Characterization of immune cell populations in the microenvironment of mice liver during the progression of liver fibrosis to hepatocellular carcinoma

A. Ajith[1], F. Smets[1], E. Sokal[1], M. Najimi[1]

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

Contact information
Ananya Ajith, ananya.ajith@uclouvain.be

EASL CONGRESS Milan, Italy 5–8 June 2024

UCLouvain IREC

Introduction

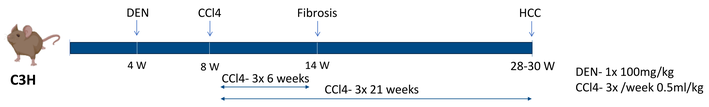
Around 90% of hepatocellular carcinoma (HCC) cases arise in the background of chronic liver diseases (CLD). Chronic inflammation is the underlying cause of CLD and development of HCC. Thus, dysregulated functions of hepatic immune cell populations plays an important role in CLD and development of HCC.

Aim

In this study we aim to characterize the different immune cell populations dysregulated during liver fibrosis and HCC development in mouse model treated with a combination of Diethylnitrosamine (DEN) and carbon tetrachloride (CCl4).

Method

Liver fibrosis and HCC animal model: A single injection of DEN was administered at 4 weeks of age followed by continuous injections of CCl4 for 6 weeks results in the development of liver fibrosis and CCl4 injections for 21 weeks resulted in the development of HCC tumors in the background of CLD.



Flow cytometry analysis: By 2 step collagenase perfusion method, whole liver was dissociated to obtain single cell suspension followed by several steps of centrifugation to separate the non-parenchymal cell (NPC) fraction. NPC were then stained with specific cell surface and nuclear antibodies to identify the different immune cell populations.

Multiplex immunofluorescence: Liver FFPE sections were stained with specific immune cell antibodies to analyse the localisation of different immune cell populations on liver during liver fibrosis and HCC.

Conclusions

Our results suggest a dynamic change in the different immune cell populations during the liver disease progression from fibrosis towards HCC.

KCs and INF macrophages occupy more or less the same area within the tumor while, INF macrophages form the predominant macrophage population in the invasive margin. T lymphocytes were observed to be increased in fibrosis and HCC livers. CD8 T lymphocyte numbers are higher within the tumor, while Tregs vastly occupy the invasive margin.

Acknowledgements

The authers would like to thank the Cytoflux Flow cytometry facility and 2IP imaging facility of IREC institute, UCLouvain for their help and Télévie FNRS for funding the project.

Results

Figure 1: Mice liver models of fibrosis liver, and hepatocellular carcinoma nodule development in the background of CLD

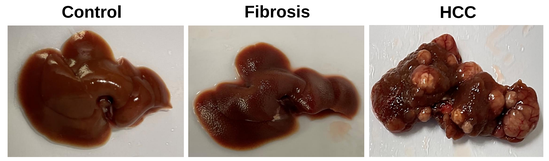


Figure 2: Sirius red staining of collagen tissue in both fibrosis liver and HCC liver with tumor nodules encapsulated by collagen

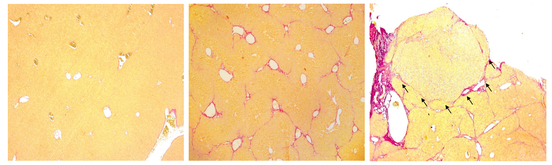


Figure 3: Significant changes in the hepatic immune cell populations of myeloid and lymphatic origins are observed during liver fibrosis progression towards hepatocellular carcinoma

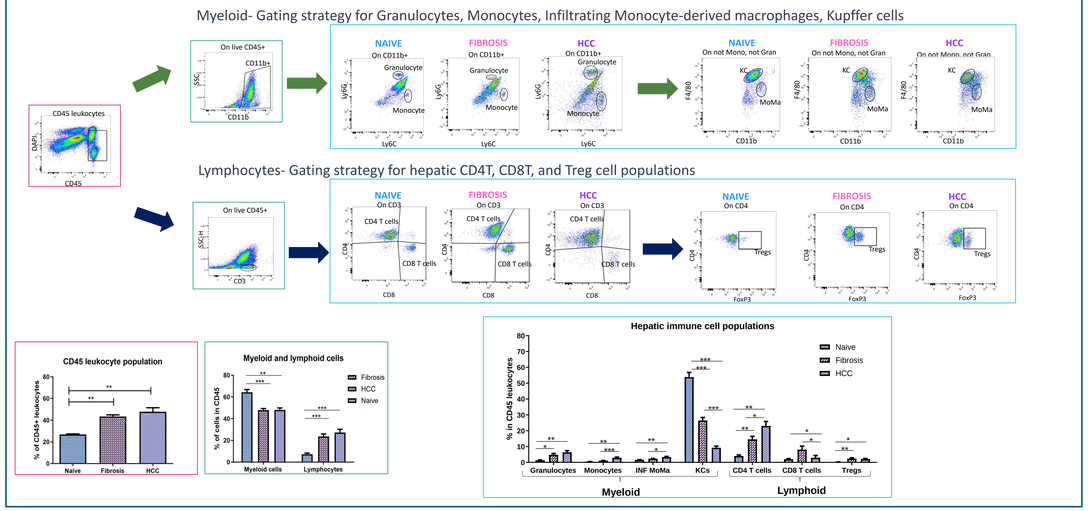
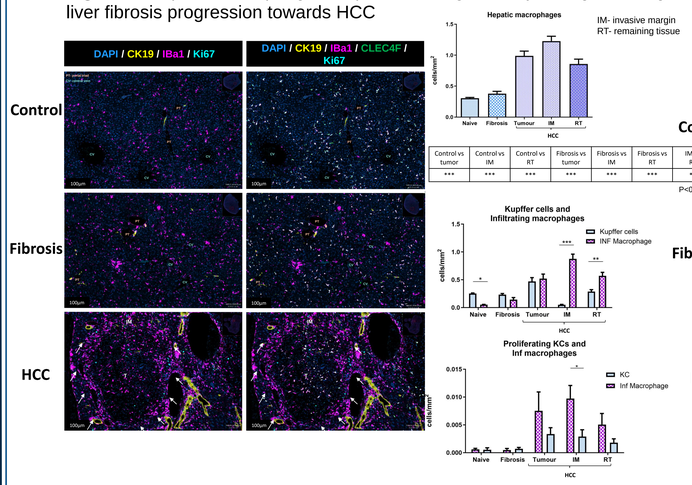
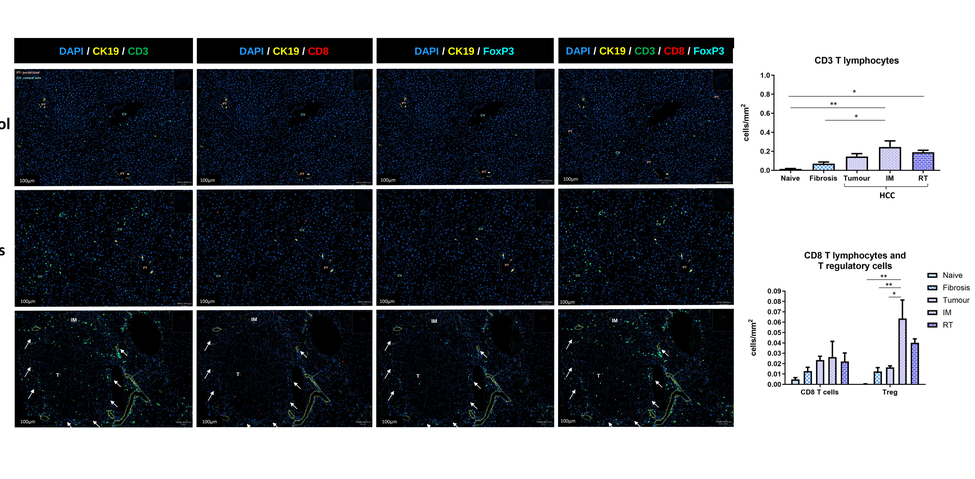


Figure 4: Hepatic macrophage composition is significantly changed during liver fibrosis progression towards HCC



IBA1⁺ CLEC4F⁺ liver resident Kupffer cells are the predominant macrophage population in healthy liver. While the infiltrating IBA1⁺ CLEC4F⁻ monocyte/macrophage occupancy is observed to be increased during liver fibrosis and HCC development.

Figure 5: Dysregulated levels of T lymphocytes are observed during liver fibrosis progression towards HCC



Increase in the overall T lymphocyte population is observed during liver fibrosis and HCC. Increased CD8 cytotoxic T cell localisation is observed in the tumor liver whereas T regulatory cells are more positioned in the invasive margin.