Flow cytometry reveals distinct changes in hepatic inflammatory cell populations in a diet-induced obese mouse model of non-alcoholic steatohepatitis

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INTRODUCTION AND AIM

Inflammation is an important component in the pathogenesis of non-alcoholic steatohepatitis (NASH). Inflammatory cell infiltrates can be characterized by immunohistochemistry, but this requires the application of multiple cell markers and stereological methods for adequate qualitative and quantitative immune cell analysis. Multiple cell markers are readily accessible with flow cytometry, which we employed to identify liver immune cell profiles in two mouse models of severely fibrotic NASH. We observed that monocytes/macrophages and Kupffer cells were strongly affected by NASH-induction in parallel to T-cells, B-cells, and neutrophils.

METHODS

Extended NASH models: ob/ob mice and C57Bl6 mice were fed either chow or AMLN diet (40% total fat kcal of which 18.5% were trans-fat kcal; 20% fructose; 2% cholesterol) for 20 and 58 weeks, respectively (Fig. 1). Animals with a fibrosis score of 3 and a steatosis score ≥ 2 were included in the studies and treated with Elafibranor (30 mg/kg), vehicle, or returned to chow diet for 8 weeks. Tissue was processed for histology (Galectin-3 IHC). Left lateral liver lobular samples were sampled and digested in collagenase A/DNAse-containing solution, and cells were stained with two sets of fluorescently labelled antibody panels to detect and quantify immune cells of lymphoid (T-cell, B-cell, and natural killer cells: CD3, CD4, CD8, CD19, CD45, B220, NK1.1) and myeloid (monocytes, macrophages, and neutrophils: Ly6G, Ly6C, CD11b, F4/80, CD14) lineage by flow cytometry.

Liver myeloid and lymphocyte populations change in ext. ob/ob NASH

Figure 3 | Study outlines for the extended DIO NASH and ob/ob NASH mouse models. To obtain fibrosis stage 3, mice were fed the AMLN diet for 58 weeks (extended DIO NASH) or 20 weeks (extended ob/ob NASH).

Liver myeloid and lymphocyte populations change in ext. ob/ob NASH

Data are mean ± SEM. **P < 0.01 vs. NASH vehicle. Right panel | Percentages of major liver immune cell populations constituted by specific cell types. Means of n = 7-8.

Hepatic inflammatory cells are modulated by Elafibranor in ext. ob/ob NASH

Figure 5 | Liver immune cells in extended ob/ob-NASH mice. Top | Liver lymphocyte populations as a percentage of total myeloid cells. Values expressed as mean of n = 7-8 + SEM. Dunnett’s test one-factor linear model. **P < 0.01 compared to NASH Vehicle.

CONCLUSION

Hepatic inflammation in extended DIO-NASH and ob/ob NASH models is characterized by:

- Recruitment of hepatic macrophages (ob/ob NASH) and activation of monocytes (DIO NASH)
- Recruitment of CD8+ cytotoxic T-cells
- Marked reduction in neutrophils and B-cells
- Some of these populations are partially restored by Elafibranor treatment

Flow cytometry-based phenotyping of hepatic immune cell populations permits detailed quantitative assessment of the anti-inflammatory effects of treatment modalities, including the identification of specific activated or regulatory immune cell sub-populations.