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Intestinal Dysbiosis Augments Liver Disease Progression Via NLRP3 in a Murine Model of Primary Sclerosing Cholangitis

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Background: There is a striking association between human cholestatic liver disease and inflammatory bowel disease. However, the implications for intestinal microbiota and inflammasome mediated innate immune response in cholestatic liver disease remain elusive. Here we investigated the functional role of gut-liver crosstalk for cholestatic liver disease in the murine Mdr2 knockout (*Mdr2*$^{-/-}$) model resembling human primary sclerosing cholangitis (PSC).

Methods: *Mdr2*$^{-/-}$, *Mdr2*$^{-/-}$ crossed with hepatocyte-specific deletion of capsase-8 (*Mdr2*$^{-/-}$/*Casp8*$^{\Delta hepa}$) and wildtype (WT) control mice were housed for 8w or 52w respectively to characterize the impact of Mdr2 deletion on liver and gut including comprehensive bile acid and microbiota profiling. To block caspase activation, a pan-caspase inhibitor (IDN-7314) was administered. Finally, the functional role of *Mdr2*$^{-/-}$ associated intestinal dysbiosis was studied by microbiota transfer (FMT) experiments.

Results: *Mdr2*$^{-/-}$ mice displayed an unfavorable intestinal microbiota signature and pronounced NLRP3 inflammasome activation within the gut-liver axis, as found by immunostaining and western blot analysis in the intestine as well as in the liver. Intestinal dysbiosis in *Mdr2*$^{-/-}$ mice prompted intestinal barrier dysfunction evidenced by reduced colonic mucus layers, reduction of tight junction expression and increased permeability evidenced by an *in-vivo* FITC-dextran assay. Loss of intestinal barrier integrity and bacterial translocation triggered the hepatic NLRP3 mediated innate immune response and fueled liver disease progression. Strikingly, transfer of *Mdr2*$^{-/-}$ microbiota into healthy WT control mice, urged intestinal barrier impairment and induced significant liver injury in recipient mice, highlighting the causal role of intestinal dysbiosis for disease progression in *Mdr2*$^{-/-}$ mice. This phenotype could not be rescued by introducing *Mdr2*$^{-/-}$/*Casp8*$^{\triangle hepa}$ indicating that hepatocytic caspase-8 activation is a downstream consequence and dispensable for the inflammatory response. In contrast, caspase inhibition via IDN-7314 dampened inflammasome activation, improved intestinal barrier function, ameliorated liver injury, reversed serum bile acid profile and cholestasis associated microbiota signature.

Conclusion: Cholestatic liver disease in *Mdr2*$^{-/-}$ mice triggers intestinal dysbiosis, which is transmissible to healthy WT mice. In turn, translocation of endotoxin into the portal vein and subsequent NLRP3 inflammasome activation contribute to higher liver injury in Mdr2$^{-/-}$ mice. This process does not essentially depend on hepatocytic caspase-8, but can be blocked by IDN-7314, highlighting the central role of the inflammasome mediated innate immune within the gut-liver axis.

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