Interleukin-1 receptor antagonist modulates liver inflammation and fibrosis in mice in a model-dependent manner

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Abstract

Background: Interleukin-1 (IL-1)β and IL-1 receptor antagonist (IL-1Ra) have been proposed as important mediators during chronic liver diseases. We aimed to determine whether the modulation of IL-1β signaling with IL-1Ra impacts on liver fibrosis. Methods: We assessed the effects of IL-1β on human hepatic stellate cells (HSC) and in mouse models of liver fibrosis induced by bile duct ligation (BDL) or carbon tetrachloride treatment (CCl-4). Results: Human HSCs treated with IL-1β had increased IL-1β, IL-1Ra, and MMP-9 expressions in vitro. HSCs treated with IL-1β had reduced α-smooth muscle actin expression. These effects were all prevented by IL-1Ra treatment. In the BDL model, liver fibrosis and Kuppfer cell numbers were increased in IL-1Ra KO mice compared to wild type mice and wild type mice treated with IL-1Ra. In contrast, after CCl-4 treatment, fibrosis, HSC and Kupffer cell numbers were decreased in IL-1Ra KO mice compared to the other groups. IL-1Ra treatment provided a modest protective effect in the BDL model and was pro-fibrotic in the CCl-4 model. Conclusions: We demonstrated bivalent effects of IL-1Ra [...]
Supplementary Figure 1. IL-1Ra does not reverse TGF-β1-dependant stellate cell activation. (A) Total protein extracts from untreated LX-2 human hepatic stellate cells (LX-2) (control), LX-2 treated with TGF-β, and LX-2 treated with TGF-β and IL-1Ra simultaneously were obtained. Protein extracts were subjected to SDS-PAGE, transferred to nitrocellulose and blotted with anti-α-SMA and vimentin. (B) α-SMA signals were quantified by densitometry and normalized using vimentin signals as a loading control.

Supplementary Figure 2. IL-1Ra knockout mice rescue with IL-1Ra treatment. Mice livers were fixed in formalin and embedded in paraffin following 2-4 weeks BDL. Groups presented in figure 3 were compared to IL-1Ra KO mice treated with IL-1Ra treated mice (50 mg/kg/day) (n=5). Liver sections
stained by sirius red (A). On sirius staining liver parenchyma appears in light yellow and fibrotic areas in red. Morphometric quantification of fibrosis was performed on multiple liver sections and expressed as percentage of liver surface area (B). Serum levels of IL-1Ra (C) and fasting insulin (D) were measured by ELISA. Scale bars, 400 µm. * p < 0.05, ** p < 0.01, *** p < 0.001, comparing two groups as indicated.

Supplementary Figure 3. Comparison between wild type (WT) and knockout (KO) baseline mRNA levels of fibrosis-related gene of interest. IL-1β, IL-1Ra, collagen type I, α-SMA, matrix metalloproteinase (MMP) 2, 9 and 13, tissue inhibitor of metalloproteinase 1 (TIMP-1) baseline mRNA levels in non-fibrotic sham wild type and IL-1Ra KO mice are represented (3-8 animals per group). * p < 0.05, ** p < 0.01, *** p < 0.001, comparing two groups as indicated.