Characterization of hepatic macrophage cell populations during liver fibrosis, a precancerous stage of chronic liver disease

Ananya Ajith[1], Jonathan Evraerts[1], Davide Brusa[2], Caroline Bouzin[3], Etienne Sokal[1], Mustapha Najimi[1]

Laboratory of Pediatric Hepatology and Cell Therapy (PEDI), Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

IREC Flow Cytometry platform, Institute de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

IREC Imaging Platform (2IP), Institute de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

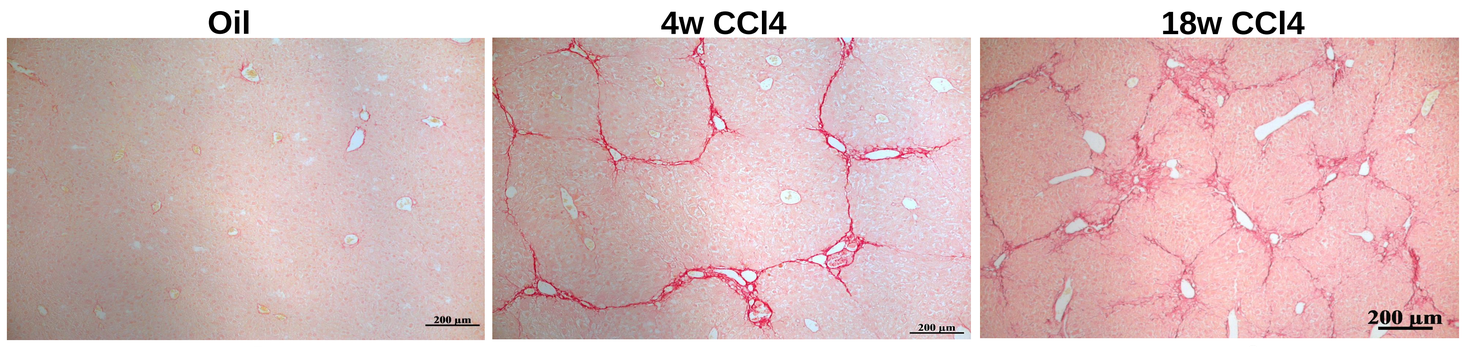
BACKGROUND

Hepatocellular carcinoma (HCC) is the 6th most prevalent cancer worldwide and 80-90% of HCC cases arise from fibrotic and cirrhotic livers. Chronic inflammation is the underlying reason for chronic liver diseases and their progression into HCC. One of the important liver cells dysregulated is the hepatic macrophage population. Liver macrophages are a heterogenous cell population that includes Kupffer cells (KCs) or liver resident macrophages and monocyte-derived macrophages (MoMFs). They play an important role in maintaining homeostasis, initiating of the inflammatory response during liver injury, sustaining liver fibrosis, and promoting its resolution. The macrophage pool largely dominated by KCs during homeostasis is replaced by the infiltrating macrophage populations during liver injury. Deciphering the miRNA- based epigenetic alterations that may explain the involvement of those macrophages in sustaining inflammation and the progression of chronic liver disease toward HCC is of interest.

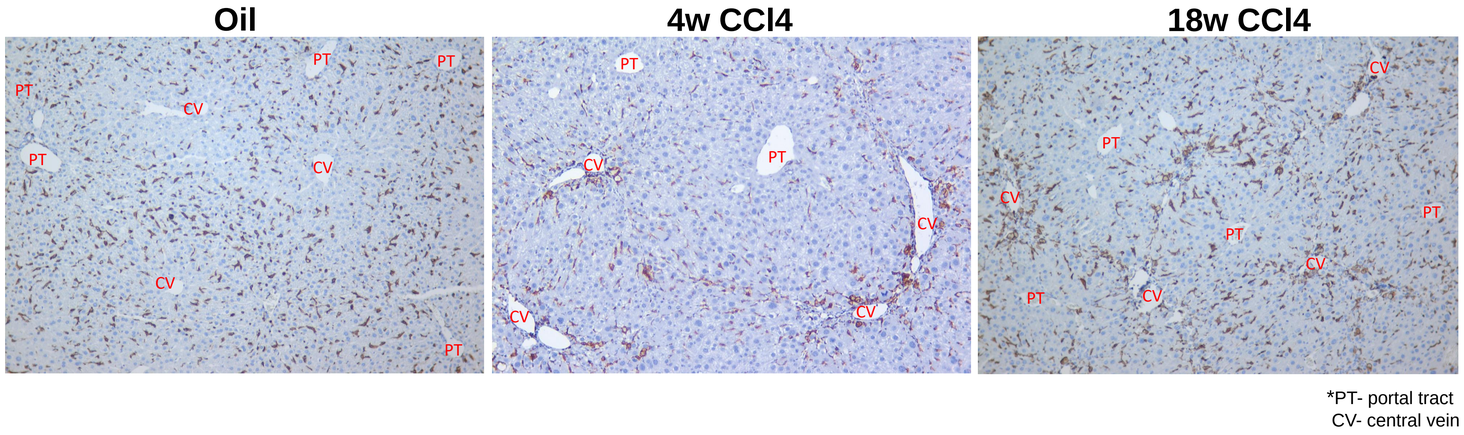
METHODS AND RESULTS

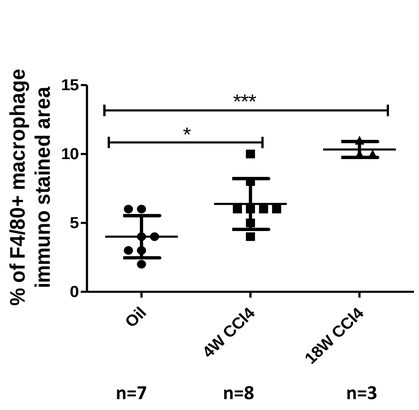
Male C57BL/6 mice were injected with 0.5ml/kg concentration of CCl4 diluted in 10x corn oil intraperitoneally twice a week for 4 and 18 weeks timepoints in order to induce different stages of liver fibrosis. Controls underwent corn oil treatments for the same time periods.

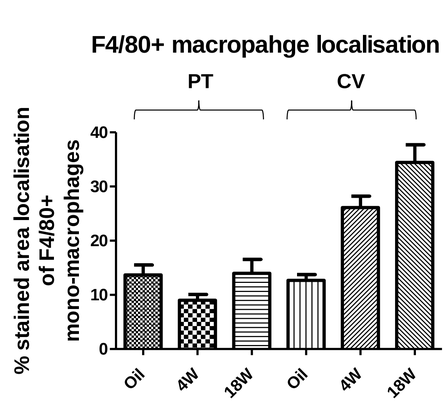
1. Sirius red-stained collagen to analyse the extent of CCl4-induced chronic liver damage



2. Immunohistochemistry and morphometry to analyze tissue distribution and levels of F4/80+ macrophages in CCl4- induced chronic liver damage



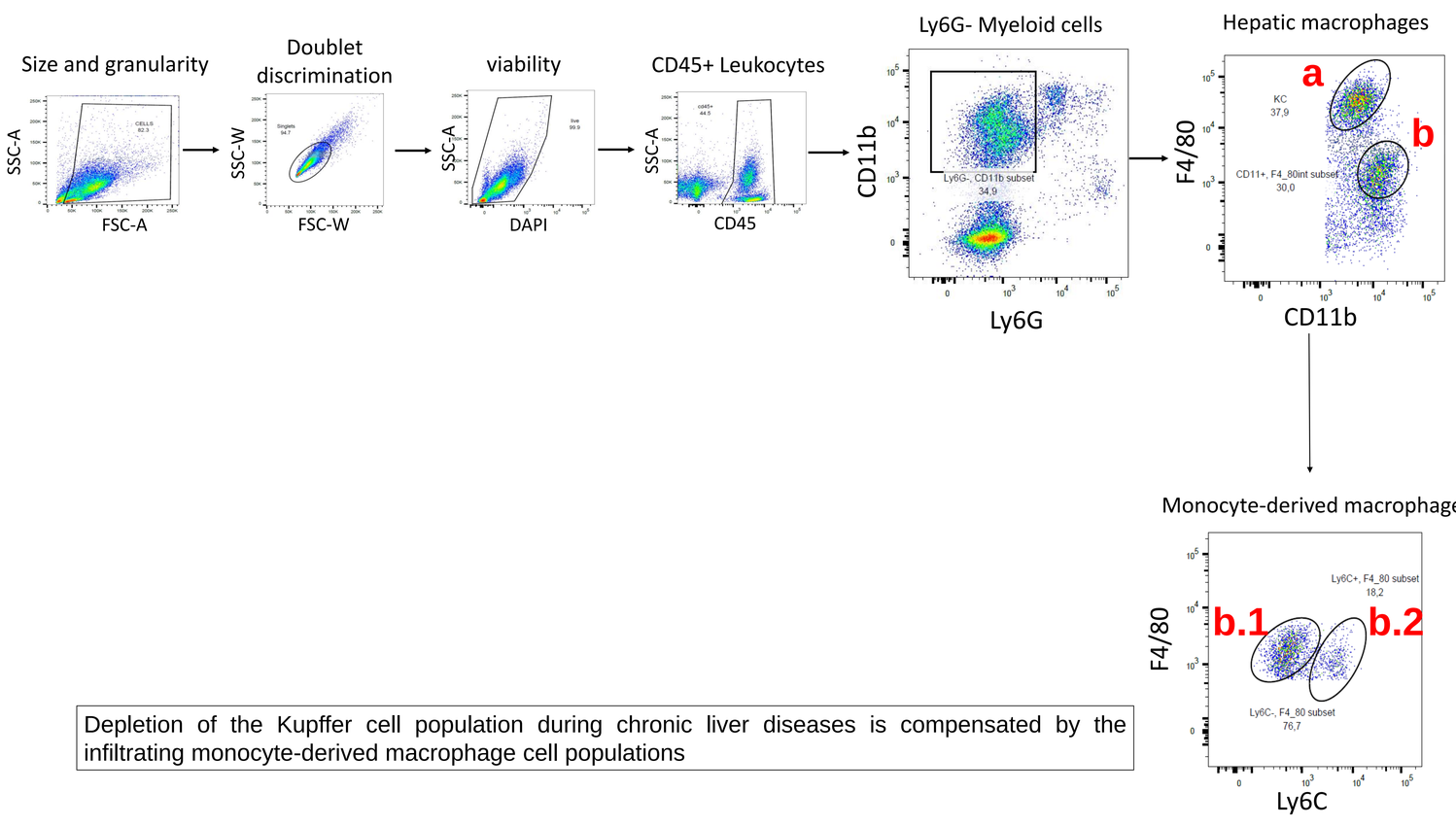


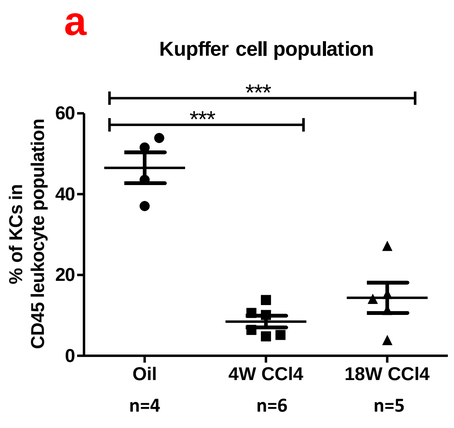


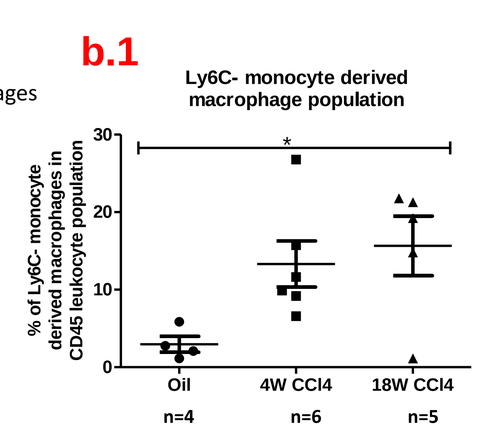
Oil PT v/s Oil CV	-
4W PT v/s 4W CV	***
18W PT v/s 18W CV	***

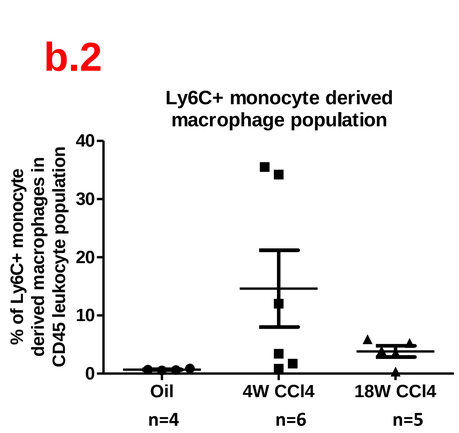
F4/80 is a pan-marker for macrophages in mice. It is expressed by both the resident and infiltrating macrophages in the mouse liver. F4/80+ macrophage cell population is increased at the central vein which is the site of inflammation after CCl4 treatments.

3. Fluorescence-activated cell sorting using specific cell surface markers from the single-cell suspension of isolated liver non-parenchymal fraction yields hepatic macrophages including Kupffer cells and infiltrating mono-macrophages









Depletion of the Kupffer cell population during chronic liver diseases is compensated by the infiltrating monocyte-derived macrophage cell populations

Conclusion & Prospects

Our preliminary results confirm the dynamic regulation of the hepatic macrophage cell populations analyzed consequently to CCl4-induced liver damage. The aim of the study is to further analyze the miRNA-based epigenetic dysregulation of both KCs and infiltrating macrophages during the disease progression from liver fibrosis, a pre-cancerous stage, towards hepatocellular carcinoma.