Poster 19: LiSyM Midterm Evaluation 2018
Bode Group (Düsseldorf)

Facts & Figures:

Area of Expertise:	Experimental
Methodes:	Immunfluorescence, Immunhistochemistry, transcriptome analysis, FACS, FACS-based cell sorting, Multiplex analysis, ELISA, animal experiments (HFD), Confocal LSM & Elyra Superresolution Microscopy
Scientific Involvement:	Pillar II and Pillar III
LiSyM Resources:	245.996 €
LiSyM Scientists:	PhD Student Sonja Kleeschulte
Collaboration:	Dooley, Drasdo, Hengstler, Höhme, Klingmüller, Knolle, Timmer

Introduction:

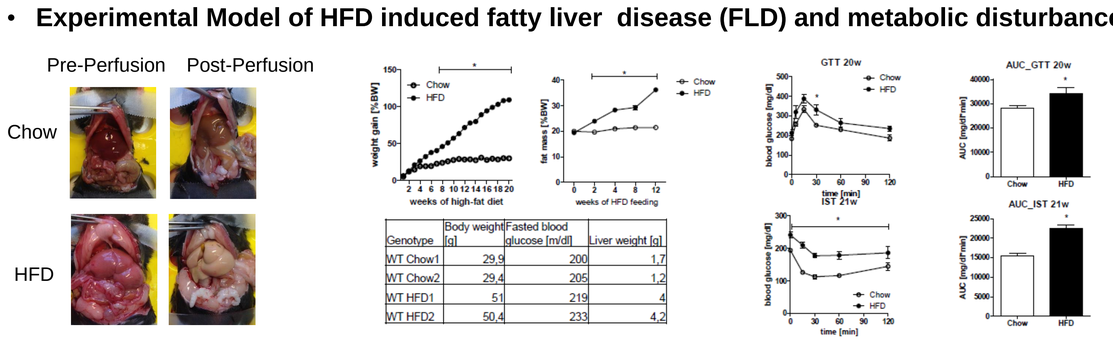
- Macrophages display a high plasticity and there polarization is strongly influenced by the local microenvironment.
- Own unpublished data indicate that hepatocyte-derived factors have a strong impact on macrophage polarization and function
- The change of macrophage polarization and stage-dependent recruitment of neutrophils during development of HFD induced fatty liver disease (FLD) and its relevance for progression to non-alcoholic steatohepatitis (NASH) and development of acute on chronic liver failure (ACLF) is not fully understood.

Aim:

- Time resolved analysis of the recruitment of macrophages and neutrophils and changes of macrophage polarization as well as assessment of metabolic disturbance during development of HFD-induced FLD and evaluation of its relevance for progression to NASH and/or ACLF development by a systems biology based approach

Progress and Results:

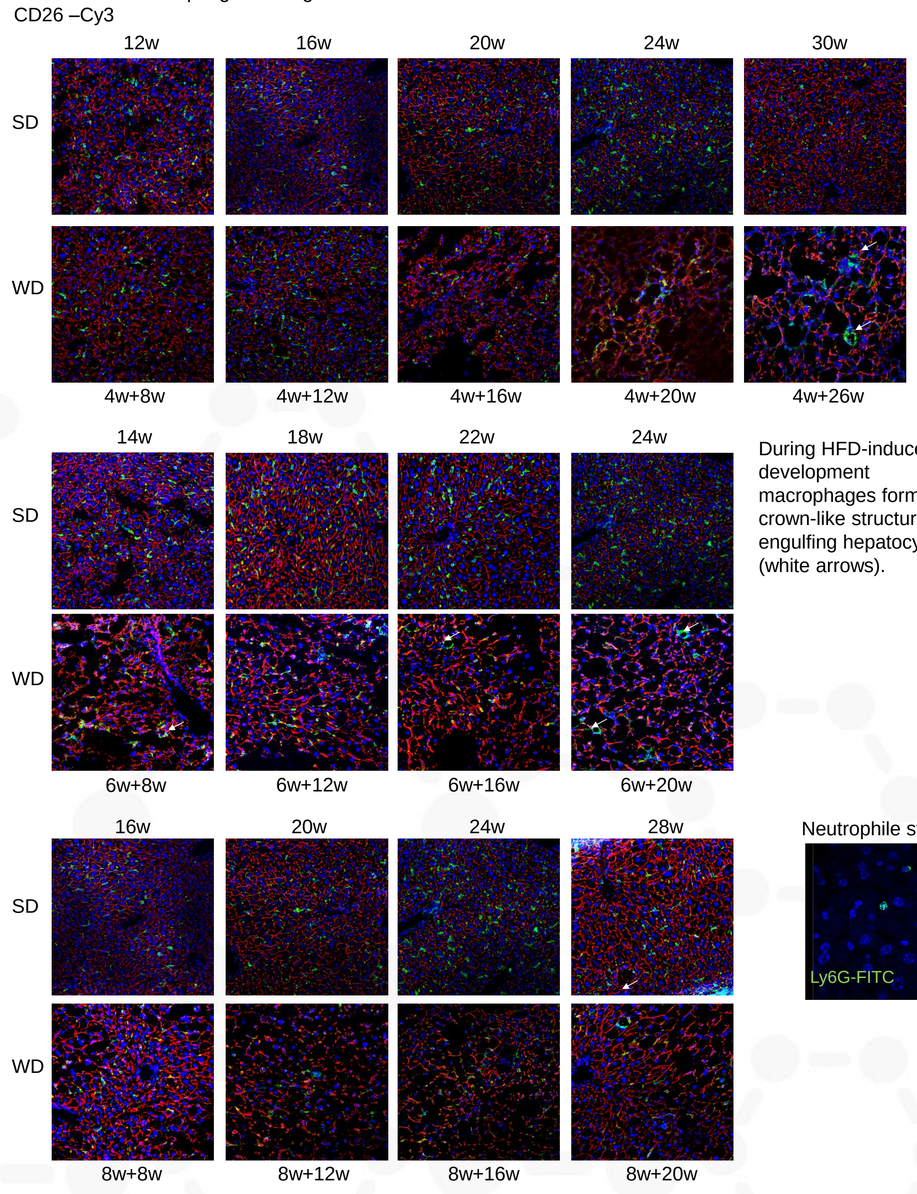
- **Experimental Model of HFD induced fatty liver disease (FLD) and metabolic disturbance**



Genotype	Body weight [g]	Fasted blood glucose [m/dl]	Liver weight [g]
WT Chow1	29,9	200	1,7
WT Chow2	29,4	205	1,2
WT HFD1	51	219	4
WT HFD2	50,4	233	4,2

➢ HFD leads to increased weight gain, increased liver weight and causes metabolic disturbance

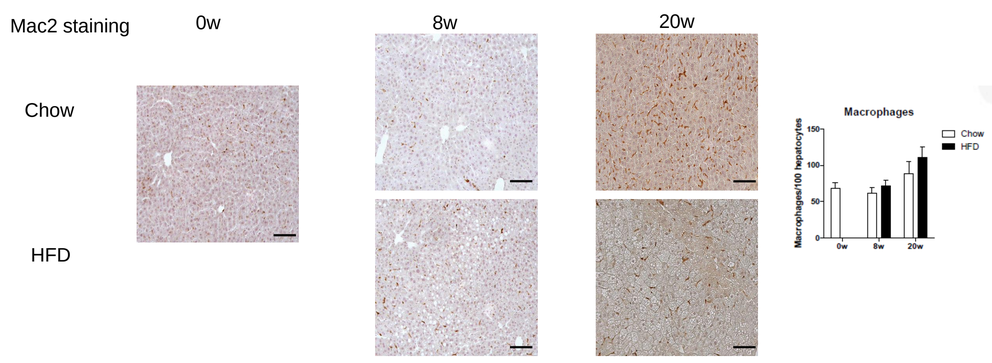
- **Changes of macrophage numbers and their morphological appearance**
  - F4/80-AF488 macrophage staining
  - CD26 –Cy3



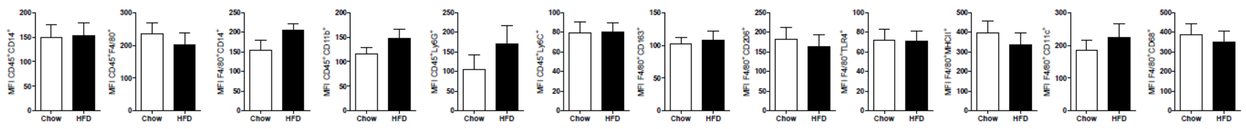
During HFD-induced FLD development macrophages form crown-like structures engulfing hepatocytes (white arrows).

Neutrophile staining

- **Recruitment of neutrophils and macrophages and characterization of macrophage polarization**

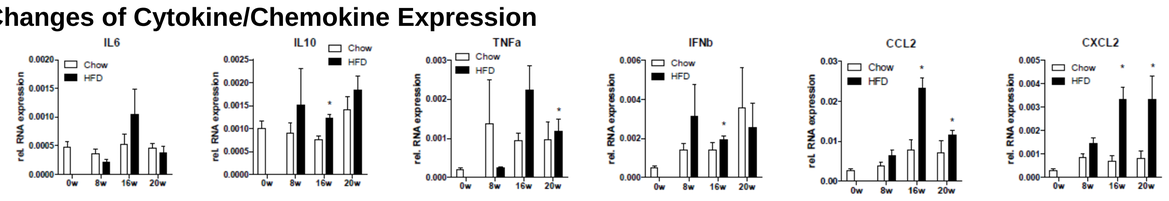


➢ Livers from HFD fed mice show lipid accumulation while the number of macrophages only slightly increases after 8 and 20 weeks of HFD (additional time points in work: 4, 8, 12, 20, 26, 30 weeks)



➢ After **16 weeks** of HFD in particular the number of neutrophil granulocytes increases when compared to chow fed animals, while recruitment of $CD14^+/F4/80^+$ macrophages is rather slightly enhanced. (additional time points in work: 4, 8, 12, 20, 26, 30 weeks)

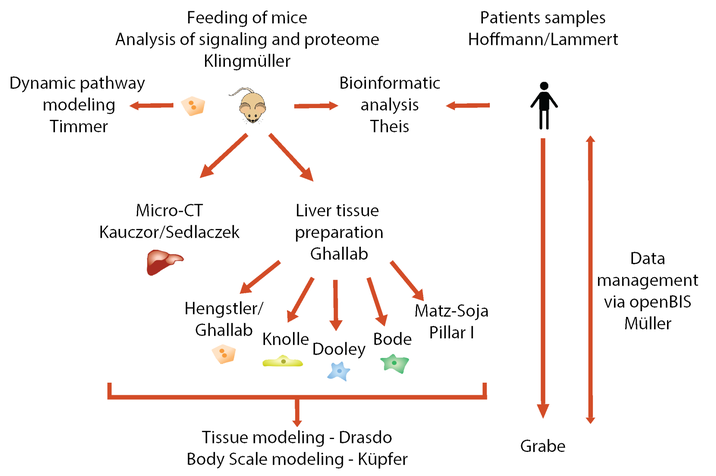
- **Changes of Cytokine/Chemokine Expression**



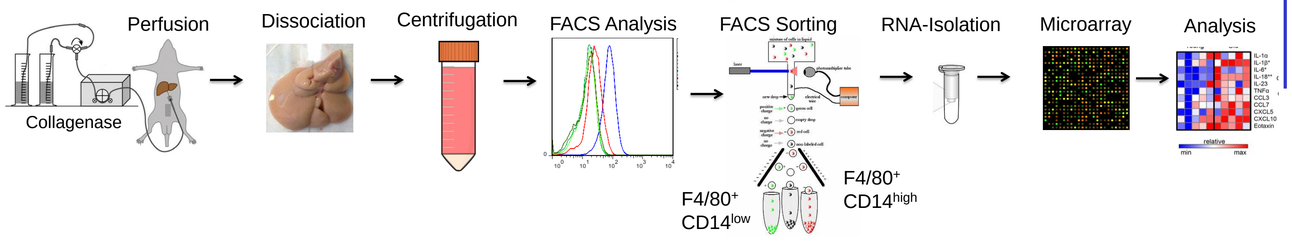
➢ In line with the observed enhanced recruitment of neutrophiles mainly the CXCR2 ligand CXCL2 is upregulated during HFD development (time points in work: 4, 8, 12, 16, 20, 26, 30)

Ongoing work & Outlook:

- **Summarizing scheme of the ongoing pilot experiment:**



- **Complete time resolved assessment of serum parameters during FLD and ACLF development:**
  - Clinical Chemistry (AST, ALT, Bili, Alb, TP)
  - Adipokine (Leptin, Insulin, Adiponektin) ELISA
  - Fetuin and Thrombin ELISA
  - Inflammatory cytokine/chemokine Multiplex
- **Complete (spatio)-temporal resolved data series on:**
  - Macrophage & neutrophile recruitment during development and progression of FLD and ACLF
  - Expression of cytokines, chemokines, growth factors in liver tissue
  - Macrophage polarization
- **Optimization of FACS-based cell sortening**
  - Different macrophage markers: CD14, CD11b, CD11c, CD163, CD206, F4/80, CD68
- **Transcriptome of sorted resident and infiltrated macrophages**



- **Integration of data into mathematical models of the recruitment of macrophages and neutrophils during FLD development and:**
  - its progression to NASH (Höhme/Drasdo/Klingmüller/Timmer/Küpfer)
  - development of ACLF (Höhme/Drasdo/Klingmüller/Küpfer/Timmer)
- **Depending of the results analysis of genetically engineered mice: $MK2^{-/-}$, $STAT3^{\Delta hep}$, $STAT3^{\Delta mac}$, $STAT3^{\Delta ec}$, $PTEN^{\Delta hep}$, $PTEN^{\Delta mac}$, $PAR4^{-/-}$, $LRP8^{-/-}$**

Publications:

- K. Breitkopf-Heinlein, C. Meyer, C. Konig, H. Gaitantzi, A. Addante, M. Thomas, E. Wiercinska, C. Cai, Q. Li, F. Wan, C. Hellerbrand, N. A. Valous, M. Hahnel, Christian Ehlting, Johannes Bode, S. Muller-Bohl, U. Klingmuller, J. Altenoder, I. Ilkavets, M. J. Goumans, L. J. Hawinkels, S. J. Lee, M. Wieland, C. Mogler, M. P. Ebert, B. Herrera, H. Augustin, A. Sanchez, Steven Dooley, P. Ten Dijke, BMP-9 interferes with liver regeneration and promotes liver fibrosis. Gut. 2017 May;66(5):939-954. doi: 10.1136/gutjnl-2016-313314. Epub 2017 Mar 23.
- S. Sobotta, A. Raue, X. Huang, J. Vanlier, A. Junger, S. Bohl, U. Albrecht, M. J. Hahnel, S. Wolf, N. S. Mueller, Lorenza D'Alessandro, S. Mueller-Bohl, M. E. Boehm, P. Lucarelli, S. Bonefas, G. Damm, D. Seehofer, W. D. Lehmann, S. Rose-John, F. van der Hoeven, N. Gretz, F. J. Theis, Christian Ehlting, Johannes Bode, J. Timmer, M. Schilling, U. Klingmuller, Model Based Targeting of IL-6-Induced Inflammatory Responses in Cultured Primary Hepatocytes to Improve Application of the JAK Inhibitor Ruxolitinib. Front Physiol. 2017 Oct 9;8:775. doi: 10.3389/fphys.2017.00775. eCollection 2017.
- Katharina Beuke, Frank A. Schildberg, Federico Pinna, Ute Albrecht, Roman Liebe, Michaela Bissinger, Peter Schirmacher, Steven Dooley, Johannes Bode, Percy A. Knolle, Ursula Kummer, Kai Breuhahn, Sven Sahle, Quantitative and integrative analysis of paracrine hepatocyte activation by nonparenchymal cells upon lipopolysaccharide induction, FEBS J 284(5) : 796

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