SENSEING OF A MICROBIAL METABOLITE BY FIBROBLASTS THROUGH THE PREGNANE X RECEPTOR RESTRAINTS INFLAMMATION AND FIBROSIS IN MICE

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**Background:** Fibrosis is a serious and irreversible complication of Crohn’s disease (CD) that can lead to intestinal obstruction and has no effective treatment beyond surgical resection. The pathogenesis of fibrosis is incompletely understood however, it is thought to involve an aberrant repair response following inflammation-associated tissue damage. The pregnane X receptor (PXR), a xenobiotic receptor, is recognized for its role in suppressing inflammation and has been show to influence fibrogenesis in the liver. In the intestine, PXR-signaling is influenced by the tryptophan metabolite indole-3-propionic acid (IPA), which can affect intestinal inflammation, in turn influencing fibrogenesis. How microbial metabolite sensing through the PXR influences intestinal fibrotic responses has not been explored.

**Aims:** To understand the impact PXR-signaling has on intestinal fibrosis and determine whether it can be modulated by IPA.

**Methods:** Intestinal inflammation was induced using DSS (3.5%) for 5 days followed by healing for 25 days. Fibrosis in the colon was assessed using Masson’s trichrome and Sirius Red histological stains in wild type (WT), PXR⁻/⁻, epithelial-specific PXR⁻/⁻ and fibroblast-specific PXR⁻/⁻ mice. Immune cell influx was measured by flow cytometry and cytokine concentrations by Luminex. Fibroblasts isolated from WT and PXR⁻/⁻ mice were stimulated with cytotoxic (TNF-α, IL-1β, and IFN-γ) or LPS in the presence or absence of IPA for 24 hours and assessed for gene expression. To examine the role of microbial metabolites in fibrosis, the microbiota was depleted using a cocktail of broad-spectrum antibiotics and some mice treated with IPA.

**Results:** Following recovery from DSS, WT mice showed clear evidence of colonic fibrosis, a response that was exacerbated in PXR⁻/⁻ mice and correlated with greater neutrophil infiltration and levels of innate cytokines (e.g. CXCL2, GMCSF, GCSF,) in the colon. This phenotype was not observed when PXR deficiency was limited to the epithelial cells, but was reproducible in mice with fibroblast-specific PXR-deficiency. Mechanistically, PXR⁻/⁻ fibroblasts were hyper-responsive to proinflammatory cytokines and LPS, producing increased levels of CXCL2, GM-CSF...
and G-CSF compared to WT cells. Importantly, the microbial metabolite IPA was able to block the expression of these cytokines. Depletion of the microbiota completely suppressed systemic levels of IPA and exacerbated intestinal fibrosis, an effect that was reversed by IPA treatment.

**Conclusions:** PXR signaling in intestinal fibroblasts restrains their inflammatory responses, reducing fibrogenesis. Luminal sensing of the bacterial derived indole, IPA, via PXR may be involved in this process, highlighting a microbial metabolite-sensing pathway in fibroblasts that could be targeted to prevent intestinal fibrosis observed in CD.

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