Study of hepatolysis induced by immune cells in murine hepatitis models: role of RIPK1

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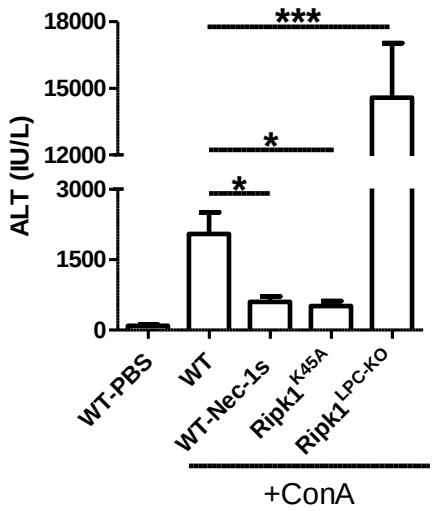
Hepatocyte death is a starting point of liver disease progression[1], and when it is massive, may be fatal for people. Moreover, failure to resolve the causes of hepatitis can lead to chronic hepatocyte death which contributes to the persistence of inflammatory and regeneration mechanisms[1,2], creating an environment conducive for hepatocellular carcinoma (HCC) development[1,3]. Inhibition of acute or chronic hepatolyis is thus considered to be a therapeutic strategy to limit liver damage and the sequences of these processes. Immune cells play a role in the induction or amplification of hepatocyte death mediated by expression and release of death ligands belonging to the TNF-α superfamily including TNF-α which is also known as a powerful pro-inflammatory cytokine[3]. However, signaling pathway downstream of the TNF-receptor family is not completely understood.

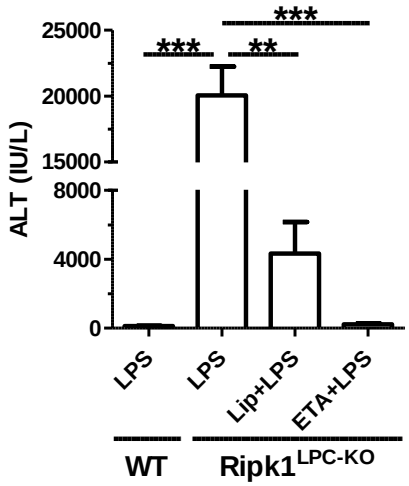
The roles of Receptor-Interacting-Protein Kinases (RIPK) during hepatitis is the subject of many debates[4], and some studies suggest that the serine/threonine kinase RIPK1 plays a role in the induction of hepatocyte death during T-lymphocyte-dependent hepatitis, induced by the Concanavalin A (ConA) in mice[5]. Through chemical and genetic approaches, we studied involvement of RIPK1 in the hepatocyte death process.



First, we confirmed that inhibition of RIPK1 kinase activity reduces hepatolysis induced by ConA administration, using RIPK1 kinase dead mice (*Ripk1*^{K45A}) and the Nec-1s, an inhibitor of the RIPK1 kinase activity. However, using mice deficient in RIPK1 only in liver parenchymal cells (LPC) (*Ripk1*$^{LPC\text{-}KO}$) we observed a sensitivity of mice to ConA-induced hepatitis (Fig. 1). Interestingly, these mice did not exhibit hepatolysis under physiological conditions, revealing that RIPK1 was necessary for the hepatocyte survival and the maintenance of hepatic homeostasis under inflammatory conditions. By dissecting *in vivo* and *in vitro* the molecular mechanisms involved in this process, we revealed that RIPK1 leads to cell survival downstream of the TNF-α. Without interfering with the NF-κB survival pathway, RIPK1 stabilizes the TRAF2 protein and thus prevents caspase activation and hepatocyte death[6].

Figure1. Double edge sword of RIPK1 during the immune-hepatitis model induced by ConA. ALT serum levels 11 h after ConA or PBS administration in WT mice pretreated eventually with Nec-1s, Ripk1 kinase dead mice (*Ripk1*^{K45A}), and mice deficient for Ripk1 in LPC (*Ripk1*$^{LPC\text{-}KO}$). Graph +/- SEM, *p<0,5 ; *** p<0.001.

Following to this mechanistic discovery, we studied the role of RIPK1 in a more physiopathological context. Chronic ingestion of alcohol or of lipid-rich products leads to the development of hepatitis but also to a permeabilization of the intestinal barrier. This latter one is known



to be responsible for an elevation of bacterial Pathogen Associated Molecular Patterns (PAMPs) in the liver. This phenomenon is known as one of the causes of acute on chronic liver failure (ACLF) known to be a very poor prognostic[7,8]. These PAMPs can be mimicked by administration of lipopolysaccharide (LPS) and unmethylated CpG-DNA. While these two bacterial motifs do not induce hepatolysis in wild-type mice, we have shown that the absence of RIPK1 in LPC induces apoptotic hepatocyte death. By eliminating macrophages using clodronate liposomes, or by TNF-α inhibition, we have been able to demonstrate that this hepatocyte death is dependent on TNF-α released mainly by PAMPs-activated macrophages[9] (Fig. 2).

Figure2. RIPK1 protects hepatocytes from death induced by TNF-a released by LPS-activated macrophages. ALT serum levels in WT mice or *Ripk1*$^{LPC\text{-}KO}$ mice, 8h30 after LPS administration pretreated eventually with clodronate-encapsulated liposomes or with Etanercept (ETA), a TNF-α inhibitor. Graph +/- SEM, *p<0,5; ** p<0.01; *** p<0.001.

These results made it possible to specify the role of RIPK1 in different acute hepatitis models. The ability of RIPK1 to control the death and the survival of hepatocytes in an inflammatory context suggests its involvement in chronic hepatitis and opens the door to work in human liver disease and studies of its inhibition as a therapeutic target.

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