Cellular & Molecular Immunology (2018) 15, 74–75

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LETTER TO THE EDITOR

Mechanisms of sterile inflammation in acetaminophen hepatotoxicity

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Cellular & Molecular Immunology (2018) **15,** 74–75; doi:10.1038/cmi.2017.49; published online 10 July 2017

Acetaminophen (APAP) hepatotoxicity is the most frequent cause of acute liver failure in many Western countries. The intracellular signaling mechanisms of APAP-induced cell death have been extensively studied, and the critical roles of key features such as reactive metabolite generation, glutathione depletion, mitochondrial oxidant stress and peroxynitrite formation, mitochondrial dysfunction, and nuclear DNA fragmentation are all well-established.[1] Not surprisingly, a substantial number of intracellular molecules, termed damage-associated molecular patterns (DAMPs), are passively released into the circulation during the resulting necrosis. DAMPs include high mobility group box 1 protein, nuclear DNA fragments and mitochondrial DNA, with most being recognized by pattern recognition receptors such as various Toll-like receptors (TLRs) on macrophages.[2] As a consequence of DAMPs binding to TLRs, macrophages generate pro-inflammatory cytokines that are responsible for the activation and hepatic recruitment of neutrophils and monocytes.[2] These events are undisputed and occur in mouse models of APAP hepatotoxicity *in vivo* and in human patients with APAP overdose.[3] However, which inflammatory mediators are critical for inflammatory cell recruitment and whether neutrophils and/or monocytes actually contribute to the injury process are highly controversial issues. One of the most debated inflammatory mediators is interleukin-1β (IL-1β). IL-1β requires TLR9 for transcriptional activation, formation of pro-IL-1β and activation of the Nalp3 (NACHT, LRR and pyrin domain-containing protein 3) inflammasome, presumably through stimulation of a purinergic receptor (e.g., P2X7), to activate caspase-1, which cleaves pro-IL-1β to the active cytokine.[4] A more detailed investigation into the role of IL-1β in APAP hepatotoxicity could confirm the transcriptional activation of pro-IL-1β and the caspase-1-dependent formation of the active cytokine,[5] but the absolute amount of IL-1β is orders of magnitude too low to have relevant effects on the pathophysiology, and neither caspase inhibitors nor knockout (KO) mice of the IL-1 receptor or any component of the inflammasome (Nalp3, ASC, caspase-1) showed any protective effects.[5,6]

In their manuscript published in *Cellular & Molecular Immunology*, Zhang *et al.*[7] confirmed the limited relevance of IL-1β and of the Nalp3 inflammasome in APAP hepatotoxicity by showing that neither mice treated with anti-IL-1β antibodies nor gene KO animals of IL-1β, caspase-1 and Nalp3 were protected, nor did they show reduced hepatic neutrophil and monocyte accumulation. In contrast, the authors provided strong evidence that macrophage-derived IL-1α formation is associated with inflammatory cell recruitment and liver injury, mainly between 6 and 24 h after APAP. Animals treated with IL-1α antibodies and IL-1α KO mice were protected.[7] Moreover, deletion of Kupffer cells with clodronate liposomes and reconstitution with macrophages from wild-type or IL-1β KO mice did not have protective effects; only the transfer of IL-1α KO macrophages was protective.[7] Likewise, no protection was observed when Kupffer cell-depleted mice were reconstituted with macrophages from wild-type and KO mice of TLR3 and TLR7/9, but protection was observed when TLR4 and MyD88 KO macrophages were transferred, which also resulted in less IL-1α formation.[7] These data were further supported by evidence that mice treated with IL-1 receptor antibodies and IL-1R KO mice experienced reduced injury in this model, as did animals pretreated with antibodies that deplete neutrophils and monocytes.[7] Together, the experiments presented by the authors make a compelling argument that IL-1α generated through activation of TLR4 signaling in macrophages, not IL-1β, is the key mediator of the activation and hepatic recruitment of neutrophils and monocytes contributing to aggravation

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Received: 18 May 2017; Accepted: 19 May 2017

of early injury in APAP hepatotoxicity. The authors' conclusions are based on a variety of interventions, including both blocking antibodies as well as several gene KO mice, which gives more confidence in the reproducibility of the results. Furthermore, the authors have verified that the early metabolism phase of APAP toxicity was not affected by these interventions. The authors have also compared their results to at least one other hypothesis, i.e., the suggested roles of the Nalp3 inflammasome and IL-1β. Thus, the current study provides the most solid and convincing evidence to date for IL-1α being a critical mediator in APAP hepatotoxicity.

However, one cannot overlook the fact that the authors' data support some previous findings but also contradict a substantial number of other studies.[2,8] For example, mice deficient in caspase-1, Nalp3, TLR3 or TLR9 have all been previously reported to be protected against APAP hepatotoxicity, while IL-1R KO mice were not.[2,8] This is part of a growing trend in which many published results cannot be reproduced by other scientists. The reasons are mostly unclear, but could include issues with strain or substrain mismatch between wild-type and KO animals, the sources of the animals, environmental issues (the gut microbiome), and other differences. In contrast to these speculative problems, the use of neutropenia-inducing antibodies 24 h before APAP treatment is a clear concern. The fact that depletion of neutrophils will result in accumulation of inactivated neutrophils in sinusoids, where they are phagocytosed by Kupffer cells, has been repeatedly reported in detail.[8] This stress of massive Kupffer cell activity causes extensive adaptation reactions, which make the livers of neutropenic animals at least temporarily resistant to future stresses, such as APAP overdose.[8] Hence, the protection seen in these mice is caused by a preconditioning effect rather than the absence of neutrophils.[8] Thus, neither the current manuscript[7] nor previous studies using similar intervention strategies reliably show the involvement of neutrophils in this process.[8] Neutrophil cytotoxicity is critically dependent on reactive oxygen formation, but the general deficiency of a functional NADPH oxidase has no effect on APAP-induced oxidant stress or liver injury.[9] In fact, neutrophil activation mainly occurs after the peak of injury and correlates more with recovery in both mice and humans.[9] Similar findings were reported for monocyte-derived macrophages.[10]

The manuscript by Zhang *et al.* provides strong evidence for the involvement of macrophage-derived IL-1α, but not IL-1β, in the pathophysiology of APAP hepatotoxicity.[7] However, the assumed contribution of neutrophils and monocytes as effector cells of IL-1α in mediating the late toxicity is questionable. Further studies are necessary to evaluate additional, inflammatory cell-independent mechanisms of toxicity for IL-1α.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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