Attenuating liver fibrosis and inflammation: blocking CCL24 inhibits recruitment of hepatic stellate cells, monocytes and neutrophils, and modulates hepatic stellate cell activation

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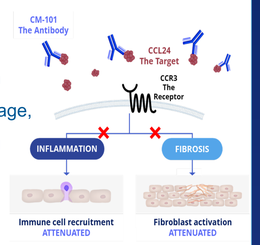
INTRODUCTION

The chemokine system plays a pivotal role in hepatic inflammation and fibrosis development. CCL24, a pro-inflammatory and pro-fibrotic chemokine, was found to be overexpressed in fibrotic liver conditions such as Primary sclerosing cholangitis (PSC)[1] and Metabolic dysfunction associated steatohepatitis (MASH)[2], contributing to liver damage. Numerous liver disease models have shown that blocking CCL24 attenuates disease progression[1-3]. CM-101 is an IgG1 monoclonal antibody that neutralizes CCL24 activity and is currently undergoing clinical development for PSC treatment.

AIM

To investigate the mechanism by which CCL24 blockade ameliorates liver damage, focusing on its impact on cell trafficking and activation of key cell populations: liver macrophages and hepatic stellate cells (HSC).

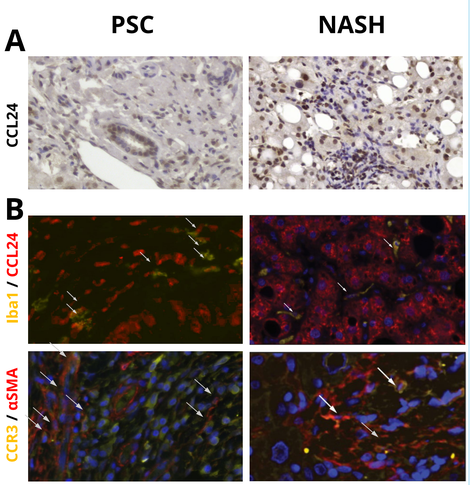


METHODS

- Liver expression: biopsies were examined for CCL24 and CCR3 expression by immunohistochemistry and immunofluorescence:
- Activation of HSC: the fibrotic marker αSMA (smooth muscle actin) was assessed by flow-cytometry in the human LX2 cell line , following 24-hour incubation with CCL24 with CM-101 or isotype control antibody.
- Immune cell recruitment: immune cell composition was examined following intraperitoneal (i.p.) injection of CCL24 by scRNA-seq or by flow-cytometry.
- Animal models: the effect of CM-101 was tested in Mdr2-/- and ANIT-fed mice models of PSC. Liver samples were analyzed by immunohistochemistry and immunofluorescence to detect collagen deposition (Sirius Red stain), cholangiocytes (PanCK), macrophages (Iba1) and neutrophils (CXCR2 or Gr1).

RESULTS

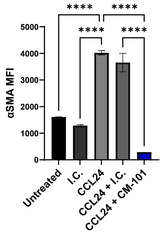
CCL24 and its receptor, CCR3, are highly expressed in PSC and NASH livers

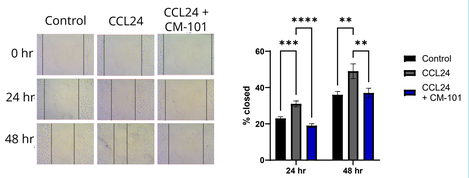


CCL24 and CCR3 expression in NASH and PSC biopsies
(A) Representative pictures of liver histological sections stained for CCL24 by immunohistochemistry. (B) Representative pictures of liver histological sections stained for CCL24 and macrophages (Iba1) by immunofluorescence (upper panels) or for CCR3 and myofibroblasts (αSMA) by immunofluorescence (lower panels). Arrows point to co-stained cells.

CCL24 directly activates HSCs

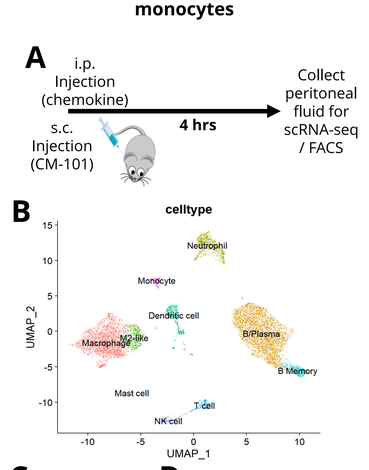
CM-101 suppressed CCL24-induced activation of HSCs in vitro.
HSC activation, tested on the LX2 cell line. α-SMA expression measured by FACS following 24 h incubation with CCL24 (50 ng/ml), with or without CM-101, or an isotype control antibody (10 μg/ml). I.C., isotype control

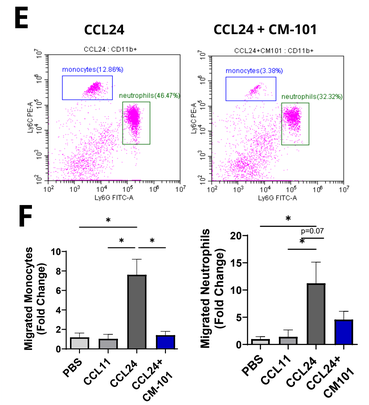




CM-101 suppressed CCL24-induced HSC motility in vitro.
Representative images (left panels) and quantification (right panels) of HSC (LX2 cell line) scratch assay. Images of scratched wells were taken at time 0 h, 24 h and 48 h. Percentage closure of scratched area was evaluated for control group or cells treated with CCL24 (25 ng/ml) with or without CM-101 (5 μg/ml).

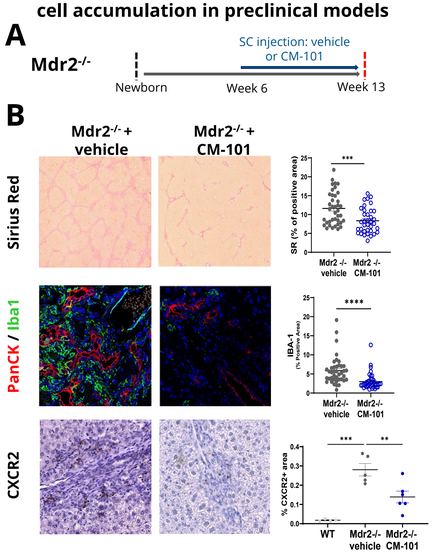
CCL24 recruits neutrophils and monocytes



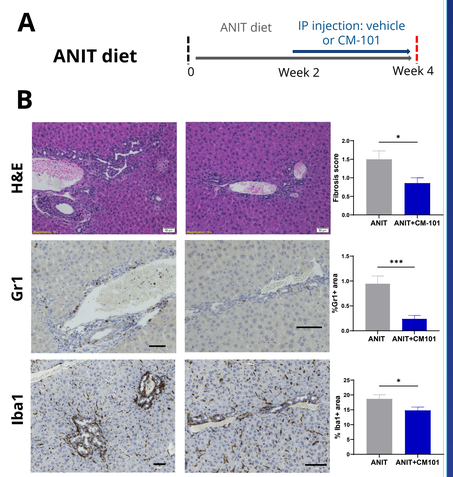


CCL24 i.p. injection recruits neutrophils and monocytes.
(A) Outline of the experimental procedure. (B–D) Identification of immune populations by scRNA-seq. (B) UMAP plot of QC-passed cells. (C) Histograms of CCL24 expression levels by cell type. (D) Stacked bar plot of the relative abundance of each cell type, per treatment. (E-F) Identification of immune populations by flow-cytometry. (E) Representative scatter plots of CD11b+ cells. (F) Quantification of relative change in cell counts for monocytes or neutrophils.

CCL24 blockade reduces fibrosis and immune cell accumulation in preclinical models



CCL24 blockade attenuates neutrophil and macrophage accumulation and liver fibrosis in Mdr2-/- PSC model. (A) Scheme of the experimental design. (B) Representative images (left) and quantification (right) of Sirius Red staining (upper panels), macrophages and cholangiocytes (Iba1 and PanCK, middle panels) and neutrophils (CXCR2, lower panels).



CCL24 blockade attenuates neutrophil and macrophage accumulation and liver fibrosis in ANIT-induced cholestasis model. (A) Scheme of the experimental design. (B) Representative images (left) and quantification (right) of H&E staining (upper panels), neutrophils (Gr1, middle panels) and macrophages (Iba1, lower panels).

CONCLUSIONS

- CCL24 induce motility and activation of HSC.
- CCL24 recruits neutrophils and monocytes to injured biliary areas.
- CM-101, a CCL24 neutralizing antibody, exhibits anti-inflammatory and anti-fibrotic effects.
- CM-101 interferes with the migration and activation of hepatic stellate cells, neutrophils and monocytes.
- Topline results from CM-101 phase 2a trial in PSC patients are expected in midyear 2024.

REFERENCES

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CONTACT INFORMATION

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