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 easily 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-cells, and natural killer cells: CD3, CD4, CD8, CD19, CD45, B220, NK1.1) and myeloid (monocytes, macrophages, and neutrophils: Ly6C, Ly6G, CD11b, F4/80, CD14) lineage by flow cytometry.

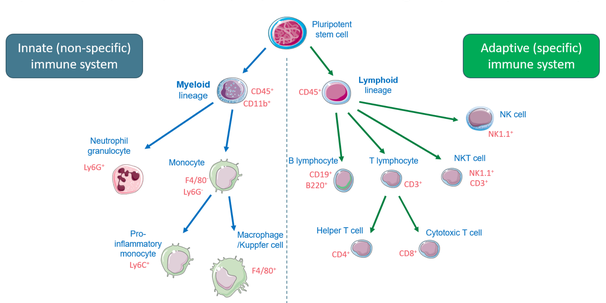

Figure 1 | Immune cell populations present in mouse NASH livers. Multi-color flow cytometry was used to quantify subpopulations of myeloid cells as well as lymphocytes. CD11b expression was used to differentiate between myeloid cells and lymphocytes. In addition, myeloid lineage cells in the liver are positive for the macrophage marker Galectin-3 as detected by IHC (Figure 2).

Livers are infiltrated with Galectin-3⁺ myeloid cells in both ext. NASH models

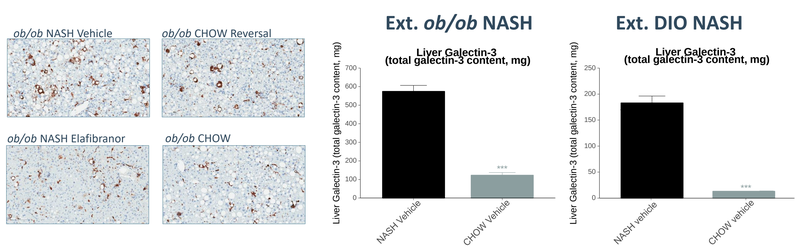

Figure 2 | *Left* | Representative images of liver stained with anti-Galectin-3 to quantify inflammatory cells at termination (magnification 20x, scale bar = 100 µm). *Right* | Image analysis quantification of Galectin-3 staining. Values expressed as mean of n = 11-14 + SEM. Dunnett's test one-factor linear model. ***: P < 0.001 compared to NASH Vehicle.

STUDY DESIGN

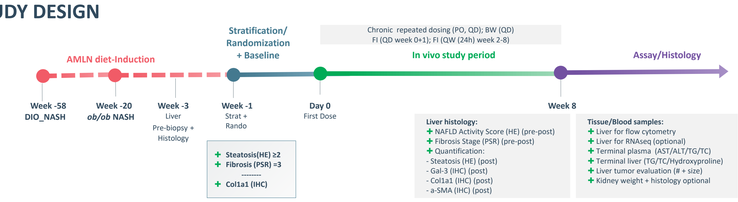

Figure 3 | Study outlines for the extended DIO NASH and *ob/ob* NASH mouse models. To obtain fibrosis stage 3, mice were fed the AMLN diet for 58 weeks (extended DIO NASH) or 20 weeks (extended *ob/ob* NASH).

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

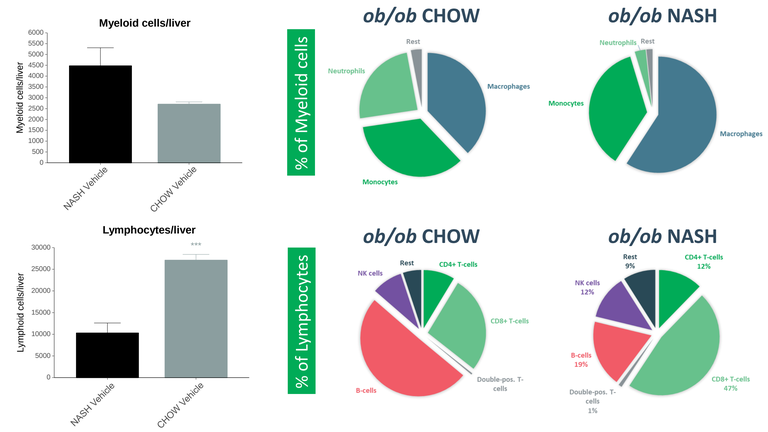

Figure 4 | *Left panel* | Major liver immune cell populations per liver quantified by flow cytometry. Data are mean + SEM. ***p < 0.001 vs. NASH vehicle. *Right panel* | Percentages of major liver immune cell populations constituted by specific cell types. Means of n = 7-8.

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

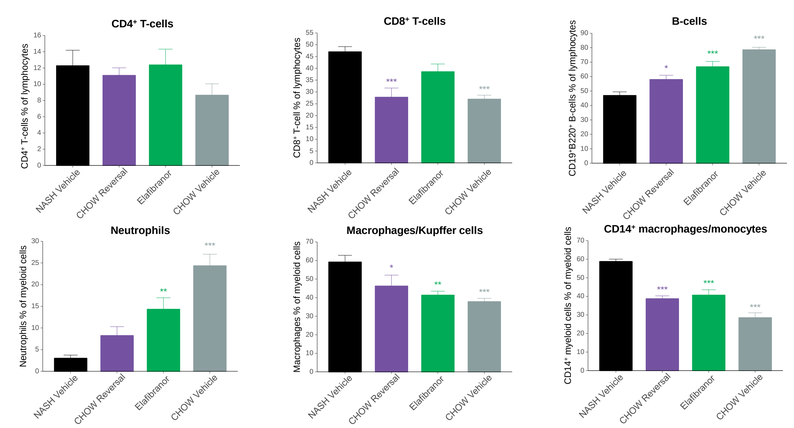

Figure 5 | Liver immune cells in extended *ob/ob*-NASH mice. *Top* | Liver lymphocyte populations as a percentage of total lymphocytes. *Bottom* | Liver myeloid cell populations as a percentage of total myeloid cells. Values expressed as mean of n = 7-8 + SEM. Dunnett's test one-factor linear model. *: P < 0.05; **: P < 0.01; ***: P < 0.001 compared to NASH Vehicle.

Neutrophil percentage is partially restored by Elafibranor in ext. DIO NASH

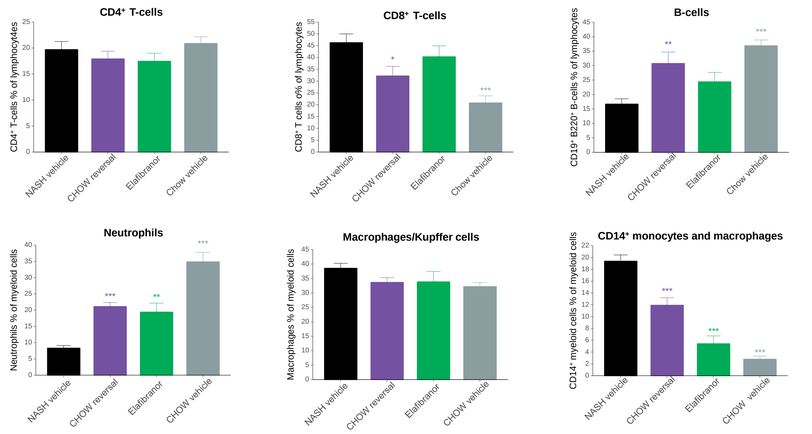

Figure 6 | Liver immune cells in extended DIO-NASH mice. *Top* | Liver lymphocyte populations as a percentage of total lymphocytes. *Bottom* | Liver myeloid cell populations as a percentage of total myeloid cells. Values expressed as mean of n = 7-8 + SEM. Dunnett's test one-factor linear model. *: P < 0.05; **: P < 0.01; ***: P < 0.001 compared to NASH Vehicle.

Early infiltration of pro-inflammatory monocytes in ext. DIO-NASH

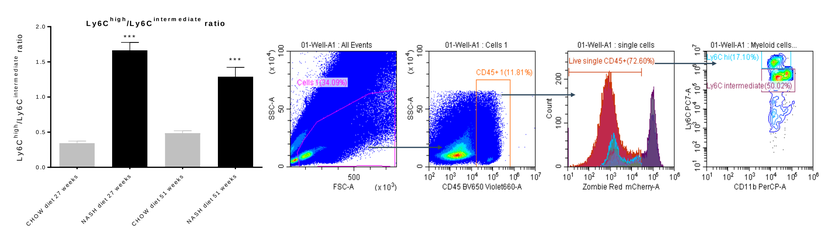

Figure 7 | *Left panel* | As an example of detailed phenotyping of hepatic immune cells, the ratio of activated monocytes (Ly6C^{high})to non-activated monocytes (Ly6C^{intermediate}) was measured. Values expressed as a mean of n = 5. Dunnett's test one-factor linear model. ***: P < 0.001 compared to NASH Vehicle. *Right panel* | Gating strategy to identify liver-infiltrating Ly6C^{high} and Ly6C^{intermediate} monocytes among the myeloid cells.

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.