.*, in 2018, suggested that pyroptosis could be one of the cell death programs involved in ConA-induced hepatitis [8]. We think that we have to reconsider the research on hepatocyte death taking into account the fact that different pathways could act in synergy and compensate each other as addressed in the new concept of PAN-optosis [9,10].

To conclude, our data demonstrate that intra-hepatocytic MLKL and then necroptosis are dispensable for hepatocyte death in this model of immune-mediated hepatitis. Moreover, our results open new insights on the involvement of other intra-hepatic cell populations in hepatocellular necrosis in this context. Thus, the

design of therapeutic approaches integrating prevention of liver tissue cell death will have to take into account the disparity of induced death types and their interconnections.

Acknowledgment

We would like to thank Prof. Warren S. Alexander (The Walter and Eliza Hall Institute of Medical Research, Australia) for the gift of the 592MLKL/Flp mice used to establish the Mkl^{LPC-KO} mouse line. We also extend our thanks to Mélanie Simoes-Eugénio, Christelle Devisme, Ghania H. Kara-Ali, Huma Hameed and Abderrahman Chafik for their technical assistance and to Dr. Muhammad Farooq for his fruitful scientific discussions. We acknowledge the “Laboratoire de Biochimie-Toxicologie” from the “CHU de Rennes” for transaminase measurements. For histological analysis and animal house facilities, we would like to thank the dedicated platforms (i.e. H2P2 and animal house platforms) of SFR Biosit – UMS 3480, US_S 018, France.

References

- [1] Petrie EJ, Czabotar PE, Murphy JM. The Structural Basis of Necroptotic Cell Death Signaling. *Trends Biochem Sci* 2019;44:53–63. <https://doi.org/10.1016/j.tibs.2018.11.002>.
- [2] Dara L, Liu Z-X, Kaplowitz N. Questions and controversies: the role of necroptosis in liver disease. *Cell Death Discov* 2016;2:16089. <https://doi.org/10.1038/cddiscovery.2016.89>.
- [3] Günther C, He G-W, Kremer AE, Murphy JM, Petrie EJ, Amann K, et al. The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. *J Clin Invest* 2016;126:4346–60. <https://doi.org/10.1172/JCI87545>.
- [4] Heymann F, Hamesch K, Weiskirchen R, Tacke F. The concanavalin A model of acute hepatitis in mice. *Lab Anim* 2015;49:12–20. <https://doi.org/10.1177/0023677215572841>.
- [5] Strilic B, Yang L, Albarrán-Juárez J, Wachsmuth L, Han K, Müller U, et al. Tumour-cell-induced endothelial cell necroptosis via death receptor 6 promotes metastasis. *Nature* 2016;536:215–8.
- [6] Filliol A, Piquet-Pellorce C, Le Seyec J, Farooq M, Genet V, Lucas-Clerc C, et al. RIPK1 protects from TNF- α -mediated liver damage during hepatitis. *Cell Death Dis* 2016;7:e2462–e2462. <https://doi.org/10.1038/cddis.2016.362>.
- [7] Filliol A, Piquet-Pellorce C, Raguénès-Nicol C, Dion S, Farooq M, Lucas-Clerc C, et al. RIPK1 protects hepatocytes from Kupffer cells-mediated TNF-induced apoptosis in mouse models of PAMP-induced hepatitis. *J Hepatol* 2017;66:1205–13. <https://doi.org/10.1016/j.jhep.2017.01.005>.
- [8] Luan J, Zhang X, Wang S, Li Y, Fan J, Chen W, et al. NOD-Like Receptor Protein 3 Inflammasome-Dependent IL-1 β Accelerated ConA-Induced Hepatitis. *Front Immunol* 2018;9:758. <https://doi.org/10.3389/fimmu.2018.00758>.
- [9] Malireddi RKS, Kesavardhana S, Kanneganti T-D. ZBP1 and TAK1: Master Regulators of NLRP3 Inflammasome/Pyroptosis, Apoptosis, and Necroptosis (PAN-optosis). *Front Cell Infect Microbiol* 2019;9:406. <https://doi.org/10.3389/fcimb.2019.00406>.
- [10] Schwarzer R, Laurien L, Pasparakis M. New insights into the regulation of apoptosis, necroptosis, and pyroptosis by receptor interacting protein kinase 1 and caspase-8. *Curr Opin Cell Biol* 2020;63:186–93. <https://doi.org/10.1016/j.ceb.2020.02.004>.

Figure 1

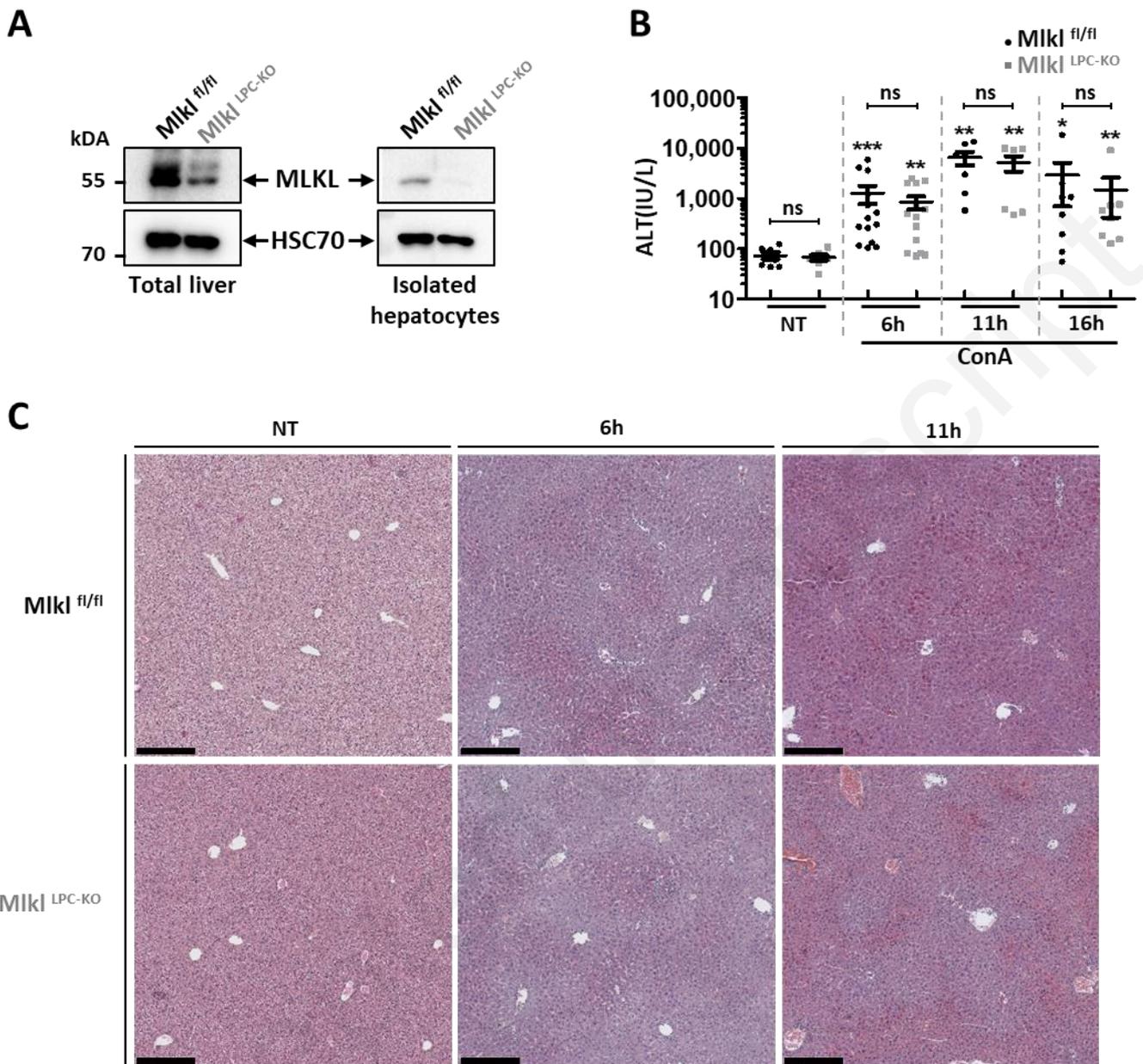


Fig.1. *Mlkl*^{LPC-KO} mice are not protected from ConA-induced hepatitis. Mice with conditional ablation of *mkl* in liver parenchymal cells (*Mlkl*^{LPC-KO} mice) were generated by crossing *Mlkl*-floxed mice with *alp*-cre transgenic mice. (A) MLKL depletion in parenchymal liver cells is efficient. Western blot analyses were performed on whole liver or isolated hepatocyte protein extracts using anti-*Mlkl* antibody and anti-Hsc70 antibody as loading control. (B and C) Lack of MLKL in parenchymal cells does not confer protection against ConA-induced hepatitis. *Mlkl*^{LPC-KO} mice and their littermate controls (*Mlkl*^{fl/fl}) were injected intravenously with vehicle (PBS, 1 mM CaCl₂, 0.5 mM MnCl₂) (NT: Non Treated; 7 mice per genotype) or with 12 mg/kg of ConA and euthanized at 6, 11 or 16h post-injection (14, 7 or 8 mice per genotype, respectively). (B) Levels of serum alanine aminotransferase. Each dot and square represent an individual; error bars are expressed as mean ± SEM; each time point of ConA-treatment was compared to the NT related mouse strain and comparisons were made between the two mouse strains at each time point. Mean differences between experimental groups were assessed using the non-parametric Mann–Whitney U-test. Significance is shown as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns: non-significant. (C) Representative pictures of liver tissue sections analyzed by hematoxylin and eosin staining. Black bars represent 250 μm.